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Melatonin and estradiol effects on food intake, body weight, and leptin in ovariectomized rats

S. Sanchez-Mateos, C. Alonso-Gonzalez, A. Gonzalez, C.M. Martinez-Campa, M.D. Mediavilla, S. Cos, E.J. Sanchez-Barcelo *

Department of Physiology and Pharmacology, School of Medicine, University of Cantabria, 39011 Santander, Spain

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Abstract

Objective: The study in ovariectomized (Ovx) rats, as a model of menopausal status, of the effects of melatonin (M) and/or estradiol (E), associated or not with food restriction, on body weight (BW) and serum leptin levels.
Methods: Female SD rats (200–250 g) were Ovx and treated with E, M, E + M or its diluents. Control sham-Ovx rats were treated with E–M diluents. After 7 weeks being fed *ad libitum*, the animals were exposed for 7 more weeks to a 30% food restriction. We measured: food intake, BW, nocturnal and diurnal urinary excretion of sulphatoxymelatonin (aMT6s), leptin in midday and midnight blood samples, glucose, total cholesterol, LDL, HDL and triglycerides.
Results: Day/night rhythm of aMT6s excretion was preserved in all cases. The increase of aMT6s excretion in M-treated animals basically affected the nocturnal period. In animals fed *ad libitum*, E fully prevented Ovx-induced increase of BW, leptin and cholesterol. Melatonin reduced food intake and partially prevented the increase of BW and cholesterol, without changing leptin levels. Under food restriction, M was the most effective treatment in reducing BW and cholesterol. Leptin levels were similar in M, E or E + M treated rats, and lower than in untreated Ovx rats.
Conclusions: Our result gives a preliminary experimental basis for a post-menopausal co-treatment with estradiol and melatonin. It could combine the effectiveness of estradiol (not modified by melatonin) with the positive effects of melatonin (improvement of sleep quality, prevention of breast cancer, etc.). The possible beneficial effects of melatonin which could justify its use, need to be demonstrated in clinical trials.

Keywords: Melatonin; Leptin; Body weight; Food intake; Food efficiency; Obesity

1. Introduction

With menopause, women experience important metabolic changes which are patent in increases in body weight, adiposity, and LDL and leptin levels [1,2]. Postmenopausal obesity has been considered responsi-

* Corresponding author at: Departamento de Fisiología y Farmacología, Facultad de Medicina, Universidad de Cantabria, 39011 Santander, Spain.
E-mail address: barcelo@unican.es (E.J. Sanchez-Barcelo).

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ble for the increased risk of many pathologies affecting these women, such as diabetes, hypertension, heart diseases, breast cancer or infectious illness among others [1,3]. Leptin, a proteic hormone synthesized mainly by adipocytes, but also in other cells such as mammary epithelial cells [4], plays a key role in the regulation of food intake, energy expenditure and body weight homeostasis [5]. The elevated serum levels of leptin in obese postmenopausal women, together with the role of this adipocytokine in proliferation, invasion and metastasis of breast cancer cells [6], has led to leptin being considered as a key to explaining the relationship between obesity and breast cancer [7,8]. Furthermore, in the context of breast cancer biology, it is interesting to point out that leptin, at concentrations similar to those detected in sera from obese women, increases the aromatase activity of adipose estromal cells from human breast fat [9] and also contributes to breast cancer as an angiogenic factor [10].

Melatonin is a pineal hormone with well-known actions on the neuroendocrine reproductive axis and circadian system as well as being an antioxidant [11]. Melatonin is also present in the gastrointestinal tract of vertebrates and its release from this source seems to be related with the periodicity of food intake [12]. Thus, melatonin could play a role in the regulation of energy balance and body weight. An age-dependent decline of melatonin secretion has been repeteadly reported [13–15]. Although studies in very elderly subjects presented no significant differences in the nocturnal urinary excretion of 6-sulphatoxymelatonin (the main melatonin metabolite) in relation to those of younger adults [16], a meta-analysis of the literature data in subjects with ages ranging from 21–82 years concluded that a decrease in melatonin excretion by approximately 36% occurs from 21–33 to 49–85 years [17]. This decline of melatonin secretion with aging has been related with the age-dependent increase of visceral fat [13]. Daily administration of melatonin to middle age male rats reverses visceral fat and circulating levels of glucose, leptin, insulin and triglycerides to the youthful values [18]. Melatonin also has oncostatic properties in animal models of hormone-dependent mammary cancer as well as *in vitro*, in cell lines of human breast cancer [19]. This oncostatic role is based on the properties of melatonin, such as its ability to counteract the effects of estradiol at the level of the estrogen-receptor [20], its capacity to inhibit the expression and activity of aromatases [21,22], and its actions as antioxidant and inhibitor of telomerase activity [23]. Furthermore, it is generally accepted that melatonin plays an important role in the mechanism of sleep onset [24], although the question of whether its beneficial effects depend either on its hypnotic or phase-shifting effects, or indeed both, is discussed [25]. In this concern, it is convenient to remember that insomnia is frequently associated with menopause [26]. Because of all the above-described properties of melatonin, and considering that no side effects have been described as a result of its therapeutic application [27] it seems justified to think that administration of melatonin to menopausal woman could produce beneficial effects.

The objective of this work is to study in ovariectomized rats, as a model of menopausal status, the effects of melatonin and/or estradiol, associated or not to food restriction, on body weight regulation and serum leptin levels.

2. Material and methods

2.1. Chemicals

Unless otherwise indicated, all chemicals used in this work were purchased from Sigma–Aldrich (Madrid, Spain).

2.2. Experiment 1

2.2.1. Animals

Three-month-old female SD rats with body weights ranging from 200–250 g, purchased from Harland (Barcelona, Spain), were caged in polycarbonate boxes (two rats per box), under a 14/10 light/dark photoperiod, and fed *ad libitum* with a standard laboratory diet (Panlab, Barcelona, Spain). Forty-eight animals were ovariectomized and, from the second day after surgery, treated with either estradiol (group Ovx + E), melatonin (group Ovx + M), estradiol + melatonin (group Ovx + E + M) or the diluents of both hormones (group Ovx). A group of animals were sham-ovariectomized and treated with the diluents of estradiol and melatonin, serving as controls (group C).

2.2.2. Treatments

Estradiol (25 μg/kg BW), dissolved in corn-oil, was injected sc twice a week. Melatonin was administered

in drinking water (20 μg/ml). It was prepared from a stock solution (1 mg/ml, in absolute ethanol) and added to water. The bottles were covered with aluminum foil to protect them from light. The water (with melatonin or ethanol 0.01% as its diluent) was changed every 2 days. The presence of melatonin in drinking water modifies neither the amount of water drunk nor the drinking rhythm (predominantly nocturnal) of rodents [18].

2.2.3. Sampling procedures

Animals were transferred weekly, for 2 days, to metabolic boxes to measure food consumption as well as to collect urine in nocturnal and diurnal separated fractions, and to be weighed. Once a week blood samples from all animals were obtained by venous puncture during the light period as well in darkness (under dim red light).

2.2.4. Determinations in urine and serum samples

Sulphatoxymelatonin (aMT6s) was measured in urine samples by using RIA kits (Stockgrand Ltd., Guildford, Surrey, UK). The total nocturnal and diurnal aMT6s excretion was calculated as the product of its concentration in urine and the amount of urine collected during each period. The serum leptin concentration was assessed by using RIA kits for rats (Linco Research, St. Charles, Missouri, USA) according to the manufacturer's instructions. Glucose, total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were measured in serum samples by enzymatic colorimetric method, with kits from BioSystems SA, Barcelona, Spain.

2.3. Experiment 2

The animals included in experiment 1 continued receiving the same treatments as described above for another 7 weeks, with only one change: instead of being fed *ad libitum*, the food given to the rats was now restricted by 30% in relation to the food intake measured in age matched controls. Sampling procedures were carried out as in experiment 1.

2.4. Statistical analysis

Changes in BW, leptin, etc. were analyzed by two-way ANOVA (time and treatment) followed by the Bonferroni test for differences between specific groups. Statistical significance was considered when $p < 0.05$ or lower.

3. Results

3.1. Experiment 1

3.1.1. Urinary excretion of aMT6s

Fig. 1 shows the total urinary excretion of aMT6s during the diurnal and nocturnal periods. In animals not treated with melatonin (groups C, Ovx, and Ovx + E) the nocturnal values were, in all cases, significantly

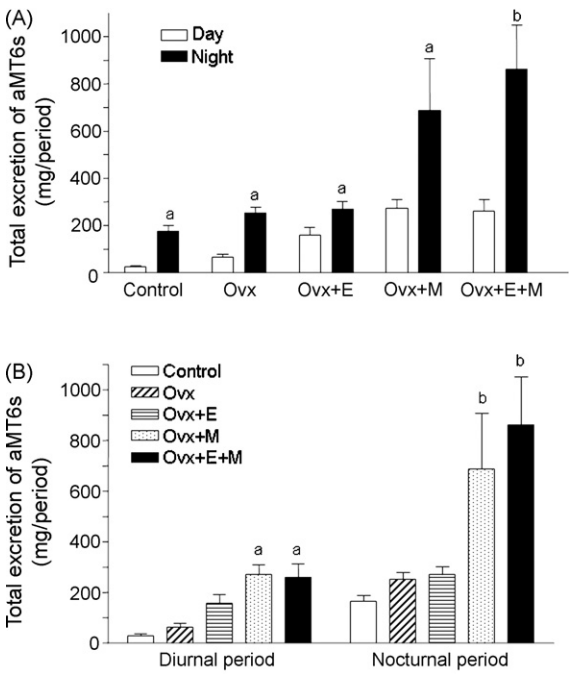

Fig. 1. (A) Total urinary excretion of 6-sulfatoxymelatonin (aMT6s) during the whole day or night periods in control female rats (C) and rats ovariectomized (Ovx) and then treated with estradiol (Ovx + E), melatonin (Ovx + M) or both (Ovx + E + M). (B) The same data as in Fig. 1A, but grouped by the time of sampling (nocturnal or diurnal).Results are expressed as mean ± S.E.M. ($n = 9$–12) and analysed by parametric two-way ANOVA, the ways being treatment and time (diurnal or nocturnal periods). Source of variation: treatment, $F = 7.44$, d.f. (degrees of freedom) = 4, $p < 0.001$; time, $F = 14.66$, d.f. = 1, $p < 0.001$. Differences between diurnal and nocturnal aMT6s excretion between animals in each group (Bonferroni post-test): $^{a}p < 0.01$; $^{b}p < 0.001$. Differences between treatments (Bonferroni post-test): diurnal values, $^{a}p < 0.05$ vs. C; nocturnal values, $^{b}p < 0.001$ vs. C, Ovx and Ovx + E.

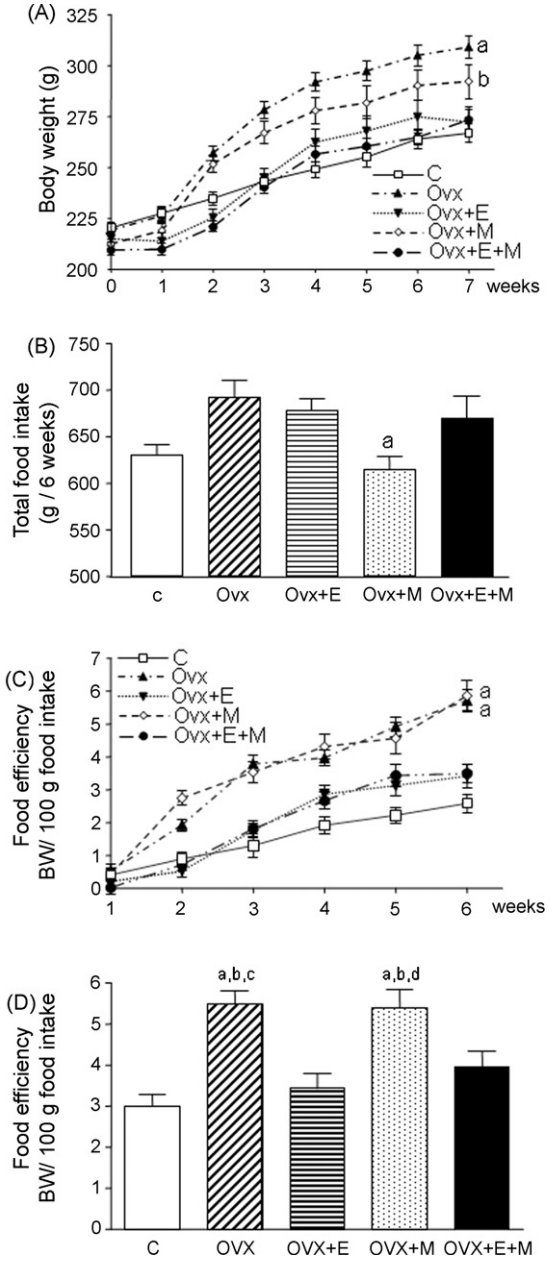

Fig. 2. (A) Time course of body weight changes in control female rats (C) and rats ovariectomized (Ovx) and then treated with estradiol (Ovx + E), melatonin (Ovx + M) or both (Ovx + E + M). The animals were, in all cases, fed *ad libitum*. Results are mean ± S.E.M. ($n = 9$–12).Two-way ANOVA (time and treatment) shown as sources of variation: time (weeks), $F = 151.5$, d.f. = 7, $p < 0.001$; treatment, $F = 60.13$, d.f. = 4, $p < 0.001$; and the interaction of both, $F = 2.696$, d.f. = 28, $p < 0.001$. Differences after 7 weeks of treatment (Bonferroni post-test) were: [a]$p < 0.001$ vs. C, Ovx + E and Ovx + E + M; [b]$p < 0.05$ vs. C and Ovx. (B) Total food intake (groups as in 2A). One-way ANOVA, $F = 2.89$, d.f. = 4, $p < 0.05$; Bonferroni post-test, [a]$p < 0.05$ vs. Ovx and Ovx + E. (C) Cumulated food efficiency [body weight reached at each week/(g of food consumed)/100].Two-way ANOVA indicated that treatment ($F = 67.24$, d.f. = 4, $p < 0.001$), time ($F = 138.7$, d.f. = 5, $p < 0.001$, $p < 0.001$) and interaction of both ($F = 3.248$, d.f. = 20, $p < 0.001$), are the sources of variation. Bonferroni post-tests: Ovx and Ovx + M vs. the remaining groups (C, Ovx + E and Ovx + E + M), [a]$p < 0.001$. (D) Total food efficiency mean ± S.E.M. ($n = 9$–12) (7 weeks). One-way ANOVA, $F = 9.364$, d.f. = 4, $p < 0.001$. Bonferroni post-test: [a]$p < 0.001$ vs. C; [b]$p < 0.01$ vs. Ovx + E; [c]$p < 0.05$ vs. Ovx + E + M; [d]$p < 0.01$ vs. Ovx + E + M.

higher ($p < 0.01$) than the diurnal ones, despite the nocturnal period (10 h) being shorter than the diurnal one (14 h). Furthermore, no differences were found either between the nocturnal or between the diurnal excretions of aMT6s of C, Ovx or Ovx + E animals. As expected, the amount of aMT6s excreted by those animals drinking water with melatonin was significantly higher than by those receiving the diluent ($p < 0.01$ in all cases). This fact indicates that the circulating levels of melatonin augmented in animals receiving the hormone exogenously. The differences in the excretion of aMT6s between animals receiving melatonin or diluent were greater during the nocturnal periods than during the diurnal ones. The reason is that the nocturnal drinking behavior of rats was not modified by the addition of melatonin. Thus, since rats drink more water at night, those animals drinking water with melatonin have increased nocturnal levels of the hormone, as well as an increased excretion of its urinary metabolite.

3.1.2. Body weight (BW) and food efficiency

Fig. 2A represents the time-course changes in BW during the 7 weeks of experiment. Ovx rats showed higher body weights than sham-operated controls (C) ($p < 0.001$). Treatment with estradiol fully prevented an increase in the BW of Ovx rats. At the end of the 7 weeks of treatment, the BW of Ovx animals receiving estradiol was similar to that of the controls (Ovx + E versus C, $p > 0.05$). Co-treatment with estradiol and melatonin gives the same results on BW as with estradiol alone (Ovx + M versus Ovx + E + M, $P > 0.05$). Interestingly, melatonin also prevented, at least in part, the increase in BW after ovariectomy. Thus, the BW of rats treated with melatonin (Ovx + M) was lower than that of Ovx animals ($p < 0.05$) although higher than those of Ovx + E, Ovx + E + M or C rats ($p < 0.05$).

The total amount of food ingested by animals during the first 6 weeks of experiment is represented in Fig. 2B. Ovariectomy, even in animals treated with estradiol or estradiol plus melatonin, slightly increased food intake in relation with controls, although differences do not reach statistical significance. Animals treated with melatonin ate significantly less than Ovx or Ovx + E rats ($p < 0.05$). Fig. 2C represents the time course changes in food efficiency (grams of increase of body weight per each 100 grams of food eaten). Food efficiency increased significantly after ovariectomy ($p < 0.001$) in relation to uncastrated controls. Whereas the treatment with melatonin did not modify the post-ovariectomy increase in food efficiency, estradiol fully prevented these effects (Ovx + E versus Ovx, $p < 0.001$). The decrease of body weight of Ovx–Mel is due to a lower food intake, since food efficiency does not change in Ovx rats because of the treatment with melatonin (Ovx + M versus Ovx, $p > 0.05$) (Fig. 2D).

3.1.3. Serum leptin concentrations

All animals, despite the treatment received, showed a clear day–night difference ($p < 0.001$) in plasma concentration of leptin (Fig. 3A). Seven weeks after surgery, Ovx rats showed diurnal and nocturnal ($p < 0.01$) serum leptin levels significantly higher than sham-operated controls ($p < 0.001$ and $p < 0.01$) (Fig. 3B); that is to say, ovariectomy induced an increase in leptin synthesis. Treatment with E_2 prevented the increase of leptin release, the nocturnal circulating levels in Ovx + E animals being even lower than those of sham-operated controls ($p < 0.001$). Melatonin had no effect on plasmatic leptin levels and, when co-administered with estradiol, did not modify the effects of the steroid (Fig. 3B).

3.1.4. Serum levels of glucose, cholesterol, LDL, HDL and triglycerides

All these metabolic indicators were measured in blood samples obtained during the diurnal or nocturnal periods (Table 1). The most relevant result was the increase of total plasma cholesterol found in diurnal samples from Ovx rats ($p < 0.001$ versus controls). Treatment with estradiol fully prevented this increase of cholesterol induced by ovariectomy (Ovx + E versus Ovx, $p < 0.001$). Melatonin also prevents the postcastration increase of cholesterol, but to a lesser extent than estradiol. Thus, the plasma cholesterol concentra-

Table 1
Modifications of serum parameters in ovariectomized (Ovx) rats treated with estradiol (E), melatonin (M), or both hormones (animals fed *ad libitum*)

Treatment	Glucose (mg/dl)		Total cholesterol (mg/dl)		HDL (mg/dl)		LDL (mg/dl)		HDL/LDL		Triglicerides (mg/dl)	
	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples
Sham Ovx (C)	171.6 ± 13.5	179.1 ± 14.8	96.2 ± 5.0	105.4 ± 10.8 (5)	24.9 ± 1.9	15.9 ± 0.5	18.8 ± 1.6	11.6 ± 3.1	1.4 ± 0.1	1.7 ± 0.3	67.4 ± 7.7	36.3 ± 4.5
Ovx	178.8 ± 15.3	221.2 ± 33.6	153.8 ± 8.7[a,b] (5)	110.8 ± 8.1	31.4 ± 4.7[c]	15.3 ± 0.7	22.6 ± 1.7	14.3 ± 2.9	1.4 ± 0.2	1.3 ± 0.3	55.2 ± 7.9	50.3 ± 3.8
Ovx + E	172.3 ± 17.5	198.3 ± 15.4	83.5 ± 12.0	92.2 ± 13.1 (5)	21.3 ± 2.1 (5)	16.0 ± 0.6 (5)	17.6 ± 1.3 (5)	20.3 ± 3.3[d]	1.2 ± 0.1	0.8 ± 0.1	58.1 ± 6.6	46.3 ± 7.2
Ovx + M	164.1 ± 5.6	215.1 ± 19.9	116.8 ± 11.4[e]	92.7 ± 10.5	22.6 ± 1.9	19.9 ± 2.5	19.9 ± 1.1	20.4 ± 3.5[d]	1.1 ± 0.1	1.08 ± 0.1	60.2 ± 8.2	58.1 ± 7.9
Ovx + E + M	171.4 ± 5.7	212.2 ± 11.9	122.5 ± 5.6[e]	115.5 ± 9.2	26.5 ± 3.9	18.8 ± 1.5	20.9 ± 3.7 (5)	12.9 ± 1.4	1.0 ± 0.2 (5)	1.6 ± 0.2	47.8 ± 3.9	43.7 ± 4.6
Statistics	NS	NS		NS		NS	NS		NS	NS	NS	NS

The number of samples was always 6, except when indicated in brackets.
[a] $p < 0.001$ vs. C and Ovx + E.
[b] $p < 0.05$ vs. Ovx + M.
[c] $p < 0.05$ vs. Ovx + E and Ovx + M.
[d] $p < 0.05$ vs. C.
[e] $p < 0.05$ vs. Ovx + E.

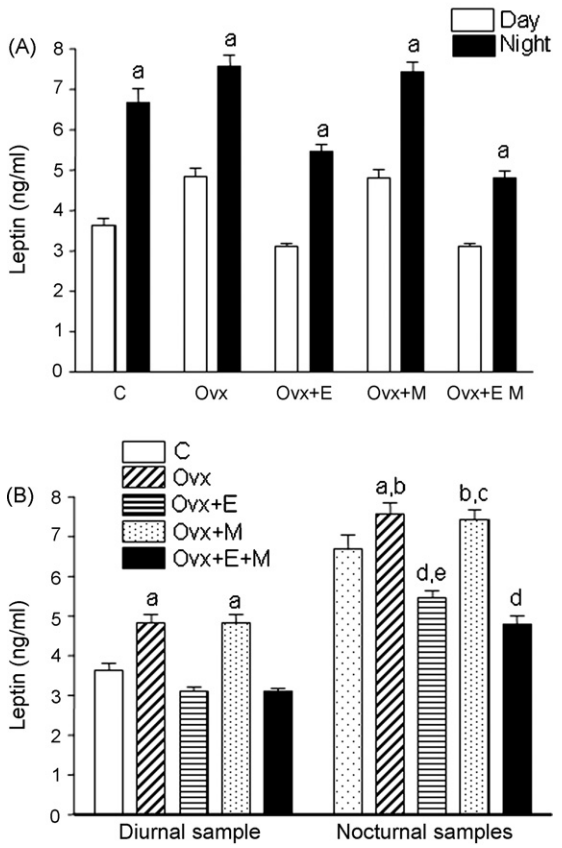

Fig. 3. (A) Serum concentration of leptin in samples collected during day and night periods in control female rats (C) and rats ovariectomized (Ovx) and then treated with estradiol (Ovx + E), melatonin (Ovx + M) or both (Ovx + E + M). (B). Same data as in Fig. 3A, but grouped by the time of sampling (nocturnal or diurnal). Data are mean ± S.E.M. ($n = 10$). Two-way (treatment and time) ANOVA. Sources of variation: treatment, $F = 49.32$, d.f. = 4, $p < 0.001$; time (day/night), $F = 360.6$, d.f. = 1, $p < 0.001$; interaction time-treatment, $F = 2.933$, d.f. = 4, $p < 0.05$. Differences between diurnal and nocturnal serum leptin concentration between animals in each group (Bonferroni post-test) $^{a}p < 0.001$. Differences between diurnal samples, $^{a}p < 0.001$ vs. C, Ovx + E and Ovx + E + M. Differences between nocturnal samples, $^{a}p < 0.01$ vs. C; $^{b}p < 0.001$ Ovx + E and Ovx + E + M; $^{c}p < 0.05$ vs. C; $^{d}p < 0.001$ vs. C; $^{e}p < 0.05$ vs. Ovx + E + M.

tion in Ovx + M rats was lower than in Ovx animals ($p < 0.05$), although higher than in Ovx + E ($p < 0.05$). HDL also increased in diurnal samples after ovariectomy ($p < 0.05$) and was normalized by treatment with estradiol or melatonin. No differences were found for the remaining parameters.

3.2. Experiment 2

3.2.1. Body weight after food restriction

Fig. 4A shows the relative changes in the BW of ovariectomized rats after a 30% reduction in the normal food intake, understood as the average amount of food eaten by control untreated age-matched animals. In all cases, body weight was decreasing, but this reduction was significantly higher in animals treated with melatonin ($p < 0.05$ versus Ovx and Ovx + E; $p < 0.01$ versus Ovx + E + M). Fig. 4B depicts the absolute body weight of rats after the 7 weeks under restriction. Ovariectomized rats, which began the period of food restriction with a higher BW than animals of the remaining groups, still have the highest body weight ($p < 0.05$ versus Ovx + M, $p < 0.01$ versus Ovx + E, $p < 0.001$ versus Ovx + E + M). However, melatonin treated rats (Ovx + M) which, before the food restriction, had BW higher than Ovx + E and Ovx + E + M rats, present, after the 7 weeks under food restriction, a BW similar to that of rats treated with E, M or both hormones (Fig. 4B). The dotted horizontal line in Fig. 4B indicates the BW of control (uncastrated) age-matched rats always fed *ad libitum* used to define the normal food intake. It serves to show that the seven-week food restriction applied to Ovx animals was enough to be able to reverse the increase in body weight reached during the previous seven week period fed *ad libitum*. Food efficiency (Fig. 4C) was negative in all cases, with significantly lower values in melatonin treated rats than in the animals of the remaining groups.

3.2.2. Serum leptin concentrations after food restriction

Food restriction did not alter the day/night rhythm of serum leptin in Ovx rats (Fig. 5). As happened among animals fed *ad libitum*, treatment with estradiol decreased serum leptin (Ovx versus Ovx + E, $p < 0.001$). Melatonin also decreased the nocturnal levels of serum leptin in relation to the animals treated with the diluent (Ovx versus Ovx + M, $p < 0.05$), but the reduction of the diurnal ones was not significant. No differences related to nocturnal or diurnal leptin serum concentrations were found among the three treatments (E, M or E + M). The observation of Figs. 3 and 5 allows a comparison to be made between serum leptin at the end of the 7 weeks in which animals were fed *ad libitum* (Fig. 3), and the concentrations measured after another

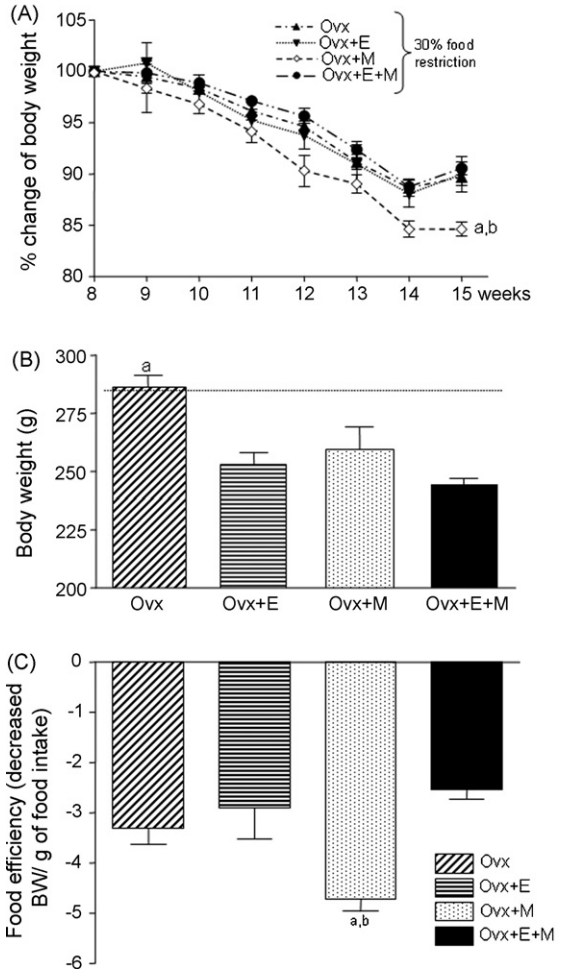

Fig. 4. (A) Time course of relative body weight changes (mean ± S.E.M., $n = 6$) in ovariectomized rats treated with estradiol (Ovx + E), melatonin (Ovx + M), estradiol plus melatonin (Ovx + E + M) or the diluent (Ovx). After 7 weeks being fed *ad libitum*, the animals were fed for 7 more weeks amounts of food equal to 70% of the average food intake of control intact age-matched rats, calculated daily. Two-way ANOVA shown as sources of variation: treatments, $F = 152.9$, d.f. = 4, $p < 0.001$; time (weeks) $F = 53.21$, d.f. = 7, $p < 0.001$; interaction of both, $F = 10.04$, d.f. = 28, $p < 0.001$. Bonferroni post-test: $^{a}p < 0.05$ vs. Ovx and Ovx + E; $^{b}p < 0.01$ vs. OvxE + M. (B) Absolute body weight (mean ± S.E.M., $n = 6$) after the 7 weeks of food restriction. The dotted line indicates the body weight of the intact animals which served to define the amount of food given to food restricted rats. One-way ANOVA: $F = 8.796$, d.f. = 3, $p < 0.001$. Bonferroni post-test: $^{a}p < 0.05$ vs. Ovx + M, $^{a}p < 0.01$ vs. Ovx + E, $^{a}p < 0.001$ vs. Ovx + E + M. (C) Food efficiency during the 7 weeks of food restriction. One-way ANOVA: $F = 6.309$, d.f. = 3, $p < 0.01$. Bonferroni post-test: $^{a}p < 0.01$ vs. Ovx + E and Ovx + E + M; $^{b}p < 0.05$ vs. Ovx.

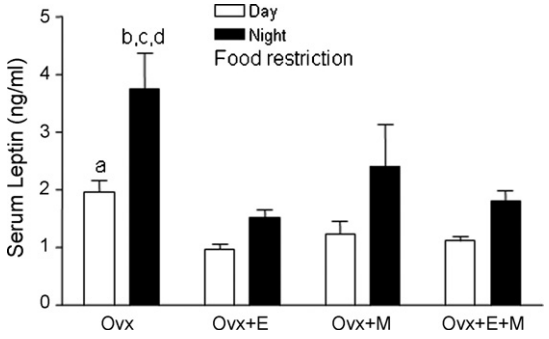

Fig. 5. Serum concentration of leptin (mean ± S.E.M., $n = 6$) in samples collected during day and night periods in rats ovariectomized and then treated with estradiol (Ovx + E), melatonin (Ovx + M), estradiol plus melatonin (Ovx + E + M) or the diluent (Ovx). After 7 weeks being fed *ad libitum*, the animals were fed, for 7 more weeks, an amount of food equal to 70% of the average food intake of control intact age-matched rats, calculated daily. Two-way ANOVA (time and treatment) shown as sources of variation: treatments, $F = 7.54$ d.f. = 3, $p < 0.001$; time (day/night) $F = 15.26$, d.f. = 1, $p < 0.001$. Differences between diurnal and nocturnal serum leptin concentration (Bonferroni post-test) were only significant between Ovx animals ($^{a}p < 0.01$). No differences were found between diurnal samples. Between nocturnal samples: $^{b}p < 0.001$ vs. Ovx + E and Ovx + E + M; $^{d}p < 0.05$ vs. Ovx + M.

7-week-period, during which the rats were under food restriction (Fig. 5). In all cases, serum leptin decreased after food restriction, probably reflecting the changes in body weight.

3.2.3. Serum levels of glucose, cholesterol, LDL, HDL and triglycerides after food restriction

Values are shown in Table 2. The only remarkable result concerned total cholesterol diurnal levels. The lowest values corresponded to Ovx rats treated with melatonin, these being significantly lower than in Ovx control animals ($p < 0.05$) as well as lower than in Ovx rats treated with estradiol ($p < 0.05$) or estradiol plus melatonin.

4. Discussion

The estrogen withdrawal in humans at menopause can be modeled by using ovariectomized rats. This model is characterized by mild obesity and is useful to study how hypoestrogenism alters adiposity [28]. The addition of melatonin to drinking water has been demonstrated to be an efficient way of administering

Table 2
Modifications of serum parameters in ovariectomized (Ovx) rats treated with estradiol (E), melatonin (M), or both hormones (animals under fed restriction)

Treatment	Glucose (mg/dl)		Total cholesterol (mg/dl)		HDL (mg/dl)		LDL (mg/dl)		HDL/LDL		Triglicerides (mg/dl)	
	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples	Diurnal samples	Nocturnal samples
Ovx	150.1 ± 9.8	130.3 ± 5.9	120.0 ± 14.2 (5)	103.1 ± 12.8	12.9 ± 1.2	12.2 ± 0.6	18.8 ± 3.3	20.5 ± 2.3	0.8 ± 0.1	0.6 ± 0.1	18.4 ± 2.4	17.0 ± 1.4
Ovx + E	168.3 ± 18.9	135.3 ± 7.8	107.2 ± 11.8 (5)	90.4 ± 7.1	14.6 ± 1.1	12.2 ± 0.6 (5)	14.7 ± 1.4 (5)	19.3 ± 1.8	1.0 ± 0.1	0.7 ± 0.1	16.0 ± 2.0	12.4 ± 1.4
Ovx + M	156.1 ± 11.5	141.1 ± 2.7	69.4 ± 4.6[a] (5)	86.2 ± 6.5	12.3 ± 0.7	12.8 ± 0.8	14.1 ± 1.8	22.9 ± 1.7	1.0 ± 0.2	0.6 ± 0.1	18.8 ± 3.1	13.1 ± 1.2
Ovx + E + M	143.1 ± 4.9 (5)	152.5 ± 13.8 (5)	107.0 ± 7.5 (5)	102.0 ± 10.3 (5)	16.1 ± 1.1 (5)	13.6 ± 1.1	14.7 ± 1.8 (5)	26.2 ± 2.7 (5)	1.2 ± 0.2 (5)	0.5 ± 0.1 (5)	21.0 ± 4.7 (5)	16.4 ± 1.8 (5)
Statistics	NS	NS		NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are means ± S.E.M. The number of samples was always 6, except when indicated in brackets
[a] $p < 0.05$ vs. Ovx, Ovx + E and Ovx + E + M.

of the hormone, since animals receiving this treatment showed significantly higher excretion of the melatonin metabolite (aMT6s) than controls. Furthermore, the fact that the increase of aMT6s excretion is limited to the night period indicates that animals drank more water at night, and that treatment with melatonin did not modify their drinking behavior [18].

In our first study, carried out on animals fed *ad libitum*, ovariectomy was followed by an increase both in body weight and in the serum leptin levels. Both effects of the ovariectomy were prevented by the administration of estradiol. These results agree with others previously reported [28–30]. In postmenopausal women, the effects of hormone replacement therapy (HRT) with estrogens upon body weight gain are controversial. Thus, some authors describe that HRT prevents postmenopausal obesity [31,32] although the numerous side effects of this therapy [33] would make it non recommendable for this sole purpose. Other studies [34] did not find any statistically significant difference in mean weight gain between women using estrogens and non-HRT users. Melatonin treatment partially prevented the increase in BW after ovariectomy. Similar effects of melatonin on body mass were described in male rats fed a high-fat diet [35,36]. Whereas the body weight decrease in Ovx rats treated with estradiol was accompanied by a decrease in serum leptin, in those animals treated with melatonin no significant changes in serum leptin levels were found. Cross-talk between melatonin and leptin is still controversial. Leptin and melatonin are secreted in a circadian rhythm with acrophases in the nocturnal period [11,37]. This data has suggested a possible interrelationship between both secretory rhythms, melatonin having a role in leptin release. Some previous studies, carried out on male rats, conclude that melatonin suppresses plasma leptin levels [18,35,36,38–41]. In seasonal animals, such as sheep and Siberian hamsters, their exposure to long days (low melatonin levels) is associated with high circulating leptin whereas in short day periods (high levels of melatonin) leptin levels are low [42]. However, like us, other authors found no changes in plasma leptin levels in male rats after pinealectomy [43] nor after i.v. administration of melatonin [44], nor in postmenopausal women [45]. These differences could depend on factors such as gender, age, or body composition of the animals receiving the treatment, as well as of the time

of sampling, because of the rhythmic secretion of leptin [37].

Another positive effect of the administration of melatonin observed in our experiments was the decrease of total serum cholesterol, especially strong in feeding restricted animals. The antilipidemic effect of melatonin has been demonstrated in experimental animals [46] and humans [47].

The time for blood sampling in our experiment was designed to study leptin, and we used the same samples to measure cholesterol, glucose, etc. Thus, although circadian rhythms have been described in plasma glucose [48], LDL [49], cholesterol [50], and triglycerides [51] in rats, our sampling procedure was not appropriate for the detection of possible changes in these circadian patterns.

In the second experiment, all animals were fed the same amount of food, equivalent to 70% of the food intake of age-matched intact rats. Obviously, the BW of these animals decreased. However, under these conditions, the treatment with melatonin was the most effective in reducing body mass and total cholesterol. Among Ovx underfed rats, melatonin was as effective as estradiol in reducing nocturnal serum leptin. As expected, parallel reduction in serum leptin was found in underfed rats, because of the strong positive correlation between body mass and circulating levels of this hormone [52]. It is, however, remarkable that modest decreases in BW (10–15%) were followed by reduction of serum leptin ranging from 50–74%. We could not measure in our animals the body composition to estimate body fat, but it is assumable that after 7 weeks with diet restriction most decreases in BW were from the fat deposits, which are those defining leptin levels.

We have demonstrated than melatonin has positive effects on ovariectomized rats which can be summarized as: (a) partial prevention of the body weight gain, by decreasing food intake, (b) reduction of total serum cholesterol levels, (c) potentiation of the effects of food restriction on body mass and leptin. However, all these effects of melatonin are common and, in most cases less strong than those obtained with estradiol, and without changes in serum leptin (except when associated to food restriction). When we obtained a reduction in BW by treatment with melatonin, without a decrease in serum leptin, it could indicate that body fat, the main determinant of serum leptin levels, did not decrease. In conclusion, the question of whether melatonin replacement therapy for postmenopausal women is justified or not, on the basis our experimental results, cannot be answered categorically. Classical estrogenic therapy seems to be more effective in terms of regulation of body mass and leptin levels, although side effects have been described. Melatonin therapy is less effective, except when associated with food restriction, but without known side effects. The co-treatment with estradiol and melatonin could give the best results since it combines the effectiveness of the steroids (not modified by melatonin) with the potential protective effects of melatonin because of the properties it possesses, commented on in the introduction to this article [19–23]. The possible beneficial effects of melatonin which could justify its use (improvement in sleep quality, prevention of breast cancer, etc.), need to be demonstrated in controlled clinical trials.

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