Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 22 (2011) 1099–1104

Journal of Nutritional Biochemistry

REVIEWS: CURRENT TOPICS

Novel molecular targets for prevention of obesity and osteoporosis☆

Srujana Rayalam[a], Jeong-Yeh Yang[a], Mary Anne Della-Fera[a], Clifton A. Baile[a,b,*]

[a]Department of Animal and Dairy Science, University of Georgia, Athens, GA 30602, USA
[b]Department of Foods and Nutrition, University of Georgia, Athens, GA 30602, USA

Received 17 June 2010; accepted 23 November 2010

Abstract

Evidence from both epidemiological studies and basic research suggests that obesity and osteoporosis are interrelated. Though there is an increase in the prevalence of these disorders, a limited number of treatments are available, one of the reasons being the complexity of the pathways involved and difficulty in identifying a single molecular target. Due to adverse effects of pharmaceuticals, intake of herbal drugs by patients without a physician's recommendation is increasing globally. Lack of success with targeted monotherapy has encouraged scientists to determine whether combinations of phytochemicals that interfere with numerous cell-signaling pathways can be a more effective approach to treat complex diseases. For example, evidence is emerging that specific combinations of phytochemicals are far more effective than single compounds in decreasing adipogenesis and promoting bone formation. Since multiple pathways are dysfunctional in obesity and osteoporosis, an ideal approach for preventing and treating these diseases may be to use a combination of phytochemicals to address several targets simultaneously.

Keywords: Vitamin D receptor; Farnesoid X receptor; Aryl hydrocarbon receptor; TGR5; H^+-ATP synthase

1. Introduction

Adipocytes and osteoblasts differentiate from a common progenitor cell, and tissue specific transcription factors regulate each differentiation pathway [1]. There is an increased interest in selective modulation of the signaling pathways; however, successful targeting of signaling pathways requires a considerable refinement of our understanding of these complex pathways and their effects on lipid metabolism and bone formation. Understanding the interactions between these pathways will help in creating novel treatment regimens for obesity and osteoporosis.

Epidemiological reports indicate the severity of the obesity problem globally. In addition, aging results in a relative increase in body fat content accompanied by an accumulation of adipocytes in bone marrow, which is directly implicated in age-related bone loss. Bone loss disorders are also on the increase worldwide. Therefore, treatments that inhibit adipogenesis in both fat depots and bone marrow, while simultaneously stimulating osteogenesis, will have significant, positive consequences for both obesity and bone health.

There are only two medications approved in the US for the long-term treatment of obesity, and both drugs have adverse side effects [2]. While a number of medications like bisphosphonates and teriparatide are available for osteoporosis prevention and therapy in the US, there is evidence for side effects with these drugs [3]. Due to potentially hazardous side effects of pharmaceuticals and dissatisfaction with high costs, a larger increasing percentage of people in the US and other countries are exploring the use of natural-product-based treatments. Natural products have been a source of drug leads for years, and in the past two decades, 61% of the new chemical entities introduced as drugs worldwide were inspired by natural products [4].

Targeting several signaling pathways simultaneously using phytochemicals to achieve synergistic effects has been investigated for cancer, but a similar concept for obesity or osteoporosis has not been as rigorously explored. We have investigated several phytochemicals alone or in combination and demonstrated enhanced effects on decreasing adipogenesis and promoting osteogenesis [5–7]. In the current review, we will discuss the novel signaling pathways that can be targeted by phytochemicals in adipocytes and osteoblasts to achieve synergistic decrease in lipogenesis and enhanced osteogenesis.

Abbreviations: AHR, aryl hydrocarbon receptor; ARNT, AHR nuclear translocator; BAs, bile acids; cAMP, cyclic adenosine monophosphate; CRE, cAMP response element; CYP24, cytochrome P450 enzyme 24-hydroxylase; CYP27B1, 1 α-hydroxylase; D2, type 2 iodothyronine deiodinase; FXR, farnesoid X receptor; FXRE, FXR responsive element; VDR, vitamin D receptor; VDRE, vitamin D responsive element; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; T3, triiodothyronine; T4, thyroxine.

☆ This work was supported in part by the Georgia Research Alliance Eminent Scholar endowment held by CAB.

* Corresponding author at: 444 Edgar L. Rhodes Center for Animal and Dairy Science, University of Georgia, Athens, GA 30602-2771, USA. Tel.: +1 706 542 2771; fax: +1 706 542 7925.
E-mail address: cbaile@uga.edu (C.A. Baile).

0955-2863/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2010.11.009

2. Vitamin D receptor

The major biological function of $1,25(OH)_2D_3$ was once perceived as to maintain normal blood levels of calcium and phosphorus, but recent research suggests that it is not just a vitamin but a hormone. Actions of $1,25(OH)_2D_3$ are mediated by the binding of $1,25(OH)_2D_3$ to a specific cytosolic/nuclear vitamin D receptor (VDR), a member of the steroid/thyroid hormone receptor superfamily, and VDR plays a key role in adipocyte biology when bound to its ligand, $1,25(OH)_2D_3$ [8]. Vitamin D receptor protein is expressed at low levels in preadipocytes, drastically increases upon initiation of adipogenesis and then gradually declines during the progression of the differentiation process and becomes barely detectable in mature adipocytes [8]. The unliganded VDR has unique functions in the presence and absence of its ligand and plays an important role in the molecular pathways governing adipogenesis, depending upon the availability of intracellular $1,25(OH)_2D_3$ [8]. A decrease in adipogenesis by liganded VDR appears focused on the C/EBPβ signaling pathway, by suppressing PPARγ up-regulation and antagonizing PPARγ activity [8]. Recently, $1,25(OH)_2D_3$ was also shown to play a role in energy metabolism *in vivo* by regulating β-oxidation and uncoupling protein expression [9]. These studies suggest that $1,25(OH)_2D_3$ and VDR may play an important role in regulation of body fat content.

Availability of intracellular $1,25(OH)_2D_3$ depends on the presence of 1 α-hydroxylase (CYP27B1), which synthesizes $1,25(OH)_2D_3$ from its precursor 25-hydroxyvitamin D_3 and cytochrome P450 enzyme 24-hydroxylase (CYP24), which hydroxylates, inactivates and decreases half-life of $1,25(OH)_2D_3$ [10]. Compounds that increase CYP27B1 and decrease CYP24 activity might therefore enhance $1,25(OH)_2D_3$'s effects, making these enzymes interesting molecular targets. In cancer cells, genistein induced CYP27B1 and reduced CYP24 activity [11]; however, no such reports are available for either adipocytes or osteoblasts. Nevertheless, genistein in combination with $1,25(OH)_2D_3$ synergistically increased VDR expression in adipocytes, leading to a synergistic decrease in lipid droplet accumulation [5]. It is interesting to note that $1,25(OH)_2D_3$ has potential to increase the expression of estrogen receptors (ERs) [12] and genistein binds to ER to mediate antiadipogenic effects [13].

Obese individuals were shown to have a greater risk for developing hyperparathyroidism, which is believed to be secondary to hypovitaminosis D [14]. $1,25(OH)_2D_3$ is also considered a bone remodeling agent and is recommended for management of postmenopausal osteoporosis [15]. The prevalence of metabolic syndrome is significantly higher in aged women with $1,25(OH)_2D_3$ deficiency than in women with normal $1,25(OH)_2D_3$ levels [16]. Interestingly, hormone replacement therapy in combination with a $1,25(OH)_2D_3$ analog and the combination of $1,25(OH)_2D_3$ with ipriflavone, a synthetic isoflavone similar to soy isoflavones, were more potent in preventing postmenopausal bone loss than $1,25(OH)_2D_3$ alone [17].

$1,25(OH)_2D_3$ can either act directly on osteoblasts or induce osteogenic differentiation of mesenchymal stem cells [18]. There are limited reports of natural compounds influencing either adipogenesis or osteogenesis via VDR signaling pathway. We have investigated the effects of phytochemicals on the expression of VDR expression in adipocytes and reported a synergistic increase in VDR protein levels when adipocytes were treated with phytochemicals in combination with $1,25(OH)_2D_3$ [5,6]. In recent preliminary studies, we have observed a significant increase in VDR messenger RNA (mRNA) levels in mature 3T3-L1 adipocytes upon treatment with $1,25(OH)_2D_3$, guggulsterone and xanthohumol (Fig. 1A, C and D), suggesting a role for VDR in the adipocyte-specific effects induced by these compounds. Thus, VDR proves to be an important target in both adipocytes and osteoblasts. Further, the reports in adipocytes strengthen our hypothesis that targeting more than one signaling pathway with phytochemicals plus $1,25(OH)_2D_3$ is more effective than targeting VDR alone.

3. Farnesoid X receptor

The farnesoid X receptor (FXR, also named RIP14 and HRP1) is an adopted member of the metabolic nuclear receptor superfamily, and two FXR isomers, FXRα and FXRβ, have been identified. Bile acids (BAs), which play an important role in the solubilization and absorption of dietary fat, act as endogenous ligands for FXR. Farnesoid X receptor down-regulates BA synthesis via suppressing the rate-limiting enzyme cholesterol 7α hydroxylase in order to prevent BA overload and toxicity [19] through a negative feedback mechanism. On the contrary, activation of FXR by BAs results in the modulation of various genes for enzymes and transport proteins involved in several cellular processes, including lipid metabolism [20].

Although the main physiological role of FXR is to function as a BA sensor in enterohepatic tissues, it plays a critical role in lipid metabolism as well. Administration of FXR agonists to normal rats and mice reduces plasma triglyceride levels. Farnesoid X receptor deficiency increases triglyceride and plasma cholesterol levels, high-density lipoprotein and circulating free fatty acids (FFAs) and also accelerates atherosclerosis [21]. The main mechanism of inhibition of triglyceride synthesis by FXR ligands in liver is inhibition of the expression of the transcription factor SREBP1-c and its lipogenic target genes, including that for fatty acid synthase [21]. Moreover, administration of BAs and synthetic FXR ligands decreased gluconeogenesis in mice fed high-fat diets, and loss of FXR function in the liver resulted in increased hepatic-lipid accumulation and elevation of FFAs in the serum, both of which contribute to impaired insulin signaling in muscle and adipose tissues [22]. This suggests that FXR also modulates insulin signaling, which has recently been supported by the finding that FXR activation reverses insulin resistance and lipid abnormalities in obese rats [23]. In adipocytes, FXR gene expression is rapidly increased in response to induction of differentiation, and FXR agonist chenodeoxycholic acid induced GLUT4 transcription [24], indicating an important role for FXR in lipid metabolism.

In addition to BAs, some compounds whose structures are much different from BAs, like GW4064 and several selective modulators like guggulsterone, have been identified as FXR ligands [25]. Guggulsterone has also been found to reduce triglyceride and cholesterol levels and has been used to treat obesity. However, FXR antagonism has been proposed as a major mechanism for the hypolipidemic effect of GS. Several other plant-derived FXR agonists and antagonists have also been discovered recently [26], but research is needed on antiobesity effects of these compounds. $1,25(OH)_2D_3$ was also reported to influence the signal transduction mediated by BA/FXR [27], and recently, we observed an increase in mRNA expression of FXR in maturing 3T3-L1 adipocytes treated with $1,25(OH)_2D_3$ (data not published). There is also evidence that VDR suppressed the transactivation driven by BA/FXR in a $1,25(OH)_2D_3$-dependent manner [27], indicating a possible cross talk between VDR and FXR [6]. Because FXR has been found to play many roles in addition to lipid metabolism, selective FXR modulators might be useful for antiobesity therapy, but a simple FXR agonist might have undesired side effects.

4. TGR5 signaling pathway

TGR5 (also known as BG37) is a novel G-protein-coupled receptor, responsive to BAs. Bile acids have received increased attention in recent years for their role in metabolic regulation. Further, administration of BAs to mice increases energy expenditure in brown adipose tissue, preventing obesity and resistance to insulin [28]. This novel metabolic effect of BAs is critically dependent on induction of the cyclic adenosine monophosphate (cAMP)-dependent thyroid

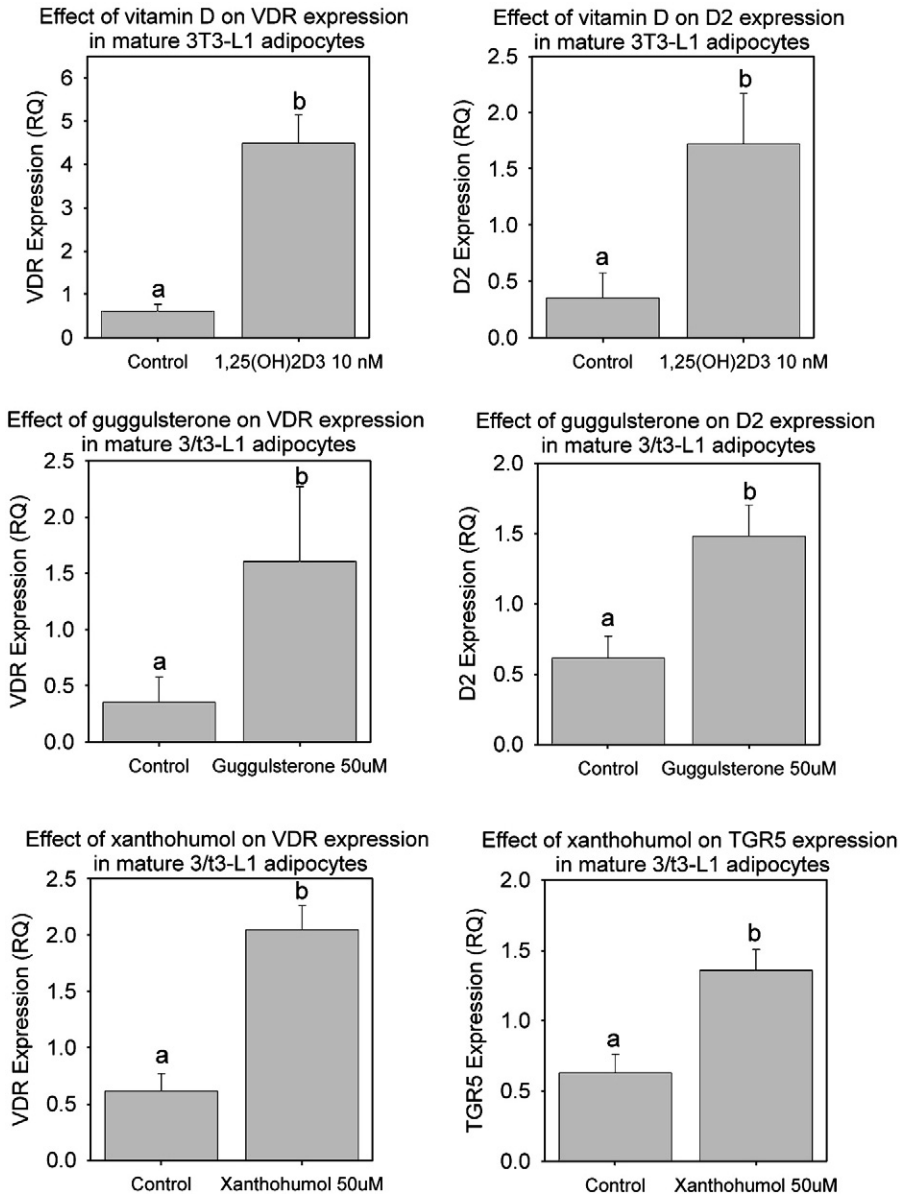

Fig. 1. Effect of $1,25(OH)_2D_3$, guggulsterone and xanthohumol on mRNA expression of VDR, D2 and TGR5 in adipocytes. 3T3-L1 adipocytes were differentiated into mature adipocytes as previously described. Mature adipocytes were treated with either 0.1% DMSO (control) or 10 nM $1,25(OH)_2D_3$, or 50 μM guggulsterone or 50 μM xanthohumol for 3 days. RNA was extracted, and real-time reverse transcriptase polymerase chain reaction was performed to quantitatively compare the expression levels of genes as described elsewhere [54]. (A and B) Effect of $1,25(OH)_2D_3$ on mRNA expression of VDR and D2. (C and D) Effect of guggulsterone on mRNA expression of VDR and D2. (E and F) Effect of xanthohumol on mRNA expression of VDR and TGR5. The experiments were performed in at least four replicates per treatment. Data are expressed as means±S.E.M. for each group and within each gene; means not denoted with a letter in common are different. ($P<.05$).

hormone activating enzyme type 2 iodothyronine deiodinase (D2). Farnesoid X receptor mainly affects enterohepatic lipid homeostasis, and TGR5 stimulates glucagon-like protein 1 production in enteroendocrine cells and activates thyroid hormone in brown adipose tissue and muscle through the stimulation of D2 [29], the enzyme that catalyzes the intracellular conversion of thyroxine (T4) to triiodothyronine (T3). These effects are independent of FXR, but are mediated through TGR5 [30]. TGR5 may also improve glucose homeostasis and, through the activation of D2, stimulate energy expenditure, thus protecting against the development of obesity. TGR5 agonists have been shown to have similar antidiabetic and antiobesity activity [31], positioning TGR5 as an attractive target for metabolic syndrome treatment.

Signal transduction by TGR5 is through Gs-protein-mediated cAMP accumulation, and cAMP-mediated signaling of TGR5 activation has been implicated in the expression of D2 in skeletal muscle and brown adipose tissue [32]. The TGR5-cAMP-D2 signaling pathway plays a critical role in metabolic control, including regulating energy expenditure in adipose and muscle tissues [33]. D2 is expressed throughout the skeleton, and its expression is increased in osteoblast precursor cells during differentiation [34]. Likewise, it is well known that T3 affects body composition by increasing energy expenditure and preventing fat mass accumulation. The role of T3 in regulation of bone turnover and mineralization for skeletal development is also well established [35]. To exert its biological activity, T4 needs to be converted to T3 by iodothyronine deiodinase. Guggulsterone and xanthohumol showed a strong thyroid stimulatory action when administered *in vivo* [36,37]. The mRNA expression of D2, which is responsible for maintaining local T3 concentration, was induced by $1,25(OH)_2D_3$ dose and time dependently [38] in primary osteoblastic

cells. Similarly, we have observed a significant 1,25(OH)$_2$D$_3$-induced increase in D2 mRNA expression in mature 3T3-L1 adipocytes as well (Fig. 1B).

Guggulsterone and xanthohumol, apart from interacting with FXR, have been identified as selective bile acid receptor modulators [39]. Since both xanthohumol and guggulsterone possess BA-like activities, we investigated the effects of these compounds on mRNA expression of TGR5 and D2 in mature 3T3-L1 adipocytes. We found that xanthohumol increased mRNA expression of TGR5 after 3 days (Fig. 1F). While guggulsterone did not modulate TGR5 expression, there was a trend for an increase in mRNA expression of D2 (P=.09) (Fig. 1E). These preliminary experiments suggest a possible role for TGR5-D2 pathway in 1,25(OH)$_2$D$_3$-, guggulsterone- and xanthohumol-induced effects in adipocytes.

5. Aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AHR) is a ligand-dependent Per-Arnt-Sim-containing transcription factor belonging to the basic helix–loop–helix family of proteins. It is most commonly called a dioxin receptor, is activated by structurally diverse synthetic and naturally occurring chemicals and is involved in the adaptation to changing environmental conditions [40]. Unliganded AHR, like the nuclear receptor, is present in cytosol in complex with chaperone proteins. Once it is bound by ligand, AHR translocates to the nucleus, dissociates from chaperones and dimerizes with AHR nuclear translocator, which associates with specific DNA sequences in promoters of target genes leading to alterations in gene expression [41].

The known ligands of AHR are environmental contaminants like 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and lack of TCDD toxicity in AHR knockout mice indicates that AHR mediates the toxic and biological effects of TCDD [42]. Natural products including herbal medicines contain various agonists/antagonists of AHR, and recently, quercetin, kaempherol, several catechins, curcumin and lutein were reported to act as antagonists for AHR [43] and protect against dioxin toxicity. Aryl hydrocarbon receptor is a constitutive inhibitor of lipid synthesis and an early regulator of adipocyte differentiation. Deletion of AHR results in elevated triglyceride synthesis in parallel with increased cytosolic GPDH activity in mouse embryo fibroblasts [44]. In addition, AHR signals have a critical effect on the differentiation of osteoblasts and influence bone modeling *in vivo* [45]. Thus, AHR plays an important role in diverse cellular functions like detoxification, adipogenesis and adipocyte and osteoblast differentiation.

Phytoestrogens like daidzein caused AHR activation in *in vitro* studies, suggesting that including AHR inducers in foods in minimal quantities may have some beneficial effects in the proliferation and differentiation of cells in animals [46]. Estrogenic actions of AHR agonists were detected in wild-type ovariectomized mouse uteri, but were absent in AHR and ER knockout ovariectomized mice, suggesting a novel mechanism by which AHR agonists, like phytoestrogens, could induce estrogenic effects [47]. On the contrary, antagonists of AHR, like resveratrol, are also beneficial, as they prevent the damaging effects of TCDD on bone formation [48]. However, AHR is still relatively poorly understood, as its mechanisms and functions remain largely unknown. Nevertheless, it is possible that the effects of AHR agonists and antagonists on adipocytes and osteoblasts may be, to some extent, mediated by AHR signaling pathway resulting in beneficial effects.

6. H^+-ATP synthase

H^+-ATP synthase is a membrane-associated protein complex that catalyzes the synthesis of ATP from ADP and inorganic phosphate, participates in ATP hydrolysis dependent processes and regulates a proton gradient across some membrane-dependent systems [49]. Of late, there is increasing evidence for the expression of H^+-ATP synthase on the surfaces of multiple animal cell types and in the membranes of mitochondria, and recent reports indicate that it is highly expressed in adipocytes as well [50]. H^+-ATP synthase is also involved in biological processes such as metabolism of lipid formation, regulation of the proliferation and

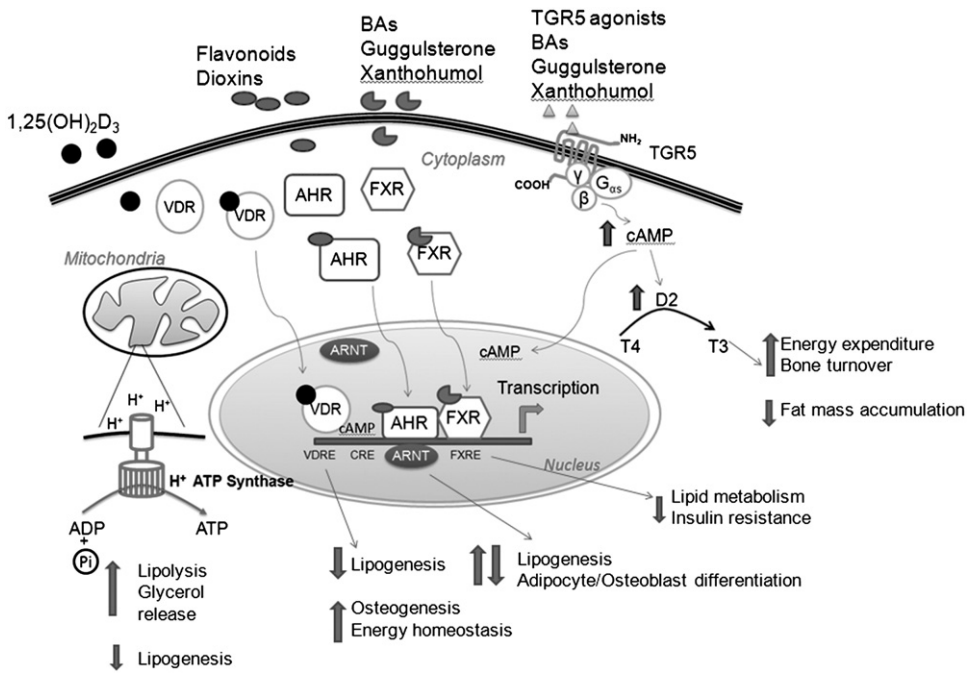

Fig. 2. An overview of novel molecular targets in a mammalian cell and their functional relevance. The synergistic effects caused by combinations of compounds like 1,25(OH)$_2$D$_3$ +genistein or 1,25(OH)$_2$D$_3$+guggulsterone or guggulsterone+xanthohumol [5–7] are likely a result of multiple pathways being triggered, as illustrated. ARNT indicates AHR nuclear translocator; CRE: cAMP response element; FXRE, FXR responsive element; VDRE: vitamin D responsive element.

differentiation in endothelial cells and recognition of immune responses of tumor cells. Owing to its multiple roles, cell surface H^+-ATP synthase is emerging as a molecular target for the treatment of various diseases [51].

ATP synthase is a target for dietary phytochemicals and polyphenolic compounds like piceatannol, resveratrol, EGCG, genistein, quercetin and curcumin, all of which have been shown to inhibit the F1 subunit complex of H^+-ATP synthase [52]. In addition, H^+-ATP synthase inhibitors stimulated glycerol release, an indicator of lipolysis, and also inhibited cytosolic lipid droplet accumulation in 3T3-L1 adipocytes [53]. Although the physiological role of cell-surface H^+-ATP synthase is not clear, by understanding the mechanisms of action of H^+-ATP synthase inhibitors in inhibiting lipid droplet accumulation, H^+-ATP synthase may emerge as a potential target for the development of antiobesity drugs.

7. Conclusions

A number of complex interconnected cell signaling pathways are involved in regulation of lipid metabolism and bone formation, and targeting multiple sites in these biosynthetic pathways may exert additive or synergistic pro-osteogenic and antiobesity effects (Fig. 2). Further, a growing number of *in vitro* and *in vivo* studies show that combinations of phytochemicals can cause significant effects at concentrations at which any single agent is not effective. Nevertheless, it is crucial to consider the impact of signal modulation on the dose-limiting side effects, and the net result must be evaluated empirically. Although the synergistic effects of dietary phytochemicals are well reported, the novel molecular pathways modulated should be explored for additional beneficial and reliable outcomes in the field of obesity and osteoporosis prevention.

References

[1] Reyes M, Verfaillie CM. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. Ann N Y Acad Sci 2001;938:231–3 [discussion 3–5].
[2] de Simone G, D'Addeo G. Sibutramine: balancing weight loss benefit and possible cardiovascular risk. Nutr Metab Cardiovasc Dis 2008;18:337–41.
[3] Gennari L, Merlotti D, Valleggi F, Nuti R. Ospemifene use in postmenopausal women. Expert Opin Investig Drugs 2009;18:839–49.
[4] Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod 2003;66:1022–37.
[5] Rayalam S, Della-Fera MA, Ambati S, Yang JY, Park HJ, Baile CA. Enhanced effects of 1,25(OH)(2)D(3) plus genistein on adipogenesis and apoptosis in 3T3-L1 adipocytes. Obesity (Silver Spring) 2008;16:539–46.
[6] Rayalam S, Della-Fera MA, Ambati S, Boyan B, Baile CA. Enhanced effects of guggulsterone plus 1,25(OH)2D3 on 3T3-L1 adipocytes. Biochem Biophys Res Commun 2007;364:450–6.
[7] Rayalam S, Yang JY, Della-Fera MA, Park HJ, Ambati S, Baile CA. Anti-obesity effects of xanthohumol plus guggulsterone in 3T3-L1 adipocytes. J Med Food 2009;12:846–53.
[8] Blumberg JM, Tzameli I, Astapova I, Lam FS, Flier JS, Hollenberg AN. Complex role of the vitamin D receptor and its ligand in adipogenesis in 3T3-L1 cells. J Biol Chem 2006;281:11205–13.
[9] Wong KE, Szeto FL, Zhang W, Ye H, Kong J, Zhang Z, et al. Involvement of the vitamin D receptor in energy metabolism: regulation of uncoupling proteins. Am J Physiol Endocrinol Metab 2009;296:E820–8.
[10] Akiyoshi-Shibata M, Sakaki T, Ohyama Y, Noshiro M, Okuda K, Yabusaki Y. Further oxidation of hydroxycalcidiol by calcidiol 24-hydroxylase. A study with the mature enzyme expressed in Escherichia coli. Eur J Biochem 1994;224:335–43.
[11] Lechner D, Bajna E, Adlercreutz H, Cross HS. Genistein and 17beta-estradiol, but not equol, regulate vitamin D synthesis in human colon and breast cancer cells. Anticancer Res 2006;26:2597–603.
[12] Ohsawa M, Mizunuma H, Kagami I, Miyamoto S, Kanuma T, Ibuki Y. Effect of 1,25(OH)2D3 on expression of estrogen receptor-alpha mRNA on rat osteosarcoma cell line (ROS 17/2.8). Life Sci 2000;66:2465–72.
[13] 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;139:4252–63.
[14] Drent ML, van der Veen EA. Endocrine aspects of obesity. The Netherlands journal of medicine 1995;47:127–36.
[15] Somjen D, Kaye AM, Harell A, Weisman Y. Modulation by vitamin D status of the responsiveness of rat bone to gonadal steroids. Endocrinology 1989;125:1870–6.
[16] Larijani B, Maghbooli Z, Hossein-nezhad A, Saghafi H, Behzadi H. The common role of vitamin D in osteoporosis and metabolic syndrome. The Endocrine Society's 90th Annual Meeting — ENDO 08; 2008 June 15–18; San Francisco, California; 2008.
[17] Mizunuma H, Shiraki M, Shintani M, Gorai I, Makita K, Itoga S, et al. Randomized trial comparing low-dose hormone replacement therapy and HRT plus 1alpha-OH-vitamin D3 (alfacalcidol) for treatment of postmenopausal bone loss. J Bone Miner Metab 2006;24:11–5.
[18] Zhou YS, Liu YS, Tan JG. Is 1, 25-dihydroxyvitamin D3 an ideal substitute for dexamethasone for inducing osteogenic differentiation of human adipose tissue-derived stromal cells in vitro? Chinese medical journal 2006;119:1278–86.
[19] Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, et al. Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. Mol Cell 2000;6:507–15.
[20] Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science 1999;284:1362–5.
[21] Watanabe M, Houten SM, Wang L, Moschetta A, Mangelsdorf DJ, Heyman RA, et al. Bile acids lower triglyceride levels via a pathway involving FXR, SHP, and SREBP-1c. J Clin Invest 2004;113:1408–18.
[22] Ma K, Saha PK, Chan L, Moore DD. Farnesoid X receptor is essential for normal glucose homeostasis. J Clin Invest 2006;116:1102–9.
[23] Cipriani S, Mencarelli A, Palladino G, Fiorucci S. FXR activation reverses insulin resistance and lipid abnormalities and protects against liver steatosis in Zucker (fa/fa) obese rats. J Lipid Res 2010;51:771–84.
[24] Shen H, Zhang Y, Ding H, Wang X, Chen L, Jiang H, et al. Farnesoid X receptor induces GLUT4 expression through FXR response element in the GLUT4 promoter. Cell Physiol Biochem 2008;22:1–14.
[25] Cui J, Huang L, Zhao A, Lew JL, Yu J, Sahoo S, et al. Guggulsterone is a farnesoid X receptor antagonist in coactivator association assays but acts to enhance transcription of bile salt export pump. J Biol Chem 2003;278:10214–20.
[26] Suzuki T, Tamehiro N, Sato Y, Kobayashi T, Ishii-Watabe A, Shinozaki Y, et al. The novel compounds that activate farnesoid X receptor: the diversity of their effects on gene expression. J Pharmacol Sci 2008;107:285–94.
[27] Honjo Y, Sasaki S, Kobayashi Y, Misawa H, Nakamura H. 1,25-Dihydroxyvitamin D3 and its receptor inhibit the chenodeoxycholic acid-dependent transactivation by farnesoid X receptor. J Endocrinol 2006;188:635–43.
[28] Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev 2009;89:147–91.
[29] Thomas C, Auwerx J, Schoonjans K. Bile acids and the membrane bile acid receptor TGR5 — connecting nutrition and metabolism. Thyroid 2008;18:167–74.
[30] Watanabe M, Houten SM, Mataki C, Christoffolete MA, Kim BW, Sato H, et al. Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. Nature 2006;439:484–9.
[31] Sato H, Genet C, Strehle A, Thomas C, Lobstein A, Wagner A, et al. Anti-hyperglycemic activity of a TGR5 agonist isolated from *Olea europaea*. Biochem Biophys Res Commun 2007;362:793–8.
[32] Foord SM, Bonner TI, Neubig RR, Rosser EM, Pin JP, Davenport AP, et al. International Union of Pharmacology. XLVI. G protein-coupled receptor list. Pharmacol Rev 2005;57:279–88.
[33] Tiwari A, Maiti P. TGR5: an emerging bile acid G-protein-coupled receptor target for the potential treatment of metabolic disorders. Drug Discov Today 2009;14:523–30.
[34] Gouveia CH, Christoffolete MA, Zaitune CR, Dora JM, Harney JW, Maia AL, et al. Type 2 iodothyronine selenodeiodinase is expressed throughout the mouse skeleton and in the MC3T3-E1 mouse osteoblastic cell line during differentiation. Endocrinology 2005;146:195–200.
[35] O'Shea PJ, Bassett JH, Sriskantharajah S, Ying H, Cheng SY, Williams GR. Contrasting skeletal phenotypes in mice with an identical mutation targeted to thyroid hormone receptor alpha1 or beta. Mol Endocrinol (Baltimore, Md) 2005;19:3045–59.
[36] Tripathi YB, Malhotra OP, Tripathi SN. Thyroid stimulating action of Z-guggulsterone obtained from *Commiphora mukul*. Planta Med 1984;50:78–80.
[37] Radovic B, Schmutzler C, Kohrle J. Xanthohumol stimulates iodide uptake in rat thyroid-derived FRTL-5 cells. Mol Nutr Food Res 2005;49:832–6.
[38] Miura M, Tanaka K, Komatsu Y, Suda M, Yasoda A, Sakuma Y, et al. A novel interaction between thyroid hormones and 1,25(OH)(2)D(3) in osteoclast formation. Biochem Biophys Res Commun 2002;291:987–94.
[39] Urizar NL, Moore DD. GUGULIPID: a natural cholesterol-lowering agent. Annu Rev Nutr 2003;23:303–13.
[40] Gu YZ, Hogenesch JB, Bradfield CA. The PAS superfamily: sensors of environmental and developmental signals. Annu Rev Pharmacol Toxicol 2000;40:519–61.
[41] Schmidt JV, Bradfield CA. Ah receptor signaling pathways. Annu Rev Cell Dev Biol 1996;12:55–89.
[42] Mimura J, Yamashita K, Nakamura K, Morita M, Takagi TN, Nakao K, et al. Loss of teratogenic response to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in mice lacking the Ah (dioxin) receptor. Genes Cells 1997;2:645–54.
[43] Zhang S, Qin C, Safe SH. Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. Environ Health Perspect 2003;111:1877–82.
[44] Alexander DL, Ganem LG, Fernandez-Salguero P, Gonzalez F, Jefcoate CR. Aryl-hydrocarbon receptor is an inhibitory regulator of lipid synthesis and of commitment to adipogenesis. J Cell Sci 1998;111(Pt 22):3311–22.
[45] Naruse M, Ishihara Y, Miyagawa-Tomita S, Koyama A, Hagiwara H. 3-Methylcholanthrene, which binds to the arylhydrocarbon receptor, inhibits proliferation and

differentiation of osteoblasts in vitro and ossification in vivo. Endocrinology 2002; 143:3575–81.
[46] Harper PA, Riddick DS, Okey AB. Regulating the regulator: factors that control levels and activity of the aryl hydrocarbon receptor. Biochem Pharmacol 2006;72: 267–79.
[47] Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, et al. Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 2003;423:545–50.
[48] Singh SU, Casper RF, Fritz PC, Sukhu B, Ganss B, Girard Jr B, et al. Inhibition of dioxin effects on bone formation in vitro by a newly described aryl hydrocarbon receptor antagonist, resveratrol. J Endocrinol 2000;167:183–95.
[49] Stock D, Gibbons C, Arechaga I, Leslie AG, Walker JE. The rotary mechanism of ATP synthase. Curr Opin Struct Biol 2000;10:672–9.
[50] Kim BW, Choo HJ, Lee JW, Kim JH, Ko YG. Extracellular ATP is generated by ATP synthase complex in adipocyte lipid rafts. Exp Mol Med 2004;36:476–85.
[51] Arakaki N, Nagao T, Niki R, Toyofuku A, Tanaka H, Kuramoto Y, et al. Possible role of cell surface H+-ATP synthase in the extracellular ATP synthesis and proliferation of human umbilical vein endothelial cells. Mol Cancer Res 2003;1: 931–9.
[52] Zheng J, Ramirez VD. Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals. Br J Pharmacol 2000;130:1115–23.
[53] Arakaki N, Kita T, Shibata H, Higuti T. Cell-surface H+-ATP synthase as a potential molecular target for anti-obesity drugs. FEBS Lett 2007;581:3405–9.
[54] Rayalam S, Yang JY, Ambati S, Della-Fera MA, Baile CA. Resveratrol induces apoptosis and inhibits adipogenesis in 3T3-L1 adipocytes. Phytother Res 2008;22: 1367–71.