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INVITED REVIEW

Dietary modification of metabolic pathways via nuclear hormone receptors

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Nuclear hormone receptors (NHRs), as ligand-dependent transcription factors, have emerged as important mediators in the control of whole body metabolism. Because of the promiscuous nature of several members of this superfamily that have been found to bind ligand with lower affinity than the classical steroid NHRs, they consequently display a broader ligand selectivity. This promiscuous nature has facilitated various bioactive dietary components being able to act as agonist ligands for certain members of the NHR superfamily. By binding to these NHRs, bioactive dietary components are able to mediate changes in various metabolic pathways, including, glucose, cholesterol and triglyceride homeostasis among others. This review will provide a general overview of the nuclear hormone receptors that have been shown to be activated by dietary components. The physiological consequences of such receptor activation by these dietary components will then be discussed in more detail. Copyright © 2012 John Wiley & Sons, Ltd.

KEY WORDS—nuclear hormone receptors; cafestol; isoflavones; probiotics; GSPE; phytosterols

INTRODUCTION

Nuclear hormone receptors

Nuclear hormone receptors (NHRs) are ligand dependent, and in some cases, ligand independent, transcription factors that mediate changes in gene expression via interaction with nuclear proteins that act as co-activators and co-repressors.[1] The human genome contains 48 members of this superfamily, whereas the mouse genome contains 49 members,[2–4] including receptors for steroid and thyroid hormones, retinoic acid, vitamin D, bile acids, oxysterols and fatty acids. NHRs are involved in key biological processes such as development, reproduction, cell growth, cell cycle progression, differentiation, apoptosis, immunity and metabolism,[5] making them necessary for all stages of life from embryonic development to whole body metabolic homeostasis in the adult. Using molecular phylogeny based on sequence homology, the receptors have been classified into seven subfamilies; namely, NR1, NR2, NR3, NR4, NR5, NR6 and NR0.[6] The majority of the members of the NHR superfamily contain five domains: a DNA binding domain (DBD), which contains two zinc finger DNA binding motifs,[7] and governs the direct interaction with NHR dimers and their corresponding response elements (which can either be located within the promoter region of their respective target genes, or for receptors such as estrogen, androgen, glucocorticoid receptors and peroxisome proliferator receptor γ, receptor binding sites can be located distally from the promoter, in intergenic and intronic regions[8]); a ligand binding domain (LBD), which forms a pocket for direct interaction with specific endogenous, or pharmacological ligands; and two activation domains: one located in the amino terminus (AF-1), and one in the carboxy terminus (AF-2) (Figure 1). The AF-2 domain is involved in ligand binding as well as co-activator binding, whereas transcriptional activation of the AF-1 domain is independent of ligand,[9] and the AF-1 region tends to be highly variable in both length and sequence between various NHRs. The N-terminal DBD targets the receptor to a response element; and coupled by a flexible hinge region to the LBD, the binding of small lipophillic hormones is facilitated, serving as a switch to initiate gene transcription.[3,10] Regulation of transcription by NHRs is a complex process that relies on ligand-dependent recruitment of different co-regulatory proteins (co-activators) to a surface on the LBD that, in turn, mediates interactions with the basal transcriptional machinery, resulting in repression or activation of transcription.[11] The binding of agonist ligand to the LBD induces a conformational change in the receptor that releases bound transcriptional co-repressor proteins and creates a binding surface for

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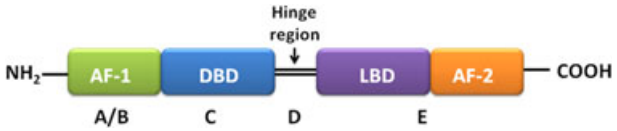

Figure 1. **Structure of nuclear hormone receptors**. DBD: DNA binding domain; LBD: ligand binding domain; AF-1: activation function 1; AF-2: activation function 2; A/B: activation domain of ligand-independent transcription; C: DBD; D: hinge region; E: LBD

co-activators.[12–14] The binding of co-activators to the receptor is mediated by a nuclear receptor interaction domain (RID) that contains a conserved LXXLL sequence.[15,16] The LBD is composed of 12 helices and several β-turns. Analysis has shown that 11 of the helices form the ligand binding pocket, whereas the c-terminal helix, helix 12, forms a flexible lid over the entrance to the ligand binding pocket.[7] Ligand-dependent NHRs are poorly ordered in the absence of ligand, and ligand-induced stabilization of the receptor has been shown to correlate with activity.[17–19]

There are three groups of NHRs that are divided as such based on their known ligands: the '*classical*' nuclear hormone receptors (also referred to as *steroid* receptors) were the first receptors to be cloned and were identified in an effort to define the mechanism of action of known hormones, for example, estrogen receptor (ER), which binds to estrogen.[20] The *classical steroid* receptors tend to bind ligand very tightly.[13] The second class are the former or '*adopted*' orphan receptors that were cloned prior to identification of their known ligand, and thus became *adopted* upon identification of their respective ligands, for example, Farnesoid x receptor (FXR), the receptor for bile acids.[21,22] These receptors bind physiological ligands with a much lower affinity and, therefore, demonstrate a greater degree of promiscuity than the *classical* receptors.[13] The third class are the '*orphan*' receptors, for which there is still no identified ligand, for example, small heterodimer partner (SHP).[23] The majority of the orphans and former orphans were isolated very simply by cross-hybridization with probes derived from conventional receptors.[24] There are on-going efforts by several groups aiming to identify ligands for those receptors that still remain orphans. Much of our current knowledge regarding NHRs and their physiological importance has resulted from the combined information obtained from pharmacological studies that involved the administration of an agonist or antagonist ligand to animal models and/or the characterization of genetically engineered mouse models, in which the expression of a particular NHR has either been induced via transgenesis, or deleted via gene targeting strategies. The cloning of newer members of the NHR superfamily combined with this physiological insight into NHR function has revealed a pattern of co-ordinated regulation of transcription by these receptors that has become a recurring theme in the control of whole body metabolism.[25]

It has become increasingly evident over the past few years that NHRs represent an important target for therapeutic intervention for multiple diseases, including cancer, inflammation as well as several metabolic diseases.[26] An active area of research has been the development and identification of synthetic compounds that can mimic the cognate ligand and induce comparable NHR activity but that can do so selectively in a cell-type or tissue-specific manner, while exerting only the desired therapeutic effects and avoiding any unwanted adverse side effects. It is interesting from a nutritional perspective that there are a variety of bioactive dietary components that can naturally act as ligands for NHRs. As described in this review, this ligand activation can be from either a beneficial or a detrimental standpoint, and there can be little manipulation regarding which ends up being the case with any given dietary component. The ability of various dietary components being able to act as ligands is based mainly on the fact that the newer members of the superfamily, unlike the *classical* NHRs, which tend to be more specific and bind ligand very tightly, were found to bind ligand with a much lower affinity and, therefore, display a broader ligand selectivity. Consequently, this allows for a multitude of different compounds that can potentially act as ligands for these NHRs. Below we will first briefly introduce each NHR that has been identified as being activated by a dietary component and then we will go on to discuss those dietary components in more detail. Although certain NHRs have been shown to be activated by herbal remedies, these will not be discussed herein, and this review will focus solely on dietary components that can activate NHRs.

Constitutive androstane receptor

Constitutive androstane receptor (CAR; nuclear receptor subfamily 1, group I, member 3, NR1I3) was first cloned in the early 1990's by David Moore's group.[24] CAR was cloned from a human liver cDNA library that was screened with a degenerate oligonucleotide based on the sequence of part of the DBD of the retinoic acid receptor/thyroid hormone receptor (RAR/TR) class of NHRs. When first cloned it was called MB67 (due to it being the 67th clone identified by the first author Myriam Baes) and was found to be highly expressed in liver. It was found to transactivate retinoic acid response elements (RARE) consisting of direct repeat hexamers separated by 5 base pairs (DR-5) that controlled expression of the genes for retinoic acid receptor β2 and alcohol dehydrogenase 3.[24] During the initial characterization of MB67, it was found to be constitutively active,[24] that is, it could bind DNA as a heterodimer with retinoid x receptor (RXR) and activate gene transcription in a constitutive manner. Subsequent studies demonstrated that the constitutive activity of CAR results from ligand-independent recruitment of transcriptional co-activators.[27] While searching for potential ligands for CAR, it was found that the steroids androstanol and androstenol inhibit this constitutive activity of CAR. However, they do not interfere with heterodimerization or DNA binding, but instead, promote co-activator release from the LBD.[27] These androstane ligands are examples of naturally occurring inverse agonists that reverse transcriptional activation by nuclear receptors. Based on this observation, MB67 was subsequently referred to as '*constitutive androstane receptor*' (CAR). Murine CAR (mCAR) was cloned by the same group in 1997[28]

and is closely related to the previously described human receptor MB67, which has subsequently been referred to as hCAR. Like hCAR, mCAR is a constitutive transcriptional activator, and this activity is dependent on the presence of the conserved C-terminal AF-2 transcriptional activation motif.[28]

In the mouse, CAR is almost exclusively expressed in the gastrointestinal system (stomach, duodenum, jejunum, ileum, colon and gall bladder) and liver, with lower levels in the seminal vesicle and eye.[29] CAR is a transcriptional regulator of cytochrome P450 (CYP) expression that couples xenobiotic exposure to oxidative metabolism. CAR is also an important regulator of Phase II enzymes (sulfotransferases, glucuronosyltransferases and glutathione S-transferases), and transporters (multidrug resistance proteins, multidrug resistance-associated proteins, and organic anion-transporting polypeptides).[30] CAR binds to DNA as a monomer or a heterodimer with RXR and regulates the transcription of target genes involved in drug metabolism and detoxification (reviewed in[31]) as well as bilirubin clearance.[32] There is significant species-specific activation of CAR, with the xenobiotic compound 1,4-Bis [2-(3,5-dichloropyridyloxy)]benzene, abbreviated to TCPOBOP, as an agonist ligand for mouse CAR.[33] TCPOBOP acts as a potent phenobarbital-type enzyme inducer in mouse liver but not in the rat or human. This is due to a substitution of Thr350 in mouse CAR with Met in the rat and human CAR,[34,35] whereas 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde*O*-(3,4-dichlorobenzyl)oxime (CITCO) was identified as a novel human CAR agonist,[36] based on the observation that it potently activated CAR in an *in vitro* fluorescence-based assay. CITCO demonstrated selectivity for CAR over other nuclear receptors, including the closely related xenobiotic pregnane x receptor (PXR). In addition, it also induced nuclear translocation of human CAR and induced the classical CAR target gene, *CYP2B6*, in primary human hepatocytes.[36] According to the general mechanism of NHR activation, the absence of an activator, such as an agonist ligand, causes helix12 to unwind. Therefore, co-repressor proteins may bind to the un-liganded receptor and recruit histone deacetylases, causing target gene transcription to be silenced.[37] Once an agonist ligand binds, this causes the ligand-dependent activation function to fold into a short helix that covers and seals the ligand binding pocket. By adopting the active conformation, helix12 displaces co-repressors and promotes co-activator binding. Generally, the ligand binding pocket when bound with ligand makes contact with helix12 via van derWaals interactions which stabilizes its' active conformation. In contrast, antagonists sterically prohibit helix12 from adopting the active conformation, thereby facilitating co-repressor binding.[38] Based on CAR X-ray crystal structures, it has been suggested that ligands entering the ligand binding pocket of CAR promote receptor translocation into the nucleus rather than inducing a conformational change in helix 12, as is commonly seen with other NHRs.[39] However, recent data also suggests that CITCO may modulate the helix 12 conformation similar to the standard model of ligand-mediated NHR activation. Although in the absence of an agonist ligand, helix 12 shows partial unfolding, and ligand binding preserves the helical shape of helix 12.[40] Although CAR was initially characterized as a xenosensor, additional evidence supports the notion that it also plays an important role in metabolic regulation by altering lipid metabolism, glucose homeostasis, and inflammation, thereby triggering pleiotropic effects on various physiological pathways (reviewed in[41]).

Farnesoid X receptor

Farnesoid x receptor (FXRα, nuclear receptor subfamily 1, group H, member 4, NR1H4) is expressed in the liver, intestine, kidney and adrenal gland. It is activated by conjugated and unconjugated bile acids,[21,22,42] as well as androgen metabolites.[43,44] FXR regulates transcription by binding as a heterodimer with RXR to DNA response elements in the regulatory regions of target genes. The main function of FXR is to control the expression of genes involved in the enterohepatic recycling and detoxification of bile acids[45–47] and is consequently sometimes referred to as the bile acid receptor (BAR). Bile acids, such as chenodeoxycholic acid (CDCA), are the natural physiological ligands for FXR,[21,22,42] and acting as the endogenous bile acid sensor, FXR plays an important role in cholesterol homeostasis[21,22,42,48]. FXR also regulates genes involved in various metabolic pathways, including lipid and glucose homeostasis, in the prevention of intestinal bacterial infection, gallstone formation and in modulating liver regeneration and tumorigenesis.[49–53] Most studies had focussed on the role of FXR in the liver and intestine, whereas utilization of a FXR-luciferase reporter mouse demonstrated that FXR signalling is active beyond the enterohepatic system in both the adrenal and kidney.[54] The studies also revealed that the terminal ileum is the primary bile acid signalling tissue in the basal state and that FXR signalling in the liver is only induced under pathological conditions, such as cholestasis.[54] Recently, an important role of FXR in diabetic nephropathy has been revealed. The pathogenesis of diabetic nephropathy is complex and involves activation of multiple pathways leading to kidney damage. One such pathway involved is the alteration in lipid metabolism that occurs via sterol regulatory element binding proteins (SREBPs). Recent studies have shown that FXR modulates renal SREBP-1 expression and that there is accelerated renal injury in a model of experimental diabetic nephropathy in FXR knockout mice.[55] Modulation of SREBP signaling is also the mechanism by which FXR regulates triglyceride metabolism in the liver.[56]

Liver X receptor

The liver X receptors (LXR) are crucial regulators of cholesterol metabolism, via regulation of genes that control cholesterol efflux in macrophages, promotion of bile acid synthesis in the liver and inhibition of cholesterol absorption.[57,58] LXRα (nuclear receptor subfamily 1, group H, member 3, NR1H3) and LXRβ (nuclear receptor subfamily 1, group H, member 2, NR1H2) are the two isoforms of this receptor, which differ significantly in their tissue distribution pattern. LXRα expression predominates in metabolic tissues

such as the liver, adipose tissue, intestine, kidney and tissue macrophages,[59] whereas LXRβ is ubiquitously expressed.[57,59] Oxysterols, which are oxidized cholesterol metabolites, are the main ligands of LXR.[58,60] In mice the Cyp7A1 gene, a member of the cytochrome P450 family encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme in the synthesis of bile acids from cholesterol, has been shown to be a direct target gene for LXR activation.[61] However, no LXR response element (LXRE) has been found in the human CYP7A1 promoter. Studies have revealed that there is a fundamental difference in the regulation of CYP7A1 *in vitro* in rodent and human hepatocytes and that there appears to be distinct molecular strategies for the regulation of cholesterol homeostasis between these two species.[62] LXR also promotes cholesterol elimination via bile and decreases absorption of cholesterol by intestinal cells via increased expression of two members of the ABC transporter family, namely ATP-binding cassette sub-family G member 5 (ABCG5) and ATP-binding cassette sub-family G member 8 (ABCG8).[63] In addition, LXRs are also involved in the promotion of reverse cholesterol transport in peripheral cells[57] via two other members of the ABC transporter family, namely, ABCA1 and ABCG1.[64] Additionally, LXR is important in the control of lipogenesis, where it regulates the co-ordinate expression of major fatty acid biosynthetic genes as well as increasing plasma triglyceride and phospholipid levels.[65]

Recently, LXRs were also found to participate in the regulation of glucose homeostasis.[66,67] Activation of LXRs by synthetic agonists in rodents represses the expression of certain genes encoding enzymes involved in gluconeogenesis in the liver, including phosphoenolpyruvate carboxy kinase and glucose 6-phosphatase, as well as inducing the expression of the glucose transporter type 4 (GLUT4) in adipose tissue.[66–68] LXRβ, expressed in pancreatic cells, facilitates insulin secretion and indirectly leads to an increase in the expression of the insulin gene[69,70] via a SREBP-1-regulated pathway. Several synthetic agonist ligands of LXR have been developed, including T0901317 and GW3965, of which, T0901317 is the often used in basic research. However, T0901317, at high levels, can also activate other NHRs, such as PXR and FXR.[71–73] Consequently, GW3965 has been shown to be much more selective agonist ligand for both LXRα and LXRβ.[72]

Peroxisome proliferator-activated receptors

The peroxisome proliferator-activated receptor (PPAR) subfamily of nuclear hormone receptors consists of three members, each of which is encoded by a separate gene: PPARα, PPARγ and PPARδ (also known as PPARβ). They are often termed the '*lipid sensor*' receptors, and PPARα was the first member of this family to be cloned in 1990.[74] PPARα was identified based on the observation that a diverse group of chemical compounds including fibrates, herbicides and industrial plasticizers triggered an increase, in size and number, of peroxisomes in cells, while also increasing fatty acid β-oxidation.[74] The three PPARs are essential for energy homeostasis and, as mentioned above, act as fatty acid sensors.[75] These receptors do not show strict ligand specificity, and most PPAR ligands so far identified are able to stimulate different PPAR receptor subtypes with varying efficiencies.[76]

Peroxisome proliferator-activated receptor alpha

Peroxisome proliferator-activated receptor alpha (PPARα; nuclear receptor subfamily 1, group C, member 1, NR1C1) is predominantly expressed in the liver but also in cardiac myocytes, renal tubular epithelial cells, skeletal muscle, large intestine epithelium, endothelial cells, smooth muscle cells, and in cells of the immune system.[75] PPARα acts principally by stimulating mitochondrial and peroxisomal β-oxidation of fatty acids, via transcriptional activation of specific target genes involved in this process.[75,77] These PPARα downstream-regulated genes include carnitine palmitoyl transferase (CPT)-1α and acyl-coA oxidase (ACO), which are the rate-limiting enzymes involved in fatty acid β-oxidation, in the mitochondria and peroxisome, respectively.[75,77] Subsequently, PPARα is required for ketogenesis and is induced by fasting. PPARα knockout mice in the fed state present with a fatty liver phenotype because of decreased expression in genes associated with fatty acid β-oxidation.[77] However, fasting causes an even more severe phenotype in PPARα knockout mice because ketogenesis is extremely impaired. The manifestations include exacerbated fatty liver, hypoglycemia, hypoketonemia, hypothermia and increased plasma free fatty acid levels.[77] Endogenous ligands of PPARα comprise saturated fatty acids, unsaturated fatty acids and certain eicosanoids, particularly leukotriene B4 (LTB4) and 8-hydroxy-eicosatetraenoic acid (8-HETE). The fibrates, including gemfibrozil, fenofibrate and clofibrate, a class of hypolipidemic agents are synthetic ligands of PPARα, and are used in the treatment of hypertriglyceridemia.[75]

Peroxisome proliferator-activated receptor gamma

Peroxisome proliferator-activated receptor gamma (nuclear receptor subfamily 1, group C, member 3, NR1C3) is predominantly expressed in the adipose tissue; however, it is also expressed at lower levels in the liver, pancreas, spleen, skeletal muscle, endothelium, vascular smooth muscle cells and cells of the immune system.[75] In white and brown adipose tissue, PPARγ has been termed the '*master regulator of adipogenesis*' because it is necessary for the differentiation of adipocytes involved in the formation and maintenance of adipose tissue.[78,79] Therefore, PPARα and PPARγ have opposing functions in the regulation of fat metabolism: PPARα promotes utilization, whereas PPARγ promotes storage.[68] Additionally, PPARγ is an important regulator of insulin sensitivity in major glucose-utilizing tissues, although the precise mechanisms of these insulin-sensitizing effects are still unknown.[80] However, it has been proposed that fatty acid accumulation in insulin-sensitive tissues, such as the liver and skeletal muscle, promotes insulin resistance.[68,80] It is known that activation of PPARγ increases the number of adipocytes, which promotes the relocalization and storage of fat in adipose tissue, thereby

helping to protect peripheral tissues from lipotoxicity, by increasing insulin sensitivity.[68,80] Furthermore, PPARγ activation regulates the production of certain adipokines such as adiponectin, resistin and tumour necrosis factor-α (TNF-α), and these hormones and cytokines have also been shown to be important in the control of insulin sensitivity.[75,77] Endogenous ligands of PPARγ mainly comprise unsaturated fatty acids and components of oxidized low-density lipoproteins, such as hydroxyoctadenoic acids (HODE).[75] The thiazolidinediones (TZDs) have been developed as synthetic ligands for PPARγ and are used as anti-diabetic medication to treat type 2 diabetes.[77]

Peroxisome proliferator-activated receptor delta

This subtype of the PPAR family has been the least studied so far. PPARδ (nuclear receptor subfamily 1, group C, member 2, NR1C2) is expressed ubiquitously, with higher levels in the large and small intestine, liver and keratinocytes.[75] PPARδ is a powerful regulator of fatty acid catabolism and energy homeostasis, with activation in adipose tissue increasing adipocyte differentiation and fatty acid oxidation.[75,77,81] Furthermore, it increases energy expenditure via induction of uncoupling proteins (UCPs) in brown adipose tissue.[82] UCPs are proton transporters located in the inner mitochondrial membrane, where they uncouple the mitochondrial proton gradient from ATP production, leading to energy being lost as heat, thereby mediating an increase in energy expenditure.[83] In skeletal muscle, activation of PPARδ also increases fatty acid β-oxidation, improves insulin sensitivity and augments the number of oxidative muscle fibers.[77,81] Transgenic mice expressing activated PPARδ were found to be protected against high-fat diet-induced obesity, muscular lipid accumulation, hyperinsulinemia, and insulin resistance.[77] Endogenous ligands for PPARδ include saturated and unsaturated fatty acids, eicosanoids and components of very-low-density lipoproteins (VLDL).[75] Although several synthetic agonists, namely, GW501516, GW0742, GW2433 and GW9578, are currently under development, they are not yet clinically available.[81]

Pregnane x receptor

The pregnane x receptor (PXR) (nuclear receptor subfamily 1, group I, member 2 and NR1I2) is a crucial regulator for the expression of drug-metabolizing enzymes and transporters involved in the response of mammals to their chemical environment.[84] PXR is primarily expressed in the liver, intestine and kidney and forms a heterodimer with RXR to regulate gene transcription.[85] Compared with other NHRs, there is a large spectrum of compounds that can act as ligands for PXR, including dietary supplements and herbal remedies, prescription drugs, endogenously produced metabolites and environmental pollutants.[84,86] PXR plays an essential role in xenobiotic metabolism, via regulation of phase I and phase II enzymes and drug transporters.[85,87] PXR induces the major phase I cytochrome P450 enzymes, which catalyse the first step in the detoxification of lipophilic compounds. Human CYP3A4 and the murine homolog Cyp3A11 are the major CYPs regulated by PXR.[88] In humans, CYP3A4 is critical for drug metabolism and is responsible for the metabolism of about 50% of all prescription drugs.[89,90] One of the major concerns regarding the promiscuity of PXR is that it can be activated by herbal remedies, and subsequently, this leads to interference with the metabolism of co-administered prescription drugs. For example, the herbal supplement St John's Wort (*hypericum perforatum*), a commonly used anti-depressant, contains the active ingredient hyperforin, which was identified as a potent activator of PXR.[91] Activation of PXR, therefore, increases the expression of CYP3A4, which can subsequently affect the half-life of co-administered prescription medication, such as immunosuppressant drugs or oral contraceptives.[85,92] The ligand-dependent activation of PXR is species specific: in humans, the antibiotic rifampicin is a potent activator of PXR, but it has little effect on mouse PXR.[93] In contrast, the synthetic molecule pregnenolone-16α-carbonitrile (PCN) activates mouse PXR, with no effect on human PXR.[87,88,93] Generation of transgenic mice expressing human PXR, in the absence of mouse PXR, has facilitated studies investigating the effect of various compounds on this receptor.[93] The endobiotic function of PXR is a novel concept, and recent studies have shown that PXR regulates the metabolism of endogenously produced toxic chemicals,[84] such as lithocholic acid (LCA), a secondary bile acid that is toxic if allowed to accumulate to high levels.[94] Studies showed that high levels of LCA activate PXR, which, via activation of CYP3A4, subsequently causes the detoxification of LCA.[94] By binding to various compounds, PXR is therefore a major regulator of both xenobiotic and endobiotic metabolism.

Vitamin D receptor

Vitamin D3, is acquired from either dietary sources or via ultraviolet irradiation of 7-dehydrocholesterol in the skin. Vitamin D3 then undergoes two hydroxylation reactions, first in the liver by the enzyme 25-hydroxylase and secondly in the kidney by the enzyme 1α-hydroxylase, to form the most biologically active metabolite, 1,25-dihydroxyvitamin D3 ($1,25(OH)_2D_3$).[95–98] $1,25(OH)_2D_3$ is then bound to the vitamin D binding protein (DBP) and is delivered systemically to vitamin D target tissues, which express the vitamin D receptor (VDR) (NR1I1 (nuclear receptor subfamily 1, group I, member 1). $1,25(OH)_2D_3$ functions to regulate cellular proliferation and differentiation and calcium homeostasis in the intestine, bone and kidney.[99] VDR acts as a ligand-inducible transcription factor via heterodimerization with RXR and binding to the vitamin D response element (VDRE) within vitamin D-inducible genes.[99] Binding of $1,25(OH)_2D_3$ to VDR induces alterations, including strong heterodimerization of VDR with RXR, as well as one or more hormone-dependent phosphorylations of VDR. Phosphorylation and reconfiguration of the AF2 domains in VDR and RXR bring their helix 12 motifs to the closed position over the ligand binding pocket.[100] The VDR-RXR heterodimer binds with very high affinity to the VDRE,

and the conformational changes and phosphorylation status of the VDR-RXR heterodimer serve to dissociate co-repressor/HDAC complexes and promote coactivator binding, facilitating histone acetylation and chromatin remodeling, and therefore subsequent gene transcription.[100]

Intestinal epithelial cells and osteoblasts represent the primary sites of VDR expression, where the receptor mediates the actions of $1,25(OH)_2D_3$ to promote intestinal calcium and phosphate absorption and bone remodeling.[101] $1,25(OH)_2D_3$ can also be produced locally in a number of cell types, including the skin, colon, pancreas, cells of the immune system and the vasculature.[100] Although the significance of these effects are not fully defined as yet, it appears that vitamin D, by co-operating with other regulators, exerts additional effects including xenobiotic detoxification, immunoregulation, antimicrobial defense, anti-cancer effects as well as the control of insulin secretion.[100]

Vitamin D receptor and bile acids

Although VDR was cloned prior to the newer members of the NHR family, that is, the former orphan receptors, it is most closely related to both FXR and PXR.[102,103] Overall, VDR and PXR are the closest relatives, and analogous to PXRE's, VDRE's possess either a motif composed of a direct repeat of two half-elements with a spacer of three nucleotides (DR3) or an everted repeat of two half-elements with a spacer of six nucleotides (ER6).[100] It is therefore not too surprising that, analogous to PXR, lithocholic acid (LCA) was recently identified as a ligand for VDR.[105] VDR is highly expressed in the colon and appears to be important in detoxification. LCA is a secondary bile acid formed by the action of anaerobic intestinal bacteria on primary bile acids, such as CDCA. Unlike the primary bile acids, CDCA and cholic acid (CA), LCA is poorly absorbed via enterohepatic recirculation and consequently passes into the colon. LCA has been shown in animal models to promote colon cancer,[106] and there are reports showing that concentrations of LCA are higher than other secondary bile acids in patients with colon cancer.[107] This is thought to be mediated by the fact that at high concentrations, LCA has been shown to induce DNA strand breaks, the formation of DNA adducts and inhibition of DNA repair enzymes.[108–110] An important route for the elimination of LCA is via catabolism, mediated by the enzyme cytochrome P450 3A4 (CYP3A4). CYP3A4 is involved in the systemic and presystemic (first pass) metabolism of many drugs and xenobiotics, converting them into more hydrophilic compounds that can then be more easily eliminated from the body.[111] Expression of CYP3A4 is regulated by PXR, which, as already mentioned, can be activated by high concentrations of LCA ($>100\ \mu mol \cdot l^{-1}$).[94,112] However, LCA-induced expression of CYP3A11 (the mouse homolog) is still present in PXR-knockout mice,[94] suggesting an alternative mechanism for activation of CYP3A by LCA. Subsequent studies identified LCA as being able to bind directly to VDR and that VDR is a more sensitive receptor for LCA and its' major metabolite, 3-keto-LCA, than PXR.[105] Additionally, the human CYP3A4 gene contains an ER6 response element corresponding to a VDR-RXR response element,[114], and studies demonstrated that VDR mediates LCA-dependent induction of CYP3A4 gene expression.[105] By binding to VDR, both LCA as well as vitamin D may activate a feed-forward catabolic pathway to increase CYP3A4 expression and subsequent detoxification of LCA. Via this mechanism, the enteric system can therefore protect itself from the potentially harmful effects of LCA,[105] and this observation may help explain the apparent protective effects of vitamin D against colon cancer.[114]

Dietary components as ligands for NHRs

As mentioned earlier, because of the promiscuous nature of the newer members of the NHR superfamily, that is, the adopted orphans, it is becoming increasingly evident that they can also be activated by '*bioactive*' dietary components. This is consequently becoming a very interesting and exciting development both in the fields of molecular endocrinology and molecular nutrition. Some of the dietary components that have been shown to mediate metabolic changes via NHRs are discussed below.

Coffee and the diterpene cafestol

Cafestol is a diterpene present in varying amounts in unfiltered coffee brews such as Scandinavian boiled (6·2 mg per cup), Turkish (4·2 mg per cup), Cafetiere/French press coffee (2·6 mg per cup) and espresso (2 mg per cup),[115] and it has been reported to be the most potent cholesterol-elevating compound known in the human diet.[116] Consumption of these unfiltered coffee brews raises triacylglycerol and low-density lipoprotein (LDL) cholesterol concentrations in humans.[115, 117,118] Human intervention trials showed that consumption of 10 mg of cafestol (corresponding to consumption of 0·91 of French press coffee) each day for 4 weeks results in a 8–10% increase in serum cholesterol levels.[118] A high intake of boiled coffee was associated with hypercholesterolemia and risk of coronary heart disease in Norway and Finland,[115,119,120] and cafestol was later identified as the factor responsible.[121–123] In addition to elevating serum cholesterol levels, cafestol may also act as an anti-carcinogen, with some studies suggesting that there is an inverse association between coffee consumption and the development of colorectal cancer,[124,125] as well as reducing the risk of hepatocellular carcinoma.[126] The basic-region leucine zipper (bZIP) transcription factor Nrf2 (Nuclear factor-erythroid 2 p45 subunit-related factor 2) controls the expression of several drug-metabolizing and anti-oxidant enzymes.[127] Consumption of coffee induces drug-metabolizing and anti-oxidant enzymes *in vivo*,[127] for example, cafestol robustly induces glutathione-S-transferase enzymes (GSTs) in mouse liver and intestine.[116] Recent studies demonstrated that induction of cancer chemopreventive enzymes, such as GSTs by cafestol is mediated via Nrf2.[127] Although the induction of GSTA1 in the small intestine is seen in the absence of Nrf2,[127] and similarly induction of GSTμ1 by cafestol in the small intestine is mediated via PXR, but this does not appear

to be the case in the liver because GSTμ1 expression remained elevated in PXR knockout mouse liver.[116] Indicating that there may be some overlap between different pathways with regard to the regulation of these drug-metabolizing enzymes.

Although it was known for several years that cafestol was the factor responsible for an increase in serum LDL cholesterol levels,[118,121] the molecular mechanism remained a mystery for some considerable time. Cholesterol homeostasis is maintained in three ways: by dietary intake, endogenous biosynthesis in the liver and disposal in the form of bile acids. Bile acids are not merely metabolic byproducts but have been shown to be essential for facilitating the absorption of dietary lipids and fat-soluble vitamins. On a daily basis, approximately 95% of the bile acids produced are returned to the liver via the enterohepatic circulation, whereas the remaining 5% are excreted. As mentioned in the previous section on FXR, bile acids have been shown to be the physiological ligands for FXR.[21,22,42] One of the problems in studying the effects of cafestol in a suitable animal model is the fact that various strains of wild-type mice, rats, gerbils, hamsters, rhesus and cebus monkeys do not show the same alterations in triglyceride levels as those seen in humans following cafestol ingestion.[128–130] ApoE3Leiden transgenic mice are an established model used in studies relating to hyperlipidemia and atherosclerosis.[131,132] A mutated form of the human APOE3 gene has been introduced resulting in an attenuated clearance of apoB-containing lipoproteins, because of the concomitant expression of ApoE3Leiden and ApoC1 in this strain. These mice display a lipoprotein profile comparable to those patients with dysbetalipoproteinemia, that is, their plasma cholesterol and triglyceride levels are increased. ApoE3Leiden mice respond to hypolipidemic drugs and dietary compounds such as cafestol[133] in a similar way to humans. In addition, cafestol was shown to repress Cyp7A1 gene expression, thereby repressing bile acid synthesis in these mice, with a concomitant increase in serum lipids similar to that observed in humans.[133] Interestingly, a disabling mutation in the CYP7A1 gene in humans results in an increase in plasma triglyceride and LDL cholesterol levels.[134] Armed with this information, we studied the molecular mechanism underlying the ability of cafestol to suppress Cyp7A1 and increase serum lipids and proposed that cafestol could mediate these effects via FXR.

Our studies showed that cafestol-treated ApoE3Leiden mice had alterations in lipid parameters similar to those observed in humans after cafestol consumption,[116] in agreement with previous reports.[133] Additionally, microarray analysis identified alterations in the hepatic expression of genes involved in lipid metabolism and detoxification,[116] many of which are known to be regulated by FXR and PXR. Consistent with this, we found that *in vitro*, using transient transfection, cafestol specifically activated FXR and PXR.[116] Initial studies utilized a Gal4-based transactivation assay, in which LBDs of different NHRs are fused to the Gal4 DNA-binding domain, and effects on expression directed by a Gal4-dependent reporter plasmid are tested. Cafestol was found to activate the ligand-binding domains of FXR and PXR, as compared with their established ligands, whereas no other NHRs tested were activated by cafestol. Additional studies utilizing the full-length receptors confirmed these initial observations, and subsequent studies demonstrated that cafestol can recruit co-activators to FXR and PXR, analogous to their known ligands. *In vivo*, we found that cafestol suppressed the expression of Cyp7A1, Cyp8B1 and NTCP expression in the livers of wild-type mice but not FXR knockout mice. One of the puzzling things during the course of these studies was that we did not see any induction in the expression of SHP in the liver following cafestol treatment, unlike with CDCA.[116] The down-regulation of Cyp7A1 expression by specific FXR activators has been well documented,[135,136] and is thought to be mediated by the induction of the negative regulator SHP.[137,138] However, several studies have demonstrated that SHP induction is not an absolute requirement for Cyp7A1 repression,[137–139] indicating the existence of additional pathways. Importantly, fibroblast growth factor 15 (FGF15) was identified to be an FXR-dependent target gene in the small intestine and is a key component of an enterohepatic feedback pathway in the production of bile acids.[140] In this pathway, the growth factor released from the gut activates its FGF4R receptor in hepatocytes, causing repression of Cyp7A1, which is dependent on both kinase activation and SHP expression.[140] This mechanism provides an elegant explanation for the seemingly paradoxical observations that cafestol results in FXR-dependent repression of Cyp7A1 and other negative target genes in the liver but fails to induce the expression of positive FXR targets in the liver. Subsequent analysis in both single FXR and PXR knockouts as well in the double FXR/PXR knockout model demonstrated that both receptors are involved in the induction of FGF15 in the small intestine by cafestol. Additionally, we found that intestinal bile acid-binding protein (IBABP) is induced in the intestine in an FXR-dependent manner, which would enhance the transportation of bile acids into the portal circulation for return to the liver. We also showed that cafestol is an intestine-specific activator of PXR, where it induces the expression of not only FGF15 but also Cyp27A1 and ABCA1, both involved in increasing cholesterol efflux to the liver; as well as inducing the drug metabolizing enzymes Cyp3A11 and GSTμ1 in a PXR-dependent manner,[116] as summarized in Figure 2. In conclusion from this study, we determined that the direct regulation of such FXR and PXR target genes in the intestine combines with indirect effects in the liver to contribute to the cholesterol-raising effect of cafestol in humans. This study revealed the complexity regarding the actions of cafestol, and it also revealed that the elucidation of detailed tissue-specific effects and biochemical mechanisms involved in the actions of dietary components can account for diverse biological effects.

There is also another diterpene present in unfiltered coffee brews, namely, kahweol. Kahweol was also tested for its ability to activate NHRs in a Gal4-NHR-LBD screen, but unlike the results seen with cafestol, no activation was

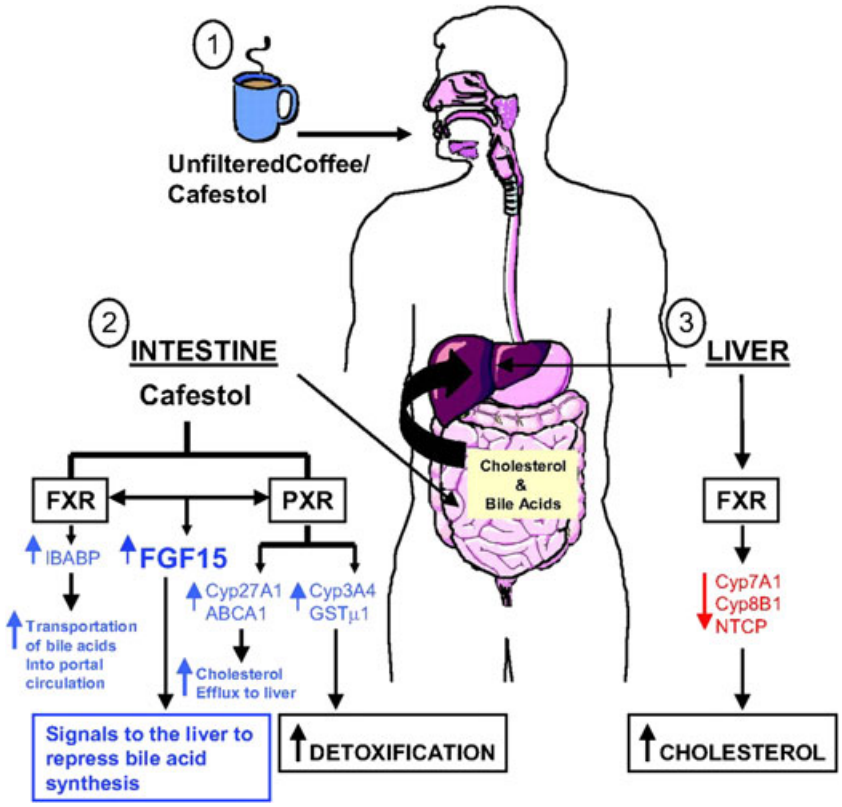

Figure 2. **Schematic representation of the proposed mechanisms by which cafestol increases cholesterol levels *in vivo***. (i) Cafestol is consumed in the form of unfiltered coffee and passes into the stomach and then into the small intestine. (ii) In the small intestine, cafestol activates FXR and PXR. IBABP is induced by cafestol in a FXR-dependent manner, further increasing the transportation of bile acids into the portal circulation. Upon activation of PXR, cafestol induces the expression of Cyp27A1 and ABCA1, resulting in an increase in the efflux of cholesterol into the portal circulation. Cafestol also induces Cyp3A11 and GSTμ1 gene expression via PXR, leading to an increase in detoxification. Cafestol acts via both FXR and PXR to induce FGF15, which signals to the liver to repress bile acid synthesis. (iii) In the liver, Cyp7A1, Cyp8B1 and NTCP expression is repressed via FXR, thereby reducing the synthesis of bile acids. The direct regulation of such FXR and PXR target genes in the intestine combines with indirect effects in the liver to contribute to the cholesterol-raising effect of cafestol in humans. Reproduced from[115] with kind permission Copyright 2007, The Endocrine Society.

observed in the presence of kahweol (Ricketts and Moore, unpublished observations).

Curcumin

The existence of nutritionally derived lipophillic ligands that might bind to and activate VDR with low affinity, similar to that seen with LCA, has been investigated.[100] Curcumin, a turmeric-derived polyphenol, abundant in a traditional Indian diet, is known to have anti-inflammatory effects and reduces inflammatory bowel disease,[141] as well as exerting chemopreventive effects in the intestine and skin, both of which are two major VDR-target tissues.[142] Studies have shown that curcumin inhibits tumour initiation by suppressing pro-inflammatory pathways and by inducing phase II conjugating enzymes including glutathione-s-transferases and sulfotransferases, thereby facilitating excretion of carcinogens.[143] Based on these observations, it was consequently hypothesized that VDR may be the mediator of the bioactions of curcumin. Subsequent studies have identified curcumin as a bonafide ligand for VDR.[144,145] Curcumin is able to bind to VDR, induce recruitment of RXR, as well as the coactivator steroid receptor coactivator 1 (SRC-1), and activate transcription of a VDR-target gene, namely CYP3A4.[144,145] Consequently, some of the bioeffects of curcumin, particularly in the colon and skin, appear to be mediated via direct binding to and activation of VDR. These new observations support the hypothesis that implicates VDR as a dietary sensor of curcumin, and there may be additional nutritionally derived lipids that can also exert tissue-specific effects both in regards to chemoprevention and drug metabolism via VDR.[145] Ultimately, identification of nutritionally derived ligands, such as curcumin, opens up avenues challenging the notion that nuclear receptors, such as VDR, solely bind to and mediate the actions of high-affinity endocrine ligands, as was previously thought.

Grape seed procyanidin extract

Consumption of dietary flavonoids on a regular basis over a long period, has been associated with reduced mortality and risk of cardiovascular disease (CVD).[146–148] Hypertriglyceridemia is an important contributing factor in the development of atherosclerosis and is an independent risk factor for CVD.[149,150] Several studies have shown beneficial effects of flavonoids in the prevention of atherosclerosis, mainly

by reducing apolipoprotein B (ApoB) and triglyceride levels.[151,152] Procyanidins, a class of flavonoids, are present in grapes, apples, red grape juice, red wine and chocolate[153] and have been shown to prevent and ameliorate atherosclerosis and other factors associated with CVD. This action has primarily been attributed to their anti-oxidant activity as well as modulating various signaling pathways in the vascular system.[154] However, the anti-atherogenic properties of procyanidins are also attributable to a reduction in plasma levels of apoB-containing triglyceride-rich proatherogenic lipoproteins, that is, intestinal chylomicrons and hepatic VLDL and LDL, as well as to an improved serum cholesterol profile. An acute dose of GSPE was shown to reduce postprandial triglyceridemia and plasma ApoB levels in normolipidemic rats.[155] This was accompanied by an increase in hepatic SHP mRNA expression. We have subsequently shown that *in vitro* in HepG2 cells, a human hepatoma cell line, GSPE requires the activity of SHP to reduce TG levels, whereas the reduction in ApoB is SHP independent.[156] Additional studies *in vivo* demonstrated that the hypotriglyceridemic effect of GSPE in wild-type mice in the postprandial state is SHP dependent because it does not occur *in vivo* in SHP knockout mice.[156] In addition, this was accompanied by the down-regulation in hepatic expression of SREBP1, a master regulator for insulin/glucose signalling and lipogenesis. Bile acids are well established potent hypotriglyceridemic agents, and the mechanism of action is mediated by the bile acid binding to FXR, resulting in the upregulation of SHP expression in the liver, which in turn represses the expression of SREBP1, which translates into diminished hepatic fatty acid synthesis and increased TG catabolism.[57] Ultimately, TG-rich lipoproteins are also cleared from the blood leading to a TG lowering effect. Based on the fact that SHP is a down-stream target of FXR, we proposed that GSPE could be acting as an agonist ligand for FXR, resulting in the observed up-regulation in SHP expression and subsequent TG-lowering effect of GSPE. Analysis both *in vitro* and *in vivo* revealed some interesting and unexpected results. First, *in vitro* transient transfection studies revealed that GSPE is unable to activate FXR alone but, instead, requires the presence of the bile acid CDCA.[157] In the presence of CDCA, GSPE is able to enhance the transactivation of FXR in a dose-dependent manner, indicating that it acts as a co-agonist ligand for FXR. Further *in vivo* studies utilizing wild-type and FXR knockout mice showed that GSPE lowers serum TG levels in an FXR-dependent manner.[157] In summary, the results showed that procyanidins enhance the activity of CDCA-bound FXR and that, like bile acids, signal through FXR to lower triglyceridemia, concomitantly inhibiting hepatic expression of SREBP1 and several SREBP1-target genes involved in lipogenesis in an FXR-dependent manner.[156,157]

Farnesoid X receptor activity is key in the regulation of triglyceridemia, as well as cholesterol and glucose homeostasis, and modulation of FXR has been proposed as a therapeutic target for the treatment of hyperlipidemia, hyperglycemia and the metabolic syndrome.[158–160] Consequently, dietary procyanidins, acting as activators of FXR, are promising natural agents for the treatment of these metabolic disorders.

Soy isoflavones

Although widely consumed by Asian populations for centuries, soy intake in the West initially occurred in subpopulations, including the Seventh-day Adventists and vegetarians about 100 years ago.[161] Since the 1970s, there has been an increase in consumption of soy by the general population.[161] Health conscious consumers were initially attracted to soy based on the fact that it was perceived to be a source of high-quality protein that is low in saturated fat and also because it was more efficiently produced than animal protein sources.[161] Soy intake has since been linked to improvements in blood lipid levels, amelioration in symptoms associated with menopause, such as hot flashes and bone loss, as well as anti-cancer properties.[162–165] In 1999, the FDA established a health claim 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*'. There has been much debate regarding the components in soy responsible for mediating these health benefits and the soy isoflavones, which include genistein, daidzein and glycitein, have been proposed to be one of the bioactive components present in soy. In addition to these equol, a metabolite of daidzein, has also received much attention as a bioactive compound with beneficial health effects.[166] During the 1980s, Setchell and colleagues showed that only about 25–30% of the Western population possesses the intestinal bacteria capable of converting daidzein into equol,[167,168] and equol has subsequently been shown to have a wide range of biological actions.[169] Other bioactive compounds present in soy that have also been the focus of active research, although to a much lesser extent, include saponins[170] and lunasin.[171–174] A more detailed discussion of the various pathways affected by both the isoflavones, equol and other bioactive components present in soy is outside the scope of this current review, and we will focus solely on the known effects exerted via nuclear hormone receptors.

The bioactive nature of the isoflavones first came to light in the 1940s when farmers in Western Australia experienced fertility problems with sheep that had been grazing on clover, which was rich in isoflavones.[175] It was discovered that the isoflavones were acting as bioactive agents disrupting estrogen action in the female sheep, which ultimately led to the term '*phytoestrogens*'. The cDNA for the estrogen receptor, now referred to as ERα, was first cloned in 1985,[20] with the later identification of a second receptor ERβ.[176] Both ERα and ERβ are classical hormone receptors that bind estradiol and synthetic estrogenic ligands with high affinity. Activated ER binds to target gene promoter sequences as homodimers or as ERα/ERβ dimers.[1] Subsequent work confirmed the ability of isoflavones to bind to estrogen receptors: genistein was demonstrated to be a more potent ER agonist compared with daidzein, whereas both isoflavones are more potent ligands for ERβ than ERα.[177–179]

Based on this observation, the soy isoflavones genistein and daidzein have been described as selective estrogen receptor modulators. The isoflavone glycitein has a much lower affinity for ER. The ability of the daidzein metabolite equol to exert hormonal effects was initially documented using estrogen bioassays, and the relative affinity of equol for both ERα and β was determined, and it was also found to preferentially bind to ERβ.[180–182]

Soy isoflavones as ligands for newer members of the NHR superfamily

Although it was known for many years that isoflavones could act as ligands for estrogen receptors, it was not known if they could activate any other NHRs. Based on the observations that soy intake has pronounced effects on lipid metabolism that seemed to resemble some of the actions resulting from activation of lipid regulating NHRs such as FXR, LXR and PPARs, studies were subsequently undertaken to determine whether soy isoflavones could indeed activate any of these other receptors.

Soy and PPARα

Both *in vitro* and *in vivo* studies have demonstrated that soy isoflavones can activate both PPARα and PPARγ.[183–188] The ability of soy isoflavones to stimulate PPARα has been related to their positive effects on lowering blood lipid levels, particularly triglycerides.[89] *In vitro* studies demonstrated that isoflavone-containing soy extracts, as well as individual soy isoflavones were potential ligands for PPARα. Studies demonstrated the ability of soy to induce the expression of PPARα (both mRNA and protein),[183,186] as well as the ability to stimulate its transcriptional activity[183,186] and to increase expression of PPARα downstream target genes, including CPT1α and ACO.[183] Studies utilizing an estrogen receptor antagonist (ICI182780) showed that the PPARα-mediated effect on genes associated with β-oxidation exerted by the isoflavones was not abolished, thereby suggesting that this effect of soy isoflavones was independent from any activation of the estrogen receptor.[183] *In vivo* studies using rodent models that were fed a high-fat pro-atherogenic diet showed that the addition of soy protein and isoflavones decreased triglyceride levels, resembling the action of fenofibrates.[184,185] Soy isoflavone consumption also resulted in an increase in mRNA levels for PPARα-related genes, namely, CPT1α and ACO.[184,185] This effect was completely abolished in PPARα knockout mice, confirming the requirement of the PPARα receptor to mediate the soy isoflavone-induced activation of genes involved in β-oxidation.[184] Considering the fact that PPARα knockout mice fed a diet containing soy isoflavones had lower triglyceride levels than those fed the control diet, it was concluded that one mechanism for the hypolipidemic effect mediated by isoflavones is independent from PPARα activation. This indicates that soy isoflavones decrease triglyceride levels not only via PPARα-dependent mechanisms but that there are also additional PPARα independent pathways mediating this effect.[184]

There is some controversy among researchers regarding which component, or components, of soybean, that is, whether it is the protein component or individual isoflavones that are responsible for the hypolipidemic effects observed following soy intake. Soy protein increased mRNA levels of PPARα in rat adipose tissue; however, it failed to enhance the activity and mRNA levels of enzymes involved in fatty acid oxidation.[185] Conversely, when isoflavones were added to a soy protein diet, PPARα expression not only increased to higher levels, but it did so in a dose-dependent manner, while also augmenting mRNA levels of enzymes involved in fatty acid oxidation, specifically ACO and CPT-Iα.[185] Furthermore, intact soy protein has been shown to decrease the activity and mRNA levels of many lipogenic enzymes such as fatty acid synthase, ATP-citrate lyase, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malic enzyme, whereas the addition of isoflavones did not reduce lipogenesis.[185] Therefore, it appears that either the consumption of intact soy protein or the addition of individual isoflavones to a soy protein diet both result in decreased blood lipid levels; however, this appears to occur via different mechanisms, that is, a soy protein diet results in modulation of lipogenesis, whereas the isoflavones increase lipolysis, via activation of PPARα.[185]

Soy and PPARγ

Activation of PPARγ has been suggested as an explanation responsible for the beneficial effects of soy isoflavones on insulin sensivity.[75,77] *In vitro* studies have demonstrated that daidzein, equol and genistein stimulate PPARγ transcriptional activity and mRNA expression, and they also enhance adipocyte differentiation and insulin-stimulated glucose uptake.[186–188] These effects were inhibited in the presence of BADGE (bisphenol A diglycidyl ether), a reversible PPARγ antagonist.[187] Genistein shows a biphasic behaviour with respect to its ability to activate PPARγ and estrogen receptors. At high concentrations, it stimulates adipogenesis and inhibits osteogenesis, concomitant with increased PPARγ mRNA expression.[188] However, at low concentrations, genistein induces ER-mediated effects, resulting in the inhibition of adipogenesis and enhancement of osteogenesis.[188] Furthermore, activation of ER by genistein down-regulates PPARγ transcriptional activity, whereas activation of PPARγ by genistein down-regulates its estrogenic transcriptional activity.[188,189] Therefore, genistein concomitantly activates two different NHRs, namely, ER and PPARγ, and the balance between their respective activation is concentration dependent.[188,189] Consequently, the ultimate biological effect of genistein on adipocytes varies with the source of the tissue and the dose of genistein used. This fact could explain why the available data regarding the role of genistein in the prevention and treatment of obesity are inconclusive.[189]

In a study using Sprague–Dawley rats that were fed a high isoflavone soy protein diet, mRNA levels for PPARγ and uncoupling proteins (UCPs) were increased in brown adipose tissue.[185] Additionally, body weight and adipose

tissue weight were significantly lower in the group receiving the soy diet.[185] The up-regulation in the expression of UCPs in brown adipose tissue via PPARγ activation may be responsible for the proposed anti-obesity effect of soy protein-rich isoflavones that has previously been proposed.[185] An additional study using obese Zucker rats demonstrated that administration of a high isoflavone-containing soy protein diet improved glucose levels in a glucose tolerance test; however, this was only observed in female rats.[186] These observations could be consistent with activation of PPARγ by the soy isoflavone diet because isoflavones have been shown to increase insulin sensitivity in obese rats, and PPARγ is a target for anti-diabetic drugs because activation of this receptor improves insulin resistance.[77]

Soy and PXR

Studies, both *in vitro*, in cells, and *in vivo* in animal models have shown that PXR is activated by soy isoflavones.[88,190] The individual isoflavones, genistein, daidzein and the metabolite equol activated human and mouse PXR in an *in vitro* transient tranfection assay using both CV-1 and HepG2 cells.[190] Additionally, isoflavones were shown to enhance the recruitment of the coactivator, steroid receptor coactivator 1 (SRC-1) to PXR in a mammalian two-hybrid assay,[190] indicating that isoflavones can act as ligands for PXR. The effect of isoflavones on the classical PXR-downstream targets in humans and mice, namely, CYP3A4 and Cyp3A11, respectively, was also studied. Genistein, daidzein and equol were found to induce the activity of a CYP3A4 promoter luciferase reporter construct.[190] Although equol was found to be the most potent activator of PXR and induced CYP3A4 mRNA and protein expression in primary human hepatocytes, genistein and daidzein had no such effect. In contrast, genistein and daidzein induced Cyp3A11 mRNA expression in primary hepatocytes from wild-type mice, whereas equol treatment did not.[190] The increase in Cyp3A11 mRNA expression was abolished in hepatocytes isolated from PXR knockout mice, indicating that this is mediated via PXR. Cyp3A11 mRNA expression was also induced *in vivo* in mice fed a soy protein and isoflavone-containing diet.[190]

These results highlight the species specific variations in activation of PXR. Equol is a more potent inducer of human PXR, whereas genistein and daidzein are more potent activators of mouse PXR.[190] Overall, isoflavone activation of PXR, and its regulated genes, that is, the CYPs, is relevant from a clinical perspective because soy isoflavone intake may affect the bioavailability of co-administered pharmaceutical drugs. Natural supplements are frequently used by patients concomitantly with prescription drugs; therefore, based on *in vitro* evidence that equol activates human PXR, clinical studies are warranted to assess the extent to which soy isoflavone intake influences the bioavailability of other co-administered prescription medication, particularly in equol-producing individuals. This is particularly important in the light of previous cases including co-consumption of the anti-depressant herbal remedy St John's Wort (SJW) and oral contraceptives. The active component in SJW is hyperforin, a known activator of PXR,[91] which via activation of PXR-induced CYP3A4 expression, subsequently altering the metabolism of the co-administered oral contraceptives, resulting in a decrease in the half-life of the drug and subsequent unplanned pregnancies in several women.[92]

Soy and LXR

As previously mentioned, LXRs are crucial regulators of cholesterol metabolism[57,58] and are also important regulators of glucose metabolism.[66,68,191] Increased LXRα mRNA and protein expression was seen in Sprague–Dawley rats fed a soy protein and isoflavone-containing diet.[192] Concurrently, mRNA expression of genes regulated by LXRα, namely, CYP7A1, ABCG5 and ABCG8, were increased as a result of isoflavone administration.[192] Supplementation with soy protein and isoflavones in the diet protected against weight gain as well as the increase in percent body fat observed in rats fed a Western diet. Isoflavones also reduced serum cholesterol levels and increased insulin sensitivity in rats fed a Western diet.[192] Activation of LXR by isoflavones, therefore, provides a plausible alternative explanation for the observed beneficial effects of soy consumption on lipid homeostasis.

In summary, soy isoflavones have been demonstrated as ligands for NHRs involved in diverse metabolic pathways, namely, PPARα, PPARγ, PXR and LXR.[88,183–190,192] These mechanistic insights help elucidate the beneficial effects of soy intake previously reported. However, there is still controversy in regard to the singular effect of soy components, gender differences and bioavailability of isoflavones in different species. Further research is therefore needed to fully understand the complex and multifactorial nature of the molecular mechanisms involved behind the observed physiological effects following soy intake.

Plant sterols and phytosterols

Phytosterols exist as naturally occurring plant sterols present in the non-saponifiable fraction of plant oils.[193] Phytosterols are plant components, which have a chemical structure similar to that of cholesterol with the exception of a substitution on the sterol side chain at the C24 position.[193] Despite the structural similarity, phytosterol absorption in humans is considerably less than that of cholesterol, and they have, in fact, been shown to reduce cholesterol absorption and, therefore, lower circulating levels of cholesterol,[193] although the exact mechanism of how this occurs is still unknown. The efficacy of phytosterols as cholesterol-lowering agents have been demonstrated when incorporated into fat spreads as well as other food matrices. In addition, phytosterols have been combined with other beneficial dietary components including fish and olive oils, psyllium and β-glucan to enhance their effect on cardiovascular disease risk factors.[193] The primary phytosterols in the diet are sitosterol, stigmasterol and campesterol.[193] Phytosterols appear not only to play an important role in the regulation of cardiovascular

disease, but they also exhibit beneficial anti-cancer properties.[194]

There has been great interest in elucidating the underlying molecular mechanism by which plant sterols reduce intestinal cholesterol absorption, as well as the molecular actions of phytosterols. One mechanism proposed to explain this hypocholesterolemic effect is that there is competition between plant sterols and intestinal cholesterol for incorporation into mixed micelles.[195] Although it appears that phytosterols do not need to be present in the intestinal lumen simultaneously with cholesterol to inhibit its absorption.[196] Consequently, recent questions have arisen as to whether plant sterols could mediate hypocholesterolemic actions via LXR.[197] It is well established that induction of LXR increases transcription of several members of the ABC gene family, including ABCA1 and ABCG5/ABCG8,[63,198,199] and that LXR is a master regulator of hepatic lipogenesis.[65,200] Interestingly, it has previously been shown that synthesized derivatives of phytosterols can act as LXR agonists.[201] A recent study investigated the effects of plant sterols in wild-type C57BL/6 J mice and found that consumption of the plant sterols reduced plasma and hepatic triglycerides, despite increasing both the expression of hepatic lipogenic regulatory genes and *de novo* lipogenesis.[202] This study found that SREBP-1c and fatty acid synthase (FAS) gene expression was significantly increased following consumption of plant sterols versus the control diet ($p < 0{\cdot}05$),[202] both established LXR-target genes involved in hepatic lipogenesis. The authors proposed that there is interference with intestinal fatty acid/triglyceride metabolism, as suggested by increased fecal fatty acid loss and reduced weight gain in the plant sterol fed mice, which may be associated with triglyceride lowering following plant sterol consumption.[202] Although it does appear that there are other pathways being affected that may over-ride the effects mediated at a transcriptional level via LXR it would be interesting to conduct a study using LXR-deficient mice to directly address the question of whether LXR plays a role.

In contrast to this beneficial effect on cholesterol and triglyceride levels, phytosterols as components of soy-derived lipids, have been proposed as exacerbants in the development of parenteral nutrition-associated cholestasis. Based on the fact that FXR is involved in cholestatic hepatoprotection, phytosterols were proposed as potential antagonists of FXR and as such, may contribute to bile acid-induced hepatocyte damage.[203] Stigmasterol is a phytosterol prevalent in soy-derived parenteral nutrition solutions. *In vitro* studies using HepG2 cells showed that stigmasterol acetate, a water-soluble derivative of stigmasterol, suppressed ligand-activated expression of FXR-target genes involved in adaption to, and protection from, cholestasis, including bile salt export pump (BSEP).[203] Additionally, in transiently transfected cells, stigmasterol acetate inhibited bile acid-activated FXR-dependent reporter gene expression; however, the most prevalent phytosterol in lipids, β-sitosterol, had no such inhibitory effect.[203] In subsequent experiments using hepatocytes isolated from both wild-type and FXR null mouse models, stigmasterol acetate antagonized FXR-target genes including SHP and BSEP in wild-type hepatocytes but not those lacking FXR.[203] This consequently demonstrates that stigmasterol can act as a potent antagonist of FXR with subsequent detrimental effects on gene transcription, which therefore inhibits the possibility of adaption to any adverse effects of bile acids on the liver, subsequently contributing to the development of cholestasis.

Probiotics and nuclear hormone receptors

Intestinal microflora has several important functions including the maintenance of intestinal epithelial cell health and regulation of immunity, inflammation and metabolism.[204] Dysregulation of the microflora has been associated with several diseases including colorectal cancer, inflammatory bowel disease (IBD) and obesity.[204] Probiotics and prebiotics have been proposed as a means to restore microflora homeostasis.[205] Probiotics are defined by the World Health Organization and the Food and Agriculture Organization of the United Nations as '*live microorganisms which, when administered in adequate amounts, confer a health benefit on the host*',[206] whereas prebiotics are defined as '*nondigestible food components that selectively stimulate the growth and/or activity of beneficial microflora within the colon*'.[207]

Probiotics have been tested as a potential therapy for the prevention and treatment of IBDs because of their beneficial effects on the gastrointestinal tract. They were demonstrated to block pathogenic bacterial effects, regulate immune responses and stimulate a protective role for intestinal epithelial cells by promoting cell survival.[205] Studies have shown that diverse molecular mechanisms mediate these beneficial effects, including via activation of PPARγ. PPARγ is highly expressed in the colon[208] and plays a role in the regulation of intestinal inflammation.[209] Low levels of expression of PPARγ have been associated with IBDs,[210] and it has been proposed that PPARγ is a therapeutic target mediating the effect of 5-aminosalicylic acid, a commonly used treatment for IBD.[211] PPARγ exerts an anti-inflammatory role, predominantly via regulation of nuclear factor kappa B (NF-κB)[212] and mitogen-activated protein kinase (MAPK) pathways.[213] PPARγ also enhances the innate immune response in the gastrointestinal tract, thereby helping to maintain the constitutive expression of endogenous antimicrobial peptides called β-defensins.[204] Defensins are peptides involved in innate immunity, with the β subfamily being the most abundant within the gastrointestinal tract.[214] The expression of β-defensins is reduced in patients with IBD, and their deficiency has been proposed to play a role in the pathogenesis of these diseases.[214] Consequently, activation of PPARγ by probiotics could be a beneficial therapeutic target for increasing β-defensins in IBDs, as depicted in Figure 3.

In vitro models using different cell lines have evaluated the effect of probiotics on PPARγ in response to proinflammatory inducers. Studies have shown that different probiotics increase colonic PPARγ mRNA and protein expression, with a concomitant decrease in interleukin-8 (IL-8) mRNA expression[215] and NF-κB activity.[216–219]

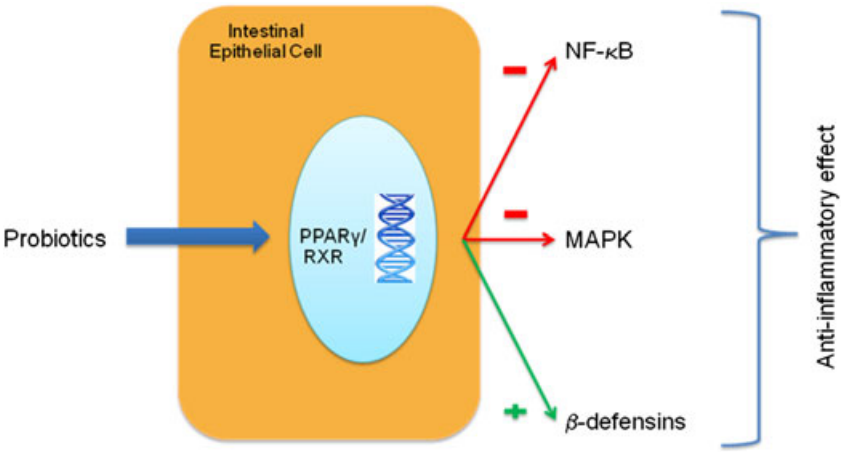

Figure 3. **Anti-inflammatory effects of probiotics mediated by PPARγ**. Probiotics have been shown to target PPARγ expressed in epithelial cells within the intestinal mucosa. PPARγ is activated by probiotics, which results in activation of anti-inflammatory downstream targets including β-defensins as well as inhibition of pro-inflammatory pathways, such as NF-κB and MAPK signaling. The overall effect is the immunomodulation of intestinal responses and the relief of inflammatory signs and symptoms in patients with IBD

When cells were treated with a specific PPARγ inhibitor, namely, prostaglandin F2α, the immunomodulatory effects of the probiotics were lost, thereby establishing a key role for this NHR in probiotic-mediated responses.[216] The effect of a particular probiotic strain, namely, *Lactobacillus crispatus* M247, on PPARγ was shown to be mediated by the production of hydrogen peroxide (H_2O_2), which acts as a signal-transducing molecule.[216]

Probiotics stimulate toll-like receptors (TLR), and in epithelial cells, different types of TLR trigger inflammatory or anti-inflammatory responses when they recognize conserved microbial structures.[204] For example, *Lactobacillus crispatus* M247 up-regulates TLR-2, an immunomodulatory receptor in the colonic mucosa.[216] However, TLR-2 stimulation has not been shown to be essential for PPARγ expression or to mediate its anti-inflammatory effects.[216] Furthermore, in the same study, *Lactobacillus crispatus* M247 failed to induce the up-regulation in TLR-2 when PPARγ was inhibited, corroborating the important role of PPARγ in probiotic-induced mucosal homeostasis.[216]

Although PPARγ-mediated probiotic effects have been widely reported, certain isolated studies have shown that probiotics may also exert anti-inflammatory effects in the intestine by enhancing the expression of other epithelial NHRs, such as PXR and FXR.[204,217,218] In an *in vivo* model in CD1 mice, colitis was induced by treatment with trinitrobenzene sulfonic acid (TNBS). VSL#3, a commercially available probiotic consisting of four strains of lactobacilli and three strains of bifidobacteria and *S. thermophilus*, was administered for 10 days, starting 5 days before the induction of colitis. TNBS-induced colitis resulted in a 80% decrease in PPARγ and FXR mRNA and protein expression, as well as a 50% reduction in the expression of PXR ($p < 0{\cdot}05$).[217] VSL#3 treatment effectively attenuated signs and symptoms of colitis, while reducing colonic expression of inflammatory mediators, such as TNF-α, interleukin-6 (IL-6) and interleukin-1β (IL-1β). Additionally, probiotics antagonized the colonic TNBS-associated down-regulation in PPARγ, PXR and FXR expression, facilitating the maintenance of the intestinal expression of these NHRs.[217]

Prebiotic oligosaccharides were also found to induce PPARγ expression in an *in vitro* Caco-2 cell model.[220] Prebiotics were found to inhibit proinflammatory cytokine production via induction of peptidoglycan recognition protein 3 (PGlyRP3).[220] This protein is part of a novel family of pattern recognition molecules that are involved in host defense as well as mediating anti-inflammatory effects, via induction of the NF-κB pathway. Antagonizing PPARγ, by culturing cells with GW9662, abolished the induction of PGlyRP3 expression and the anti-inflammatory effect of prebiotics.[220] Therefore, prebiotic oligosaccharides exert an anti-inflammatory effect via activation of PPARγ, which then induces the immunomodulatory PGlyRP3 pathway.[220]

Additionally, probiotics have also been demonstrated to reduce cholesterol absorption by inhibition of Niemann-Pick C1-like 1 protein (NPC1L1), via a LXR-mediated pathway.[221] NPC1L1 is localized in jejunal enterocytes and is critical for intestinal absorption of dietary and biliary cholesterol.[222] Cholesterol absorption is reduced by 90% in NPC1L1 knockout mice or when NPC1L1 is bound by ezetimibe, a hypolipidemic drug.[222] *Lactobacillus acidophilus* (ATCC4356) treatment in Caco2 cells resulted in substantial reduction in the expression of the NPC1L1 protein, which was mediated via enhanced LXR mRNA expression.[221] When LXR expression was abolished, by transfecting cells with a short interfering RNA (siRNA), the expression of NPC1L1 was no longer decreased by the addition of the probiotics, and no reduction in cholesterol absorption was observed.[221]

Nuclear hormone receptors, therefore, represent a novel mechanism for the action of probiotics. This is an interesting area of current research, and further investigation into the

complex interaction of NHRs, probiotics and immunomodulatory pathways is therefore warranted.

NHRs and a high fat diet

Nuclear hormone receptors play an integral role in metabolism, and each receptor regulates metabolism uniquely. A useful model for studying the role of NHRs in metabolism is to challenge mice with a high-fat or Western diet, which has been shown to induce obesity, dyslipidemia and glucose intolerance.[223] As mentioned earlier, the role played by a particular receptor of interest is often determined by either modifying up-regulation or silencing of that particular NHR. Silencing is often the preferred method because ligands for all NHRs are not currently known. This section will cover the response of particular NHR knockout mouse models following challenge with a high-fat diet and its subsequent effect and physiological consequence. It is interesting to note that, although diet-induced obesity in a mouse model results in changes in the expression of drug-metabolizing enzymes, such as Cyp3A11, which can ultimately alter drug pharmacodynamics,[224] no such studies utilizing PXR knockout mice challenged with a high fat diet have so far been conducted. Consequently, because of lack of current knowledge regarding the role, if any, of PXR in regards to a physiological response to a high-fat diet, and because of the lethality of RXR knockouts, these receptor knockout models will not be discussed.[225] The results discussed below are summarized in Table 1.

Constitutive androstane receptor. Constitutive androstane receptor (CAR) is best known for xenobiotic effects with respect to drug metabolism; however, it also has an important role in metabolic regulation. When CAR knockout mice were challenged with a high-fat diet, they were found to be resistant to an increase in serum triglyceride (TG) levels, clearly seen in their wild-type counterparts.[226] An important finding in CAR knockout mice is that the lower serum TG level was not associated with an increase in hepatic TG levels. Interestingly, this is believed to be as a result of the increase in TG oxidation in the liver, resulting from increased activity of PPARα. There is an inverse relationship between CAR and PPARα,[226,227] whereby CAR knockout mice demonstrate increased expression of PPARα-downstream targets. Additionally, when wild-type mice were treated with the CAR agonist, TCPOBOP, a decrease in PPARα downstream targets was observed.[226] Subsequent studies have also shown that CAR is also important in the regulation of thyroid hormone, whereby treatment of wild-type mice with TCPOBOP results in a decrease in the circulating levels of thyroxine (T4).[228] This is due to the fact that CAR knockout mice lack repression of T4 by CAR under fasting conditions, leading to an increase in basal metabolic rate.[228] However, when CAR knockouts are challenged with a high fat diet, any increase in metabolic rate, as a result of the increased circulating T4, is not sufficient enough to protect against diet induced obesity.[226] The regulation of T4 by CAR is likely to result in the down regulation of thyroid hormone when it is high, but it does not appear to be capable of increasing T4 and, therefore, potentially increase thyroid hormone production sufficiently to protect against diet-induced obesity.

Farnesoid x receptor. In the Farnesoid X receptor (FXR) knockout model, there is a marked increase in serum cholesterol (twofold increase) and plasma triglyceride levels (1·6-fold increase) as compared with wild-type mice.[229] Challenging FXR knockout mice with a high-fat diet resulted in further increases in both cholesterol (twofold) and plasma triglyceride levels (threefold increase) compared with wild-type mice.[230] These increases were unexpectedly found to be the result of reduced hepatic absorption of high-density lipoproteins (HDL), increased synthesis of apoB lipoprotein and increased absorption of cholesterol and triglycerides in the stomach.[229]

Liver x receptor. When LXR α/β double knockout mice were challenged with a high-fat diet, they were found to be resistant to diet-induced obesity.[231] The resistance observed in the double knockout revolves around altered expression of many genes, including but not limited to SREBP1c.[231] SREBP1c is involved in cholesterol synthesis and triglyceride metabolism and is the one of the main downstream targets of LXR. In support of this, it was shown that SREBP-1 knockout mice were not resistant to diet-induced obesity.[231] The LXR knockouts were found to have increased expression of Cyp7a1, as well as Abcg5/Abcg8, which are involved in bile acid excretion. The LXR knockout also has decreased expression of SREBP-1 and other genes involved in fatty acid and triglyceride synthesis, including fatty acid synthase (Fas), acetyl-CoA carboxylase

Table 1. **The physiological effect of challenging nuclear hormone receptor-specific knockout mouse models with a high-fat diet**. Each NHR knockout mouse model has a unique response to challenge with a high-fat diet, showing that specific NHRs play different roles in metabolism

Nuclear hormone receptor knockout	Response to high-fat diet	Reference
CAR	Normalizes high TG levels	225
FXR	High TG and cholesterol levels	229
LXR	Resistant to HFD-induced obesity	230
PPARα	Protected from insulin resistance	235
PPARδ (adipose tissue-specific knockout)	Increased adiposity and TG levels, lower total body weight	238
PPARγ (adipose tissue-specific knockout)	Resistant to HFD-induced obesity, glucose intolerance and insulin resistance	233

(CAR: constitutive androstane receptor; FXR: farnesoid x receptor; LXR: liver x receptor; PPAR: peroxisome proliferator-activated receptor; HFD: high-fat diet; TG: triglyceride)

(Acc1), stearoyl-coenzyme A desaturase 1 (Scd-1) and Spot14.[231] More specifically, it has been shown that LXRα has a greater role in cholesterol regulation than LXRβ.[61,232]

In individual knockouts, the LXRα knockout model resulted in higher hepatic and plasma cholesterol levels compared with the wild-type counterpart when challenged with a high-cholesterol diet.[61] In comparison, the LXRβ knockout mice were not found to be significantly different than their wild-type counterparts when challenged with a high-cholesterol diet.[232] In addition to lipid metabolism, it has been shown that the LXR α/β double knockout mice are resistant to diet-induced obesity, resulting from an increase in basal metabolic rate. This increased energy expenditure was dependent on the presence of cholesterol in the diet, which is required for the expression of type 2 iodothyronine deiodinase (Dio2) in the liver.[231] Dio2 is responsible for the conversion of inactive T4 to active thyroid hormone (T3) in the tissue, as opposed to Dio1, which is responsible for T4 conversion in plasma. Importantly, in LXR knockouts, an increase in only Dio2 is seen, and subsequently, plasma levels of T3, T4 and thyroid stimulating hormone (TSH) were found to be no different than FXR knockout mice fed a cholesterol deficient diet.[231] Overall, the LXR knockout model is resistant to diet-induced obesity by a combination of impaired fat storage and increased energy expenditure.

Peroxisome proliferator-activated receptors. The PPAR family has a wide variety of roles in metabolism, particularly lipid homeostasis. PPARγ, which is predominantly localized to adipose tissue, is one of the most studied of the family, although it has proved to be more difficult to study PPARγ because whole body knockouts are embryonically lethal.[233] To overcome this, conditional knockouts and localized knockouts have been developed. PPARγ knockouts vary in response depending on the age of the mice, when studies were undertaken, that is, whether it was during postnatal development or in an adult mouse, and the location, that is, in which tissue PPARγ is knocked out. When challenged with a high-fat diet, in general, the knockouts have demonstrated resistance to diet-induced obesity but not dyslipidemia. This is mainly because of an impaired ability of adipose tissue to function and differentiate in the absence of PPARγ.[234] In most PPARγ knockouts, there is whole body insulin resistance and glucose intolerance; however, in the adipose tissue-specific PPARγ knockout, this was not observed. This relates to the ability of adipose tissue-specific PPARγ knockouts to retain normal PPARγ function in the liver, leading to protection against global insulin resistance and glucose intolerance.[234] The adipose-tissue specific PPARγ knockout does have localized insulin resistance in skeletal muscle; however, this is compensated for by the expression of PPARγ in the liver of these mice. PPARα, mainly present in the liver, has also been shown to play a major role in lipid homeostasis.[235] PPARα knockout mice are not embryonically lethal, unlike PPARγ whole body knockouts,[235] and when challenged with a high-fat diet, PPARα knockouts were found to be protected against insulin resistance.[236] The results of this finding have been contested; however, because of the non-apparent protection of PPARα knockouts to insulin resistance in times of non-fasting, unlike the protection observed following a 2-h fast previously reported.[237] Further studies using PPARα knockouts need to be conducted to definitively elucidate the real physiological consequence of this knockout model with respect to insulin sensitivity. PPARδ is the third major PPAR, and because it is ubiquitously expressed, whole body PPARδ knockouts are embryonically lethal.[238] Like PPARγ, adipose tissue-specific PPARδ knockouts were found to be resistant to diet-induced obesity, albeit with increased adiposity and serum triglyceride levels.[234,239]

SUMMARY AND CONCLUSIONS

In summary, there is considerable evidence that nuclear hormone receptors are important mediators in the ability of bioactive dietary components to mediate changes in various metabolic pathways. The former orphan receptors are interesting targets for pharmacological manipulation to aid in the development of treatment strategies for metabolic disorders, including the metabolic syndrome. It would therefore be useful to identify natural treatments that could be used without any adverse side effects, such as inadvertent activation of PXR. Although diet and exercise, either individually or combined, often provide the best means for metabolic control, individuals who have either genetically, pathologically or behaviourally dysregulated metabolic function may benefit from natural treatments that activate NHR-mediated metabolic pathways. Consequently, by improving insulin sensitivity and lipid profiles, or synergistically by combining exercise regimes, individuals may be better situated to avoid a downward spiral of insulin resistance and lipid accumulation or other metabolic dysfunction via utilization of known bioactive dietary components. Studies highlighted in this review have shown that there is potential for great complexity in the actions of bioactive dietary components when they act via NHRs, for example, cafestol. However, this is an exciting field, and current on-going research will undoubtedly provide a new insight into the molecular actions of dietary components as well as provide new insights into NHR action.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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