



## Effects of melatonin in perimenopausal and menopausal women: a randomized and placebo controlled study

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### Abstract

In aging humans, night levels of melatonin (MEL) decline progressively. Also thyroid and gonadal functions decline during aging while gonadotropins (luteotropic hormone (LH) and follicle stimulating hormone (FSH)) steadily increase. A desynchronization of pineal circadian cyclicality as expressed by the progressive decrease of the MEL night peak may be permissively linked to the onset and progression of menopause. We studied the effects of exogenous, evening administration of MEL on the level of hormones which are known to be involved in the genesis and progression of menopause.

Perimenopausal and menopausal women from 42 to 62 years of age with no pathology or medication were selected. MEL was measured in saliva to divide them into low, medium and high-MEL patients. Half of them took 3 mg MEL and half of them Placebo at bedtime (10–12 p.m.) in a fully randomized and double-blind fashion. Three and six months later blood was taken for determination of pituitary (LH, FSH), ovarian, and thyroid hormones ( $T_3$  and  $T_4$ ). All women taking MEL with low basal level of MEL and/or Placebo for three and six months showed a significant increase in levels of thyroid hormones. Before initiation of the study, a negative correlation was found in all women between LH, FSH and basal MEL levels. Within six months of treatment, MEL produced a significant diminution of LH in the younger women (43 to 49 year-old), while no effect was seen in the older women (50–62 years old). A decrement of FSH was observed in MEL-treated women with low basal MEL levels. In addition, most MEL-treated women reported a general improvement of mood and a significant mitigation of depression. MEL decline during aging may thus signal the derangement of pineal and pituitary-controlled ovarian cyclicality and the progressive quenching of

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fertility in women. These findings seem to show a recovery of pituitary and thyroid functions in MEL-treated women, towards a more juvenile pattern of regulation. © 2001 Elsevier Science Inc. All rights reserved.

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## 1. Introduction

A progressive reduction of nocturnal serum melatonin (MEL) concentration is observed in aging healthy humans (Iguchi et al., 1982). It is demonstrated that the pineal gland is responsible for the development and for the maintenance of neuroendocrine and sexual functions. In fact, the identification of the pineal with regard to its key role in the control of reproductive biology, emerged from the observation of precocious puberty in children carrying pineal tumors (Zrenner, 1985). In non-human mammals as, e.g. the sheep, seasonal fertility and reproduction during young, reproductive age, are strictly dependent on night levels of MEL, which vary according to the seasons (temperature and photoperiod). In this case MEL exerts an inhibitory action on gonadotropins and thyroid hormones (Tamarkin et al., 1985; Arendt, 1995; Vriend and Steiner, 1988). The effect of MEL seems to be related, at least to a large extent, to regulation of high-affinity MEL receptors in the neurohypophysis and on modulation of luteotropic hormone-releasing hormone (LH–RH) receptor density in the hippocampus and in the brain in general (anterior hypothalamus, suprachiasmatic nuclei, preoptic area a.o.) (Reppert et al., 1988; Morgan et al., 1992; Weaver et al., 1993). MEL administration at high, pharmacological doses, inhibits gonadal function in male hamsters but this effect is especially evident when MEL is given at daytime rather than with nocturnal periodicity (Reiter, 1980; Arendt, 1986). MEL at extremely high doses and for periods of years has also been extensively given to thousands of young women in the attempt to develop a new kind of contraceptive pill, with no early or late side effects (Cohen et al., 1996). MEL thwarts the growth of hormone-sensitive tumors such as certain breast cancers, and can increase the density of hormone receptors on cancer cells (Regelson and Pierpaoli, 1987).

In the course of aging, age-related illnesses and disabilities are clearly linked to a progressive decay of reproductive-sexual functions both in man (andropause) and in women (menopause). The progressive quenching of this basic function leads also to severe side-effects of psychosomatic nature and to a chain of negative events which depend on the close relationship between neuroendocrine and immune capacity and functions. In fact, it has been demonstrated that LH–RH is a powerful immuno-regulating and -enhancing hormone both in ontogeny and in adult life (Marchetti et al., 1998).

Findings from our laboratory have demonstrated that both administration of MEL in the night hours and also transplantation of pineal glands from young into older mice and rats maintain juvenile thyroid function and significantly delay their aging and/or prolongs their life (Pierpaoli et al., 1991; Pierpaoli and Regelson, 1994). A clear-cut activity of MEL and pineal, young-to-old grafting in the maintenance of gonadal and sexual functions have also been shown. A most remarkable maintenance of juvenile levels of LH–RH receptors both in the brain (hippocampus) and in the gonads is achieved by transplantation of the pineal gland from a young donor rat into older recipients (Pierpaoli et al., 1997). This effect

becomes more pronounced in the course of months after grafting, which demonstrates that, in rats, not only an arrest of sexual aging is achieved, but even a progressive reversal of sexual functions to more juvenile levels (Pierpaoli et al., 1997). The general conclusion derived from others' and our own work is that circadian, nocturnal administration of MEL may postpone endocrine aging and maintain or reconstitute more juvenile sexual functions at a time of life (e.g. between 40 and 60 years of age in women) when changes of ovarian cyclicity become evident (pre-, peri-menopausal and menopausal age). The complete absence of noxious side-effects of MEL and the many beneficial somatic and psychologic effects observed in humans, suggest to clinically evaluate the use of MEL in perimenopausal and menopausal women. Many peri- and post-menopausal women undergo a hormone replacement therapy (HRT) in the attempt to correct levels of ovarian steroids, such as estrogens and progesterone, to pre-menopause values. HRT also protects against the psychosomatic side-effects of menopause and against heart disease and osteoporosis. Even if MEL could not, by itself, prevent completely menopause and maintain ovarian cyclicity, it may enhance and improve the efficiency of HRT and thus add more years to a woman's fertile and healthy life. It is thus worth trying MEL alone before discarding this opportunity for prevention, delay or mitigation of menopause and its related psychosomatic problems. An early intervention with night administration of MEL before the initiation of ovarian dysfunction in relatively young women may indicate to us whether or not MEL can modify the onset or the course of menopause in women.

## 2. Patients and methods

### 2.1. Patients and treatment

A formal and fully documented application for the conduction of the clinical trial was delivered to the Ministry of Public Health, Rome, Italy. All women joining the clinical trial were asked to sign an informed consent. They were given detailed information on the character, duration and aims of the research. Women were carefully selected with no relevant pathologies, taking no drugs, hormones or herbal preparations and conducting a normal lifestyle with typical Mediterranean diet rich of carbohydrates and fresh vegetables, with the normal sleep habits of the population of the region south of Rome. All women were non-smoking or alcohol-abusing.

All women were also given a questionnaire in which life habits, physiological data, data concerning previous pathologies, treatment-related side-effects, treatment-induced alterations, effects on perimenopausal symptoms (neurovegetative, sleep, psychological) were recorded at time 0, and at 3 and 6 months of MEL or Placebo treatment. The questions concerned duration and character of their menstrual cycle and/or psychosomatic, peri- and menopause-related symptoms such as irritability, morning mood and depression, insomnia, night sweatings, headache, stypsis, amnesia, hot flushes, palpitations and body weight increase.

The recruited premenopausal (25 women), perimenopausal (36 women) and post-menopausal (18 women) women, whose age ranged from 42 to 62 were divided in two age groups (42–49 and 50–62) in which women with similar age and peri- or post-menopausal

Table 1

Effect of circadian, evening administration of melatonin on thyroid function in perimenopausal and menopausal all women (42–62 years old) grouped according to their basal melatonin night levels (T3 and T4 were measured in blood samples taken under standardized conditions (fasting, 8–10 a.m.) before initiation (0 time) and at 3 and 6 months after beginning of placebo or melatonin oral intake. Mean  $\pm$  SD; \* $p$  < 0.05 when compared to 0 time; \*\* $p$  < 0.0001 when compared to 0 time; <sup>a</sup> $p$  < 0.05 when compared to placebo; <sup>b</sup> $p$  < 0.0001 when compared to placebo, <sup>NS</sup> = not significant when compared to placebo; <sup>c</sup> $p$  < 0.05 when compared to medium levels of melatonin; <sup>d</sup> $p$  < 0.05 when compared to high levels of melatonin. Statistical analysis was performed using ANOVA and Student's  $t$ -test. Levels of melatonin in saliva: High = 300 pg/ml; Medium = >20 pg/ml, <300 pg/ml; Low = <20 pg/ml)

Melatonin levels in saliva	T3 (ng/ml)			T4 (ng/ml)			TSH ( $\mu$ U/ml)		
	0	3	6	0	3	6	0	3	6 months
<i>Melatonin</i> (N = 39)	1.44 $\pm$ 0.19 <sup>NS</sup>	1.58 $\pm$ 0.21 <sup>*a</sup>	1.65 $\pm$ 0.22 <sup>**a</sup>	85.2 $\pm$ 9.3	90.2 $\pm$ 8.3 <sup>*</sup>	94.1 $\pm$ 8.6 <sup>**b</sup>	1.05 $\pm$ 0.48 <sup>NS</sup>	1.03 $\pm$ 0.56	1.11 $\pm$ 0.65 <sup>NS</sup>
High (N = 13)	1.41 $\pm$ 0.23 <sup>NS</sup>	1.56 $\pm$ 0.28	1.67 $\pm$ 0.33 <sup>*</sup>	86.9 $\pm$ 9.7 <sup>NS</sup>	88.9 $\pm$ 11.0	90.2 $\pm$ 10.3	1.02 $\pm$ 0.39 <sup>NS</sup>	1.01 $\pm$ 0.37	1.24 $\pm$ 0.53
Medium (N = 12)	1.43 $\pm$ 0.12 <sup>NS</sup>	1.44 $\pm$ 0.13	1.56 $\pm$ 0.08 <sup>*</sup>	86.4 $\pm$ 6.6 <sup>NS</sup>	90.5 $\pm$ 7.2	95.6 $\pm$ 8.7 <sup>*</sup>	0.95 $\pm$ 0.45 <sup>NS</sup>	1.04 $\pm$ 0.68	1.13 $\pm$ 0.64
Low (N = 13)	1.39 $\pm$ 0.20 <sup>NS</sup>	1.61 $\pm$ 0.17 <sup>*c</sup>	1.60 $\pm$ 0.15 <sup>*</sup>	84.8 $\pm$ 7.1 <sup>NS</sup>	92.8 $\pm$ 4.6 <sup>*</sup>	95.4 $\pm$ 6.2 <sup>**a</sup>	1.06 $\pm$ 0.39 <sup>NS</sup>	1.00 $\pm$ 0.42	1.14 $\pm$ 0.42
<i>Placebo</i> (N = 40)	1.43 $\pm$ 0.20	1.48 $\pm$ 0.22	1.54 $\pm$ 0.19 <sup>*</sup>	85.5 $\pm$ 9.2	86.1 $\pm$ 11.3	86.4 $\pm$ 9.6	1.08 $\pm$ 0.54	1.10 $\pm$ 0.66	1.16 $\pm$ 0.69
High (N = 11)	1.40 $\pm$ 0.14	1.47 $\pm$ 0.19	1.71 $\pm$ 0.11 <sup>**</sup>	85.3 $\pm$ 9.1	85.1 $\pm$ 9.1	93.0 $\pm$ 9.2	1.09 $\pm$ 0.52	1.10 $\pm$ 0.30	1.21 $\pm$ 0.39
Medium (N = 14)	1.47 $\pm$ 0.22	1.49 $\pm$ 0.25	1.55 $\pm$ 0.25	86.8 $\pm$ 7.4	90.3 $\pm$ 11.2	88.2 $\pm$ 10.9	0.97 $\pm$ 0.38	1.06 $\pm$ 0.44	0.99 $\pm$ 0.46
Low (N = 13)	1.39 $\pm$ 0.07	1.53 $\pm$ 0.23	1.52 $\pm$ 0.18 <sup>d</sup>	86.4 $\pm$ 5.8	85.7 $\pm$ 13.7	85.4 $\pm$ 8.9	1.05 $\pm$ 0.36	1.20 $\pm$ 0.45	1.19 $\pm$ 0.36

Table 2

Effect of circadian, evening administration of melatonin on LH levels in perimenopausal and menopausal women (42–62 years old) grouped according to their age. Melatonin produces a more pronounced decrement of LH in the younger women (N1 = number of women taken into consideration; N2 = number of women where the increment ( $\Delta\%$ ) of LH after six months of treatment is higher or equal to 10%; % = percent of women where LH increases; \* $p < 0.05$  when compared to placebo; <sup>NS</sup> = not significant when compared to placebo; <sup>b</sup> $p < 0.05$  when women treated with melatonin (43–49 years old versus 50–62 years old) are compared ( $\chi^2$ -test))

Treatment	Age (years)	N1	N2	%
Melatonin	43–49	21	6	28.6* <sup>b</sup>
Placebo	42–49	18	11	61.0
Melatonin	50–62	16	12	75.0 <sup>NS</sup>
Placebo	50–59	21	10	47.6
Melatonin	43–62	37	18	48.6
Placebo	42–59	39	21	53.8

symptoms were equally distributed. In fact, in most countries and latitudes perimenopausal symptoms and onset of menopause do not depend precisely on the age but rather on individual, environmental and genetical variability. The two groups of women were given plastic bottles with 60 MEL capsules of 3 mg (MEL, synthetic, 100% purity) or Placebo at the beginning of each two-month period. The bottles with MEL or Placebo were certified as a GMP product and

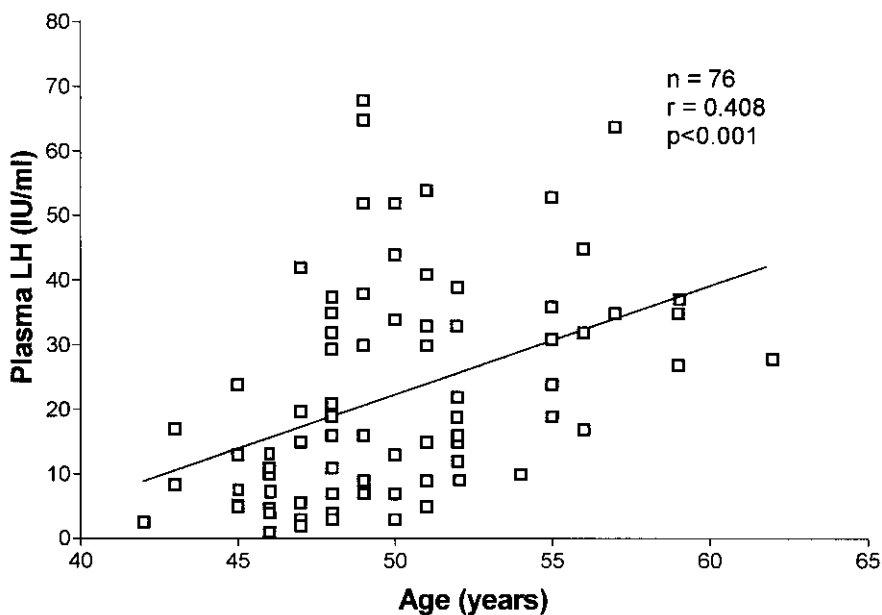


Fig. 1. The correlation between the age of all women (42–62 years old) and basal levels of LH before initiation of melatonin or placebo treatment is significantly positive (LH increases in the course of aging).

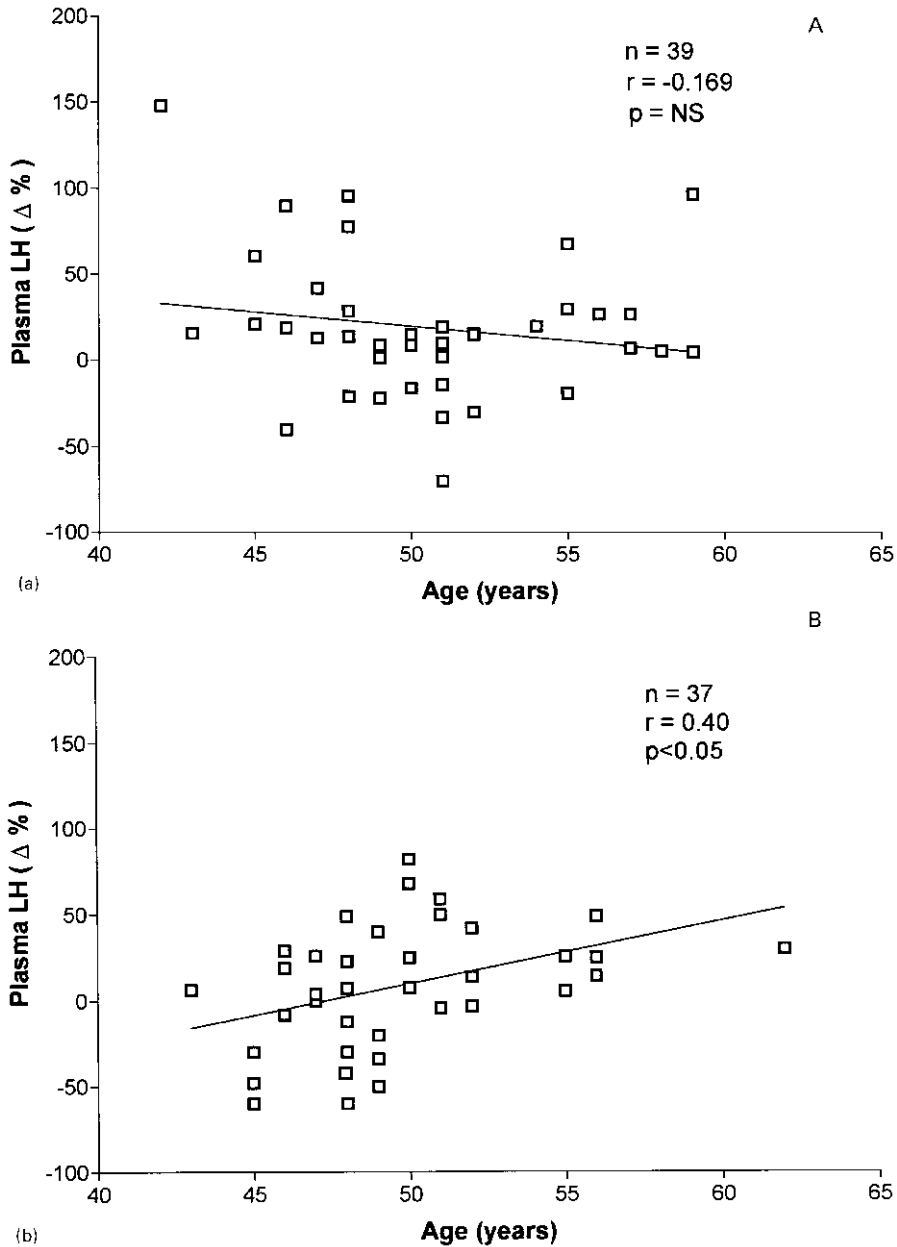


Fig. 2. (A) The correlation between the age of all women (42–59 years old) treated with placebo and the increment ( $\Delta$ %) of LH after six months of treatment is not significant. (Placebo has no effect in the maintenance of low LH levels in the younger women.) (B) The correlation between the age of all women (42–62 years old) treated with melatonin and the increment ( $\Delta$ %) of LH after six months of treatment is significant. (The effect of melatonin in the maintenance of low LH levels is progressively higher in the younger women.)

were a gift from EUROCHEM Ltd, Munich, Germany. They were only marked with a code number. The opaque white capsules of MEL and Placebo were identical. It was unknown to the women and to the doctor which of the two groups received MEL or Placebo. In fact the key of the code number (MEL or Placebo) was unknown to the patients and to the doctor and was opened only at the end of the first six-month trial.

All women were asked to take the capsules at bedtime, between 10 and 11 p.m. For obvious ethical reasons, administration of Placebo was restricted to the initial six months of this ongoing clinical study.

At the end of the six-month trial, final results were collected from a total of 79 women, with small number variations in the group studied depending on minor failure of some determinations of MEL in saliva, T3, T4, LH and follicle stimulating hormone (FSH), as shown in Tables 1 and 2 and Figs. 1–3.

## 2.2. *Methods*

A correlation exists between blood and salivary levels of MEL (Nowak et al., 1987). In order to evaluate the basal levels of MEL in the recruited women, MEL was measured in the saliva of all women immediately before the initiation of the trial (late summer 1997). All women under test were given a commercial kit for the quantitative determination of MEL in saliva (DIAGNOS-TECHS Inc., Kent, WA, USA). Also a detailed leaflet was given for the proper use of the kit. For night sampling of saliva, all women were instructed to keep out of light. Samples of saliva for measurement of MEL were taken at 2 p.m., 10 p.m., 2 a.m., 8 a.m. and 10 a.m. For testing MEL in saliva, 500  $\mu$ l of standards, controls and unknown patient saliva samples were dispensed into labeled tubes. In a second step, fixed amounts of assay buffer, MEL antiserum and  $I^{125}$ -MEL tracer were added to the tubes and incubated for 36–48 h at room temperature. In this reaction the unlabeled MEL of standards, controls and patients samples and the  $I^{125}$ -MEL compete for the binding sites of the MEL antibody. In a final step the antibody-bound fraction is precipitated by the addition of a second antibody. Samples were incubated for another 30 min and centrifuged. The supernatant was decanted and the pellet was counted in a Gamma counter. The concentration of MEL of the unknown patient was read from the calibration curve. The sensitivity of the assay is approx. 1.5 pg/ml.

A negative correlation was found between levels of MEL in saliva and age (see Section 3). The correlation between basal MEL levels and menopause was not evaluated.

For determination of hormones in blood, heparinized blood was taken from all fasting women between 8 and 10 a.m., at time 0, 3 and 6 months irrespective of menstrual cyclicity. The following hormones were assayed by standard laboratory techniques: thyroid hormones (TSH, total T3 and T4), LH, FSH, prolactin (PRL), estrone, estradiol and progesterone.

## 2.3. *Statistics*

Results are expressed as mean  $\pm$  SD. The significance between the means was assessed using paired Student's *t*-test. ANOVA test (one-way) was used where appropriate. Correlations were determined by linear regression analysis by the least square method. The differences between the various regression lines were evaluated by analysis of

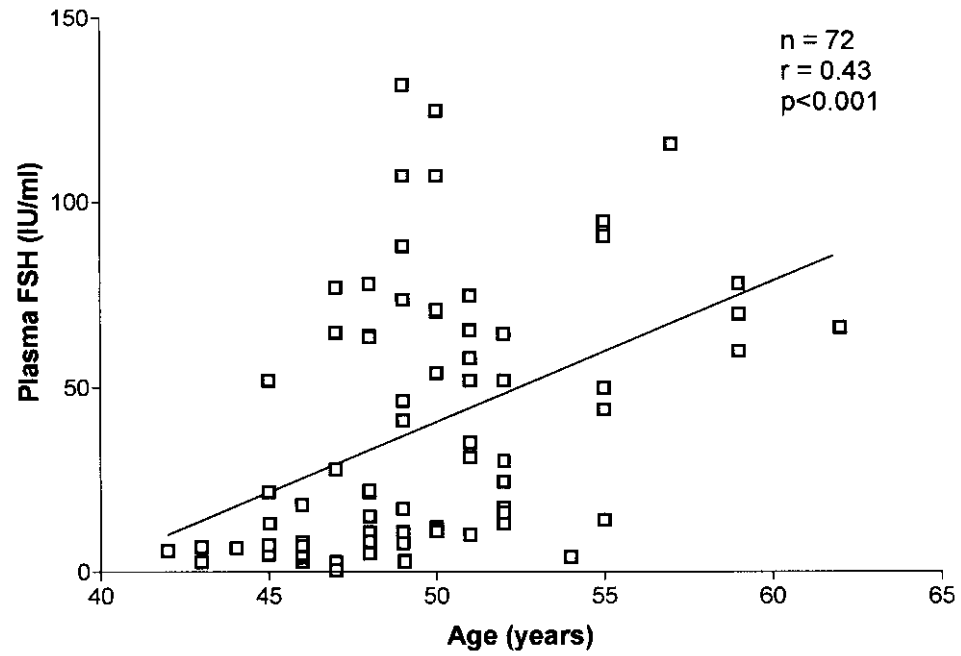


Fig. 3. The correlation between the age of all women (42–62 years old) and basal levels of FSH before initiation of melatonin or placebo treatment is significantly positive (FSH increases in the course of aging).



covariance.  $\chi^2$ -test was used to establish significance of LH increment in MEL-treated women (Table 2). Differences were considered statistically significant when  $p < 0.05$ .

### 3. Results

#### 3.1. Basal levels of MEL in saliva

As mentioned above, basal levels of MEL in the saliva were measured in all women before initiation of the clinical trial. The women were divided into three groups according to their average, nocturnal MEL levels between 10 p.m. and 2 a.m. Low levels were considered those below 20 pg/ml, medium levels were those higher than 20 and lower than 300 pg and high levels those around 300 pg/ml. In spite that the age range of the women in our trial was relatively short (42–62 years of age), a negative correlation was clearly present between age of the women and basal night levels of MEL ( $n = 76$ ;  $r = -0.263$ ;  $p < 0.05$ ).

#### 3.2. Effects of MEL on blood levels of thyroid hormones

At time 0, before initiation of the trial, a positive correlation was found between basal levels of total T3 and MEL in all women pooled together ( $n = 76$ ;  $r = 0.350$ ;  $p < 0.05$ ). This correlation was absent between MEL and total T4 ( $n = 76$ ;  $r = 0.076$ ;  $p = 0.594$ ). In the course of the initial six-month period of MEL or Placebo administration, significant changes of thyroid hormone levels were observed in the MEL-treated women. As shown in Table 1, evening administration of MEL produced a significant increase of total T3 and T4 after 3 and 6 months of MEL administration. When compared to Placebo-treated women, the increment of T3 was significant after 3 and 6 months of treatment while T4 only after 6 months (Table 1). There were no differences in TSH levels. However, a slightly significant enhancement of T3 was measurable also in Placebo treated women after 6 months of treatment. When thyroid hormone levels were evaluated according to the basal levels of MEL which had been determined in the saliva of all women before initiation of MEL or Placebo treatment, it was found that the effects of MEL on the increase of T3 and T4 were particularly significant in women with low (below 20 pg/ml) night MEL levels, when T3 and T4 were measured at 3 and at 6 months after the initiation of MEL administration (Table 1). A minor effect was seen in women with medium (above 20 and below 300 pg/ml) night levels of MEL after six months of treatment (Table 1). Only T3 was positively modified after six-month treatment in women with high (300 pg/ml) basal levels of MEL. An increase of T3 could be observed also in Placebo-treated women after six months of treatment (Table 1), which was significant in women with high basal levels of MEL. No effects were observed on levels of TSH. MEL administration also produced a significant increment of total T4 in women with low basal level of MEL, when compared to Placebo-treated women (Table 1).

#### 3.3. Effects of MEL on blood levels of LH

As it is known, progression of aging in women leads to increased blood levels of LH

(Ahmed-Ebbiary et al., 1994). A consistent increase of basal LH in relation to the increasing age of women was in fact visible at time 0 (Fig. 1). Also a clear-cut negative correlation between basal levels of MEL and LH ( $n = 76$ ;  $r = -0.314$ ;  $p < 0.05$ ) was found in all women at time 0. When the levels of LH were evaluated in the younger or older age groups (42–49 and 50–62 years of age) in relation to the percentage of women displaying an increment of LH, it was seen that MEL produces a significant decrease in plasma LH only in the younger and not in the older women (Table 2). In fact, in the Placebotreated women, no correlation was found between the increment ( $\Delta\%$ ) of LH level and the age of the women after six months of treatment (Fig. 2A), while in MELtreated women a significant and positive correlation was found between age and LH increment (Fig. 2B). This demonstrates that the effects of MEL in controlling and maintaining low levels of LH are much more pronounced in the younger women (between 43 and 49 years of age) than in the older women (between 50 and 62 years of age) (compare Fig. 2A and B).

#### 3.4. *Effects of MEL on blood levels of FSH*

FSH was measured in all women before initiation of Placebo or MEL treatment. As predictable (Ahmed-Ebbiary et al., 1994) and shown in Fig. 3, also basal levels of FSH increase with age. Similar to LH, a negative correlation exists between basal levels of MEL and FSH at time 0 in all women ( $n = 72$ ;  $r = -0.322$ ;  $p < 0.05$ ). Treatment with MEL for six months produced a significant decrease ( $\Delta\%$ ) of FSH especially in women with low basal MEL levels ( $n = 35$ ;  $r = 0.468$ ;  $p < 0.05$ ). After six months of treatment, no correlation was seen between increment of FSH ( $\Delta\%$ ) and basal levels of MEL in the Placebo-treated women ( $n = 37$ ;  $r = -0.057$ ;  $p = 0.795$ ).

#### 3.5. *Effect of MEL on estrogens, progesterone and PRL*

The large variability of the values measured in blood samples taken at different times of the menstrual cycle and in menopausal women with age varying from 42 to 62 years of age, did not allow to evaluate any significant difference between MEL or Placebo-treated women within the six-month period of MEL treatment.

#### 3.6. *Menstrual cyclicity*

Within the relatively short period of MEL administration, only episodic improvement of regularity and duration of menstrual cycles were reported. However, six menopausal women (at 1 and 2 years after total cessation of the menses) reported a re-acquisition of normal (bleeding and duration) menstrual cycles.

#### 3.7. *Other psychosomatic and neurovegetative changes*

From the analysis of all answers obtained from the questionnaire after the initial six months of MEL or Placebo treatment, the evaluation of different typical perimenopause or menopause-related symptoms or alterations did not disclose a clear-cut difference between the two groups, with the notable exception of a very significant improvement of mood and complete disappearance of morning depression in the MEL-treated women. Only 6.7% of MEL-treated women reported on maintenance of morning depression compared to 21% of

Placebo-treated women ( $p < 0.05$ ,  $\chi^2$ -test). Although not significant, many women reported a tendency to amelioration of hot flushes, palpitations and improvement of quality and duration of sleep.

#### 4. Discussion

The purpose of this initial trial on possible effects of nocturnal MEL administration in perimenopausal women was solely that of verifying whether or not MEL may by itself modify levels of hormones and produce changes of any kind, independently of age (42–62 years of age) and of the stage of the menstrual cycle. It is in fact undisputed that a close link exists between the pineal gland, MEL and human reproduction (Reiter, 1998) and that a relationship exists between adenohipophyseal and steroid hormones and MEL during the ovarian cycle, perimenopause and menopause (Fernandez et al., 1990).

The preliminary findings emerging from this ongoing clinical trial help to focus and to restrict our attention on the significant changes of thyroid, and of some hypophyseal hormones, namely LH and FSH, after evening administration of exogenous, oral MEL in the course of six months. In fact, no statistically significant changes were observed in the levels of gonadal steroids and PRL. This may depend on the different age of women, on the short treatment period (six months) and because the blood samples were taken with no regard of menstrual cyclicity.

The initial measurement of MEL in the saliva of all women allowed to establish a criterion for dividing the women in low, medium and high-MEL subjects, in order to see whether or not MEL can produce endocrine changes depending on the basal, individual level of MEL. It was also important to divide all women in two age groups (42–49 and 50–62 years of age) in order to verify if MEL can affect neuroendocrine functions in relation to the age of the subject. In fact, these separations allowed to discriminate between MEL-reactive and -unreactive women and to disclose an evident higher sensitivity of younger women to MEL treatment. This is extremely relevant in view of the fact that prevention of perimenopausal and menopause-related endocrine changes may be facilitated by knowledge of the age and timing when MEL treatment or replacement therapy may be initiated in women.

The most important and largely unexpected finding was the clear-cut effect of MEL on thyroid function (Table 1). Our trial was initiated in late summer and was concluded in late winter, a season when physiological levels of thyroid hormones are higher (Nicolau et al., 1992). In spite of the obvious variability of T3 and T4, which depends on changes of season, temperature and photoperiod (Maes et al., 1997), clearly the MEL-treated women showed a remarkable increase of T3 and T4 values (Table 1). When the effect of MEL was evaluated in relation to the basal levels of MEL in the saliva, it can be seen that MEL produced a significant increase of T3 and T4 in low- and medium-MEL women but only T3 increased in high-MEL women. Apparently this effect of MEL on thyroid function can be exerted only when the pineal gland produces less MEL while, in the Placebo-treated women, endogenous levels of MEL in the high-MEL women seems to affect per se and positively thyroid function (Table 1). The interpretation of this Placebo-dependent change could also be attributed, in the high-MEL women, to the physiological changes of thyroid

function in relation to temperature and season (Nicolau et al., 1992; Maes et al., 1997). It is thus clear that low MEL levels can be suggestive of low thyroid function and that administration of MEL may prevent and cure the decay of thyroid function in perimenopausal women (Vriend, 1983). Our findings are confirmed by the positive correlation existing between basal levels of MEL and T3 at time 0 in all women (see Section 3). These results seem also to suggest that thyroid deficiency may be a common and apparently latent endocrine disorder for initiation and progression of menopause in women, and also an unsuspected cause for at least some of the psychosomatic and psychic, neurovegetative symptoms. That MEL can restore a deranged thyroid function in perimenopausal women is not a surprise if we consider that MEL modulates the 5' thyroid deiodinase (Reiter and Guerrero, 1992), that MEL or pineal grafting improve thyroid function in old rodents (Pierpaoli and Regelson, 1994) and that the pineal gland contains relatively large amounts of thyrotropin releasing hormone (TRH) (Lew, 1989), which has been suggested to be a key element for pineal function (Vriend, 1978; Pierpaoli and Yi, 1990). These findings are in contradiction with the recent assertion that a MEL replacement therapy is unjustified in postmenopausal women (Brzezinski, 1998), while they are in full agreement with an investigation in which MEL was measured in 77 females around 50 years of age (30–75) (Vakkuri et al., 1996). MEL was found to decline significantly from premenopause to postmenopause and to correlate negatively with serum FSH, indicating a sharp decline of nocturnal MEL far before menopause, and suggesting that MEL may be determinant for the initiation of menopause (Vakkuri et al., 1996). FSH may as well represent an early endocrine marker of reproductive aging (Vakkuri et al., 1996). In our trial the administration of MEL produces significant changes in LH and FSH levels which seem to be related, not only to the original basal levels of MEL but also to the age of the women (see Section 3, Table 2, Fig. 2A and B). In fact, in accordance with our data and those in the literature, LH and FSH progressively increase in perimenopausal women (Figs. 1 and 3). Basal levels of MEL do not significantly affect LH in MEL or Placebo-treated women (data not shown). However, while MEL produces a remarkable decrease of LH in the younger women (Table 2, Fig. 2B), Placebo does not produce any change (Fig. 2A). MEL depresses the production of FSH only in women with low MEL levels. The apparent partial recovery of more juvenile gonadotropin function (decrease of LH and FSH) in the MEL-treated women is consonant with the notion that the progressive increase of levels of LH and FSH in aging women signals an increased central hypothalamic resistance and insensitivity to feed-back regulation, with increasing number of LHRH receptors in the hippocampus and consequent compensatory increase of LH and FSH secretion (Pierpaoli et al., 1997). This effect of MEL in the restoration of reproductive functions is evident in aging experimental animals (Pierpaoli et al., 1997).

Our results indicate that a cause-effect relationship between the decline of nocturnal levels of MEL and onset of menopause may in fact exist (Fernandez et al., 1990). However, we have no element to associate the effects observed with a direct action of exogenous MEL administration in the regulation of the menstrual cycle. We are confident that the continuation of our trial will show in the course of time (1–2 years) whether or not MEL can at least partly abrogate hormonal, menopause-related neurovegetative disturbances and restore menstrual cyclicity and fertility in perimenopausal or menopausal women. At present we can assert that the six-month treatment with MEL produced a

remarkable and highly significant improvement of thyroid function, positive changes of gonadotropins towards more juvenile levels and the abrogation of menopause-related depression.

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