

Long-term effectiveness and safety of GnRH agonist plus raloxifene administration in women with uterine leiomyomas

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BACKGROUND: Our aim was to evaluate the long-term effectiveness and safety of GnRH agonist plus raloxifene administration in women with symptomatic uterine leiomyomas. **METHODS:** Fifty pre-menopausal women with uterine leiomyomas were treated with leuprolide acetate depot at dose of 3.75 mg/28 days and raloxifene hydrochloride at 60 mg/day for 18 cycles. At admission and after each six cycles of treatment, bone mineral density (BMD), uterine, leiomyoma and non-leiomyoma dimensions, serum bone metabolism markers, lipid, glucose and insulin levels were evaluated. Leiomyoma-related and climacteric-like symptoms were assessed using a daily diary. **RESULTS:** Throughout the study, no significant change in BMD or in any bone metabolism markers was observed. A significant decrease in uterine, leiomyoma and non-leiomyoma sizes was detected in comparison with baseline already after 6 months. No other significant change was observed at the successive follow-up visits. No significant change in lipid and glucose profile was detected throughout the study. The treatments were well tolerated. All treatment withdrawals (16%, eight out of 50) were due to lack of compliance, and none to drug-related adverse experiences. **CONCLUSION:** GnRH agonist plus raloxifene administration is an effective and safe treatment for pre-menopausal women with uterine leiomyomas.

Key words: bone loss/GnRH a/leiomyomas/metabolism/raloxifene

Introduction

The hypoestrogenic state induced by continuous administration of GnRH agonist is effective in the treatment of various gynaecological sex hormone-related diseases (Surrey *et al.*, 1995) causing climacteric-like symptoms (Sherwin and Tulandi, 1996; Palomba *et al.*, 1998, 1999, 2002a, 2003).

Several drugs have been used in association with GnRH agonists (i.e. 'add-back therapy') to reduce these side effects, but only a few did not compromise the effectiveness of the analogue alone in women with uterine leiomyomas (Carr *et al.*, 1993; Friedman *et al.*, 1994; Palomba *et al.*, 1998, 1999, 2002a, 2003; Nakayama *et al.*, 1999). In addition, only tibolone seems to be an effective add-back therapy when administered together with the analogue at the start of the treatment (Palomba *et al.*, 1998, 1999).

Raloxifene hydrochloride is a synthetic non-steroidal drug derived from benzothiophene and afferent to selective estrogen receptor modulators (SERMs). Raloxifene administration pre-

vents post-menopausal bone loss in women without osteoporosis and significantly reduces the incidence of bone fractures in post-menopausal osteoporotic women (Clemett and Spencer, 2000; Cranney *et al.*, 2002). In addition, pre-clinical (Black *et al.*, 1994; Bryant *et al.*, 1996; Fuchs-Young *et al.*, 1996; Porter *et al.*, 1998; Walker *et al.*, 2000) and clinical data (Palomba *et al.*, 2001a, 2002b; Jirecek *et al.*, 2004) have suggested that raloxifene may have a beneficial effect on uterine leiomyomas.

Recently, in a prospective parallel single-blind placebo-controlled study, we have demonstrated that raloxifene prevents the bone loss related to GnRH agonist administration (Palomba *et al.*, 2002c). A benefit in terms of reduction of uterine and leiomyoma dimensions was also observed when raloxifene was administered in association with analogue (Palomba *et al.*, 2002d). No significant change in lipid or glucose metabolism was detected in women who received the GnRH agonist plus raloxifene treatment (Palomba *et al.*, 2004). On the contrary, women who received the analogue alone

showed a worsening of the lipid profile and an increase of insulin resistance indexes (Palomba *et al.*, 2004).

With this in mind, the present study was carried out to investigate the long-term effectiveness and safety of GnRH agonist plus raloxifene administration as medical treatment for pre-menopausal women with symptomatic uterine leiomyomas.

Materials and methods

The procedures used in this study were in accordance with the guidelines of the Declaration of Helsinki on human experimentation. The protocol was approved by the Local Ethic Committees. Before entering the study, the purpose of the protocol was explained to each woman attending the Departments of Gynecology of the University of Catanzaro, Messina and Naples 'Federico II'. Written informed consent was obtained from all subjects.

One hundred pre-menopausal women affected by symptomatic uterine leiomyomas initially were enrolled in a wide randomized controlled trial (RCT) with multiple end-points of 6-month follow-up (Palomba *et al.*, 2002c,d, 2004). The exclusion criteria have been reported previously (Palomba *et al.*, 2002c,d).

The present study is the extension of a further 12 months follow-up regarding the arm treated with leuprolide acetate depot (LAD) plus raloxifene hydrochloride.

LAD (Enantone, Takeda, Rome, Italy) was administered at a dose of 3.75 mg/28 days and raloxifene hydrochloride (Evista, Eli Lilly, Sesto Fiorentino, Italy) at a dose of 60 mg/day p.o.

At the beginning of the study and after every six cycles of treatment, we assessed in each subject bone mineral density (BMD), bone, lipid and glucose metabolism, Ca intake, alcohol consumption, physical activity, uterine and leiomyoma size, and endometrial thickness.

The BMD was determined by dual energy X-ray absorptiometry (Dexa QDR 1000, Hologic, Waltham, MA) at the posterior-anterior lumbar spine (vertebrae L1–L4) and at the hip (trochanter and femoral neck) as previously reported (Palomba *et al.*, 2002c), and the results of absorptiometry were examined by a single observer blind with respect to the different treatment regimens.

A blood sample, was collected from each woman, immediately centrifuged, and stored at -80°C until analysis in duplicate, and the mean of two assays was used. A urine sample was also obtained. Blood was sampled between 08.00 and 09.00 h with the subject resting in bed, after overnight fasting and a 3 day 300 g carbohydrate diet. Patients were also asked to refrain from eating foods containing fat or gelatin for 12 h prior to their clinic visit. At study entry, the blood samples were collected during the early follicular phase of the menstrual cycle (Palomba *et al.*, 2004).

Bone metabolism was evaluated by determining the serum levels of osteocalcin (OC) and bone alkaline phosphatase (BAP), and urinary creatinine (Cr)-corrected free deoxypyridinoline (DPD) and pyrilinks-D (PYD) with commercial kits as previously reported (Palomba *et al.*, 2002c). Serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) were measured with an autoanalyser using commercially available kits as previously reported (Palomba *et al.*, 2004). Serum LDL-C was evaluated using Friedewald's formula: $\text{TC} - \text{HDL-C} - 1/5 \text{ TG}$ (Friedewald *et al.*, 1972).

Plasma glucose and insulin levels were measured as already reported (Palomba *et al.*, 2004). The homeostasis model assessment (HOMA) score, which is a measure of insulin resistance, was calculated for all subjects with the formula: $\text{fasting serum insulin}$

$(\mu\text{U/ml}) \times \text{fasting plasma glucose (mmol/l)} / 22.5$ (Matthews *et al.*, 1985).

The uterine and leiomyoma sizes were obtained with the use of ultrasonographic scans equipped with a 7.5 MHz transvaginal probe. The ultrasonographic assessments were performed by the same experienced operator blinded to treatment. To evaluate the effect of treatments on the non-leiomyoma tissue, the difference between uterine and leiomyoma volumes (Δ size) was calculated in each subject (Palomba *et al.*, 2001a, 2002b,d).

The subjects were instructed to report in a personal daily diary the characteristics of their menstrual cycle (length and severity of uterine bleeding) as well as the onset of any adverse experience (Palomba *et al.*, 2002d).

The severity of leiomyoma-related symptoms, such as menorrhagia, pelvic pressure and pain, urinary frequency, and constipation was also reported. The severity of symptoms was carefully recorded by each woman using a rank scale ranging from 1 to 10 (Palomba *et al.*, 2001a, 2002b,d).

Every three cycles, each subject also underwent a standard clinical evaluation and laboratory analyses, including haematological, renal function and liver function tests, and microscopic examination of sediment from midstream urine specimens.

At baseline and at 3 month intervals during treatment, Ca intake, alcohol consumption and physical activity were evaluated as previously described (Palomba *et al.*, 2002d).

No dietary restriction or changes were implemented during the study. To ensure adequate Ca intake, all patients with a Ca intake <1000 mg received daily supplements of elemental Ca in the form of an effervescent tablet composed of calcium carbonate (Cacit, Procter & Gamble, Rome, Italy).

All women agreed to use barrier contraception during the study.

Repeated measures of analysis of variance (ANOVA) was used to evaluate changes during the study in age, body mass index (BMI), BMD, endometrial thickness, uterine and leiomyoma sizes, and Δ size. ANOVA was also used to compare biochemical data. The differences in length and severity of menstrual cycles within the study group were compared at entry and after each six cycles of treatment, using ANOVA and Wilcoxon's signed rank tests, respectively. Wilcoxon's signed rank test was also used to compare within-group cigarettes smoked, alcohol consumption, Ca intake and physical activity. Data were analysed with the use of the intention-to-treat method. The statistical analysis was performed using SPSS 11.0 (SPSS Inc., Chicago, IL). Data were normally distributed and expressed as mean \pm SD.

Results

In Table I, we report the demographic, clinical and biochemical data of the study population.

BMD measurements and bone turnover markers

Throughout the study, lumbar spine, trochanter and femoral neck BMD did not differ from baseline values (Figure 1). Serum OC and BAP levels, and urinary DPD and PYD excretion were also unchanged during the overall treatment period in comparison with baseline values (Figure 2).

Uterine and leiomyoma sizes, and Δ size

At the sixth cycle of treatment, a significant ($P < 0.05$) decrease in uterine and leiomyoma size was obtained (Figure 3). A significant ($P < 0.05$) change in Δ size was also observed in

Table I. Demographic, clinical and biochemical data of the 50 patients initially enrolled

Demographic data	
Age (years)	49.1 ± 4.2
Parity (no.)	1.8 ± 1.4
BMI (kg/m ²)	23.8 ± 3.2
Cigarettes smoked (no./day)	6.4 ± 5.6
Calcium intake score ^a	2.5 ± 0.8
Alcohol consumption score ^b	1.1 ± 0.6
Physical activity score ^c	1.9 ± 0.9
Women receiving Ca supplements (%)	17 (34.0)
BMD (g/cm ²)	
Lumbar spine	1.077 ± 0.069
Trochanter	0.881 ± 0.050
Femoral neck	0.769 ± 0.047
Biochemical markers	
OC (µg/l)	8.41 ± 1.33
BAP (IU/l)	23.3 ± 7.6
DPD/Cr (nmol/mmol)	4.41 ± 1.34
PYD/Cr (nmol/mmol)	27.2 ± 5.3
TC (mmol/l)	3.98 ± 0.13
HDL-C (mmol/l)	1.81 ± 0.16
LDL-C (mmol/l)	2.87 ± 0.21
TG (mmol/l)	1.27 ± 0.20
Glucose (mmol/l)	5.41 ± 1.55
Insulin (µU/ml)	6.47 ± 1.33
HOMA score	1.62 ± 0.44
Ultrasonographic findings	
Uterine size (cm ³)	473 ± 112
Leiomyoma size (cm ³)	197 ± 61
Δ size (cm ³)	259 ± 67

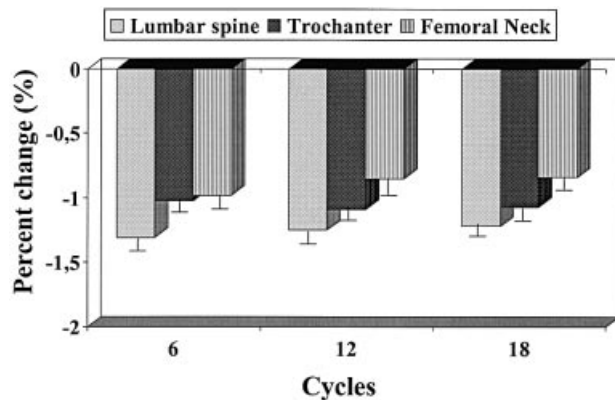
Values are reported as mean ± SD.

BMI = body mass index; Ca = calcium, OC = osteocalcin, BAP = bone alkaline phosphatase, DPD = deoxypyridinoline, HOMA = homeostasis model assessment, PYD = pyrilinks-D, Cr = creatinine.

^a1 = <500 mg/day, 2 = 500–1000 mg/day, 3 = >1000 mg/day.

^b1 = <1000 mg/day, 2 = 1000–2000 mg/day, 3 = >2000 mg/day.

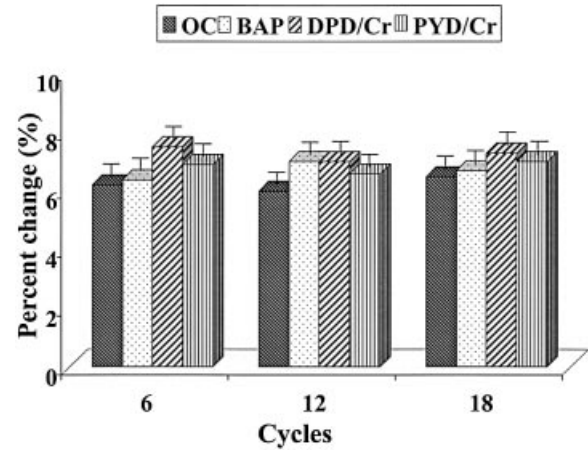
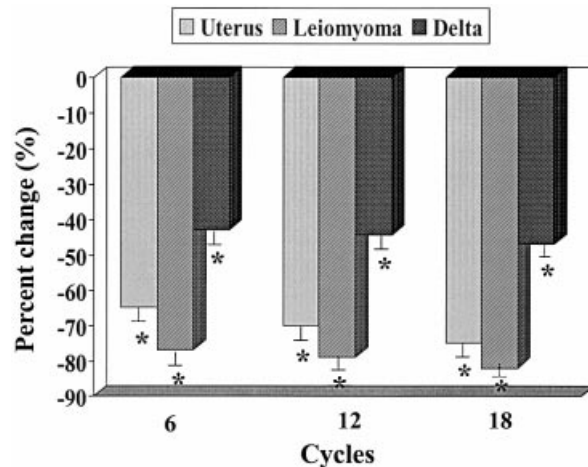
^c1 = low, 2 = moderate, 3 = high.

**Figure 1.** Percentage change versus baseline in bone mineral density (BMD) throughout the study. Data are reported as mean ± SD.

comparison with baseline (Figure 3). After the sixth cycle and throughout the study, no other change in uterine and leiomyoma size, and in Δ size was observed (Figure 3).

Leiomyoma-related and vasomotor symptoms

The changes in the intensity of leiomyoma-related symptoms are shown in Table II. Already after six cycles of treatment, a significant ($P < 0.05$) decrease in severity of all leiomyoma symptoms was observed in comparison with baseline. After the

**Figure 2.** Percentage change versus baseline in biochemical markers of bone turnover throughout the study. Data are reported as mean ± SD.**Figure 3.** Percentage change versus baseline in uterine and leiomyoma size, and Δ size throughout the study. Data are reported as mean ± SD. * $P < 0.05$ versus baseline.

sixth cycle and throughout the study, no other change in the intensity of leiomyoma-related symptoms was observed (Table II).

The percentage of patients with bleeding decreased constantly throughout the first cycles of treatment. At the sixth, twelfth and eighteenth cycle of treatment, three out of 47, two out of 44 and two out of 42 women were bleeding, respectively. In all cases, the uterine bleedings had a 'spotting' pattern.

In Figure 4 we show the mean number of hot flushes per day. A significant ($P < 0.05$) increase was observed after 15 days from the start of the treatment and remained constant for all successive cycles (Figure 4).

Lipid and glucose metabolism

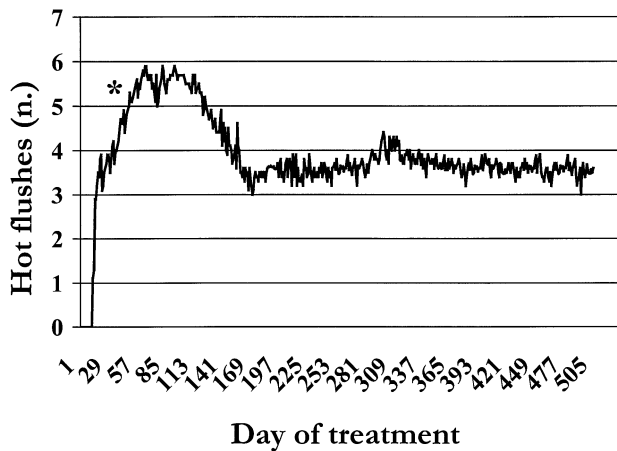
After six cycles of treatment, serum TC, HDL-C and TG levels were significantly ($P < 0.05$) higher than baseline (Figure 5). No other significant variation was detected throughout the study.

Table II. Leiomyoma-related symptoms (severity and number of subjects affected) in women treated with GnRH agonist plus raloxifene

Symptom	Baseline	6th cycle	12th cycle	18th cycle
Menorrhagia	7.6 ± 1.6 (50/50)	– (0/47) ^a	– (0/44)	– (0/42)
Pelvic pressure	6.8 ± 1.4 (44/50)	3.7 ± 1.0 (4/47) ^a	4.0 ± 1.0 (3/44) ^a	3.7 ± 0.6 (3/42) ^a
Pelvic pain	7.0 ± 1.8 (36/50)	4.0 ± 0.9 (5/47) ^a	3.8 ± 0.8 (4/44) ^a	3.7 ± 1.1 (3/42) ^a
Urinary frequency	5.9 ± 1.7 (31/50)	2.3 ± 1.5 (3/47) ^a	2.5 ± 0.7 (2/44) ^a	2.5 ± 0.7 (2/42) ^a
Constipation	5.2 ± 1.5 (11/50)	– (0/47) ^a	– (0/44)	– (0/42)

^a*P* < 0.05 versus baseline.

Values are reported as mean ± SD.

**Figure 4.** Mean number of hot flushes per day throughout the study. Data are reported as mean ± SD. **P* < 0.05 versus baseline.

During the study, glucose and insulin levels did not differ in comparison with baseline (Figure 6). HOMA scores were also not significantly different in comparison with baseline values.

Lipoprotein, glucose and insulin levels were within the normal range in all women.

Side effects and drop-outs

Throughout the study, the treatment schedule was well tolerated.

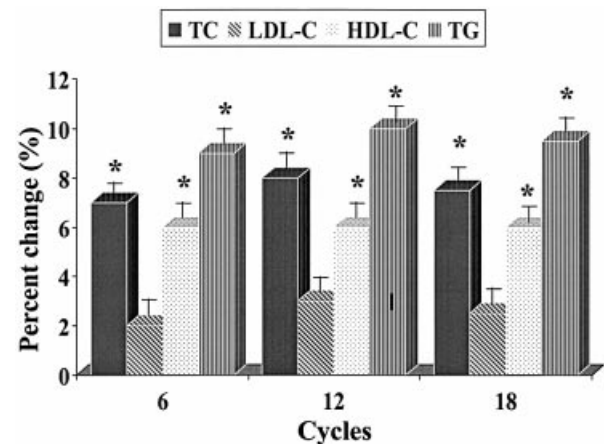
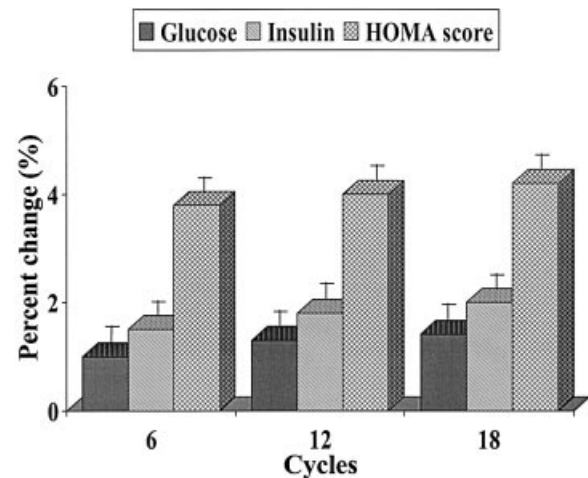
No serious adverse experience was reported during the study. The incidence of clinical effects and/or laboratory abnormalities was very low.

Eight out of 50 subjects (16%) dropped out from the study throughout the 18 cycles of treatment. Specifically, three, three and two patients dropped out due to lack of compliance with the treatment after 6, 12 and 18 cycles, respectively.

Three, four and five patients did not undergo a BMD and ultrasound-guided examinations with a transvaginal probe for personal reason at the sixth, twelfth and eighteenth cycle of treatment, respectively. No drop-out was due to drug-related adverse experiences.

Discussion

The present study is the first evaluating the long-term safety and effectiveness of administration of LAD plus raloxifene in women with uterine leiomyomas. Only two other studies

**Figure 5.** Percentage change versus baseline in serum lipoprotein levels throughout the study. **P* < 0.05 versus baseline.**Figure 6.** Percentage change versus baseline in serum glucose and insulin levels, and HOMA score.

(Friedman *et al.*, 1994; Palomba *et al.*, 1999) with a long-term follow-up of 2 years are available in the literature evaluating progestin or estro-progestin and tibolone addition to GnRH agonist as add-back therapy in women with uterine leiomyomas.

The bone loss is the main and most studied side effect related to GnRH agonist use (Surrey, 1995). The continuous administration of GnRH agonist induces a significant bone loss depending on the dose of the analogue, on the length of the

treatment and on the degree of induced hypoestrogenism (Monroe *et al.*, 1986; Broekmans *et al.*, 1996a; Palomba *et al.*, 2002a). In addition, the bone loss does not seem to recover significantly after treatment withdrawal (Matta *et al.*, 1988; Revilla *et al.*, 1995; Taga and Minaguchi, 1996) and it is not known whether the add-back therapy completely preserves the bone tissue (Pierce *et al.*, 2000). A randomized trial (Pierce *et al.*, 2000), in fact, suggested that the bone loss is not totally recovered after long-term treatment with GnRH agonist for 6 years, notwithstanding the use of add-back therapy (Pierce *et al.*, 2000). Recently, we observed that post-menopausal women previously treated with long-term GnRH agonist plus tibolone administration have a reduction in BMD and in bone turnover markers similar to that observed in surgically post-menopausal women, suggesting that GnRH agonists exert a direct adverse effect on the bone (Palomba *et al.*, 2002a).

Our data show that the use of raloxifene as 'add-back therapy' during GnRH agonist administration protects the bone for the overall period of the treatment. In fact, no significant variations in BMD were detected at the axial and appendicular bone sites. The positive effect of raloxifene on bone metabolism was also confirmed by the lack of significant change in biochemical parameters of bone formation and reabsorption.

The percentage change in lumbar BMD observed during the present study was similar to that obtained with GnRH agonist plus tibolone in another study with a long-term follow-up of 2 years (Palomba *et al.*, 1999). Furthermore, at present, it is not known if the addition of raloxifene or tibolone only reduces the deleterious effects of GnRH agonist-related hypoestrogenism on bone metabolism or also completely protects the bone from direct analogue damage (Palomba *et al.*, 2002a).

The ideal add-back therapy would not only prevent the bone loss, but also preserve the efficacy of the analogue. In this view, while the add-back therapy is well established in women with endometriosis (Surrey, 1999), in patients affected by uterine leiomyomas the addition of progestins or estrogen-progestins at the start of GnRH agonist administration seems to reduce the effectiveness of the analogue on the uterine and leiomyoma size (Carr *et al.*, 1993; Friedman *et al.*, 1993, 1994; Pickersgill, 1998), and only tibolone (Palomba *et al.*, 1998, 1999) or raloxifene (Palomba *et al.*, 2002d) addition alone may be used at the start of the analogue treatment.

In the present study, we show that the use of GnRH agonist and raloxifene association is an effective long-term treatment for women with symptomatic uterine leiomyomas. After 6 months of treatment with GnRH agonist plus raloxifene, we already observed a significant reduction in uterine and leiomyomas size in comparison with analogue alone (Palomba *et al.*, 2002d). The continuation of the treatment for another 12 cycles maintained suppression of the uterine and leiomyoma dimensions, and no other significant decrease was observed throughout the study. The raloxifene-related reduction in leiomyoma dimensions observed in the present study was higher in comparison with that obtained during administration of GnRH agonist plus tibolone (Palomba *et al.*, 1999) or in comparison with other studies (Pickersgill, 1998). These results may be due to the effect of raloxifene, but could also be due to the careful selection of women without hypoechoic or

calcified leiomyomas. Certainly, a more accurate evaluation should be performed using magnetic resonance imaging (Carr *et al.*, 1993; Broekmans *et al.*, 1996b).

In the same way, a significant reduction in leiomyoma-related symptomatology was observed already after six cycles of treatment, and during the successive 12 cycles no other change was detected.

Several studies (Gerhard *et al.*, 1992; Palomba *et al.*, 1998, 1999; Al-Omari *et al.*, 1999, 2001; Cheung *et al.*, 2000; Somekawa *et al.*, 2001) have shown an alteration in lipid profile in unselected populations treated with GnRH agonist. Recently, to evaluate specifically the effect of GnRH agonist administration on lipid, glucose and homocysteine metabolism, we studied a sample of patients (Palomba *et al.*, 2004) with normal weight and waist-hip ratio (<0.8) without clinical/biochemical hyperandrogenism (Efstathiadou and Tsatsoulis, 2001). GnRH agonist administration induced an alteration in serum TC, HDL-C and TG levels, and the addition of raloxifene did not prevent this side effect. However, the increase in TC and LDL-C concentrations was significantly less in women who received raloxifene addition than in women who received GnRH agonist alone (Palomba *et al.*, 2004). Also, in the present long-term study, performed on an unselected population, we observed a significant change from baseline in TC, HDL-C and TG levels. Furthermore, throughout the study, the HDL-C/LDL-C ratio was not changed after GnRH agonist plus raloxifene, confirming the beneficial effect of raloxifene on the lipid pattern and on the cardiovascular risk in post-menopausal women (Walsh *et al.*, 2000; De Leo *et al.*, 2001).

Only one study has been performed to evaluate glucose metabolism in women treated with a GnRH agonist (Palomba *et al.*, 2004). A significant increase in serum insulin levels and in HOMA scores after six cycles of GnRH agonist treatment was observed, whereas these parameters remained unchanged in the women treated with GnRH agonist plus raloxifene (Palomba *et al.*, 2004). According to this last study (Palomba *et al.*, 2004), no significant change was observed throughout the 18 cycles of GnRH agonist plus raloxifene administration. Raloxifene, in fact, seems to reduce circulating insulin plasma values in hyperinsulinaemic women by increasing fractional hepatic insulin extraction and improving peripheral insulin sensitivity (Cucinelli *et al.*, 2002).

During the overall study period, few side effects were detected and the raloxifene treatment was well tolerated. In particular, a low rate of uterine bleeding was observed. The rate of uterine bleeding in women was similar to that observed after long-term treatment with GnRH agonist and tibolone. In fact, in post-menopausal women, raloxifene and tibolone have shown no proliferative effect on the endometrium, inducing a high percentage of 'not bleeding' cycles (Palomba *et al.*, 1999; Goldstein *et al.*, 2000; Modelska and Cummings, 2002).

A high percentage of women treated with GnRH agonist plus raloxifene reported hot flushes. Unfortunately, raloxifene administration did not reduce the vasomotor symptoms related to GnRH agonist. In contrast, a significant reduction in mean number of hot flushes per day was observed when adding tibolone to GnRH agonist treatment (Palomba *et al.*, 1998,

1999, 2001b). Notwithstanding the presence of this side effect, a low rate of treatment withdrawal (eight out of 50, 16%) was observed after 18 cycles of treatment, confirming the long-term acceptability of the combined regimen.

Finally, in pre-menopausal women with symptomatic uterine leiomyomas, the long-term administration of GnRH agonist plus raloxifene is safe and effective in reducing the size of the uterus and of the leiomyomas. The use of raloxifene in women who receive GnRH agonist prevents the analogue-related bone loss and lipid and glucose alterations during the overall period of treatment.

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