# Supplementation with specific micronutrients reduces the adverse effects of combined oral contraceptive treatment

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***ABSTRACT — OBJECTIVE:*** *Discontinuation and poor compliance are leading causes of oral contraception failure. Because of possible adverse effects that impact their quality of life, women skip their daily dose of contraceptive, or opt for different contraception methods. This study aims to assess whether a specifically formulated dietary supplement helps to reduce the occurrence of these unwanted side effects in women prescribed an estroprogestinic contraceptive.*

***PATIENTS AND METHODS:*** *This is a controlled pilot prospective study, involving 52 healthy female volunteers who were prescribed an estroprogestinic contraceptive. The study group (n=26) received a dietary supplement containing Centella Asiatica (C.A.) extract, vitamins and minerals, the control group (n=26) received only the estroprogestinic pill. Baseline values were measured at enrollment, and the outcomes assessed after 3 months of intervention. Bodyweight, BMI and extracellular body water were measured with a bioelectrical impedance analysis (BIA) scale. Evaluation of cellulite, breast tenderness, leg swelling, mood, fatigue, migraine, vaginal discharge and hair dryness was self-reported by patients through a dedicated questionnaire.*

***RESULTS:*** *After 3 months of supplementation, patients reported a noticeable 56% decrease in cellulite level and a 4% decrease in water retention with respect to the control group. Significant improvements were observed also for the remaining outcomes. Overall, supplementation counteracts the negative effect the estroprogestinic contraceptive by significantly limiting water retention, bodyweight and BMI increase. Moreover, all other outcomes improved compared to both control group and baseline values.*

***CONCLUSIONS:*** *The results of this study indicate that specific supplementation significantly limits frequency and severity of adverse effects during oral contraception therapy. C.A. extract helps to reduce water retention, counteracting the consequent bodyweight increase, leg swelling and breast tenderness. The combination of vitamins and minerals contributes to the reduced occurrence of migraine, increased energy levels, and to a generally better mood.*

***KEYWORDS***
*Centella Asiatica, Cellulite, Water retention, Contraception, Female health.*

## INTRODUCTION

With over 100 million users worldwide, combined oral contraceptive (COC) pills are probably the most widely prescribed drugs[1]. In Italy over 2.5 million women use COCs and the trend of first-time users

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is on the rise. COCs contain a progestin and an estrogen, typically ethinyl estradiol. They suppress the release of gonadotropins, preventing ovulation. Originally designed for birth control, COCs are currently used in hormone-replacement therapies (HRTs) during menopause and in the treatment of medical conditions such as polycystic ovary syndrome, endometriosis and painful menstruation.

Despite numerous health benefits, including reduced risk for ovarian and endometrial cancer, COC treatments have always suffered from poor compliance due to the onset of adverse effects, or the fear of them[2]. The result is contraception failure, with the occurrence of unwanted pregnancies. Novel formulations allow to tailor therapies on patients' characteristics, greatly improving both safety and tolerability[3]. Nevertheless, current discontinuation rates still exceed 20% within the first months of treatment[1].

The negative effects of COCs, which lead to altered body image and mood disturbances, include increased cellulite and water retention – yielding to leg swelling, breast tenderness and bodyweight increase – but also migraine and lack of energy, which affect the quality of users' life. Cellulite, in particular, is the macroscopic manifestation that results from alterations of blood and lymphatic vessels. As well as other physical blemishes, cellulite represents also a worrisome social issue, encompassing psychological and socio-cultural aspects. Indeed, altered body image causes insecurity and low self-esteem, often resulting in undergoing dangerous and invasive surgical cosmetic procedures[4].

Reducing the occurrence and the magnitude of such negative outcomes is crucial to improve patients' compliance to COC treatment. Current methods mainly consist in adjusting the formulation to the patients' specific needs, but fail to prevent discontinuation. Easy management of COC nuisance side effects through the use of supplements would be a highly desirable approach that, to the best of our knowledge, has yet to be tested.

In this study we report the administration of a dietary supplement to patients under COC treatment, evaluating its efficacy in the management of therapy adverse effects and its impact on the outcomes that affect the quality of life.

## PATIENTS AND METHODS

This was a pilot prospective study on a group of female volunteers. A total number of 52 patients were enrolled and examined, from January to December 2018, at Centro Polispecialistico Anteo – Terni, Italy. After explanation of the study purpose, all the patients included gave their written consent to participate. This study was conducted following the Ethical principles of the Declaration of Helsinki and the national laws. Inclusion criteria were: age 18-39 years, Body Mass Index (BMI) in the range 18-30 $Kg/m^2$, prescription for COC treatment. Exclusion criteria were: diabetes, previous or existing breast pathologies, hypertension, obesity, smoking habits and cardiovascular disorders. Participants were 1:1 randomized in two groups using block excel program: group A (control, 26 patients), treated with a combined estroprogestinic oral contraceptive (dienogest, 2 mg – ethinyl estradiol, 30 mcg); group B (supplemented, 26 patients), subjected to the same estroprogestinic oral contraceptive therapy but with the addition of a combined dietary supplement (magnesium, 300 mg; Centella Asiatica, *C.A.*, extract 150 mg; vitamin C, 80 mg; vitamin E, 12 mg; zinc, 10 mg; vitamin $B_2$ (riboflavin), 1.4 mg; vitamin $B_6$, 1.4 mg; folic acid, 300 mcg; selenium, 55 mcg; vitamin $B_{12}$, 2.5 mcg – Zyxelle®, Lo.Li. Pharma Srl, Rome, Italy).

Primary outcomes included reduction of cellulite, water retention, leg swelling; secondary outcomes included reduction of bodyweight/BMI, breast tenderness, fatigue, migraine, hair dryness, vaginal discharge, and improved mood.

Baseline characteristics of patients were collected at enrollment ($T_0$). Participants were asked to fill a self-assessment questionnaire, ranking the following symptoms from 0 (absent) to 4 (frequent): cellulite, leg swelling, breast tenderness, fatigue, migraine, mood, hair dryness and vaginal discharge. Weight and the amount of extracellular body water were measured using a bioelectrical impedance analysis (BIA) scale.

During the treatment, patients were instructed to take a single contraceptive pill, at the same time every day for 21 days, starting from the first day of menstruation. Pill administration was suspended for the following 7 days (scheduled bleeding was generally observed 2-3 days after the last administration) before starting a new cycle.

After 3 months of treatment ($T_1$), outcomes were evaluated with the same baseline modalities. All patients in both groups attended the follow-up visit, but 2 subjects from group A were excluded from the study because of unsatisfactory COC therapy compliance.

The sample size was calculated based on the effect of cellulite. In order to observe a statistically significant 25% reduction in group B, minimum enrollment of 34 patients (+15% of possible dropouts) is necessary to reach the power of 80% (a = 0.05; b = 0.02).

### Statistical Analysis

Data are compared using the two-tailed unpaired *t*-test. Differences are considered statistically significant when $p \leq 0.05$. Data are reported as mean values, with standard deviation of the mean in parentheses.

**Table I.** Characteristics of patients at the baseline. Mean values are reported, with standard deviations in parentheses; data analyzed with the unpaired *t*-test. *Group A*: patients under COC treatment; *Group B*: patients under COC *plus* supplement treatment.

| Entries | Group A (n=26) | Group B (n=26) | *p*-value |
|---|---|---|---|
| Age [years] | 27.8 (6.4) | 27.2 (6.8) | 0.72 |
| Height [cm] | 161.8 (5.8) | 163.0 (6.6) | 0.49 |
| Body Weight [Kg] | 62.1 (6.0) | 60.8 (5.9) | 0.43 |
| BMI [Kg/m$^2$] | 23.7 (2.2) | 23.0 (2.6) | 0.24 |
| Cellulite | 1.3 (0.7) | 1.2 (0.7) | 0.58 |
| Breast tenderness | 1.3 (0.8) | 1.6 (0.6) | 0.11 |
| Fatigue | 1.0 (0.8) | 1.2 (0.8) | 0.50 |
| Leg swelling | 1.5 (0.8) | 1.8 (0.8) | 0.17 |
| Mood | 0.8 (0.7) | 1.2 (0.8) | 0.06 |
| Hair dryness | 0.5 (0.5) | 0.7 (0.8) | 0.21 |
| Vaginal discharge | 0.2 (0.4) | 0.3 (0.5) | 0.54 |
| Migraine | 1.3 (0.8) | 1.4 (0.6) | 0.58 |
| Extracellular body water [%] | 42.7 (6.0) | 40.1 (3.5) | 0.06 |

## RESULTS

Table 1 reports the patients' characteristics at the baseline. The two groups exhibited homogeneous physical characteristics and ranking of symptoms, with none of the entries having significant differences.

After the follow-up visit at $T_1$ (3 months), we evaluated the differences observed between $T_0$ and $T_1$ within the single groups, and those between the two groups at $T_1$ (Figures 1-3).

### Cellulite, extracellular body water, leg swelling and breast tenderness

Patients in group A observed a significant increase of cellulite levels with respect to baseline ($p$<0.001), while those in group B reported an essentially unvaried situation (except a small, non-significant reduction). At $T_1$, the mean values were 2.3 for group A (SD: 1.0) and 1.0 for group B (SD: 0.7), $p$<0.0001 (Figure 1).

Water retention increased in both groups, though non-significantly in either cases. At $T_1$, the mean value was 44.5% for group A (SD: 5.7) and 40.7% for group B (SD: 3.5), $p$<0.0001 (Figure 2).

At the end of the treatment period, group A experienced significant worsening in leg swelling ($p$<0.001). On the other hand, group B reported a significant average reduction ($p$<0.001), with ranking improved by 1.4 points. The difference at $T_1$ between group A and group B is also highly significant (Figure 1).

We observed analogous results for breast tenderness, with significant increase in group A ($p$=0.01) and significant decrease in group B ($p$<0.001) compared to baseline values. The values at $T_1$ between the two groups are significantly different (Figure 1), with episodes of breast tenderness occurring less often and with lower intensity in group B.

### Bodyweight and BMI

COC intake caused weight gain in group A, although the average increase at $T_1$ is non-significant. This adverse effect is reduced in group B at $T_1$, with a non-significant average bodyweight increase of 0.4 Kg (SD: 1.6 Kg). As a consequence, BMI remains

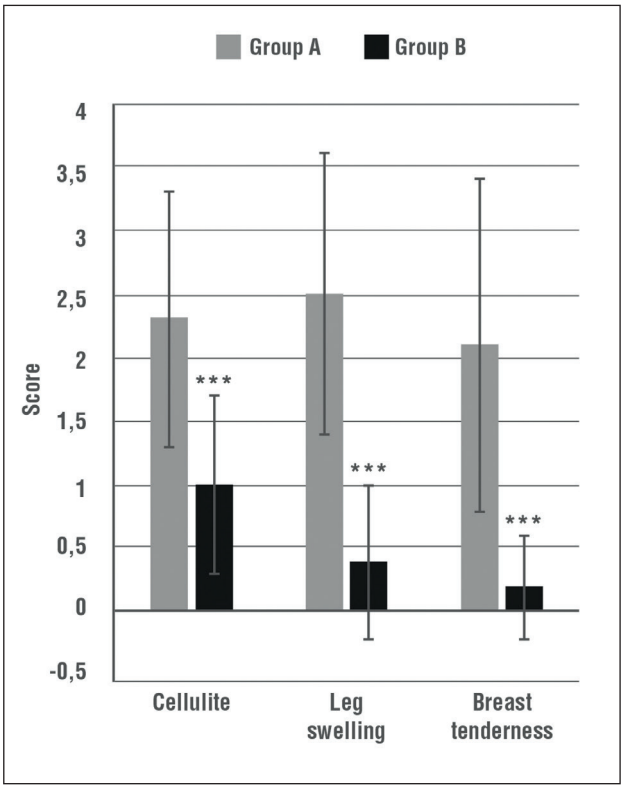


**Figure 1.** Values at $T_1$ for cellulites, leg swelling and breast tenderness. *Group A (gray bars)*: patients under COC treatment; *Group B (black bars)*: patients under COC *plus* supplement treatment. Error bars are ±SD. Significance: *, $p$≤0.05; **, $p$<0.001; ***, $p$<0.0001.

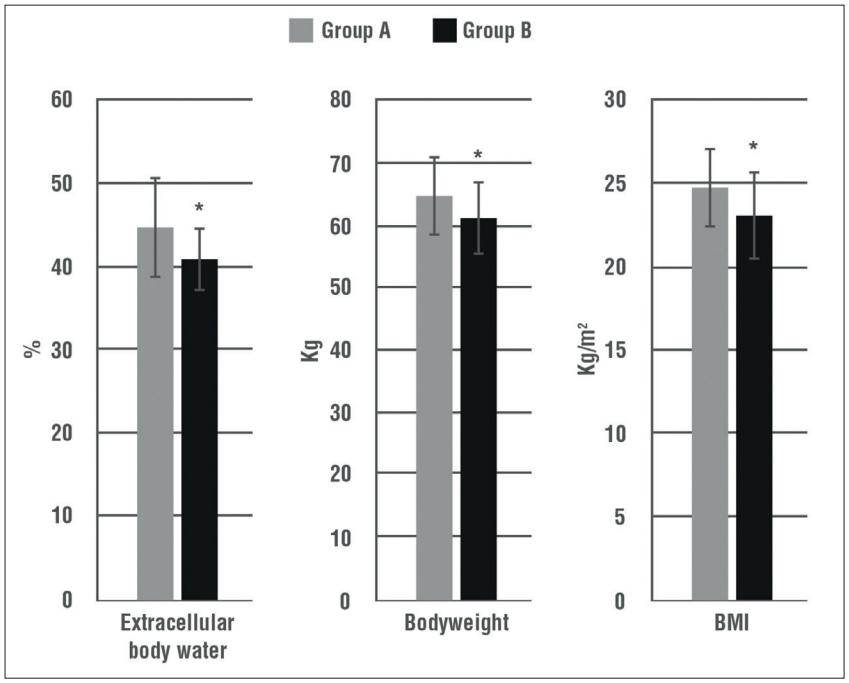


**Figure 2.** Values at $T_1$ for extracellular body water, bodyweight and BMI. *Group A (gray bars)*: patients under COC treatment; *Group B (black bars)*: patients under COC *plus* supplement treatment. Error bars are ±SD. Significance: *, $p \leq 0.05$; **, $p < 0.001$; ***, $p < 0.0001$.

basically unaffected in group B, while it reaches the upper limit of the healthy range in group A, with a statistically significant difference between the two groups at $T_1$ (Figure 2).

### Mood, migraine and fatigue

Mood score remained unchanged in group A at the end of the treatment, but patients in group B reported significantly improved mood compared to the baseline ($p<0.001$). The difference between the two groups at $T_1$ is also significant (Figure 3).

We observed a significant increase in migraine episodes in group A during this study ($p=0.01$) and a significant average reduction by 1.2 points in migraine ranking in group B ($p<0.001$). The difference between the two groups at $T_1$ is also highly significant (Figure 3), with fewer cases of migraine attacks in group B compared to group A.

Fatigue significantly increased amongst patients in group A ($p=0.01$). On the other hand, group B experienced significantly increased energy levels ($p<0.001$). At $T_1$, the difference between the two groups is significant (Figure 3), with very rare episodes of fatigue (rank=1, or below) occurring in group B.

### Hair dryness and vaginal discharge

Patients in group B reported a significant reduction of hair dryness ($p<0.001$) and vaginal discharge ($p=0.002$) with respect to baseline, while those in group A experienced non-significant small variations. The differences at $T_1$ between groups are significant (Figure 3). At the end of the observation period, only one patient in group B reported experiencing hair dryness (rank=1) and vaginal discharge was completely absent among patients of the same group.

## DISCUSSION

We have found that supplementation with *C.A.* extract, vitamins and minerals during COC treatment, reduces the magnitude of unwanted effects of the hormonal therapy.

Differences at $T_1$ between the two groups indicate that specific supplementation counteracts the negative effects of COC intake, with reduced cellulite ranking by about 56% and reduced extracellular body water by about 4%. Most interestingly, this supplementation results in relieved perception of symptoms in group B in comparison both with group A and with baseline scores, with overall improved quality of patients' life.

### Body fluid retention

Increased retention of body fluids is a major adverse effect of hormonal therapies[5]. Estrogens, indeed, activate the renin-angiotensin system and increase the levels of angiotensin II, aldosterone and sodium in plasma[6-8]. Elevated sodium concentrations induce the release of arginine vasopressin and the reabsorption of water in the kidneys. In COCs, estradiol is the main responsible for fluid retention,

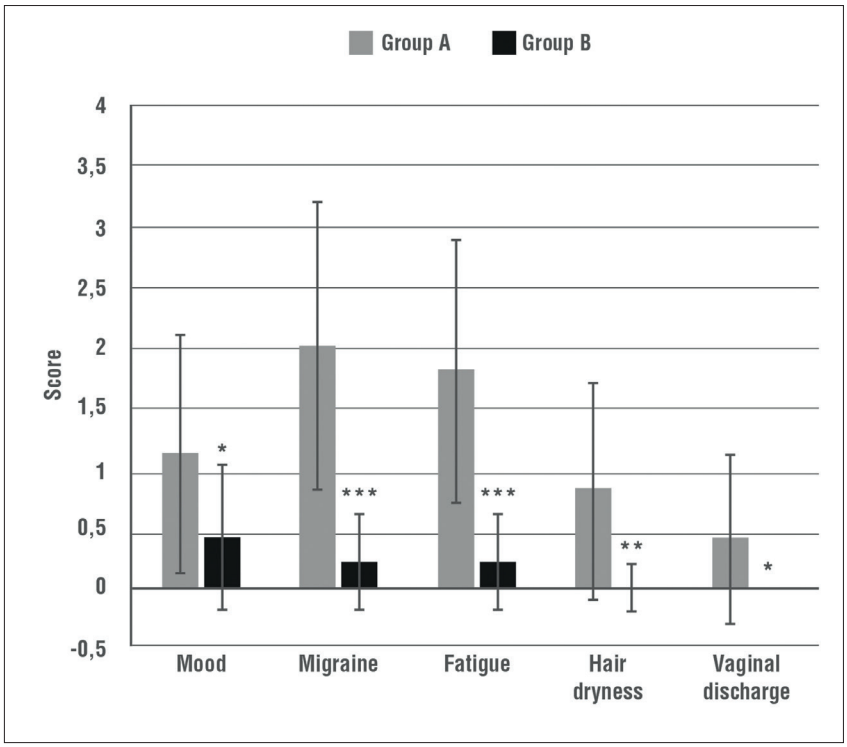


**Figure 3.** Values at $T_1$ for extracellular mood, migraine, fatigue, hair dryness and vaginal discharge. *Group A (gray bars):* patients under COC treatment; *Group B (black bars):* patients under COC *plus* supplement treatment. Error bars are ± SD. Significance: *, $p \leq 0.05$; **, $p < 0.001$; ***, $p < 0.0001$.

while the influence of progestin appears negligible[9]. Progestins with anti-mineralocorticoid activity, such as drospirenone[10], may be used to counteract this effect[11,12]. But the choice of progestin, however, must be tailored to patients' specific needs, and anti-mineralocorticoids are contraindicated in case of renal impairment or hepatic dysfunction[13]. Besides, dienogest, which has no anti-mineralocorticoid activity, is widely used in COC therapies for its safety and tolerability, which increase the compliance[14]. COCs users may experience several symptoms correlated with increased extracellular water retention, including increased body weight, breast tenderness, leg swelling, and ultimately increased cellulite.

We have found that the administration of a supplement containing *C.A.* extract effectively counteracts the adverse effects related to fluid retention due to estradiol intake. *C.A.* is a plant commonly used in Ayurvedic and Chinese medicine as "adaptogen", meaning that it has non-specific beneficial effects in restoring physiological functions[15]. It is rich in triterpenic saponins, known as *centelloids*, which account for 1-8% of *C.A.* extract. The main pharmacologically active components include asiaticoside and madecassoside, but *C.A.* extract contains also bioflavonoids as well as other triterpenic acids and their glycosides[16,17]. More recently, *C.A.* has found applications in western traditional medicine, especially for treating skin ailments such as burns and wounds[17], and in cosmetics because it stimulates the synthesis of collagen and mucopolysaccharide, with beneficial effects on scars and stretch marks.

Asiaticoside and bioflavonoids in *C.A.* extract stimulate angiogenesis and increase the elasticity of blood vessels[18], ameliorating local microcirculation. Effects include reduction of adipocytes diameter[19] and inhibition of cellulite progression, with improvement of skin conditions[17]. Increased angiogenesis improves perfusion of the lower limbs[19], preventing edema and leg swelling. It also reduces water retention, with positive effects on plasma pressure and breast tenderness[20]. Vitamin $B_6$ and vitamin E as well seem to be involved in the management of breast tenderness. Indeed, they relieve premenstrual syndrome (PMS) symptoms, including mammary pain[21], although further validation of these results is warranted.

## Reduced absorption of micronutrients

Administration of COCs reduces the absorption of some fundamental exogenous nutrients[22]. Folate uptake pathway, for instance, results partially impaired in patients under hormonal therapy, often causing a deficiency state. Folic acid (vitamin $B_9$) and its metabolites are essential for one-carbon chemistry processes in cells and participate in the biosynthesis of nucleic acids and proteins. Folate deficiency affects highly replicative cells, such as erythrocytes, causing megaloblastic anemia in the first place. Low folate intake also correlates with increased serum levels of homocysteine, a condition (known as *hyperhomocysteinemia*) that leads to reduction of female fertility[23] and to higher risk of cardiovascular diseases.

Adequate levels of folates are crucial in the periconceptional period and during early pregnancy[24]. Folates, indeed, are involved in the correct closure of the neural tube, which generally occurs by the fourth week of gestation in humans. Deficiency at this stage causes the development of Neural Tube Defects (NTDs), the most frequent malformations that occur during pregnancy, affecting the spine (*spina bifida*) and the brain (*anencephaly*)[25]. Frequently, women taking COCs have insufficient levels of serum folate[26], and complete replenishment is achieved after at least three months from the suspension of hormonal treatment[27]. Supplementation with folic acid, during COC treatment, reduces the occurrence of NTDs if pregnancy is sought immediately after treatment suspension[28], or in the case of unplanned pregnancy due to poor treatment compliance[29-31].

Folate metabolism comprises multiple reactions highly dependent on other vitamins. Exogenous folic acid is converted first to tetrahydrofolate (THF) and then to 5-methyl tetrahydrofolate (5-MTHF), the methylated form that exerts the role of C1 donor. The latter transformation is a two-step process mediated by enzymes that require vitamins $B_6$ and $B_2$ as cofactors[32]. The absorption of both these vitamins is reduced in women under COC treatment[33-35], and supplementation is recommended in such cases[36]. Low levels of vitamin $B_6$, in addition, may expose COC users to an increased risk for thromboembolism, independently of their folate status[37].

Vitamin $B_{12}$ also influences plasma folate concentration. In the physiological conversion of homocysteine to methionine, a methyl group is transferred from 5-MTHF to cobalamin (cofactor of Methionine synthase), and then to homocysteine. Reduced levels of vitamin $B_{12}$ yield to accumulation of 5-MTHF, a "methyl trap" that formally causes folate deficiency[38]. Supplementation of vitamin $B_{12}$, during hormone therapy, is recommended not only for appropriate folate metabolism, but also because deficiency of vitamin $B_{12}$ is an independent risk factor for NTDs[39] and anemia, both megaloblastic and pernicious (*vitamin $B_{12}$-deficiency*). Even though vitamin $B_{12}$ and folate have a common metabolic pathway, different factors are responsible for their deficiencies, and restoring the physiological status of one fails to compensate the other[40]. The causes of low serum levels of vitamin $B_{12}$ in COC users are still debated. Homeostasis of vitamin $B_{12}$, as well as tissue concentrations, are indeed unaltered in these women. However, low transcobalamin I levels and reduced blood binding capacity of cobalamin may account for depleted vitamin $B_{12}$ statuses[41].

Hormonal treatment may also lead to depleted serum levels of α-tocopherol (vitamin E)[42], a radical scavenger that protects tissues against the damage of oxidative stress. *In vivo*, vitamin E is constantly regenerated by ancillary antioxidants, such as vitamin C[43], and protects the integumentary system (namely nails and hair) from aging[44,45]. Protection from oxidative damages is also exerted by selenoproteins like Glutathione peroxidases[46], whose homeostasis is maintained with adequate levels of selenium. Selenium deficiency states feature nail affections and impaired hair growth[47], conditions that should be treated with supplementation[48,49].

## Further adverse effects

COCs seem to affect multiple factors that influence the mood of women under therapy[50]. Indeed, management of the adverse effects of hormonal treatment and altered body image, along with reduced absorption of micronutrients (namely, vitamin $B_6$ and magnesium) negatively impacts the psychological state of users[51].

We observed that supplementation with B vitamins and magnesium significantly improves the mood and the overall quality of life in women using COCs.

Vitamin $B_6$ is involved in the biosynthesis of neurotransmitters, in particular it is necessary for the conversion of tryptophan to niacin and serotonin. Deficiency of vitamin $B_6$ results in depleted serotonin levels, enhanced aggressive behavior and negative consequences on the mood[52]. In addition, supplementation with vitamin $B_6$ seems to relieve nausea and dizziness, which may occur during hormonal therapy and cause poor compliance[53]. Low serum values of vitamin $B_6$ is fairly common among COC users[54]. Several studies report that women under COC therapy exhibit low tryptophan concentrations[55,56], which can be restored to physiological levels with vitamin $B_6$ supplementation[57-59].

Adequate intake of minerals prevents adverse mood states. Depleted magnesium levels are common in COC users[60], causing the onset of several pathological states[61] including anxiety disorders and depression[62]. Indeed, low concentrations of magnesium are associated with a systemic inflammation state[63] and to the onset of depression[64]. Magnesium supplements proved to relieve symptoms of depression in patients with chronic fatigue syndrome[65] and PMS[66].

Selenium contributes to maintain the physiological turnover rate of some neurotransmitters[67] and prevents the onset of negative mood states. Lack of selenium, indeed, leads to increased incidence of depression, anxiety, confusion and hostile behavior[68].

Also *C.A.* has positive effects on the management of anxiety disorders[69], reducing stress and relieving depression symptoms[70]. As confirmed by *in vitro* studies on rat brains[71], *C.A.* extracts modulate the synthesis of gamma-aminobutyric acid (GABA) by stimulating the enzyme Glutamic acid decarbox-

ylase. GABA is a neurotransmitter that acts on the central nervous system in mammals, and depleted levels may lead to anxiety and depression[72].

Reduced concentrations of magnesium and vitamins $B_2$ and $B_6$ may cause the onset of common side effects experienced by women under COC therapy[51]. These include fatigue and recurrent migraines[73], which indirectly affect the mood of patients, lower the quality of their life and reduce therapy compliance. Magnesium is essential in the mitochondrial production of ATP, the main cellular energy source. Indeed, it is a cofactor of ATP synthase[74], the enzymatic complex that catalyzes the biosynthesis of ATP at the end of the cell respiratory chain, a multistep process that involves numerous enzymatic complexes and requires also vitamin $B_2$[75]. Magnesium is necessary for ATP metabolism as well. Indeed, ATP is stable and biologically available only when bound to a magnesium ion, and the enzyme Adenylate cyclase requires magnesium to transform ATP into cyclic AMP[76], the second messenger of many biological processes. Furthermore, reduced magnesium concentrations are involved in the etiology of migraine[77], a nuisance for women under COC therapy. Researchers, indeed, demonstrated that up to 50% of patients with recurrent migraine has depleted serum magnesium levels[78]. Oral supplementation with magnesium has a remarkable effect in lowering the frequency of migraine attacks, with tendency to reduce duration and intensity[79]. Also, intravenous perfusion with solutions of magnesium salts demonstrated great efficacy in rapidly halving the pain during acute migraine episodes[80].

Reduced mitochondrial metabolism is an etiological factor of migraine[81]. As previously mentioned, vitamin $B_2$ participates in the electron transport chain and cell energy production, and supplementation proved effective in migraine prophylaxis[82]. Intake of vitamin $B_2$, indeed, reduces the frequency, the intensity and the duration of headaches, as well as the need for drugs[83].

Nitric oxide (NO), a common biological cellular messenger, represents another independent cause for the onset of migraine attacks[84,85]. Vitamin $B_{12}$ acts as NO scavenger[86], and supplementation demonstrated effective in preventing headache episodes, halving their frequency in over 50% of migraineurs[87].

During this study we observed that supplementation significantly improves two other conditions caused by COC treatment, hair dryness and the occurrence of vaginal discharge. Evidences support the hypothesis that hair dryness can be caused by a zinc deficiency state[88], which is likely to occur during COC therapy[89]. Vaginal discharge, on the other hand, is a common sign of infections, and vitamin C seems to have beneficial effects in the treatment of non-specific vaginitis[90].

## CONCLUSIONS

Despite improved formulations and the use of novel progestogens, COC therapies still exhibit some side effects that may lead to poor compliance and discontinuation. A negative influence on physical appearance, consequent to cellulite and bodyweight increase, is particularly relevant for its repercussions on psychological and social aspects. Our findings demonstrate that properly formulated supplements limit water retention, which causes alterations in bodyweight during COC treatments. Patients reported a lower ranking in cellulite levels by over 50% compared to the control group, and significantly decreased leg swelling and breast tenderness. Through patients' self-evaluation, we proved that vitamins, minerals and the extract of *C.A.* significantly ameliorate their quality of life, improving the mood and reducing the occurrence of migraine episodes in comparison both to the control group and to the baseline characteristics. These results strongly suggest that this supplementation may be successfully used to increase tolerability in COC users – with greater adherence to the treatment. Future investigations, including a study with a placebo control group, are required to further validate these findings. In our opinion, data from a larger cohort of patients, considering their socio-economic and cultural background, would be necessary to assess the effect on therapy continuation rates.

**Conflicts of interest:**
The Authors declare that there are no conflicts of interest.

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