



Estromineral

Soy Isoflavones enhanced by Probiotic
Lactic Acid Bacteria,
with Calcium, Vitamin D₃ and Horsetail for
Well-being and Bone Health in Menopause

by

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Preface

Every woman facing the menopause has a dream: to age with dignity. This means having the longest possible health expectancy and remaining mentally alert and physically independent in complete autonomy. To realize this desire, every woman is motivated to use all the resources medicine has to offer, without taking any extra risk.

Hormone replacement therapy (HRT) with conjugated equine estrogens (CEE) extracted from the urine of pregnant mares or with synthetic estrogen hormones provides undoubted benefits to climacteric and postmenopausal women: they are relieved from hot flashes, sweats, insomnia, mood swings and irritability that typically occur in climacteric and in early menopause, due to the deficiency of ovarian hormones.

More questionable is the risk/benefit ratio of long-term treatments aimed at reducing cardiovascular risk. Observational studies suggest positive results both in terms of cardiovascular prevention and osteoporosis risk reduction. Unfortunately, the first large prospective controlled clinical trial published so far did not confirm the previous positive data on cardiovascular prevention, although it confirmed the positive impact on bone protection. The Women's Health Initiative (WHI), that included more than 27,000 generally healthy postmenopausal women - aged 50 to 79 - randomly assigned to receive HRT or placebo, was stopped after 5 years because the risks of the long-term combined HRT (0.625 conjugated estrogens and 2.5 medroxyprogesterone acetate, MAP) were not compensated by benefits on the general health. The extra risk was present only in the arm with CC and MAP, whilst the arm with CC only is still ongoing. The impact of WHI results, that indeed confirmed previous observational data with the only exception of the cardiovascular prevention, has been dramatic both on physicians and women. Fear of cancer and of cardiovascular negative effects has been overemphasized.

The wave of negative emotions about HRT is still ongoing. Frightened women want something helpful but not steroid hormones. Physicians are becoming more and more cautious. The medical need of intervention in the management of late menopausal disorders, such as the increase of cardiovascular risk, osteoporosis, atrophy of the genital organs, deficiency of cognitive functions, is open for a different approach, that can be either symptomatic or etiologically oriented. Possibly "natural": this is the core of women's request. Can we, Western women, learn something useful from different cultures and different lifestyles with regards to a better health in aging?

Indeed, there are populations in the World, e.g. in China and in Japan, who have a much lower incidence of cardiovascular disease, of osteoporosis, and of genital and breast tumors, compared with women in the Western hemisphere. This is interpreted as the result of genetic, environmental, behavioral, cultural and dietary factors. Soy, which is an essential component of Chinese and Japanese diet, was identified as the leading factor of Eastern health-protective diet. The epidemiological evidence suggests that soy, thanks to its content of isoflavones, can be considered an important health factor for the control of early and late menopausal disorders. Actually, the North American Menopause Society in its Consensus Opinion has encouraged the dietary use of soy and of soy isoflavones, together with a healthy lifestyle and a low cholesterol diet, in order to reduce cardiovascular risk.

Soy isoflavones have a strong biological rationale: they elicit light estrogenic effects because they interact with estrogen receptors, that are present in most tissues of the human body and are classified as alpha-receptors and beta-receptors. Alpha-receptors are present in the breast and in the endometrium, whereas beta-receptors are found mainly in the endothelium of the blood vessels, in the brain, kidney, intestine, urethra and bladder and in bone. In general, alpha-receptors mediate proliferative and reproductive actions, whilst beta-receptors mediate antiproliferative and reparative cell processes. Thanks to the higher affinity for the beta-receptors, soy isoflavones have only very slight effects on the uterus and breasts, much smaller than that of estradiol and of other exogenous steroid estrogen hormones. The biological action of the soy isoflavones is therefore milder, more respectful of the delicate hormonal balance in women, compared with the action of the steroid estrogens. Therefore, for many women with mild menopausal symptoms, soy isoflavones may represent an interesting alternative to HRT.

Soy isoflavones, as well as those from other plants, are bound by glycosidic links to different sugars. As such, they are very large molecules and cannot be easily absorbed in the digestive tract. To be absorbed and to become bioavailable, the active fraction of the molecule must be split off from the sugar moiety, a process that is accomplished by special enzymes, the glycosidases. Our gastrointestinal juices, however, do not contain glycosidases and are unable to transform the isoflavones into bioavailable molecules. This process is provided by the intestinal flora and in particular by lactic acid bacteria, which produce the glycosidases required for the absorption of the isoflavones. Indeed, a notable contribution to understand the correct use of soy isoflavones was provided by KDR Setchell and by his coworkers, who pointed out the importance of intestinal flora for the absorption of these substances. Since the full bioavailability and efficacy of the isoflavones depend on the presence in the intestine of lactic acid bacteria, it appears quite rational to combine soy isoflavones with lactic acid bacteria to assure their bioavailability.

ESTROMINERAL is based on this rational combination. The addition of calcium and vitamin D supplements enhances the mineralizing effects of the preparation, and provides some essential natural bricks for aging in better health.

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Climacteric and menopause.

Clinical aspects

Menopause is the cessation of physiological menstruation (from the Greek *menos* = month, and *pausis* = cessation). The start of menopause is defined retrospectively, i.e. as absence of menstruation for 12 months. Menopause occurs between the age of 44 and 53, in average at 49 years.

Climacteric (from the Greek *klimaktéros* = critical transition) is the transition from the physiological conditions of a woman's fertile years to the new conditions that are established after the menopause. Climacteric generally begins 3-5 years before menopause and ends 4-5 years after menopause and is characterized by the appearance of the symptoms of menopause.

Perimenopause is the period between the start of climacteric symptoms and one year after the menopause.

General considerations

Our cells, tissues, organs, systems, functions and psyche are profoundly influenced by **hormones**, which are produced in specialized glands called **endocrine glands**. Among the hormones, the sex hormones are of primary importance for the expression of the somatic and psychic characteristics that are typical of the two sexes and differentiate men and women.

In women of childbearing age, there are two main sex hormones, estradiol and progesterone, both produced by the ovaries. Their production follows the monthly cycles that determine the menstrual cycle. **Estradiol** exerts a trophic action on the whole genital system and especially on the mucosas of the vagina and of the uterus (the endometrium). The trophic action extends to the mammary glands, to the endothelium of the blood vessels, to the skin and to the skeleton. Estradiol also has a profound influence on a woman's psyche, conferring the characteristics of femininity, i.e. those that ultimately orientate a woman towards her procreative function (attraction to a partner, desire to mate, libido, care and protection of newborn, etc.). **Progesterone**, on the other hand, acts primarily on the endometrium, preparing it for implantation of the fertilized ovum. It also has effects on the psyche, including a particular depressive effect that afflicts many women during the second half of the menstrual cycle.

At around 50 years of age, the ovarian function ceases almost completely. All the stimuli exerted by estradiol and progesterone will therefore be missing and consequently the monthly hormonal changes of the menstrual cycle. A quiet hormonal homeostasis follows. In many women, however, the hormonal changes cause disturbances at various levels, known as **menopausal symptoms**.

The complete cessation of hormone production by the ovaries is never sudden, but occurs after some years of irregularity and adaptation

to the new situation. This period of transition is called the **climacteric** and is characterized by the appearance of **climacteric symptoms**.

The deficiency of ovarian hormones exposes a woman to other types of hormones. One of these is estrone, produced mainly in the adipose cells that utilize androstenedione, a steroid synthesized by the adrenal glands. Estrone is also present before menopause as a derivative of estradiol, but is balanced by the larger quantity of estradiol, which disappears almost completely after menopause. In addition, a woman is exposed also to androgenic hormones (androgens) produced by the adrenal glands, which, before menopause, are balanced by the estrogen hormones produced by the ovaries. The prevalence of androgen hormones has masculinizing effects on the skin and on the hair-producing system.

These changes are completely natural and physiological for this new phase of the woman's life. Nevertheless, many women find living through the climacteric and the menopause a bad experience, partly because of traditional, cultural, psychological and social conditioning. Presently, however, western women are facing this stage of life calmly, with vigor and continuity, now liberated from the menstrual cycles and from pregnancies, and perform important political, social, cultural, creative and managerial activities. The discomfort and the psychic and organic symptoms that occur during the climacteric and the menopause are also easily overcome, thanks to a healthy lifestyle and the natural and pharmacological remedies that are presently available and which, among other things, make it possible to maintain full sexual function.

Symptoms of climacteric and of menopause

Many menopausal symptoms already start in perimenopause, i.e. 3-5 years prior to the menopause, and are the first signs of diminished ovarian function and of the consequent irregularities in secretion of the sex hormones. These symptoms are called **climacteric symptoms**, and characterize the **climacteric**. The climacteric symptoms then continue for 4-5 years after the menopause, i.e. until the body has adapted to the new hormonal balance. The type and intensity of the climacteric and menopausal symptoms depend also on non-hormonal factors as a result of the interaction of the new biological conditions with the individual, social and cultural situation. From an operational standpoint, however, the symptoms that are closely connected with the decrease in levels of estrogens and that receive an obvious benefit from hormone replacement therapy are defined **specific climacteric and menopausal symptoms**.

Symptoms of climacteric and of early menopause

Hot flashes

Hot flashes are the main symptom of climacteric and consist of a



feeling of heat that lasts a few minutes affecting the face first, and then spreading to the whole body. They occur in 85% of women in the premenopause and in the early menopause. During a hot flash there is an increase in heart rate and in skin temperature, leading to considerable sweating.

Sweats

Hot flashes are often associated with sweats that occur mostly at night and cause frequent and troublesome awakening.

Insomnia

Frequent awakening and difficulties in getting to sleep often trouble menopause.

Anxiety and depression

In climacteric and in early menopause a higher incidence of anxious and depressive states is observed. Hormone replacement therapy or treatment with phytoestrogens alleviates or suppresses these disturbances.

Disturbances of sexual function

These may be of psychological origin, such as decrease in sexual desire, excitement and sexual pleasure. They may have an organic basis, such as dyspareunia secondary to vaginal atrophy and dryness.

Medium-term menopausal symptoms

Vaginitis, vulvitis and dyspareunia

The estrogenic hormones are essential for the trophism of the genital organs. Deficiency of these hormones leads to dystrophy and to atrophy of the uterus, of the Fallopian tubes, of the vagina and of the vulva. The symptoms that are most visible and troublesome are those involving the vagina and the vulva. The vaginal mucosa becomes thinner and loses resistance to mechanical stresses. This phenomenon, associated with vaginal dryness, shrinking of the vagina and of the introitus, makes sexual intercourse difficult, painful, and is often accompanied by bleeding, with consequent dyspareunia. In the vaginal mucosa, the cyclic process of maturation of the epithelium ceases, and with it the production of glycogen in the surface layers. Glycogen is the nutrient substrate of a certain vaginal flora (especially of Döderlein's bacillus: *Lactobacillus acidophilus*), which produces lactic acid and maintains the pH of the vagina at values of 5-6. The vaginal acidity prevents colonization by pathogenic microorganisms (for example those originating from the colon) that require an alkaline environment. The atrophic vagina therefore becomes very susceptible to infections that cause itching, burning and discharges.

Urinary incontinence

The estrogenic hormones also exert a trophic action on the mucosa of the bladder and urethra. Deficiency of these hormones causes atrophy of the mucosa with consequent frequent urgency of miction and susceptibility to urinary incontinence on exertion, coughing, laughing, etc.

Skin ageing, hypertrichosis and alopecia

The estrogenic hormones exert a trophic action on the skin, espe-

cially on the deep layers (dermis) that are rich in elastic fibers and collagen, proteoglycans and capillaries, which give the skin elasticity, turgor and firmness. At menopause the lack of estrogenic hormones causes atrophy of the dermis, which is manifested as decrease in thickness and loss of elasticity of the skin, with the consequent formation of wrinkles, dryness, desquamation, itching and increased cold sensitivity. Furthermore, the androgenic hormones, no longer balanced by the estrogenic hormones, cause an increase in hair growth (hypertrichosis), altering its distribution, which becomes more masculine, and causing seborrhea and hair loss.

Symptoms and diseases in the advanced menopause

Osteoporosis

Osteoporosis is defined by WHO as "disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures".

Skeletal bones are not inert tissues, but are subject to a continuous process of **remodeling**, in which old bone is replaced with new tissue. Remodeling comprises local resorption of bone by special cells, the **osteoclasts**, and subsequent apposition of new bone by other cells, the **osteoblasts**. Bone turnover involves the organic components of the bone, i.e. collagen fibers and proteoglycans, and the mineral components, i.e. calcium, phosphorus and magnesium. The turnover is positive in the first two decades of life. The **peak bone mass** is reached between the 20th and 30th year. After the age of 30, the osteoclastic processes predominate over the osteoblastic ones, with a slow and progressive loss of bone mass. The estrogenic hormones inhibit the osteoclasts and stimulate the osteoblasts, enabling a satisfactory bone mass to be maintained. After the menopause, the deficiency of estrogenic hormones accelerates the process of bone resorption, thus causing rapid loss of calcium from the bones. Therefore, after the menopause, the requirement for calcium increases to compensate the larger losses of calcium due to the deficiency of estrogenic hormones. In the most severe cases, the predominance of the processes of resorption of bone calcium leads to **postmenopausal osteoporosis**, with increased bone fragility, so that fractures can easily occur as a result of minimal trauma, especially affecting the vertebral bodies.

The most obvious symptoms are hunchback (kyphosis) and decrease in height, which are typical characteristics of persons affected by osteoporosis.

Diagnosis of osteoporosis is based chiefly on computerized bone mineralometry (CBM) which measures the so-called **T-score**. A T-score less than or equal to -2.5 indicates the presence of **osteoporosis**, which is defined as **serious** in the presence of fractures. If CBM gives a T-score between -2.5 and -1.0, this is called **osteopenia**. A T-score above -1.0 is regarded as **normal**.



Cardiovascular diseases

The risk of a woman suffering a heart attack before menopause is 44% relative to the risk for a man. After the menopause the risk increases, and after the age of 60 years it is the same as for men of the same age.

The estrogenic hormones protect against cardiovascular diseases by several mechanisms. They lower blood cholesterol and LDL (low-density lipoproteins, constituting the “bad” cholesterol) and lower also blood triglycerides (fats). They have a protective effect on vessel walls. They exert beneficial effects on the metabolism of polysaccharides and lipids and on the clotting factors, they protect against arterial hypertension, etc. The increase in cardiovascular risk after menopause therefore results from a combination of factors due to deficiency of female sex hormones.

Decline in cognitive ability – Alzheimer’s disease

Alzheimer’s disease is the commonest cause of senile dementia. The symptoms begin insidiously even 5-10 years before the disease is obvious and its prevalence increases exponentially after the age of 65 years, affecting women more often than men. Numerous data suggest that the lack of estrogens that is characteristic of the menopause represents one of these factors. The estrogens control the response to stress, increasing cerebral blood flow and increasing the utilization of glucose in the brain. The estrogens probably also reduce the formation of beta-amyloid substance that is typical of Alzheimer’s disease. It was shown that the hormone replacement therapy definitely improves the cognitive disturbances occurring in Alzheimer’s disease.

Therapeutic provisions

The treatment of menopause has the following objectives.

- **To eliminate or alleviate the acute menopausal symptoms**, primarily the vasomotor and neurovegetative disturbances.
- **To treat the disturbances that arise in the medium term** connected with dystrophy of the tissues, especially of the urogenital tract.
- **To prevent and treat the diseases of advanced menopause**, important on account of their inherent morbidity and mortality, in particular the fractures due to osteoporosis and the risk of myocardial infarction.
- **To improve the quality of life.**

Hormone Replacement Therapy (HRT)

Excellent therapeutic results are obtained correcting the deficiency of endogenous sex hormones that occur in menopause by the administration of these hormones (hormone replacement therapy - HRT), using hormones of synthetic origin.

The HRT is definitely require in the most severe cases of menopau-

sal syndrome, and must be prescribed by the gynecologist, to establish the most suitable therapeutic regimen for the patient and to assess possible contra-indications.

HRT is very effective because it uses very potent hormones, but it must be carefully adjusted for the individual patient in order not to cause excessive disturbance of the delicate endocrine balance of postmenopause. Moreover, for safety reasons, it is a good rule not to continue HRT for longer than 5 years.

Natural remedies

The incidence of diseases like osteoporosis, atherosclerosis and breast and uterine tumors is far lower in some oriental populations, especially the Chinese and the Japanese. The women in these populations complain less often of the typical disturbances of menopause, such as hot flashes, vaginal dryness and atrophy of the tissues of the genital system. These epidemiological findings have been attributed to the dietary habits of these populations, in particular the considerable use of soy, prepared in various ways. In fact, it has been demonstrated in numerous studies that, in addition to proteins and lipids of high nutritional grade, soybeans also contain substances that have effects similar to those of the estrogenic hormones, though with lower activity. These substances were identified as **isoflavones**, nonsteroidal natural compounds, structurally similar to the hormonal estrogens. In view of the vegetal origin and of the estrogenic effects, these substances have been called **phytoestrogens** (from the Greek *phyton*, plant). The action of the soy phytoestrogens is milder, more balanced and more respectful of the delicate hormonal balance in women, compared with the action of the hormonal estrogens. Therefore in many cases the phytoestrogens represent a valid alternative to HRT also because they do not have the hormonal effects that can in some cases constitute a contra-indication for hormonal estrogens.

Phytoestrogens are also found in other plants besides soy (alfalfa, rue, skullcap, red clover, peppermint, sassafras, dwarf palm, ginkgo biloba, black cohosh). However, the soy phytoestrogens are certainly the best-studied and the best known with regard to their biological effects and safety in use.

The soy isoflavones of ESTROMINERAL

Chemistry and kinetics

The phytoestrogens contained in ESTROMINERAL are soy isoflavones (SIs) obtained from purified and titrated extracts of soybeans (*Glycine max L.*), not genetically modified (GMO-free). Used is a special extraction process that is specific for the flavone fraction of soybeans. This enables to preserve the naturalness of the SIs, i.e. to maintain the same proportions in which the SIs occurs in the soybeans, and to get the benefit of the natural synergy of the active principles. The extracts are purified from inert components to obtain a concentration of SIs of 40%. Extraction processes that could alter the chemical structure



and the natural ratio of the SIs are carefully avoided.

ESTROMINERAL is manufactured in oval tablets (*caplets*). Each caplet contains 150 mg of soybean extract, equal to 60 mg of SIs, and in particular equal to 30 mg of **genistin** and 30 mg of **daidzin**. **Genistin** (MW 432.22) is the β -glucoside of **genistein** (MW 270.24) (Fig. 1). **Daidzin** (MW 416.23) is the β -glucoside of **daidzein** (MW 254.24) (Fig. 2).

Genistin and daidzin are relatively large molecules; they are very water-soluble and are highly polar. Therefore they are not easily absorbed from the gastrointestinal tract. For them to be absorbed and rendered bioavailable, they must be hydrolyzed to *aglycons*, i.e. without the glucoside component [15]. This takes place by the action of special enzymes, the *glycosidases*. Our gastrointestinal tract does not produce glycosidases and therefore we cannot hydrolyze the isoflavones and make them bioavailable. Conversely, some of the intestinal flora that colonizes our intestine produces the glycosidases required for the mentioned hydrolytic process that is necessary for the absorption of the iso-

flavones [6, 25]. The aglycons are absorbed in the small intestine. However, conversion of glucosides to aglycons mainly occurs in the colon, which has the greatest population of glycosidase-producing intestinal flora. Therefore the peak blood levels of aglycons are observed 4-6 hours after ingesting the soy extracts, i.e. when the glucosides are hydrolyzed under the action of the enzymes of the colonic flora [26].

The flora that populates the intestine comprises more than 500 microbial species and amounts to more than a hundred thousand billion living cells. The composition is not the same in all individuals and varies even in the same individual, depending on the diet, any drugs that are being taken, especially antibiotics, diseases in progress, etc. Therefore glycosidase activity is variable and in certain conditions it may even be absent, compromising the bioavailability of the isoflavones [28]. In ESTROMINERAL this problem has been solved by the addition of a lactic acid bacterium, the *Lactobacillus sporogenes*, that produces glycosidase and balances the intestinal flora.

Following absorption, genistein and daidzein undergo various

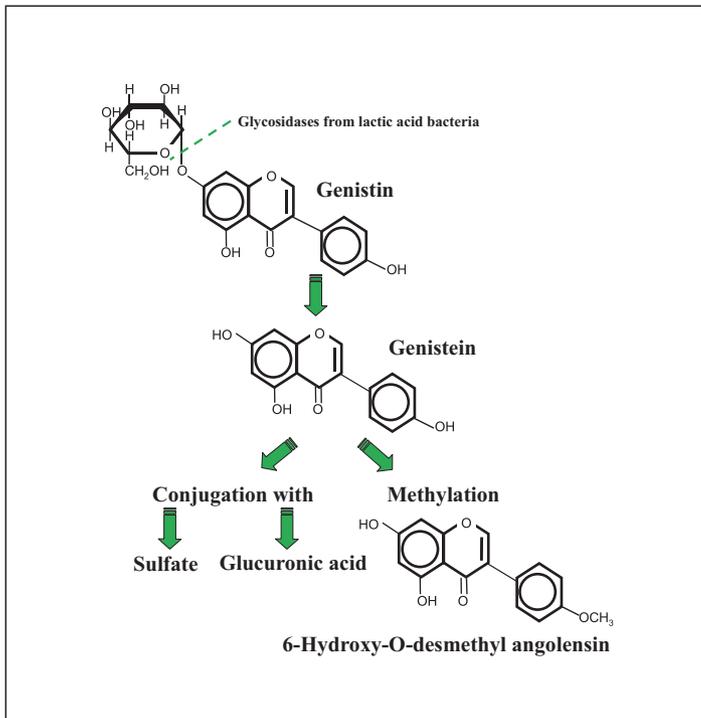


Fig. 1. Genistin: biotransformation and metabolism. The glycosidases of the intestinal flora and chiefly those of the lactic acid bacteria disrupt the glycosidic bond, releasing the aglycon, i.e. genistein. This is absorbed and metabolized to various active metabolites. Genistein and the various metabolites are finally conjugated with glucuronic acid and with sulfate, to be excreted in the bile and in the urine.

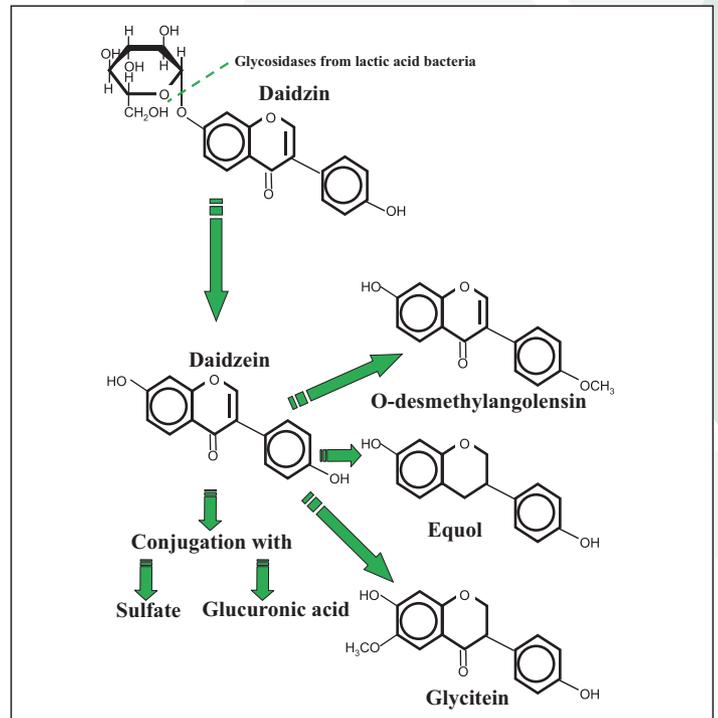


Fig. 2. Daidzin: biotransformation and metabolism. The glycosidases of the intestinal flora and chiefly those of the lactic acid bacteria disrupt the glycosidic bond, releasing the aglycon, i.e. daidzein. This is absorbed and metabolized, mainly to equol, which is the most active metabolite. Daidzein and its metabolites are finally conjugated with glucuronic acid and with sulfate, to be excreted in the bile and in the urine.



metabolic processes, mainly taking place in the liver [28]. They are conjugated with glucuronic and sulfuric acid, making them hydrophilic, and thus easily eliminated by Phase II detoxication through the renal and biliary route. Genistein and daidzein undergo also to various processes of drug metabolism, mainly in the liver, such as demethylation, dehydroxylation, reduction and ring cleavage.

The principal metabolite of genistein is hydroxy-O-demethylangolensin (Fig. 1). The principal metabolites of daidzein are O-demethylangolensin, glycitein and equol (Fig. 2). Equol is very important because its estrogenic activity is about 5 times greater than that of daidzein [1] and twice as high as that of genistein [31]. This remarkable efficacy is probably due to the similarity of the three-dimensional arrangement of the equol molecule with that of estradiol (Fig. 3) [27]. The formation of equol, which is so important for the estrogenic activity of the SIs, requires the fermenting activity of particular strains of lactic acid bacteria of the intestinal flora [27] that convert equol from its precursor daidzein. In this connection, particularly important is the diet. In fact a diet rich in carbohydrates that favors the growth and colonization of lactic acid bacteria, leads to notable amounts of equol (Fig. 4). ESTROMINERAL is enriched by *Lactobacillus sporogenes* a lactic acid bacterium which integrates the intestinal flora and maximizes the biotransformation of daidzein into equol.

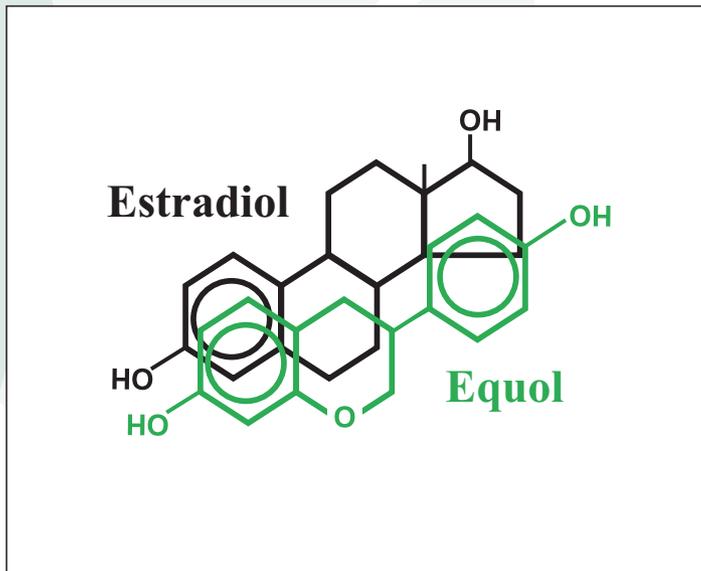


Fig. 3. Structural similarity of equol and estradiol [27].

The isoflavones and their metabolites are eliminated in the bile and then reabsorbed, entering an enterohepatic cycle. Notable amounts are eliminated in the urine [14, 26] and in breast milk [10].

The processes of glucoside hydrolysis required for the absorption of genistein and daidzein and the successive biotransformations of these isoflavones, are summarized in Fig. 5.

Biological activity of soy isoflavones

Soy phytoestrogens have various biological effects. One of the most important effects is binding to estrogen receptors and stimulating them, thus producing estrogenic effects.

Estrogen receptors

The estrogen receptors (ERs) belong to the superfamily of nuclear (and hence intracellular) hormone receptors, of which there are more than 150 entities. ERs stimulate the trophism of the tissues that contain them. There are two types of ERs, the alphaERs (α ERs) and the betaERs (β ERs), which have different distribution in the tissues. The α ERs are present in the endometrium, in the stroma of the ovaries and in the mammary gland. The β ERs, on the other hand, are present in the cells of the granulosa of the ovarian follicle, in the endothelial cells of the blood vessels, in brain, kidney, in the cells of the bladder and urethra, in the

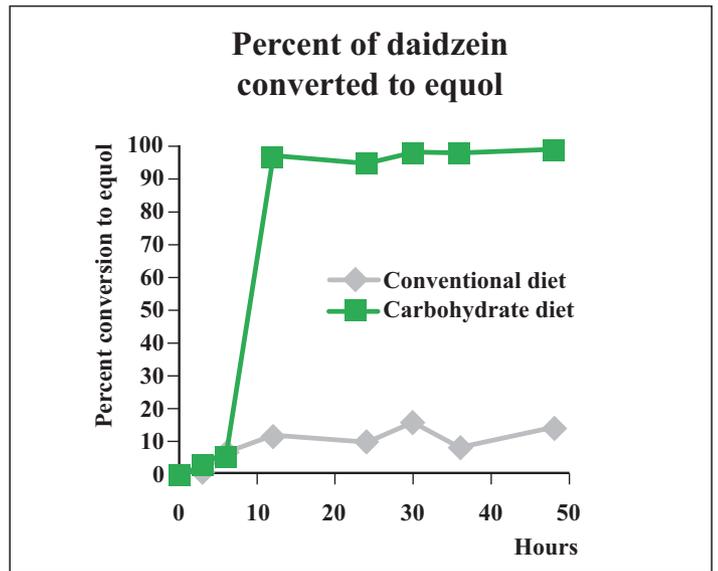


Fig. 4. Biotransformation of daidzein in to equol in the colonic material. In subjects with a conventional diet, only 16% of daidzein was converted into equol. In the material obtained from subjects with a carbohydrate-rich diet (prebiotic of the lactic acid bacteria) conversion was greater than 90% [27].

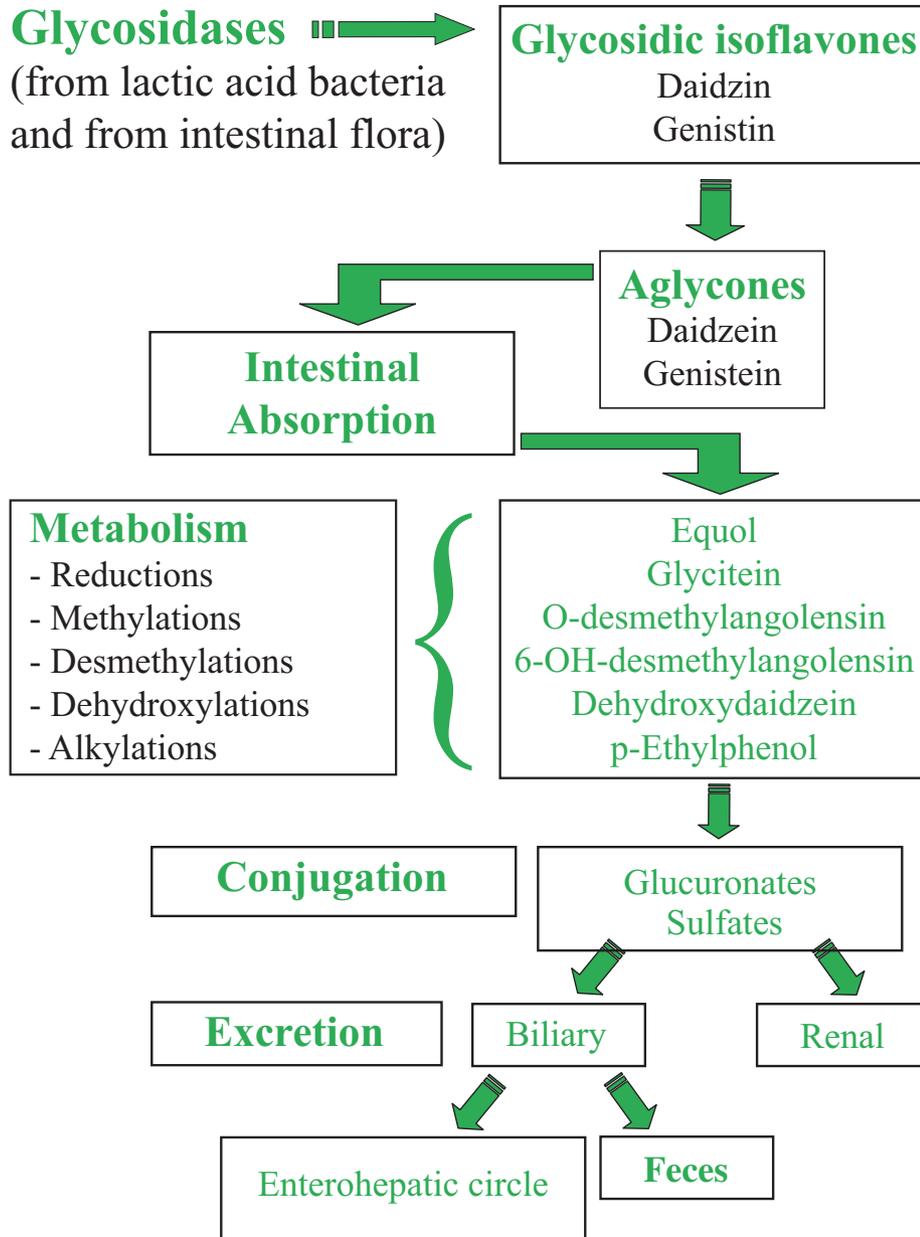


Fig. 5. Biotransformations, metabolism and excretion of soy isoflavones.



cells of the intestinal and pulmonary mucosas and in those of the bone [12]. It follows that the effects resulting from stimulation of the α ERs are different from those resulting from stimulation of the β ERs. Estradiol, the main physiological estrogen hormone, mainly stimulates the α ERs, eliciting the well known effects on the endometrium and on the breast. Genistein, daidzein and their metabolites mainly stimulate the β ERs and have hardly any effects on the endometrium and on the breast, whereas they have various favorable effects on various symptoms of the menopause.

It must be remembered that the affinity of the SIs for the ERs is 500-10000 times lower than that of the estrogenic hormones, depending on the molecular or cellular model used for comparing their potency. Their action of the SIs is therefore always milder than that of the estrogenic hormones, and hence more respectful of the delicate hormonal balance that is established after the menopause. Furthermore, the SIs stimulate the synthesis of the blood globulin that binds the sex hormones (sex hormone-binding globulin, SHBG) [19]. It has been speculated that increase in SHBG subtracts estradiol from the circulation, exerting an anti-estrogen effect and neutralizing certain undesirable effects of estradiol on the endometrium and on the breast [31].

Effects of soy isoflavones on climacteric symptoms and on symptoms of the early menopause

Prospective double-blind, placebo-controlled clinical studies docu-

ment significant favorable effects of soy on the vasomotor climacteric symptoms [2, 18]. Even more obvious effects were obtained with 50 mg/day of SIs investigated in a double-blind, placebo-controlled study lasting 12 weeks on 177 postmenopausal women, randomized into two parallel groups [32]. The SIs were very effective in reducing the intensity of hot flashes (Fig. 6) and the number of night sweats (Fig. 7), with indisputable benefit for the quality of life of the women treated. In fact, the women treated with placebo woke on average 1.89 times because of flashes and sweats, but this was reduced to 1.52 in those treated with SIs, an improvement of 124%. The daily dose of 50 mg of SIs did not cause significant changes in FSH or in the thickness of the endometrium. However, a slight, but not significant, increase in SHBG was observed. There was a slight decrease in vaginal pH. No woman complained of breast troubles (whereas in the placebo group one subject complained of pain in the breast). The daily dose of 50 mg of SIs did not give rise to undesirable secondary estrogenic effects.

These results were confirmed in another double-blind study lasting 5 weeks, on 39 menopausal women randomized into two groups, who received 50 mg/day of SIs or a placebo [22]. Again in this study it was possible to document a progressive decrease in the number of hot flashes per week, significantly greater in the group treated with SIs than in the group receiving placebo (Fig. 8). In this study too, treatment with 50 mg/day for 5 weeks did not cause any secondary effect, either non-spe-

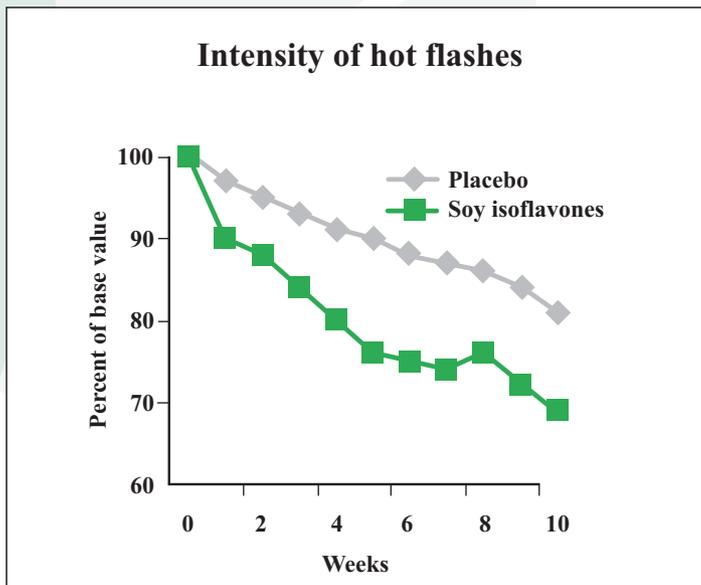


Fig. 6. Intensity of hot flashes recorded by women treated with placebo and with 50 mg/d SIs. At the 10th week of treatment with placebo the intensity had decreased by 19%, whereas with the SIs it had decreased by 31%, hence with a benefit greater than 160% [32].

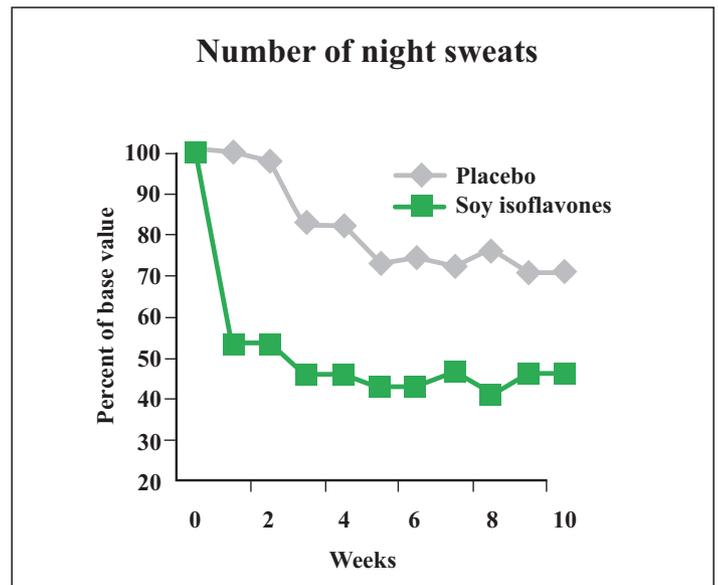


Fig. 7. Number of night sweats that caused waking in women treated with placebo and with 50 mg/d SIs. By the 10th week of treatment with placebo the number had decreased to 65% of the base value, whereas with the SIs it had decreased to 36% of the base value, hence with a benefit greater than 180% [32].



cific, or correlated with any estrogenic effects (on the endometrium, on vaginal cytology, or on the hormonal parameters). On the other hand, one woman treated with placebo complained of nausea and headache. The authors conclude that treatment with SIs is tolerated well and is effective against climacteric symptoms. Therefore it can be recommended in women for whom HRT is contra-indicated, or who do not want it for personal reasons.

Soy isoflavones also bring an overall improvement of the quality of life of menopausal women. For instance, the use of foods rich in SIs for 12 weeks significantly improved the climacteric symptoms (hot flashes, night sweats, insomnia, depression, vaginal dryness and dyspareunia) versus a control group of women who had their usual diet [7].

Similar results on climacteric symptoms (Kupperman index) were described with the use of SIs orally for 12 months [33]. Particularly interesting results were obtained in this study for some skin parameters, which demonstrated a significant improvement in skin trophism.

The doses of SIs used in these studies were 50-60 mg per day. These doses are effective, and higher doses should not be used, in order not to trigger undesirable estrogenic effects [22].

Effects on bone metabolism

The incidence of fractures ascribable to osteoporosis is lower in Asia than in western countries. This difference has been attributed to the extensive use of soy-based foods [13]. The efficacy of the SIs on the trophism of the bones in the postmenopause was demonstrated in a double-blind prospective study lasting 6 months, on 66 women whose meals were combined with SIs-enriched soy proteins [20]. Dual-energy X-ray absorptiometry (DXA) showed that there was a significant increase in

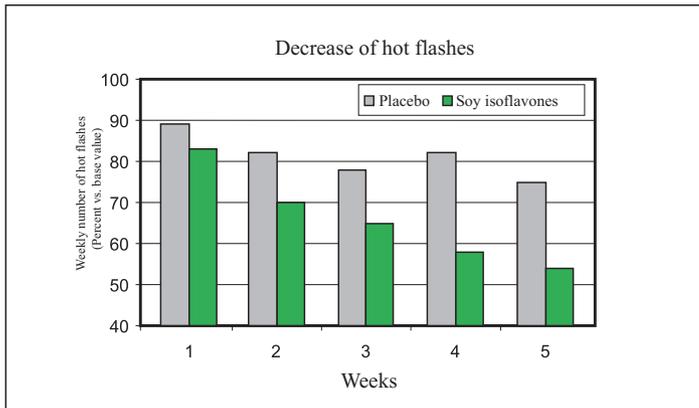


Fig. 8. Decrease in weekly number of hot flashes in women treated daily with 50 mg of SIs and with placebo [22].

bone mineral density (BMD) in L2-L4 in comparison with women whose meals were combined with non-enriched food or with food enriched with casein (Fig. 9). The doses of SIs were rather high in this study (90 mg/d). Even so, the results are very interesting, as they were obtained in just 6 months, which is the minimum time for being able to find a significant increase in BMD with HRT or with drugs specifically used for osteoporosis [11]. The efficacy of the SIs on bone metabolism has also been confirmed in other clinical studies [3, 17, 24] and is supported by studies in ovariectomized rats [9].

The published trials show that SIs effectively reduce the risk of postmenopausal osteoporosis, and this despite all the technical difficulties inherent in research relating to studies of efficacy in postmenopausal osteoporosis and the practical impossibility of conducting prospective, double-blind studies of the minimum duration of two years necessary for documenting clinical efficacy in this field [11]. The SIs, moreover, also reduce the risk of osteoporosis with another mechanism of action, because they inhibit the osteoclastic activity [5] and therefore potentiate the anti-resorption effect exerted by their estrogenic activity.

Cardiovascular effects

A soy-based diet appears to reduce the risk of coronary heart disease (CHD) [1]. In fact, SIs have various effects on lipid metabolism which may explain the decrease in cardiovascular risks: for example, they cause a rise in HDL (high-density lipoproteins) and so cause a decrease in the ratio of total cholesterol to HDL cholesterol, as has been demonstrated in hypercholesterolemic menopausal women (Fig. 10) [20]. Soy and its extracts also exert various other actions that explain their cardiovascular benefits, as summarized by the Group of Experts of the North American

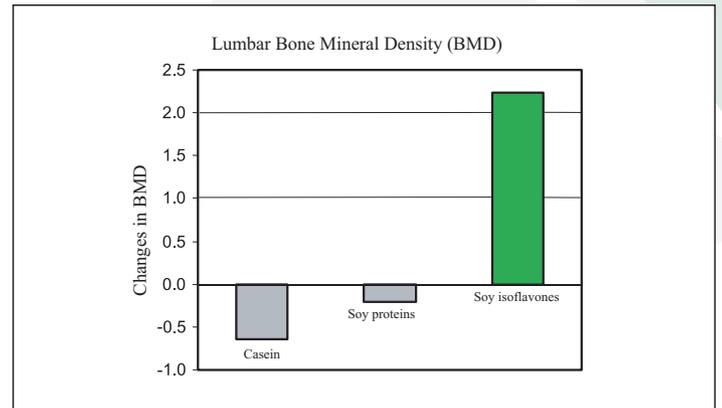


Fig. 9. Percentage increase in lumbar (L2-L4) bone mineral density (BMD) in women who ate a diet supplemented with casein, with soy proteins or with SIs, for 24 weeks. Only supplementation with SIs produced an increase in BMD. With the other two supplements a (non-significant) decrease in BMD was observed [20].



Menopausal Society [8]. In fact they:

- Decrease the diastolic arterial pressure.
- Lower the total cholesterol, the “bad” cholesterol (LDL-C) and the triglycerides.
- Elicit an antiplatelet action.
- Reduce the progression of atherosclerosis plaques (study conducted in ovariectomized cynomolgus monkeys).
- Improve the compliance (elasticity) of the arteries.
- Elicit an antioxidant action and suppress free radicals, opposing to the liperoxidation of lecithin and of LDLs that yields the end products which have a high affinity for the arterial walls and provoke the atherosclerosis plaques.

On the basis of these results, the Food and Drug Administration of the USA approved in 1999 the claim that soy, in conjunction with a diet low in saturated fats and cholesterol, can reduce the risk of CHD.

Effects on cognitive functions

It is known that HRT can have favorable effects on cognitive impairment and dementia in elderly women [4]. It has therefore been suggested that phytoestrogens might also exert similar effects [16].

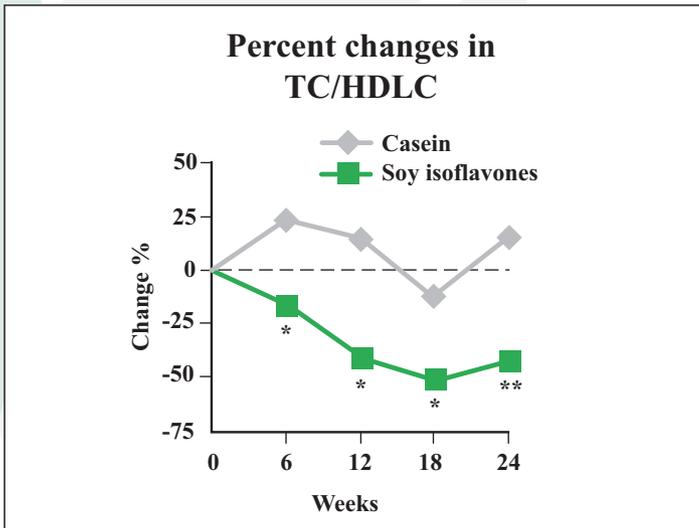


Fig. 10. Ratio of total cholesterol to HDL cholesterol (TC/HDLC) found in a double-blind clinical study conducted for 24 weeks on 66 hypercholesterolemic menopausal women, whose diet (CNFDM Step 1) was supplemented with casein or with SIs. After just 6 weeks there was a significant decrease ($p < 0.05$) in the TC/HDLC ratio in women with supplementation with SIs, and it was more than 50% in the 18th and 24th week, with a significant difference ($p < 0.05$) compared to the control group of women with casein supplementation [20].

Reduced risk of hormone-dependent tumors

The incidence of certain types of hormone-dependent tumors (of the endometrium, of the breast and of the ovaries) varies considerably from one population to another, and is very low in Asian women. This epidemiological observation has been attributed to the soy-rich diet [21]. The clinical and experimental studies demonstrating the efficacy of the phytoestrogens in reducing the risks of endometrium, breast and ovary cancer were summarized by Sirtori [29] and in the aforementioned Consensus Opinion [8]. The results are not completely consistent and further studies are required in order to draw a conclusion that SIs can effectively prevent these tumors. Certain is that SIs do not increase the risk of tumors, even though they have estrogenic effects, probably because SIs mainly stimulate the β ERs, which are scarce in the organs that are most susceptible to estrogen-dependent tumors.

Possible adverse effects of soy isoflavones

Soy and SIs are generally well tolerated, apart from some rarely occurring light gastrointestinal disturbances [30]. Soy belongs to the Fabaceae family. Therefore subjects who are allergic to legumes might also suffer allergies after ingesting foods obtained from soy (tofu, soy milk, soy flour, etc.). However, these reactions are due to the proteins present in soy and have never been recorded with deproteinized extracts as those used in ESTROMINERAL [30]. In contrast to conventional HRT, SIs do not increase the risk of cancer of the breast or of the endometrium [35].

Summary of the activities of soy isoflavones

The SIs:

- Prevent, reduce or eliminate menopausal symptoms of light or medium severity.
- Prevent and reduce the risk of osteoporosis.
- Prevent and reduce cardiovascular risks and have a eulipidizing action (improvement of the quality of blood lipids).
- Probably prevent and reduce the risk of cancer of the breast, endometrium and ovaries.
- Have an antioxidant action and scavenge free radicals.
- Probably prevent the decline in cognitive functions in the elderly.

Uses of soy isoflavones

Due to their balanced efficacy, safety, ease of use and manageability, the soy phytoestrogens are classified as “dietary supplements” and do not require a medical prescription. They are, however, very effective remedies and it is therefore always advisable to consult a physician before taking them, and also for having a guidance regarding the quality of the commercial preparations, which, for some products, may be poor and uncontrolled.



Summarizing, SIs are particularly useful:

- For the symptoms of premenopause, climacteric and early menopause of light to moderate intensity;
- In the advanced postmenopause, in women over 60 years;
- In women for whom the use of conventional HRT is contra-indicated;
- In women who refuse HRT;
- In women with a family history of tumors of the uterus or breast;
- In women without acute symptoms, but who wish to prevent the risk of osteoporosis and cardiovascular disorders;
- In women with an impaired lipid metabolism and/or with a family history of atherosclerosis;
- In women with premature senile neurosclerotic disorders.

The *Lactobacillus sporogenes* of ESTROMINERAL

General information

Each caplet of ESTROMINERAL contains 500 million spores of *Lactobacillus sporogenes* (L.s.), which, in the digestive tract, germinate to the vegetative forms, i.e. to the viable bacilli able to multiply and perform all their fermentative activities.

L.s. produces lactic acid by a *homofermentative* process (therefore it is a true *lactic acid bacterium*), and in particular it produces exclusively L-lactic acid. This aspect is important because other strains of lactic acid bacteria produce both isomeric forms, i.e. D-lactic acid and L-lactic acid. Only L-lactic acid is used by our body for the energetic metabolism and is finally excreted as CO₂. D-lactic acid cannot be metabolized, it accumulates in our body and provokes acidosis with the related adverse reactions.

To produce L-lactic acid, L.s. utilizes the undigested fragments of sugars, proteins and fats contained in our diet. In this way it subtracts nutrients for other components of the intestinal flora that produce substances that are harmful to our body, for example those derived from the anaerobic processes of putrefaction. By this mechanism, and through the lowering of pH resulting from the production of L-lactic acid, L.s. **improves and balances** the intestinal flora. This is very useful, especially when the intestinal flora is deficient or unbalanced, as a result of incorrect diet, of drugs (especially antibiotics), of climatic stresses, of digestive disorders or deficiencies. In fact, these conditions cause diarrhea, constipation, flatulence, maldigestion, asthenia and malaise due to the toxic substances produced by the predominance of the putrefactive over the fermenting flora.

L.s. eliminates dysmicrobisms, also because it produces special antibacterial substances, the *bacteriocins*, which impede the growth of the “bad” flora. Moreover, L.s. colonizes (grows and multiplies on) the gut wall, forming a barrier against pathogenic microorganisms, such as *Candida albicans*, *Salmonellae*, *Escherichiae*, *staphylococci*, *clostridia* etc., which produce toxins and can cause intestinal infections. Finally, it

has been demonstrated that L.s. stimulates the immune defenses of the gastrointestinal tract, helping to defend our body against infections and toxic allergens.

As already mentioned, ESTROMINERAL contains L.s. in the form of *spores*, i.e. the naturally encapsulated form of the microorganism, enabling L.s. to withstand unfavorable environmental conditions. In fact, the spores are resistant to the gastric, biliary and intestinal juices, which inactivate other strains of lactic acid bacteria that are not in the form of spores. Once in the stomach, the spores of L.s. give rise to the *germinative form*, i.e. to *vegetative cells* that are able to multiply and to produce substances that are useful for the well-being of the whole body. The transformation of the spores into germinative forms takes about two hours.

The spores of L.s. are also very resistant to adverse environmental conditions and ensure the stability and viability of L.s. throughout the shelf life of ESTROMINERAL, even at room temperature. Therefore it is not necessary to keep ESTROMINERAL in a refrigerator.

Substances produced by *Lactobacillus sporogenes*

L.s. produces various substances that are very useful to our body. For that reason L.s. is called *probiotic* (from “pro bios”, promoting life).

One of the substances produced by L.s. is the previously mentioned L-lactic acid (the lactic acid isomer recommended by the World Health Organization).

L-lactic acid acidifies the intestinal environment and creates favorable conditions for the absorption of calcium, of iron and of phosphorus that are present in food.

L.s. produces vitamin K, a factor that is necessary for blood clotting. It also produces other B group vitamins and many enzymes (lipases and proteases), which assist the digestive processes.

Soy isoflavones and *Lactobacillus sporogenes*: a rational combination

Particularly important among the enzymes produced by L.s. are the *glycosidases*, enzymes that break the glycosidic bonds of the soy phytoestrogens, thus releasing the active principles, genistein and daidzein, and making them available for absorption in the intestine. In this way the L.s. contained in ESTROMINERAL ensures the bioavailability and efficacy of the soy isoflavones. In addition, L.s. is involved in the metabolism of the SIs, converting daidzein to equol, a metabolite that is more active than daidzein itself and is responsible for most of the activity of the SIs [31].

Summary of the activities of *Lactobacillus sporogenes*

L.s. produces:

- **L-lactic acid**, which is the lactic acid isomer recommended by the WHO. L-lactic acid acidifies the intestinal environment and creates conditions that favors the absorption of calcium, iron and phosphorus.



- **Vitamin K and other group B vitamins.**
- **Lipases and proteases**, which aid the digestive processes.
- **Glycosidases**, enzymes that break the glycosidic bonds of the soy phytoestrogens and make daidzein and genistein bioavailable.
In addition, L.s.:
- **Converts daidzein to equol**, the metabolite mainly responsible for most of the estrogenic activity of the SIs.
- **Improves the intestinal flora** by various mechanisms.
- **Protects against infection** by *Candida albicans*, salmonellae, escherichiae, staphylococci, clostridia etc.
- **Stimulates the immune defenses** of the digestive tract and defends our body against infections and toxic allergens.

ESTROMINERAL is formulated for a specific remineralizing action

In order to potentiate the remineralizing activity of the SIs, ESTROMINERAL contains also calcium, vitamin D₃ and extract of horsetail, components that have specific actions on the trophism and mineralization of the bones, as hereunder described.

Calcium

The normal amount of calcium in an adult woman is about 1000 g, 99% of which is stored in the bones. Only 1% of the calcium is in the other tissues, where, however, it performs vital functions, for example in blood clotting, in muscle excitation and contraction, and in neurotransmission. Calcium is also essential for the integrity of the cell membranes and for the function of many hormones.

The skeletal bones form our main reservoir of calcium. In the bones calcium is in the form of **hydroxyapatite** [Ca₁₀(PO₄)₆(OH)₂] deposited on the collagen fibrils. This mineral confers to the bones their characteristic rigidity and mechanical strength.

The skeletal bones are not static tissues, but are subject to intensive processes of **remodeling**. These consist of continuous erosion of small portions of bone (remodeling units) through the action of special cells, the **osteoclast**, alternating with the deposition of new bone by the action of other cells, the **osteoblasts**. The turnover of calcium is positive in the first two decades of life, and the peak of calcium in the skeleton is reached between the age of 20 and 30 years. Then gradual loss of calcium begins. Calcium loss is slow in men, and is also slow in women up to menopause, owing to the action of the estrogenic hormones that stimulate the activity of the osteoblasts. After menopause, however, the deficiency of the ovarian hormones leads to a net prevalence of the osteoclastic processes of calcium resorption, with rapid loss of calcium from the bones. Therefore more calcium is required, to compensate the larger losses of calcium due to the deficiency of estrogenic hormones and for this reason ESTROMINERAL was supplemented with calcium.

In more severe cases, the predominance of the processes of resorption of skeletal calcium leads to **postmenopausal osteoporosis**, with increased fragility of the bones, so that fractures may easily occur through minimal trauma, especially of the vertebral bodies. The most obvious symptoms of vertebral fractures is a decrease in height and hunchback, typical of persons affected by osteoporosis. Fracture and compression of the vertebrae cause pain, breathing difficulties (through distortion of the thoracic cage) and gastrointestinal disorders (through compression of the abdominal contents). Fractures of the vertebral bodies are irreversible. Therefore it is absolutely essential to prevent postmenopausal osteoporosis.

To supplement the calcium contained in food, each caplet of ESTROMINERAL contains 141 mg of calcium in the form of calcium carbonate and calcium phosphate. The latter supplements also the requirement for phosphorus, needed for the synthesis of hydroxyapatite, the mineral that imparts rigidity to the skeletal bones.

Vitamin D₃

Our stock of calcium results from the balance between the calcium absorbed from food and the calcium lost in the feces and urine. Calcium homeostasis is closely controlled by endocrine mechanisms (mainly parathyroid hormone) and by vitamin D.

Vitamin D and especially vitamin D₃ (cholecalciferol), which is one of the active metabolites, regulates the intestinal absorption of calcium and its deposition in the skeletal bones in the form of hydroxyapatite. Vitamin D₃ is derived from 7-dehydrocholesterol, which is converted to vitamin D₃ in the skin, by the action of ultraviolet radiation. In the kidney, vitamin D₃ is in its turn hydroxylated to 1,25-dihydroxycholecalciferol (calcitriol), which promotes the absorption of calcium in the gut, its renal reabsorption and the fixation of calcium in the bones.

At menopause there is often a deficiency of vitamin D₃, leading to insufficient absorption of calcium from food. ESTROMINERAL supplements the vitamin D₃ requirement, supplying 100% of the Recommended Daily Allowance (RDA).

Horsetail (*Equisetum arvense*)

ESTROMINERAL contains dry extract from the stem of Horsetail, standardized in silicic acid according to the French Pharmacopoeia X.

The horsetail of ESTROMINERAL is quite rich in silicon, which represents 12.5% of the dry extract. Much of the silicon consists of organic salts of monosilicic acid, which is soluble and therefore can easily be absorbed in the gastrointestinal tract. Horsetail also contains phytosterols, phenolic acids and vitamin C.

Horsetail is known for its **remineralizing** action. Silicon is in fact involved, together with phosphorus, fluoride, magnesium and boron, in



the processes of deposition of the crystals of hydroxyapatite on the collagen fibrils, i.e. on the organic structures on which the mineral component of the bones is built up. Silicon stimulates the osteogenic function of the osteoblasts. These actions have been demonstrated both *in vitro* and *in vivo*.

Silicon is also necessary for the formation of the proteoglycans of the bones and cartilage, essential molecules for imparting strength, elasticity and flexibility to the skeletal structures [34].

Horsetail also contains *equisetoinin*, a substance with mild diuretic action. This can prove useful for correcting water retention, which often occurs during the menopause and manifests itself as swelling of the lower limbs and increase in body weight.

Conclusions

ESTROMINERAL is formulated in oval tablets (caplets) containing balanced amounts of soy isoflavones, probiotic lactic acid bacteria, calcium, vitamin D₃ and extract of horsetail. These ingredients have been proportioned so as to be especially useful for balancing the hormonal deficiencies that occur during the climacteric and at the menopause. In addition, they are useful when there is increased requirement for minerals or a decrease in their supply in the diet. ESTROMINERAL promotes trophism of bones, especially in conjunction with phenomena of decalcification and of osteoporosis.

The **Soy Isoflavones** contained in ESTROMINERAL are natural substances extracted from non-GM soy, with estrogen-like action, and are therefore natural “phytoestrogens”. They prove particularly useful during the climacteric and menopause for compensating the hormone deficiencies that occur in this stage of life and which cause effects of cardiovascular lability (hot flashes, sweats, palpitations), nervous tension, irritability, progressive atrophy of the tissues of the urogenital system, and ageing of the skin. The natural phytoestrogens of ESTROMINERAL compensate the hormonal deficiencies of the menopause and are thus able to reduce the disturbances associated with the menopause, nevertheless “respecting” woman’s delicate hormonal balance.

Lactobacillus sporogenes (L.s.) is contained in ESTROMINERAL in the form of spores, and is therefore resistant to the gastric and biliary juices that normally destroy the majority of ordinary lactic acid bacteria. In the intestine the spores give rise to germinative forms that colonize the gut wall and especially the walls of the colon. Here, L.s. performs its probiotic action, i.e. it produces enzymes, group B vitamins and other substances that are beneficial to the intestinal functions and to the whole body (hence the term “probiotic”). L.s. breaks the glycosidic bond of the soy phytoestrogens, releasing the active aglycons, i.e. genistein and daidzein. This process is essential for their absorption and efficacy. In addition, L.s. promotes the biotransformation of daidzein into equol. The latter, on account of its structural similarity to estradiol, is the most active phytoestrogen substance. L.s. balances the intestinal flora because it pro-

duces bacteriocins, special antimicrobial substances that counteract the growth and intestinal colonization of pathogenic microorganisms, such as *Candida albicans*, *salmonellae*, *escherichiae*, *staphylococci*, *clostridia* etc., preventing intestinal infections and the production of toxins. Furthermore, L.s. stimulates the immune defenses at the intestinal level, helping to protect the organism against infections and toxic allergens.

The **calcium** and **vitamin D₃** contained in ESTROMINERAL supplement the dietary calcium, promote its absorption in the gut and its deposition in the skeletal bones. These components promote the trophism of the bone tissue, especially in conjunction with phenomena of decalcification and osteoporosis.

The extract of **Horsetail** contained in ESTROMINERAL supplies monosilicic acid, which is important for the maintenance of cartilage and connective tissue and for the trophism of the bones. Horsetail also has a mild diuretic action, which corrects water retention, which often occurs during the menopause and manifests itself as swelling of the lower limbs and increase in body weight.

Tolerability and dosage of ESTROMINERAL

ESTROMINERAL is generally well tolerated and does not cause an increase in body weight.

The recommended dosage is one caplet per day, to be taken preferably far from meals.



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