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Original Research Article

The effect of overweight and obesity on proliferation and activation of AKT and ERK in human endometria

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ABSTRACT

Objective. To examine whether overweight and obesity could lead to increased endometrial proliferation and activation of AKT and ERK1,2 in cycling premenopausal women.

Methods. Endometrial and blood samples were obtained from women with normal endometrial histology, and allocated into three groups—normal-weight, overweight and obese—according to the subject's body mass index (BMI). Samples from obese patients with type-I endometrial cancer (EC) were included as a control. Cell proliferation was measured by immunohistochemical detection of Ki67 and phosphorylated histone H3 (p-H3). AKT and ERK1,2 activation was assessed by Western blot. Circulating steroids, leptin and insulin were measured by immunoassays.

Results. In endometrial samples with normal histology, epithelial cell proliferation was higher in the overweight and obese groups versus the normal-weight set ($P<0.05$). Proliferation indexes were positively correlated with the subject's BMI and serum levels of estrogen, leptin and insulin ($P<0.05$). Increased phosphorylated AKT (pAKT) (1.6-fold) and ERK1,2 (pERK1,2) (8.7-fold) were observed in endometria from obese with respect to normal-weight subjects ($P<0.05$). Similarly, increased phosphorylation of AKT (0.7-fold) and ERK1,2 (2.3-fold) was detected in endometria from overweight as compared with the normal-weight group ($P<0.05$). In women with EC, we found a significant increase in endometrial proliferation, and in pAKT and pERK1,2 expression levels when compared to patients with normal endometrial histology.

Conclusion. These results show correlation between obesity (and overweight) and increased endometrial cell proliferation, and the activation of AKT and ERK1,2. These features could be related with the higher risk to develop type-I EC in overweight and obese women.

Introduction

The function of human endometrium is mainly regulated by sexual steroids, other circulating hormones, and cytokines [1–4]. In this context, it has been recognized that adipose tissue is a secretory organ with the ability to influence the function of many tissues [5]. Moreover, epidemiological studies suggest that overweight and obesity, which are characterized by an abnormal profile of hormone and adipokine production, could favor endometrial carcinogenesis in both premenopausal and postmenopausal women [6,7].

Endometrial carcinoma (EC) can be divided into two groups: type-I (accounts for 80% of all ECs, is estrogen-related, and is often preceded by atypical hyperplasia) and type-II (is not estrogen-related and arises from atrophic endometria) [8]. Evidence suggests that overweight and obesity are important risk factors for type-I EC [6,9–13]. Moreover, the International Agency for the Research on Cancer reported that the increase in risk ranges from 2- to 3.5-fold in overweight and obese women, respectively [14,15]. Given the epidemiological association between overweight, obesity and type-I EC, the search for a molecular link between these conditions is of great interest. An understanding of these links is relevant for the design of prevention and treatment strategies for obesity-associated type-I EC.

One of the main features of cancer is the alteration of signal transduction pathways as a consequence of accumulation of gene mutations [16]. Type-I EC is closely associated with abnormal signaling of PI3K/AKT and MAPK/ERK1,2 pathways. Mutation of tumor suppressor PTEN appears to result in PI3K/AKT activation, whereas activation of the proto-oncogene K-Ras causes MAPK/ERK1,2

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activation [17–23]. These are early events in the endometrial carcinogenesis.

Some investigators have reported that estradiol stimulates AKT and ERK1,2 activation in normal endometrial cells [24,25] and in endometrial carcinoma cell lines [26,27]. It has also been found that leptin can promote cell proliferation in endometrial carcinoma cell lines by a mechanism that involves activation of AKT and ERK1,2 [28]. Moreover, at physiological levels, insulin is likely to play a role in the regulation of energy metabolism in the endometrium. However, in hyperinsulinemic states, insulin may activate cellular mitosis in endometrium via the PI3K and MAPK pathways, and predispose this tissue to hyperplasia and/or cancer [29]. Thus, activation of these signal transduction pathways, which are important drivers of cell survival and proliferation, could be potentially involved in the higher risk to develop type-I EC in overweight women and mostly in obese women who show high circulating levels of estrogen, leptin and insulin.

Therefore, in the present study we examined in serum and endometrial samples obtained from women with different body mass index (BMI) (with normal endometrial histology), and from obese women with type-I EC (i) circulating levels of steroids, leptin and insulin; (ii) endometrial cell proliferation determined by the Ki67 marker (which is expressed in G1, S, G2 and M phases of the cell cycle) [30,31] and phosphorylated histone H3 (p-H3) (which is expressed during the M phase of the cell cycle and complements the Ki67 index, by providing a specific count of cells that actually completed division) [32]; and (iii) endometrial activation of AKT and ERK1,2.

Materials and methods

Hormone levels were determined using commercial kits: serum estradiol (E_2), estrone (E_1), testosterone (T), androstenedione (A_4), progesterone (P_4) and insulin by radioimmunoassay (Diagnostic System Laboratories, Webster, TX); sex hormone-binding globulin (SHBG) and leptin concentration by immunoradiometric assay (DPC, Los Angeles, CA). The rabbit monoclonal antibodies for ERK1,2, pERK1,2 (Thr202/Tyr204) and pAKT (Ser 473) were purchased from Cell Signaling (Danvers, MA), and the rabbit monoclonal antibody for Ki67 was purchased from Thermo Scientific (Fremont, CA). The rabbit polyclonal anti-human AKT and p-H3 antibodies were purchased from Cell Signaling and Upstate (Temecula, CA), respectively. The mouse monoclonal antibody for β-actin was obtained from Sigma (Saint Louis, MO). Secondary antibodies (anti-rabbit IgG and anti-mouse IgG) were purchased from GE Healthcare (Piscataway, NJ). Protease-inhibitor cocktail was obtained from Roche (Mannheim, Germany), BCA protein assay kit from Pierce (Rockford, IL), and labeled streptavidin-biotin kit from Dako (Carpinteria, CA).

Subjects

Blood and endometrial samples were obtained during the proliferative phase of the menstrual cycle, from three groups of cycling premenopausal women, undergoing routine hysterectomy to treat leiomyomas with conserved endometrial cavity (excluding submucosal leiomyoma) at the San Borja-Arriarán Clinical Hospital. The hysterectomy specimens were collected by an experienced histopathologist from the fundus and corpus of the uterus by curettage of the endometrial tissue. The proliferative phase was confirmed according to the histological criteria described by Noyes [33]. The groups were defined according to BMI, in agreement to The National Institutes of Health definition [34]: normal-weight group (BMI = 18–24.9 kg/m^2, $n = 10$), overweight group (BMI = 24.9–29.9 kg/m^2, $n = 9$), and obese group (BMI ≥ 30 kg/m^2, $n = 12$). None of the women received hormonal therapy or other medications within 3 months prior to recruitment into the study, and the endometria used (from premenopausal women) all showed normal morphology. Hence, the selected patients for this study satisfied the inclusion criteria and were accrued consecutively.

Also, we obtained samples from 10 obese patients with type-I EC at Dr. Luis Tisné Hospital, and were used as a control in this study.

None of the patients included in the present study had clinical or familial history of diabetes or thyroid problems. They were non-smokers. The clinical and endocrinological characteristics of the subjects are shown in Table 1. The institutional and Health Service review boards approved this study, and informed written consent from patients was obtained before surgery.

Immunohistochemistry

Immunostaining for Ki67 and for p-H3 was performed on 5-μm sections of formalin-fixed paraffin-embedded endometrial samples. Tissue sections were deparaffinized, hydrated and incubated in antigen retrieval solution (10 mM sodium citrate buffer). Endogenous peroxidase activity was prevented by incubating the samples in 10% hydrogen peroxide for 5 min. Nonspecific antibody binding was blocked with 4% phosphate buffered saline- bovine serum albumin (BSA) for 1 h. Primary antibody of Ki67 (1:100 dilution) and p-H3 (1 μg/ml) was added to the samples and incubated during 1 h at room

Table 1
Clinical and endocrinological characteristics of the four groups of subjects.

	Normal endometrial histology			Type-I EC
	Normal-weight ($n = 10$)	Overweight ($n = 9$)	Obese ($n = 12$)	Obese ($n = 10$)
Age (years)	43.2 ± 1.90	44.8 ± 1.05	44.6 ± 0.60	66.0 ± 4.1[a,b,c]
BMI (kg/m^2)	22.1 ± 0.80	27.2 ± 0.50[a]	35.7 ± 1.20[a]	37.8 ± 2.9[a,b]
A_4 (ng/ml)	1.34 ± 0.21	1.33 ± 0.16	1.45 ± 0.14	1.75 ± 0.1[a,b,c]
T (ng/ml)	0.28 ± 0.05	0.31 ± 0.03	0.35 ± 0.03	0.57 ± 0.1[a,b,c]
SHBG (nmol/l)	54.8 ± 8.77	40.6 ± 4.28	32.9 ± 5.46[a]	32.1 ± 5.8[a,b]
Free androgen index	1.88 ± 0.32	2.44 ± 0.30	3.37 ± 0.42[a]	6.85 ± 1.3[a,b,c]
E_2 (pg/ml)	28.0 ± 7.21	43.0 ± 13.76	66.7 ± 15.90[a]	26.4 ± 6.7[c]
E_1 (pg/ml)	34.3 ± 14.0	58.6 ± 10.6[a]	66.1 ± 10.3[a]	71.2 ± 8.2[a,b]
E_2/P_4 (ng/ml)	0.029 ± 0.004	0.030 ± 0.008	0.074 ± 0.002[a,b]	0.035 ± 0.007[c]
E_1/P_4 (ng/ml)	0.026 ± 0.006	0.065 ± 0.009[a]	0.099 ± 0.011[a,b]	0.107 ± 0.007[a,b]
Leptin (ng/ml)	3.14 ± 0.30	5.12 ± 0.40[a]	14.48 ± 1.62[a,b]	14.36 ± 2.1[a,b]
Insulin (μIU/ml)	2.92 ± 0.30	3.34 ± 0.51	8.17 ± 1.91[a,b]	7.78 ± 1.86[a,b]

Note. The values are mean ± SEM.
The intra- and interassay coefficients of variation of the hormonal measurements were, respectively, 3.2% and 6.1% for A4, 7.0% and 11% for T, 3.9% and 6.9% for SHBG, 4.1% and 6.7% for E2, 8.4% and 9.1% for E1, 4.8% and 7.2% for P4, 4.6% and 6.2% for leptin, and 3.8% and 4.7% for insulin.
[a] $P<0.05$ compared to normal-weight group.
[b] $P<0.05$ versus overweight group.
[c] $P<0.05$ compared to obese group.

temperature. Negative controls were analyzed on adjacent sections incubated without primary antibody. The second antibody was a biotinylated anti-rabbit immunoglobulin. The reaction was developed by the streptavidin-peroxidase system, using 3,3′ diaminobenzidine as chromogen. Counterstaining was carried out with hematoxylin. The slides were observed in an optical microscope (Nikon, Inc., Melville, NY). Immunohistochemical evaluation was determined as the percentage of positive stained cells. The proteins were evaluated in the functional layer by three independent observers and blinded to patient category, and the positive staining was assessed in at least 2000 cells per sample.

Western blotting

Western blotting was performed in 41 fresh tissue specimens (normal-weight group = 10; overweight group = 9; obese group = 12; obese patients with type-I EC = 10). The tissues were homogenized and lysed on ice using a cell lysis buffer consisting of 20 mM Hepes, 2 mM EDTA, 2 mM EGTA, 1% Triton X100, 5 mM PMSF, 50 μM Na_3VO_4 (Sigma) supplemented with a protease inhibitor cocktail. After centrifugation at 10,000 × g for 20 min at 4 °C, protein concentrations were determined using the BCA protein assay kit. Total proteins (50 μg per sample) were denatured in Laemmli buffer, fractionated using 7.5% one-dimensional SDS-PAGE, and transferred to PVDF membranes (Pierce, Rockford, IL). Blots were blocked for 1 h in TBST (20 mM Tris, pH 7.6; 137 mM NaCl; 0.1% Tween 20) containing 5% BSA. Subsequently, blots were washed in TBST and then incubated overnight with antibodies against human AKT (1:2000 dilution) and ERK1,2 (1:1000 dilution), in a rocking device at 4 °C. Next, the blots were washed followed by incubation for 1 h at room temperature with anti-rabbit IgG peroxidase-linked species-specific whole antibody (1:5000 dilution). The blots were washed again, developed by chemiluminescence and exposed to light sensitive films. The membranes were then stripped and reproved with anti-pAKT and anti-p-ERK1,2 antibodies (1:2000 dilution, overnight at 4 °C), and with an antibody directed against β-actin to control for protein loading. Band intensity was quantified by scanning densitometry utilizing the UN-SCAN-IT software, Automated Digitizing System, version 5.1.

Statistical analysis

The number of subjects in this study was calculated assuming an $\alpha = 0.05$ and $\beta = 20\%$, and a difference between means of 0.3 and a standard deviation of 0.200 according to our previous studies. One-way ANOVA test was used. Pearson's correlation coefficient was used to evaluate the association between (i) proliferation markers Ki67 and p-H3, (ii) BMI and proliferation markers, (iii) pAKT/AKT or pERK/ERK1,2 expression and proliferation markers, and (iv) serum levels of E_2, E_1, leptin, insulin and proliferation markers. *P* values less than 0.05 were considered significant. Statistical tests were performed using SPSS for Windows version 10.0 (SPSS, Inc., IL).

Results

Clinical and endocrinological characteristics of the subjects

As shown in Table 1 women allocated into the normal-weight, overweight and obese groups (with normal endometrial histology) were of similar age. Obese women with normal endometrial histology exhibited high free androgen index, likely as a consequence of decreased blood SHBG levels. Serum levels of E_2 and E_1 unopposed by P_4 were higher in these obese women than in normal-weight women (61% and 74%, respectively, $P<0.05$). Circulating levels of E_1/P_4 were also elevated in overweight women (60%, $P<0.05$) compared with normal-weight patients. In addition, obese women showed a higher E_2 or E_1 to progesterone ratio when compared to the overweight group ($P<0.05$). Circulating levels of leptin were elevated in overweight (39%, $P<0.05$) and obese (79%, $P<0.05$) women with respect to the normal-weight group. Obese women exhibited high levels of this adipokine compared with the overweight group ($P<0.05$). Increased concentration of insulin was observed in obese women with respect to overweight and normal-weight groups (145% and 180%, respectively, $P<0.05$).

The clinical and endocrinological characteristics of the obese patients with type-I EC, used as a control in our study, were inherent to their postmenopausal status.

Effect of overweight and obesity on proliferation of endometrial cells

We observed that the Ki67 antigen, a marker of the proliferative state of the cells, was expressed in the nucleus of endometrium epithelial cells, in the four groups under study (Fig. 1A, a–d). Among women with normal endometrial histology, the percentage of Ki67 positive cells was 9.9- fold higher ($P<0.05$) in the overweight group as compared with the normal-weight group (Fig. 1B). Obese patients showed up to 12.6-fold ($P<0.05$) higher proliferation than the normal-weight group (Fig. 1B). Positive staining for p-H3 protein was found in endometrial cells, particularly in the nuclei of epithelial cells (Fig. 1A, e–h), highlighting those in the M phase of the cell cycle. The percentage of cells in mitosis was 0.8- ($P<0.05$) and 1.5-fold ($P<0.05$) higher in overweight and obese women (with normal endometrial histology), respectively, than in normal-weight group (Fig. 1C). Moreover, p-H3 protein expression was significantly augmented in obese women (40%, $P<0.05$) compared with overweight women (Fig. 1C). A significantly larger percentage of epithelial cells with positive staining for Ki67 and p-H3 was detected in women with type-I EC versus women with normal endometrial histology ($P<0.05$) (Figs. 1B and C). In addition, the Ki67 index correlated directly with the p-H3 index ($R^2 = 0.47$, $P<0.05$) in the studied endometrial samples. Interestingly, the Ki67 and p-H3 indexes correlated with the subject's BMI ($R^2 = 0.78$ and $R^2 = 0.86$, respectively, $P<0.05$), and with serum levels of E_2 ($R^2 = 0.69$ and $R^2 = 0.76$, respectively, $P<0.05$), E_1 ($R^2 = 0.77$ and $R^2 = 0.93$, respectively, $P<0.05$), leptin ($R^2 = 0.62$ and $R^2 = 0.71$, respectively, $P<0.05$), and insulin ($R^2 = 0.46$ and $R^2 = 0.57$, respectively, $P<0.05$).

Effect of overweight and obesity on AKT activation

To gain an insight into the intracellular signaling mechanisms involved in the increased proliferation found in endometria from overweight and obese women, we first examined the basal level of pAKT (in Ser473) in these endometrial samples (Fig. 2A). Increased phosphorylation of AKT was observed in endometrial samples obtained from overweight women (70.5%, $P<0.05$) with respect to normal-weight group (Fig. 2B). In addition, we observed higher levels of pAKT in obese women compared to the normal-weight and overweight groups (163% and 54.5%, $P<0.05$, respectively; Fig. 2B). Furthermore, an increase in pAKT was detected in women with type-I EC compared with women with normal endometrial histology ($P<0.05$) (Fig. 2B). Overweight and obesity had no effect on total AKT protein levels. Interestingly, a high correlation of pAKT/AKT with Ki67 ($R^2 = 0.85$, $P<0.05$), and pAKT/AKT with p-H3 ($R^2 = 0.95$, $P<0.05$) was evidenced in the studied endometria.

Effect of overweight and obesity on ERK activation

Western blot analysis was performed for total ERK1,2 and pERK1,2 (Fig. 3A). No variations were observed in the endometrial expression of total ERK1,2 in the groups under study. We detected an increase in the relative abundance of pERK1,2 protein in overweight and obese women (with normal endometrial histology) compared with the normal-weight group (2.3- and 8.7-fold, respectively; $P<0.05$; Fig. 3B). Moreover, p-ERK1,2 was augmented in obese women (1.9-

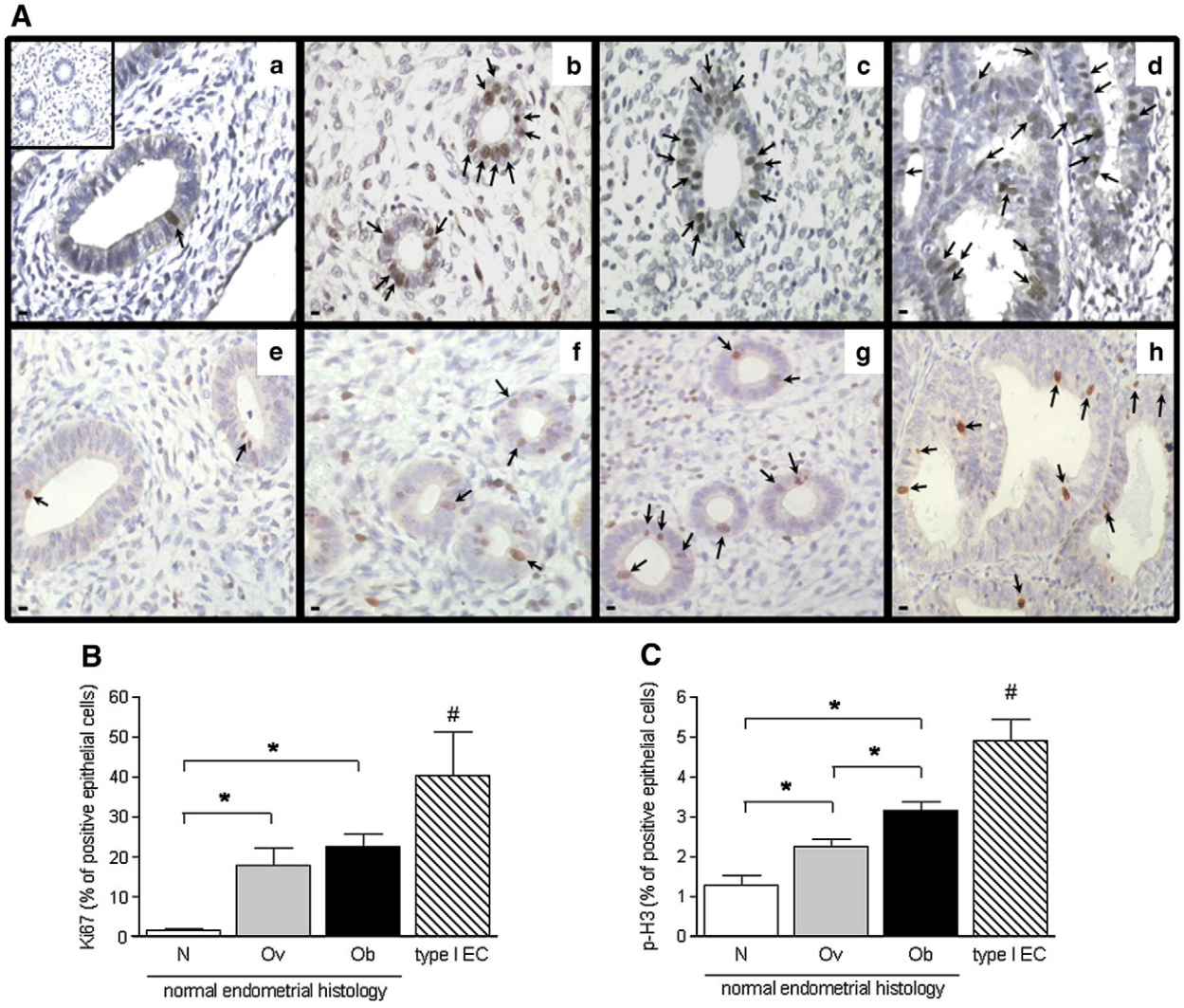

Fig. 1. Immunohistochemical detection of Ki67 and p-H3 in paraffin wax sections of endometrial tissue obtained from premenopausal women with different BMI (with normal endometrial histology) and from obese women with type-I EC. (A) Expression of Ki67 (a–d) and p-H3 (e–h) in representative images of normal-weight (a and e), overweight (b and f), and obese (c and g) women with endometrial normal histology, and in women with type-I EC (d and h). Arrows highlight some epithelial cells with dark nuclei, indicating Ki67 or p-H3 expression. As a negative control (insert in a), the primary antibody was omitted. Magnification in all panels is 400× and the scale bar represents 10 μm. (B) Evaluation of Ki67 and (C) p-H3 protein expression in epithelial cells of the normal-weight group (N, $n=10$), overweight group (Ov, $n=9$), obese group (Ob, $n=12$) and type-I EC group ($n=10$). The values are expressed as percentage of positive cells (mean ± SEM). $^{*}P<0.05$ in overweight (Ov) and obese (Ob) groups compared with the normal-weight group (N). $^{*}P<0.05$ in Ob group with respect to Ov group. $^{\#}P<0.05$ in type-I EC group vs. groups with normal endometrial histology.

fold, $P<0.05$) compared with overweight women (Fig. 3B). In addition, we found high levels of pERK1,2 in women with type-I EC when compared with women showing normal endometrial histology ($P<0.05$) (Fig. 3B). ERK1,2 phosphorylation correlated directly with Ki67 ($R^2=0.89$, $P<0.05$) and p-H3 ($R^2=0.74$, $P<0.05$).

Discussion

The human endometrium undergoes architectural modifications during each menstrual cycle. These changes include proliferation, differentiation, and shedding, all of which are regulated by estrogens and progesterone. The timing and concentration of these hormones dictate the balance between endometrial growth and transformation [35]. Overweight and obesity can alter serum levels of sex hormones and thus, increase the risk for type-I EC [36]. However, the epidemiological association between BMI and type-I EC risk cannot be fully explained by overweight- or obesity-related changes in serum levels of sex hormones. Since overweight and obesity are accompanied by an increase in the systemic secretion of insulin [36] and adipokines such as leptin [5], this may have an additional influence on the biological characteristics of the endometrium.

In the present study, we examined if overweight and obesity are associated with increased proliferation of the endometrial cells (mainly epithelial cells, because it is known that type-I EC originates from this cell type) from premenopausal women having normal endometrial histology. We found an increased proliferation index in endometria from obese and overweight women, as evidenced by the high abundance of Ki67 and p-H3 in epithelial cells, and a positive correlation between these two proliferation markers. The detection of the Ki67 antigen, which is expressed in all phases of the cell cycle, has been extensively used as a measure of proliferative activity in various tissues [37, 38] including endometrial biopsies [39]. However, the time that cells spend in G1 is highly variable, and may be affected by the hormonal or neoplastic state of the tissues [40]. Because mitosis is one of the shortest and least variable phases of the cell cycle, the p-H3 detection provides a more precise measurement of the proliferative rate and adds information on the number of cells that have actually completed the cell cycle, thus reinforcing the information provided by the Ki67 marker.

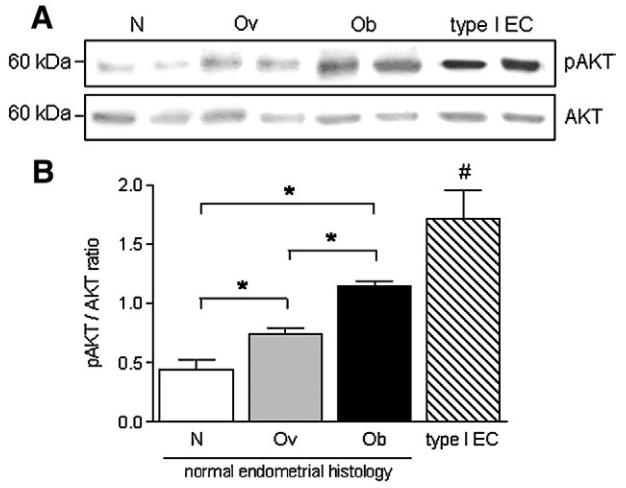

Fig. 2. Results of Western blotting analysis for pAKT and AKT expression in endometrial samples from women with normal endometrial histology (normal-weight [N], overweight [Ov] and obese [Ob] groups) and in endometria from patients with type-I EC. (A) Equal amounts of endometrial protein were loaded in each lane. pAKT and AKT were detected as bands with a molecular mass of 60 kDa. (B) pAKT and AKT band intensities were quantified by scanning densitometry and normalized to β-actin. The values shown are means ± SEM in N ($n=10$), Ov ($n=9$), Ob ($n=12$) and type-I EC ($n=10$). $^{*}P<0.05$ in overweight (Ov) and obese (Ob) groups compared to normal-weight group (N). $^{*}P<0.05$ in Ob group with respect to Ov group. $^{\#}P<0.05$ in type-I EC group vs. groups with normal endometrial histology.

The results mentioned above are associated with the elevated levels of leptin and E_1/P_4 detected in overweight and obese patients, and with high levels of E_2/P_4 and insulin and decreased levels of SHBG found mainly in obese women (as compared to the normal-weight group). Estrogen typically stimulates cell proliferation [41,42] and is well known that the proliferative phase is under the dominant effect of E_2. Proliferation markers Ki67 and p-H3 reach undetectable levels in endometrial cells after the onset of P_4 production, which is followed by cell disintegration and menstruation [32,43,44]. Although the subjects included in this study were cycling regularly, it is known that overweight and obesity can lead to periods of anovulation, devoid of regular shedding of the endometrium, therefore increasing the chances for malignant transformation to occur [45]. Consequently, increased endometrial proliferation and activation of AKT and ERK1,2 associated with obesity would be specially relevant in those women experiencing anovulation.

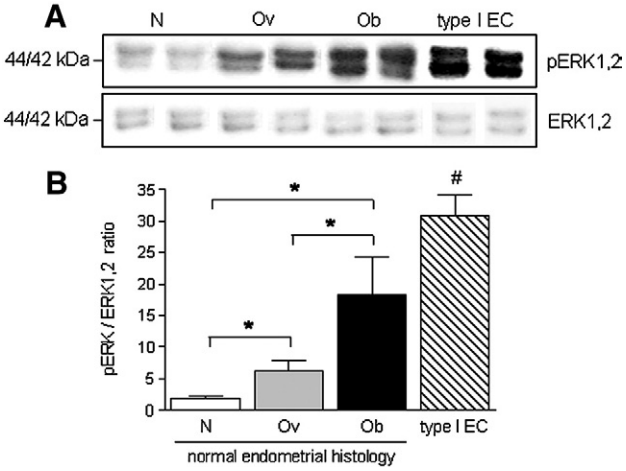

Fig. 3. Western blot analysis of pERK (44/42 kDa) and ERK (44/42 kDa) expression in human endometria obtained from premenopausal women with different BMI (with normal endometrial histology) that were in the proliferative phase of their menstrual cycle and from women with type-I EC. Equal amounts of endometrial protein were loaded in each lane (A). pERK and ERK band intensities were quantified by scanning densitometry and normalized to β-actin. The values shown are means ± SEM in N ($n=10$), Ov ($n=9$), Ob ($n=12$) and type-I EC ($n=10$) (B). $^{*}P<0.05$ in overweight (Ov) and obese (Ob) groups compared to normal-weight group (N). $^{*}P<0.05$ in Ob group with respect to Ov group. $^{\#}P<0.05$ in type-I EC group vs. groups with normal endometrial histology.

In a first approach to investigate the proteins involved in the high proliferation indexes detected in overweight and obese women, we examined the activation of AKT, which is a hallmark of activated PI3K signaling in type-I EC [46–48]. Our experiments clearly showed that overweight and in particular obesity (in women without and with type-I EC) associate with increased pAKT. It is known that activated AKT provides a survival signal to the cells and it is implicated in mediating several biological responses, including cell growth and proliferation [49–51], which is consistent with the high cell proliferation index detected in endometria from overweight and obese women.

Activation of ERK1,2 protein in endometrial cells was also investigated. We observed high levels of pERK1,2 in overweight and obese women, suggesting that not only the PI3K/AKT but also the MAPK/ERK1,2 pathway is involved in endometrial proliferation in these subjects.

Previous reports have shown the expression of leptin receptor in human endometria [52–54]. In addition, we have observed higher expression of leptin receptor in epithelial endometrial cells from overweight and obese women with respect to normal-weight set (not shown). Furthermore, Sharma et al. showed that leptin induces phosphorylation of AKT and ERK1,2 in EC cells, thus activating two key signal transduction pathways associated with cell growth [28]. In addition, inhibition of these pathways prevented phosphorylation of the respective proteins, and blocked EC cell proliferation [28]. On the other hand, increased insulin levels have been associated with EC [6]. It has been suggested that these tumorigenic effects of insulin could be directly mediated by insulin receptors expressed in endometria [55–57], or indirectly caused by changes in endogenous hormone metabolism, secondary to hyperinsulinaemia [6,58–60]. Data reported by Lathi et al. [29] support a primary role of the PI3K pathway in insulin signaling in endometria. At high insulin concentrations the participation of the MAPK pathway has been also shown [29]. Therefore, endometrial activation of these pathways and increased endometrial proliferation observed mainly in obese women could be linked with the high leptin and insulin serum levels found in these patients.

In addition, it has been reported that ligand-activated estrogen receptors (ER) may activate PI3K, resulting in increased levels of pAKT [61]. We have detected a higher expression of ER in endometria from overweight and obese women than that from women with normal BMI (not shown), which is in agreement with a previous report [62]. Therefore, because of increased expression of ER in endometria from overweight and obese women, estrogen-dependent activation of the PI3K/AKT signaling pathway is likely enhanced, and it could lead to deregulation of endometrial homeostasis.

In brief, these results show correlation between obesity (and overweight) with increased endometrial cell proliferation and the activation of AKT and ERK1,2. On the basis of these results, we think that the overweight/obesity condition could provide the opportunity for the endometrial cells from these women (without type-I EC) to accumulate mutations, escape the normal control of cell proliferation and become neoplastic at a later stage.

The study of these processes is important, because EC therapies mainly rely on surgery, which results in impairment of women's reproductive capacity and reduce their quality of life. Therefore, new tools to identify overweight and/or obese women who are more susceptible to develop type-I EC could eventually lead to early interventions and reduce the need for highly invasive surgery.

To the best of our knowledge, this is the first report on increased cell proliferation and activation of AKT and ERK1,2 in endometria from women with different BMI. This study represents an initial step towards unravelling the mechanisms that underlie type-I EC promotion in overweight and obese women. Additional investigations will be required to gain an in depth understanding on this complex process.

Conflict of interest statement
The authors declare that there are no conflicts of interest.

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