

INTERACTION BETWEEN THE REPRODUCTIVE AND VITAMIN D-HORMONAL SYSTEMS IN THE WOMEN'S LIFE CYCLE

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Summary

The existence of an interaction between the reproductive system and the vitamin D-endocrine system was recently suggested by studies, both from our laboratory and other investigators. Current data indicate that estrogen controls the sensitivity of bone forming cells (osteoblasts), duodenal mucosa and the colonic mucosa cells to vitamin D. This interaction has potential pathophysiological and clinical implications on the pathogenesis of at least two major conditions linked to the life cycle of women: postmenopausal osteoporosis and an increase in the incidence of colorectal cancer in the postmenopausal period.

An Overview of Calcium Homeostasis and the Pathogenesis of Osteoporosis

Calcium Homeostasis

Calcium plays an important role in a variety of biological systems, including biological-membrane stabilization, trans-membrane ion exchange regulation, intracellular signal transduction, as co-factor in enzymatic reactions and most notably as a major component of the mineral component of the skeleton. Extracellular concentrations of calcium are tightly controlled within narrow limits by an endocrine system involving parathyroid hormone (PTH), vitamin D and the calcium-sensor receptor (CaR) as its major components¹. Calcium flows into the extracellular space from intestinal absorption, bone resorption and increased calcium re-absorption in the renal tubuli and can be removed from the extracellular space mainly through excretion in the urine and to a lesser extent by incorporation into the skeleton². Alterations in extracellular calcium concentrations are sensed by the CaR located in the outer membranes of parathyroid cell, renal tubular cells and a wide variety of other cell types³. When hypocalcemia takes place, it stimulates PTH secretion by the parathyroid glands, which in turn stimulates activation of vitamin D (see below). In addition, PTH increases renal tubular calcium absorption and stimulates bone resorption. 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃], the most active metabolite of vitamin D, acts primarily to increase calcium absorption

in the small intestine. CaR itself stimulates calcium re-absorption in the renal tubuli in response to reduced calcium concentrations. Hypercalcemia, on the other hand, suppresses PTH secretion by the parathyroid glands, which removes the main stimulus for vitamin D activation. In addition, increased calcium concentration directly suppresses production of $1,25(\text{OH})_2\text{D}_3$ and promotes urinary calcium excretion in response to increase in extracellular calcium concentrations via tubular CaR activity.

In summary, calcium homeostasis is tightly regulated by physiologic interaction between various components of the PTH-vitamin D-endocrine system and the calcium sensor-receptor, organized in multi-level negative feedback loops².

Postmenopausal Osteoporosis

Osteoporosis is a condition characterized by progressive decrease in bone mass and deterioration of its micro-architecture, increased bone fragility and susceptibility to fractures, which are an important cause of morbidity and mortality in the elderly population. Postmenopausal women are particularly prone to develop osteoporosis as a result of the decline in their estrogen levels. Intestinal calcium absorption, which also declines in the postmenopausal period⁴, may play an additional important role in the pathogenesis of postmenopausal osteoporosis.

Vitamin D: Mode of Action, “Classical” and “Non-Classical” Functions

In humans there are two main sources of vitamin D. Vitamin D can be obtained from the diet and from de-novo synthesis in the skin, in a process that requires exposure to solar U.V. light. To gain its full activity, vitamin D undergoes sequential hydroxylations; first in the liver to produce the relatively inactive metabolite 25 hydroxyvitamin D₃ [$25(\text{OH})\text{D}_3$], and subsequently in the kidneys to form the biologically most active derivative $1,25(\text{OH})_2\text{D}_3$ ⁵. While production of $25(\text{OH})\text{D}_3$ is primarily regulated by substrate availability, regulation of $1,25(\text{OH})_2\text{D}_3$ production is tightly regulated.

Regulation of calcium homeostasis and bone mineralization is the best recognized, so-called "classical", function of $1,25(\text{OH})_2\text{D}_3$. Intestinal calcium absorption is based on two major processes: passive diffusion, which depends on the concentration gradient across the intestinal mucosa, and active absorption, which is a complex, incompletely understood process⁶.

Active intestinal calcium absorption involves transfer of calcium into the intestinal mucosal cells, its transition through the cellular cytoplasm and finally extrusion against a concentration gradient into the extracellular space⁷. All these steps are

kept under $1,25(\text{OH})_2\text{D}_3$ control following binding to dedicated receptors, both at genomic and non-genomic levels⁸.

Classical vitamin D receptors (VDR) are ligand activated transcription factors which belong to a superfamily of genes encoding receptors for vitamin A, steroid and the thyroid hormone as well as a variety of orphan receptor proteins, for most of which the ligands remain to be determined. Genes encoding proteins involved in mineral metabolism are a principal focus of $1,25(\text{OH})_2\text{D}_3$ activity through VDR^{9,10}. The concentration of VDR in target cells is a primary determinant of the biological response to $1,25(\text{OH})_2\text{D}_3$, as previously demonstrated in cell lines^{11,12}. More recently, non-genomic activities of vitamin D have been described and attributed to putative membrane-bound vitamin D receptors¹³.

Vitamin D receptors (VDR) have been detected in a variety of cell-types, which are not necessarily involved in calcium regulation. In addition to its role in mineral homeostasis, $1,25(\text{OH})_2\text{D}_3$ is now known to be involved in the regulation of genes playing key roles in processes of cellular proliferation and differentiation^{14,15}.

Estrogen, Bone and Mineral Metabolism

The skeleton is an important target for gonadal hormones, which participate in maintaining skeletal integrity. The role of estrogen in this regard is well recognized and well documented¹⁶⁻¹⁸.

Homeostasis of the adult human skeleton is maintained through interaction between osteoblast and osteoclast activity. This so-called coupling between bone formation and bone resorption is shifted towards increased resorption in pathophysiological processes that produce net bone resorption and osteoporosis.

Following menopause a sharp decline in estrogen levels takes place and is followed by an increase in the rate of bone loss. This facilitated bone resorption may result in increased bone fragility and increases the risk of fractures, a condition known as "postmenopausal osteoporosis". Estrogen-deficiency states such as menopause, post-menopausal osteoporosis¹⁹ and the post-ovariectomy state²⁰ are associated with decreased circulating estrogen and concomitant decrease in calcium absorption.

Estrogen acts on its target cells through specific estrogen receptors (ER) which belong to the same superfamily as VDR and similarly act as transcription factors for target genes. Recent studies revealed the existence of at least two ER subtypes, ER α and ER β . Binding affinity of estrogen to the different ER subtypes is similar, however the gene expression that results from this binding differs between tissues and ligand types. Different tissues characteristically express predominantly one or

both types of ER²¹. ER has been detected both in osteoblasts and osteoclasts, indicating that bone is a legitimate target tissue, which may be directly influenced by estrogen. We have recently shown that rat osteoblast-like cells (ROS 17/2.8) express Erβ but not ERα²².

In an original study by our group²³, we have shown that osteoblast-like cells (ROS17/2.8) responded to 1,25(OH)₂D₃ by decreasing cell proliferation and increasing production of the bone matrix protein osteocalcin. Incubation of the cells with estrogen concurrently increased binding of 1,25(OH)₂D₃ to its specific receptor in the osteoblasts, and the bio-responsiveness of the cells to 1,25(OH)₂D₃. Others have shown that estrogen increases VDR gene transcription²⁴⁻²⁶. Thus, estrogen was shown to modulate VDR gene expression, VDR protein expression and sensitivity of osteoblastic cells to 1,25(OH)₂D₃, which in turn promotes osteoblast differentiation and activity.

Estrogen and Intestinal Calcium Absorption

As previously mentioned, calcium intake plays an important role in retarding the osteoporotic process in postmenopausal women. Intestinal calcium absorption declines with age, both in humans²⁷⁻²⁹ and in rats^{30,31}. A widely held hypothesis suggests that the decrease in intestinal calcium absorption results from a sequence of events initiated by low estrogen levels causing increased bone resorption; released calcium increases extracellular space calcium concentration, which suppresses PTH secretion, followed by subsequent decrease in 1,25(OH)₂D₃ production, decrease in 1,25(OH)₂D₃ plasma concentration, and finally results in decreased intestinal calcium absorption⁶.

Nevertheless, there is evidence that estrogen may be more directly involved in determining intestinal calcium absorption. Estrogen receptors³²⁻³⁴, as well as estrogen-receptor associated proteins, pS2 antigen^{35,36} and ER-D5³⁷, have been consistently demonstrated in the mucosa along the alimentary tract, suggesting a specific physiological role for estrogen in the intestine. Menopause, postmenopausal osteoporosis³⁸ and the post-ovariectomy state^{39,40} are associated with decreased circulating estrogen and concomitant decrease in calcium absorption. Moreover, available data indicates that the decrease in the basal levels of 1,25(OH)₂D₃ could not solely account for the decrease in calcium absorption, suggesting that the intestines of elderly or ovariectomized women are resistant to 1,25(OH)₂D₃^{41,42}. In addition, estrogen administration was shown to effectively restore the normal responsiveness of the intestine to 1,25(OH)₂D₃ in ovariectomized premenopausal women⁴² and in postmenopausal women⁴³. Studies have indicated an age-related decrease in intestinal VDR^{44,45}.

We have recently obtained unequivocal results indicating up-regulation of VDR following estrogen treatment in the duodenal mucosa of ovariectomized female rats. The increase in VDR expression was associated with an increase in the vitamin D-related biological responses, alkaline phosphatase activity and calbindin-9k gene expression, markers of VDR activity⁴⁶. While alkaline phosphatase activity is a relatively non-specific marker of vitamin D activity, expression of the calcium binding protein, calbindin-9k, is almost exclusively vitamin D dependent^{47,48}. Chen et al⁴⁹ have demonstrated a concurrent increase in VDR expression and increased calcium absorption in response to estrogen.

Taken together, these results provide an explanation for the previously observed resistance of the aging intestine to vitamin D^{50,51}, suggesting that estrogen may influence sensitivity to vitamin D including calcium absorption through an increase in VDR expression and that the contrary may happen in estrogen-deficiency states. This effect of estrogen may account at least in part for the protective effect of estrogen against osteoporosis.

It should be noted that some evidence also exists for a direct involvement of estrogen in intestinal calcium absorption, independent of its effect via VDR⁵².

In summary, available evidence suggests that estrogen may influence bone metabolism through direct influence on bone cells and indirectly through its influence on promoting intestinal calcium absorption by increasing duodenal mucosa sensitivity to 1,25(OH)₂D₃. The increased sensitivity for vitamin D in target organs involved in maintenance of skeletal integrity is not an exclusive mechanism and it combines with additional effects of estrogen on bone, which act in concert to maintain bone integrity. The loss of these mechanisms is the basis for the pathogenesis of postmenopausal osteoporosis.

Vitamin D and Colorectal Carcinogenesis

Colorectal cancer is one of the commonest malignancies and the incidence is rising⁵³. Environmental factors such as diet are believed to be important in the etiology of sporadic cases⁵⁴⁻⁵⁷. In the majority of cases interactions between environmental and genetic elements causes a series of somatic mutations, which leads to malignant transformation as described by Ilyas et al⁵⁸. Primary prevention of this disease is still not feasible despite major advances in our understanding of the mechanisms at the genetic, germline and somatic levels. In recent years there has been interest in the role of dietary measures such as vitamins, minerals, flavonoids and fibers in prevention of colorectal cancer^{59,60}.

The possibility of using dietary micronutrients as putative anticarcinogens to prevent colon cancer has long been considered, and in this regard calcium and

vitamin D have received much attention. A number of epidemiological, clinical and experimental studies suggest a role for supplemental dietary calcium in the prevention of colon cancer⁶¹.

It is well established that $1,25(\text{OH})_2\text{D}_3$ is involved in the regulation of cellular processes not directly related to mineral and skeletal homeostasis. $1,25(\text{OH})_2\text{D}_3$, the most active metabolite of vitamin D, restrains proliferation and promotes differentiation in a variety of cancer cell lines, including human colonic adenocarcinoma cells⁶²⁻⁶⁴. $1,25(\text{OH})_2\text{D}_3$ and other vitamin D metabolites have been shown to impede tumor formation in murine models of colon carcinogenesis induced by the potent procarcinogen 1,2-dimethylhydrazine (DMH)^{65,66}. The rat colonic mucosa was shown to possess a single class of high affinity vitamin D-receptors. It was shown that vitamin D delivered prior to the administration of the colonic carcinogen DMH markedly reduced (by 50%) the number of emerging colonic adenocarcinomas⁶⁷.

We have recently conducted a study designed to gain further insight into a putative anticancer effect of dietary vitamin D₃ (cholecalciferol) in DMH-treated rats⁶⁸. Rats were maintained on a stress diet containing high (20%) fat, reduced calcium content, a high P/Ca⁺² ratio and either low or high vitamin D₃ content. The stress diet produced a time-dependent increase in colonic thymidine kinase (TK) activity concomitant with an increased proliferation rate of the colonic epithelium. Rats treated with the carcinogen DMH and maintained on the standard diet presented a marked increase in the proliferative indices of colonic epithelium and an expansion of the crypt proliferative compartment. TK activity and the crypt mitotic zone were significantly augmented in the animal group presented with the stress diet. Supplementary vitamin D₃ abrogated the stress diet-induced colonic responses to the carcinogenic insult. Colon tumor growth in the stress diet rat group was five-fold greater compared to that scored in animals maintained on a standard diet. These results clearly indicated that dietary vitamin D₃ mitigated the neoplastic process in the colon in response to the carcinogen, supporting the view that changes in dietary components and micronutrients, particularly vitamin D, have an effect on the pathogenesis of colorectal cancer.

A protective effect of $1,25(\text{OH})_2\text{D}_3$ in human colon carcinogenesis has also been demonstrated in epidemiological studies^{69,70}. However, the data on the role of vitamin D in the pathogenesis of human colorectal cancer is still conflicting and inconclusive. We have recently conducted an additional study⁷¹ in which we compared serum $1,25(\text{OH})_2\text{D}_3$, 25-OH-D₃, and PTH levels in colorectal carcinoma patients with those of healthy controls. The results obtained showed that 25-OH-D₃ serum levels were higher in the cancer patients irrespective of the stage of the

disease. However, serum $1,25(\text{OH})_2\text{D}_3$ decreased with advancing disease, and concomitantly PTH levels were significantly higher in this group of patients. These findings further support the notion that circulating $1,25(\text{OH})_2\text{D}_3$ may influence proliferation of colonic epithelial cells in humans, and that decreased serum levels may facilitate the growth of more aggressive colorectal carcinomas.

Estrogen and Colorectal Cancer

Epidemiological studies suggest a protective, antineoplastic influence of estrogens on the intestinal mucosa. This is implied by a lower incidence of gastrointestinal cancer in premenopausal women, which increases after menopause; protection against gastrointestinal cancer conferred by parity; and by an overall favorable prognosis for colorectal cancer in females⁷²⁻⁷⁵. Moreover, the use of oral contraceptive pills by premenopausal women^{76,77} and the use of estrogen replacement therapy (HRT) by postmenopausal women⁷⁸⁻⁸⁰ are associated with a significant decrease in colonic polyps, overall risk of colorectal cancer and a decrease in the risk of fatal colonic cancer. Since the number of intestinal estrogen receptors in men and women is similar⁸¹, the permissive role of circulating estrogen to enable its effect on the gastrointestinal mucosa is emphasized. Jacobs et al⁷⁸ have shown that use of hormone replacement therapy (HRT) by postmenopausal women was associated with decreased risk of colon cancer. This was particularly evident among women with more than five years of use. Any protective effect of HRT against colon cancer would be important as part of the continuing debate over the potential risks and benefits of HRT.

In *in vivo* studies conducted in animal models, the administration of estradiol was shown to decrease ^3H -thymidine incorporation into colonic mucosa of ovariectomized mice⁸² and to decrease the number of emerging colonic tumors induced by DMH in mice⁸³ and rats⁸⁴. In *in vitro* studies, estradiol was also shown to suppress proliferation of a colon cancer cell-line⁸⁵.

The putative role of estrogen in preventing gastrointestinal tumorigenesis is further suggested by studies indicating a significant decrease^{86,87} or total absence of estrogen receptor expression⁸⁸⁻⁹⁰ in neoplastic colonic tissue compared to the normal colonic mucosa. Inactivation of the estrogen receptor gene via a methylation of a specific estrogen receptor CpG island sequence and silencing of gene transcription was observed with aging and in a large number of colorectal cancers, suggesting that it could predispose to colorectal neoplasia via gene silencing⁹¹. We have recently shown that estrogen specifically protects against methylation of sequences close to the promoter area of the VDR gene⁹², thus preventing silencing of this pro-differentiative gene.

We studied the effect of estrogen on VDR expression in the model of DMH-induced colorectal cancer in four groups of ovariectomized female rats: untreated/control, estrogen-treated, DMH-treated, and combined estrogen and DMH-treated. Exposure to estrogen was associated in all cases with a marked increase in VDR mRNA content and VDR protein expression in the colonic mucosa. In tumor extracts VDR expression was considerably lower compared to the apparently normal mucosa. Analysis of the mechanism involved in VDR down-regulation indicated that a significant CpG methylation in the VDR gene was observed in colonic tissue DNA harvested from DMH-treated rats, but not in colonic mucosa DNA from DMH-treated rats with estrogen. The highest frequency of CpG methylation was detected in colorectal tumors. In addition, we have shown that estrogen administration to ovariectomized female rats, which up-regulated the expression of VDR in the colonic mucosa, was associated with a 70% decrease in appearance of colonic carcinoma following DMH administration. These results suggest that increased VDR activity could be one of the mechanisms by which estrogen protects against neoplastic transformation in the colon.

All these findings led us to hypothesize that estrogen may interfere with the process of CpG DNA methylation in the colonic mucosa to prevent silencing of the VDR gene, which in turn causes up-regulation of both VDR gene transcription and protein expression. Alternatively, estrogen may control gene expression by direct nuclear genomic interaction following binding to DNA sequences.

A wealth of evidence has been accumulated lately indicating that estrogen may induce rapid intracellular responses, similar to those evoked by the binding of peptide hormones to their respective membrane receptors. Since there is yet no evidence for an estrogen responsive element (ERE) within the VDR promoter, it is possible that estrogen either influences VDR expression indirectly, through a yet unknown mechanism that prevents gene silencing or through the putative non-genomic signal transduction pathway.

Estrogen, Vitamin D-Receptor Expression and Colon Cancer

We have recently produced clear evidence that the colonic mucosal VDR gene transcript, colonic mucosal VDR protein expression and VDR response to endogenous $1,25\text{-(OH)}_2\text{D}_3$ are markedly augmented in response to estrogen⁹²⁻⁹⁴. These particular findings were demonstrated employing a variety of experimental methods (Northern blot, ligand binding, immunohistochemistry and Western-blot analysis). All results showed that *in vivo* administration of estrogen up-regulates VDR expression in the colonic mucosa. In addition, VDR-related bioresponses, i.e., mucosal alkaline phosphatase activity and the calbindin-9k steady state mRNA content in colonic mucosae from estrogen-exposed rats, were significantly

increased compared with estrogen-deprived animals. As alluded to earlier, alkaline phosphatase is associated with the differentiated phenotype of enterocytes and colonocytes^{95,96}, and calbindin-9k represents a biological marker of Vitamin D action^{97,98}. Up-regulation of VDR could not be attributed to changes in vitamin D metabolites or in PTH concentrations since they were similar in the various treatment groups. The increase in mucosal alkaline phosphatase activity and calbindin-9k in estrogen-exposed animals indicates that in addition to up-regulation of VDR expression, *in vivo* exposure to estrogen markedly sensitizes the colonic mucosa to the influence of endogenous 1,25-(OH)₂D₃.

The effects of estrogen on VDR expression in the colonic mucosa are consistent with similar effects of estrogen on VDR in other tissues and cell types⁹⁹⁻¹⁰². Chan et al¹⁰³ and Chen et al⁴⁹ have demonstrated a positive effect of estrogen on intestinal VDR expression in the intestine but did not attempt to correlate this effect with markers of vitamin D activity.

Further studies are required to elucidate the detailed mechanism(s) by which estrogen stimulates VDR expression in cells. Data from our recent studies^{92,93} as well as data previously obtained in osteoblast-like cells²³ indicate that estrogen increases total VDR mRNA content, suggesting that it may act at the genomic level by stimulating VDR gene transcription or otherwise stabilizing the VDR mRNA transcript.

Our studies^{92,93} support the possibility that the intestinal response to estrogen could be mediated, at least in part, via stimulation of VDR expression and bioresponse. Estrogen may thus play an important physiological role in regulating colonic mucosal VDR, which in turn influences cell proliferation; which may at least partially account for the protective effect of estrogen against colorectal cancer.

We have recently performed further *in vitro* studies to assess the nature of the cross-talk and/or the interactive mechanism(s) between estrogen and VDR. The studies were conducted on rat osteosarcoma cells (ROS17/2.8) and on human colon cancer cells (HT-29)^{22,104}. HT-29 and ROS17/2.8 cells were exposed to estrogen and 1,25(OH)₂D₃ at physiological concentrations, or to the combined hormonal treatments. In both cell lines a highly significant growth inhibition was observed especially in the combined treatments, concomitant with up-VDR expression. The selective estrogen-receptor modulator tamoxifen, widely used in the treatment of breast cancer, was shown to exert estrogen-like effects on VDR expression when administered alone or in conjunction with vitamin D and produced an anti-estrogenic effect when administered in combination with estrogen. The combined tamoxifen and vitamin D treatments inhibited cell proliferation both in HT-29 cells and in ROS17/2.8 cells, with concomitant up-regulation of VDR expression. These

findings further support our view that estrogen or compounds with estrogen-like activity tightly control VDR expression.

In these cell systems we studied whether signal transduction pathways are involved following exposure to estrogen. Our preliminary results revealed a rapid induced activation of p42/44, a mitogen activated protein kinase (MAPK), in HT-29 and ROS17/2.8 cells, both depending on estrogen receptor activation. When estrogen was supplied together with tamoxifen, the antiestrogen partly suppressed the activation of MAPK activity in HT-29 cells while completely abolishing it in ROS17/2.8 cells. Moreover, suppression of MAPK activation by suramin in HT-29 cells, in contrast to ROS17/2.8, suggests that the estrogen-mediated signal transduction pathway in colon cancer cells is probably linked to membrane receptors bearing tyrosine kinase activity. On the other hand, in ROS17/2.8 cells the estrogen-mediated signal transduction pathways could be associated with interaction of estrogen receptors with a G-protein family member or with intracellular proteins leading to MAPK activation. Additional studies are being conducted in these cellular systems to further clarify the nature of the interactions between estrogen and VDR.

Compounds with Estrogen-Like Activity

SERMs, Selective Estrogen-Receptor Modulators

SERMs, Selective Estrogen-Receptor Modulators, are compounds characterized by an ability to imitate estrogen activity in certain tissues, while producing anti-estrogenic effects in others¹⁰⁵. In recent years raloxifene, a SERM used in prevention and treatment of osteoporosis, drew considerable attention because it seems to fulfil the requirements of an ideal SERM. It is estrogenic in bone and with respect to markers of cardiovascular protection, while avoiding undesired estrogenic side effects in breast tissue and in the endometrium. Tamoxifene, a different SERM with anti-estrogenic and partial intrinsic estrogenic activity (a different spectrum of activity compared to raloxifene), was previously shown to possess estrogenic activity in the intestine and to protect against induced colon cancer¹⁰⁶ and breast cancer¹⁰⁷.

Based in our findings on the role of estrogen in different target tissues^{46,92-94}, we are conducting a series of experiments designed to examine the correlation between the putative anti-neoplastic activities of SERMs in the colon and their effect on VDR expression. This series of experiments is expected to provide additional insight to the role of VDR and the vitamin D-endocrine system as mediators for the antineoplastic effect of estrogen in the colon. If, as is the case for estrogen, SERMs

are found to up-regulate VDR expression, it will further support the idea of an anti-neoplastic activity of estrogen and its analogs in the colon. Moreover, if we demonstrate that the effect of SERMs is mediated via activation of the vitamin D-endocrine system, it could provide a basis for the use of specific vitamin D derivatives in combination with estrogen or its analogs as a mean of preventing colon cancer. Parallel observation of up-regulation of VDR in the duodenum in response to SERMs will provide an important clue regarding at least one aspect of the mechanism by which SERMs protect against osteoporosis.

Natural Estrogen-Receptor Modulators, Phytoestrogens

Phytoestrogen (plant estrogen) is a generic name used to define classes of compounds that have high structural similarity to estrogen. They are either of plant origin or derived from *in vivo* metabolism of precursors present in plants and/or eaten by mammals¹⁰⁸. There are many plant hormones that act as estrogens in the body. The most common types of phytoestrogens are flavones, lignans, and isoflavones, with the latter being the most potent of the three. In plants, phytoestrogens protects against ultraviolet radiation effects, control normal plant growth and protect from stress¹⁰⁹.

Phytoestrogens, like synthetic estrogenic and anti-estrogenic compounds, bind estrogen receptors in mammals¹¹⁰. They significantly differ from the synthetic estrogen-receptor modulators in their ability to undergo metabolism¹¹¹. This remarkable characteristic of high turnover and short half-life provides that the phytoestrogens are not stored in tissues. Phytoestrogens are able to selectively mimic the effects of estrogen in certain tissues. Data indicates that phytoestrogens may offer protection against a wide range of human conditions, including breast, bowel, colon, prostate, brain and other cancers; cardiovascular disease; osteoporosis and menopausal symptoms¹¹²⁻¹¹⁶. Molecular and cellular biology experiments, animal studies and human clinical trials suggest that isoflavones in particular may confer specific health benefits related to cardiovascular diseases, cancer, osteoporosis, menopausal symptoms and other diseases. Thus, populations consuming diets rich in phytoestrogens were shown to be more protected against breast cancer, heart disease, menopausal symptoms and osteoporosis than populations consuming less of these products. An enriched phytoestrogen-diet, such as the Asian diet, contains 35 mg/day of isoflavones compared with 2mg/day in a typical Western diet^{117,118}.

Soy products are a common dietary source of isoflavones. The two major isoflavones in soy products are genistein and daidzein, which have been shown to possess anticarcinogenic properties¹¹⁷. Soy also contains genistin and daidzin,

which are sugar-containing isoflavones molecules. During digestion, intestinal bacteria cleave the sugar moiety to produce more genistein and daidzein.

Isoflavones are very similar in structure to estrogen and are able to bind estrogen receptors at 1/100th to 1/1000th the affinity of estradiol. Since high levels of estrogen have been linked to breast cancer and other hormone-related cancers, it has been suggested that isoflavones may bind estrogen receptors and block some of the untoward effects of the natural estrogen, thus functioning as antiestrogens, similarly to the mechanism of action suggested for tamoxifen.

The complicated issue of tissue specific responses and pharmacology of synthetic as well as natural estrogenic compounds is at least partially explained by the discovery of ER isoforms, expressed selectively in different tissues. The best characterised ER isoforms are estrogen receptor alpha (ER α) and the more recently discovered estrogen receptor beta (ER β)^{21,119}. Evaluation of the binding activities of a large number of phytoestrogens and other natural estrogenic compounds demonstrates that their binding affinity to ER β is significantly higher than to ER α .

Other non-hormonal mechanisms have also been proposed for the anti-cancer effects of phytoestrogens. Genistein has been shown to significantly affect growth of cancer cell lines in vivo and in vitro through inhibition of protein tyrosine kinases, a family of enzymes associated with stimulation of cancer cell growth¹²⁰. In addition, it was demonstrated that genistein promoted differentiation of cancer cells, possibly by interfering with the cell cycle¹²¹. Moreover, genistein has been shown to inhibit the formation of new blood vessels (angiogenesis), an effect directly related to reduced blood supply to growing tumors¹²². Diets containing the isoflavone genistein have been shown to decrease the number and size of carcinogen-induced tumors in experimental animals¹²³.

Our aim, as for SERMs, will be to examine the correlation between the putative anti-neoplastic activity of phytoestrogens in the colon and its effect on VDR expression. These series of experiments are expected to provide additional insight to the interaction between the reproductive and vitamin D-endocrine systems and the putative antineoplastic effect of natural compounds with estrogenic activity in the colon. As for estrogen, parallel observations of up-regulation of VDR in the duodenum in response to phytoestrogens will provide an important clue regarding at least one aspect of the mechanism by which phytoestrogens also protect against osteoporosis.

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