AFRAGIL® IN THE TREATMENT OF 34 MENOPAUSE SYMPTOMS: A PILOT STUDY

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Afragil® in the treatment of 34 menopause symptoms: a pilot study

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Aim. A combination of calcium, vitamin D3, lycopene, astaxanthin and Citrus bioflavoids (MF Afragil®) was administered for a period of 8 weeks to 65 women to determine its effects in reducing signs/symptoms of climacteric status.

Methods. Two groups of women were compared in a registry study (33 treated with MF Afragil® and 32 with no treatment). The climacteric condition was determined by using the 34-symptom questionnaire MSSQ, which was filled out at inclusion in the trial and after 8 weeks of treatment. The MSSQ signs/symptoms scores (Common Symptoms, Changes and Pains) of the two groups were comparable at inclusion, whereas after 8 weeks a significant reduction of many variables was found in the group treated with MF Afragil® and in the control group.

Results. Following the treatment with MF Afragil®, the total MSSQ score was reduced by more than 45%. There was a significant reduction in hot flashes, CNS symptoms (depression, anxiety and panic disorders), incontinence and joint pain, which are among the most frequent symptoms of climacteric status. Osteoporosis was not analyzed due to the short period of treatment.

Conclusion. MF Afragil® was found to be efficient in reducing climacteric symptoms following a short-term administration. More prolonged treatment and more cases are under analysis to also determine its effect on osteoporosis.

Key Words: Menopause - Calcium - Antioxidants - Climacteric.

Menopause, the permanent cessation of menstruation due to the loss of ovarian and follicular activity, is the major event in the aging process for women in their early 1950s. Many women spend almost one third of their lives in menopause. Menopause is diagnosed one year after the final menstrual period. However, shortly before that point, when clinical features begin, a menopausal transition period known as perimenopause takes place. This period, which continues during the year following menopause and ends with the menopausal phases, is defined as climacteric status.

The primary symptoms of perimenopause are characterized by vasomotor symptoms (e.g., hot flashes, night sweats), menstrual changes (e.g., oligomenorrhea, amenorrhea), vaginal dryness, and painful intercourse (dyspareunia). However, there are many other secondary symptoms such as urinary incontinence, mood changes and somatic complaints that are also frequent and may have an impact on the quality of life.

Therapies directed at primary and secondary symptoms include treatment with estrogen either by itself or with progestins, androgens, bioidentical hormones, and also antidepressants, phytoestrogens and botanicals. According to a Cochrane review, oral hormone therapy is highly effective in the control of vasomotor symptoms, 1 which are the major complaints of menopausal transition. However, the use of hormone therapy has declined since the results of the Women’s Health Initiative studies, 2, 3 which found a more pronounced risk of thromboembolism, stroke and breast cancer using these products, despite a reduction of hip fractures and colorectal cancer.
Other alternative therapies should be studied to improve health maintenance and make menopause manageable. Therapy based upon phytoestrogens showed no evidence of effectiveness other than some sporadic effect on hot flashes and night sweats. A strong placebo effect was also found in most of the trials with reduction of episodes up to more than 50%.

One of the most used remedies is black cohosh, which contains a preparation that has been found to improve vasomotor symptoms; but further studies are needed due to the conflicting results of treatment with this herbal preparation.

Since there is a current gap in the treatment of menopausal symptoms, several new therapies are under way that may help to alleviate the unmet needs. One new therapy that has shown some positive activity in preliminary studies (data on file) is based upon the combination of calcium, vitamin D and three antioxidants: lycopene, astaxanthin and bioflavonoids. Calcium and vitamin D are known to be beneficial in the reduction of cardiovascular events, for bone health, and for quality of life. The antioxidants used were bioflavonoids, which acted as circulating antioxidants, and two carotenoids (lycopene and astaxanthin), which acted as membrane antioxidants.

Bioflavonoids were included because some of them have been found to be beneficial in the management of osteoarthritis in the past and in more recent clinical trials. In particular, citrus bioflavonoids have been approved by the European Food Safety Agency (EFSA) for the healthy maintenance of joints. Lycopene was added to the formula because its levels in postmenopausal osteoporosis are lower, and it was shown to decrease bone resorption. Furthermore, it is the most concentrated carotenoid in lymphocytes and may help in the reduction of inflammation processes.

Astaxanthin was included in the formula because it is the most active carotenoid. This molecule acts as a hydroxyl substitute in aro position to the carbonyl group in each of the two iononic groups of the extremity of the isopropyl chain (in other words, a dihydroxy-carotenoid). This peculiar structure allows astaxantine to bind and inactivate transition metals that can oxidize the membranes of lipoproteins and cells.

The aim of the present study was to determine the activity of MF Afragil, a combination of calcium, Vitamin D and the listed antioxidants, to modify symptoms after its administration for a period of 8 weeks in a group of women in the climacteric status.

### Materials and methods

This registry study consisted of 65 healthy women that were divided into two groups and followed for eight weeks. A group of 33 women was treated once daily with two tablets of Afragil, and another group...
of 32 women took no treatment. Both groups were controlled for a period of eight weeks evaluating signs/symptoms related to menopause.

The criteria of inclusion were: age >40 and <50, and the absence of any other clinical disease or habitual use of any drug. Thyroid function (T3, T4 and TSH) and morphology were normal, and no previous significant disease requiring medical treatment or surgery had been recorded in the 12 months before the inclusion. Ovaries and uterus were normal for age, and all women had been pregnant (normal pregnancy) and had delivered at least one healthy baby. All women had regular job activity, and their blood pressure (<130 systolic and <85 diastolic) and body-mass index (BMI) were normal (by inclusion <25). No handicap was present. The criteria of inclusion in the treatment group or in the control group was randomized (casual choice of 33 yes and 33 no in a glass bowl).

Questionnaire

The Menopause Symptoms Questionnaire (34 MSSQ-http://www.34-menopause-symptoms.com/) (Table I) was chosen by the participants instead of the 11-item Menopause Rating Scale (MRS) because the description of signs and symptoms was considered more complete. The MSSQ is characterized by three main blocks of symptoms: common symptoms (6 items), changes (17 items) and pains (11 items). Osteoporosis, which is the 34th item (last item of the pains block), was not taken into consideration due to the short period of treatment.

Women were instructed to fill in the questionnaire according to a score between 0 (absent) and 4 (very severe). The complete questionnaire is reported in Table II. Each woman was given a complete written explanation of every symptom/sign of the MSSQ (12 pages available on the internet). All the signs and symptoms were discussed after the compilation of the questionnaire to clarify any items that were unclear to the participants.

Products

The product was prepared as chewable tablets (2 tablets/day) to be taken either during breakfast or in the evening before sleeping. The control group was not treated with any product. A total of 120 tablets was given to the treated women (three boxes of 50 tablets), and compliance was determined by the count of residual tablets after the 8-week period. The active product formula is reported in Table III.

Analysis of the activity

The activity of the treatment was based upon the severity of each score. The evaluation was blind.

Statistical analysis

The data were analyzed for the determination of average and SD (standard deviation). The difference between values at inclusion and after 8 weeks was calculated according to t test for interdependent data, and the difference between the product and no treatment was determined using the t test for independent data. Hot flashes were chosen as a guide parameter for sample size determination. For an α value of 0.01 and a value of 1-β of 1-0.90, a difference of 1 SD in groups of 25 cases gives a power >0.9. In the hypothesis of a 30% dropout rate, groups of at least 32 cases should be compared. Since the variables were measured with a 0–4 score, the level of P<0.01 was chosen as a cutoff for a significant difference between the two treatments.

Results

The two groups were comparable for age and signs/symptom (Table II). All the women completed the
study except one in the control group that did not come to the control after eight weeks.

Ns=t test for independent data P>0.05 (Table II) for details.

From the data reported in Table II, it is evident that the average value of the three blocks of symptoms are very similar, the only difference being the SD, which are much lower in the control group due to a minimal fluctuation of the items compared to the treated group.

Treatment was well tolerated since complaints regarding taste were reported in just a few cases, and the compliance was >97%.

The complete set of signs/symptoms is reported in Table II. Because of the differences in the SD at the baseline, the total scores of the three blocks of symptoms were not considered in the evaluation of the activity, and the difference between the two groups was based upon each sign/symptom only.

The controls did not show any evident “placebo” effect, since very few items were favorably modified (night sweats in particular, and minimally vaginal dryness, muscle tension and itchy skin), during the study. On the contrary, most of the items had the tendency to worsen.

The total average score (all three blocks of variables) was reduced by about 48% in the treatment group, whereas in the control group there was an increase of about 10%.

A significant modification of the variables in almost all the signs/symptoms was shown with the treatment with MF Afragil® compared to controls. However, only those exceeding the cut-off of P>0.01 were considered as a “positive effect”. Modification of night sweats, hair loss, dizziness, brittle nails and burning tongue were similar for both groups.

Despite the fact that the sample size was determined by the hot flashes score, most of the variables have a sufficient statistical power (<0.9). In the treatment group, the signs/symptoms that were modified by at least two points were: hot flashes, incontinence, CNS symptoms (depression, anxiety, panic) and joint pain.

A few of the signs and symptoms were present in less than 20 cases in both groups (hair loss, bloating, brittle nails, burning tongue); consequently the differences between the product and control do not have a sufficient statistical power (<0.9).

**Plasma-free radicals**

Table IV shows the variations in PFR. There was a significant reduction in PFR in the treated subjects at four and eight weeks. At eight weeks the decrease was sustained. In controls there was a significantly lower variation (not significant). These results indicate the important protective effects of the product on oxidative stress.

### Discussion

The results of this pilot study indicate that it is possible to improve signs and symptoms related to the climacteric state by using natural products. The use of a preparation containing black-cohosh (*Cimifuga racemosa*) was recently shown to be effective in the treatment of some of the common symptoms of the climacteric period, including hot flashes, profuse sweating and sleep disturbances. However, conflicting results are reported in the literature, and there is also concern about its safety with long-term administration, because a “natural product” does not necessarily mean a “safe product”. For instance, this plant contains mainly triterpens glycosydes (*e.g.*, cycloartanes), and also some unknown compound with serotonergic activity, which could be responsible for the CNS action. However, the activity can be different according to the part of the plant that is used, and if preparation is not properly standardized.

All the ingredients present in MF Afragil® are known and safe. Calcium and vitamin D₃ supplementation stimulates fatty acid oxidation and suppresses lipogenesis and fatty acid absorption. Together, these effects were found to be of benefit in the prevention of weight gain. The activity of calcium, particularly with the combination of diet plus supplement, is effective in improving bone mineral density. Calcium lactate particularly seems to be effective in lowering blood markers of bone degradation. In this experience a significant reduction of muscle tension was shown, which can be determined by a poor calcium intake with food.

A more complex effect can be determined in general by antioxidants.

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Treatment</th>
<th>P</th>
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<tbody>
<tr>
<td>PL free radicals</td>
<td>Inclusion</td>
<td>388.4;28.3</td>
</tr>
<tr>
<td>Carr Units</td>
<td>4 weeks</td>
<td>321.3;33.4</td>
</tr>
<tr>
<td></td>
<td>8 weeks</td>
<td>311;23.2</td>
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Aromatase expression and circulating C19 precursor of estrogens are essential for estrogen synthesis in various tissue to partially substitute the synthesis of the ovarian granulose cells no longer efficient in the climacteric status.

In many tissues (e.g., breast, bone, vasculature, lipids) a fundamental step of synthesis is represented by the cytochrome CYP19, which needs NADPH, and the flavoprotein NADPH-cytochrome P450 as cofactors. This means that ATP and reducing equivalents are needed; in other words, antioxidants may improve the residual synthesis of estrogens.

A similar effect can be determined by the protection of the cellular membrane from oxidative stress in the case of hot flashes. The pathogenesis of hot flashes is still under discussion and poorly defined. However, it seems that hot flashes are determined by dysfunction of the central thermoregulatory center (CTC) due to the reduction in estrogen levels, which induces a negative feedback of the pituitary gonadotropins. Hypothetically, this dysregulation of the CTC can be due to a reduction of cerebral blood glucose that induces a compensative vasodilation to increase the glucose available for the uptake of the GLUT 1 receptors. This vasodilation is considered capable of increasing the temperature of the CTC of the minimal amount that can trigger the peripheral reaction as hot flashes. These GLUT 1 receptors are membrane receptors that can become less efficient in case of oxidation of their membrane phospholipids, or even by the oxidation of cell membrane phospholipids where they are located. Lycopene and astaxantin are known to be powerful membrane antioxidants that can preserve from phospholipids oxidation (both on cellular membranes or GLUT receptors), with the final result to make the GLUT 1 more efficient.

*Citrus* bioflavonoids have been recently approved by the EFSA (31 July 2009 EFSA; Claim ID 1799) as supplements that “may help to keep joints healthy”, and in this experience a confirmation of this activity was shown in the reduction of joint pain and muscle tension. In general, the combination of all the ingredients in the MF Afragil® formula seems to reduce a significant number of symptoms that in part can be correlated. A reduction of pain and muscle tension may improve sleep in concomitance with the reduction of CNS symptoms such as anxiety, depression and panic. The same can be forecast for the amelioration of incontinence due to less muscle tension and anxiety reduction.

Finally, the MSSQ was found to be helpful in determining the climacteric condition because it is so simple to fill out. To our knowledge, this is the first time that this questionnaire has been used in a parallel study comparing treatment with no treatment. All participants filled out all the variables, and very few questions were posed about the meaning of the items.

An important element can be drawn from these results, which are related to low dosages of antioxidants contained in MF Afragil®. The term MF means “modulator” and indicates also that the combination of many ad hoc natural products such as flavonoids, lycopene, and astaxanthin, at low dosages, can result in a synergistic activity. With these ingredients administered at these dosages, side effects are not expected and, if they do occur, they will not be severe or harmful.

Separate elements such as calcium plus vitamin D3, or antioxidants only were not found as efficient (data on file under publication) in reducing the climacteric burden.

The added benefits of this product on PFR that were observed are also important since we do not know how many signs/symptoms associated to menopause are related to increased oxidative stress or may be dependent on increased PFR.

### Conclusions

In conclusion, in a parallel short term registry study the combination of calcium, vitamin D3 and ad hoc antioxidants was found to reduce significantly the symptoms of the climacteric status and to improve the quality of life. More extensive studies and a longer period of treatment are needed to determine the continuity of the activity and the improvement of osteoporosis.

### References

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