Aberrant autophagy and proinflammatory cytokines may reduce endometrial decidualization: a essential role of obesity on infertility

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Research

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Abstract

Background: Obesity is associated with reproductive disorders and infertility. Autophagy is upregulated during decidualization, but is diminished in obesity models with impaired decidualization. Defects in autophagy are related to the occurrence of inflammatory diseases, but its role in endometrial decidualization with obese patients is unknown.

Methods: The levels of autophagy-related factors (LC3B-II and Beclin 1), and autophagy-related proteins which have a correlation with endometrial decidualization (ATG3, ATG5, ATG7 and foxo1), as well as autophagy-related inflammatory cytokines in endometrium of lean and obese patients was assessed. We then investigated the the role of autophagic flux during decidualization in 'obese'- vs 'lean'- treated cells from matched patients.

Results: Alteration in autophagic flux and the expression of autophagy-related genes revealed impaired autophagy in obese patients compared with lean. Autophagy-related proinflammatory cytokines were upregulated in the uterine cavity of obese patients, who also showed impaired decidualization.

Conclusions: Obesity downregulates the expression of autophagy-related genes and impairs endometrial stromal cell autophagic flux, and induces inflammation. These alterations in autophagy are associated with increased activation of autophagy-related proinflammatory cytokines might decrease basic uterine receptivity.

Introduction

Obesity, defined as a body mass index (BMI) of > 30 kg/m^2, is now a growing problem worldwide. Among US adults, more then 36% were considered to have obesity [1]. There is growing concern that obesity is a preventable risk factor for diabetes, musculoskeletal disorders, stroke, cardiovascular disease, and certain types of cancers [1]. It has become common knowledge that obesity is associated with reproductive disease [2], such as infertility, poor oocyte quality, ovulation defects, increased early pregnancy loss and pregnancy complications, declined fertility rate even when normal ovulation [3], low implantation and pregnancy rates with assisted reproductive technology [4]. However, according to statistics, more than 30% of reproductive-aged females are considered overweight [5].

Although the pathologies underlying the decrease in pregnancy rates and miscarriage in obese women are not entirely clear, a number of studies have shown that that

obesity lead to endometrial dysfunction. One research indicated implantation and live birth rate decreased along with the BMI increased even with donor oocytes were used. Moreover, downregulation of certain endometrial receptivity related genes in the endometrium with obese women [6]. Additionally, obese patients have a higher rate of recurrent early pregnancy loss, despite having euploid embryos, than normal-weight women [7]. Diet-induced obesity impairs endometrial stromal cell decidualization, a critical adaptation required for pregnancy [8]. Both obese women and animal models showed significant

reductions in placental dysfunction, placental lesions, and spiral artery remodeling, which seem to be associated with reduced invasion of trophoblast [9–10]. In general, these findings reveal an important role of the endometrial environment in obesity-related reproductive impairment.

There is growing evidence that autophagy is associated with endometrial health. Autophagy is induced during decidualization and regarded as a influential factor in implantation. Defects in autophagy and changes in expression of autophagy related genes are closely related to the occurrence of inflammatory diseases. The present study aimed to assess autophagy in obese patients during the menstrual cycle and to investigate whether autophagy-related cytokines and dysregulation of autophagic flux by obesity affects endometrial function.

Materials And Methods

Ethical approval

This study was approved by the Institutional Ethics Committees at Beijing Chaoyang Hospital. Written informed consent was obtained from each patient prior to tissue collection and after explicit explanation of the project by a research doctor.

Tissue collection

Human endometrial tissues were collected from fertile or infertile women with no known endometrial pathology (age range, 23-38 years), and collected by curettage across the menstrual cycle. They were divided into two groups according to BMI : lean, BMI < 25 kg/m^2, n = 11; obese, BMI > 30 kg/m^2, n = 7. Gynecological pathologists determined the menstrual cycle stage by standard histological dating. The endometrial tissue samples were divided into two parts: one part was immediately fixed in 10% neutral buffered formalin at 4 ℃ for immunofluorescence analysis; the other part was snap-frozen in liquid nitrogen and stored at –80 °C for western blotting analysis and RT-qPCR.

Immunoblotting

Proteins were extracted from cells or endometrial tissues were stored at −80°C until further use. Proteins were separated were separated by 10–12% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes at 100 V for 90 minutes. The membranes were blocked at room temperature for 1 h in 5% nonfat dry milk in TBS-T, washed, and incubated with primary antibodies overnight at 4 °C. The next day, the membranes were washed and incubated with appropriate secondary antibody at room temperature for 1 hour. Signals were detected using enhanced chemiluminescence.

Cell culture

Human endometrial ECC-1 were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA). The cells were cultured in Dulbecco's modified Eagle's/F12 medium (DMEM/F12; Gibco-BRL, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Uxbridge, UK). Cells were

incubated at 37°C in 5% CO2/95% air and were passaged passaged following standard laboratory procedures to maintain logarithmic growth. ECC-1 cells were cultured for 24 h with Earle's Balanced Salt Solution (EBSS) medium, and then treated with10 nM estradiol (Sigma, USA) and1 μM progesterone (Sigma) for 9 days. Then cultures were treated with 'lean' (2000 nmol/mol lysine) or 'obese' (8000 nmol/mol lysine) from matched patients for 72 h and removed for 24 h before analysis. Additionally, ECC-1 cells were cultured with the addition of 0.1 μM bafilomycin A1 (Sigma) to inhibit autophagic cell death.

Quantitative real-time reverse transcription PCR (RT-qPCR)

Total RNA was extracted from the human endometrial tissues using RNAlso Plus (Takara, Shiga, Japan) according to the manufacturer's protocol. RNA was reverse-transcribed into complementary DNA(cDNA) using the PrimeScript™ RT reagent kit with gDNA Eraser (Takara). Quantitative real-time-PCR was performed to amplify the target genes using SYBR Premix Ex Taq™ (Takara). Relative gene expression was calculated with the comparative Ct ($^{2-\Delta\Delta}$CT) method, and the housekeeping gene *GAPDH* was used to normalize to the expression of target genes.

Immunofluorescence analysis

Tissues were fixed with 10% paraformaldehyde at 4 °C overnight. The tissues were then dehydrated, paraffin embedded, and sectioned at 5 μm. Rehydrated paraffin-embedded sections were washed in PBS-T, blocked in 5% normal goat serum at room temperature for 1 hour, incubated with primary antibodies (in 2.5% normal goat serum) at 4 °C overnight, then washed three times in PBS-T, incubated with the appropriate secondary antibodies (in 2.5% normal goat serum) for 1 hour at room temperature. To visualize the nuclei, cells were counterstained with DAPI.Then imaged under a microscope.

Results

1. Autophagy and Apoptosis are induced in the human endometrium during the menstrual cycle

We investigated whether autophagy is induced during the menstrual cycle in the human endometrium from ovarian endometriotic cysts. We measured the levels of microtubule associated protein 1 light chain 3 alpha (LC3), microtubule associated protein 1 light chain 3 beta phosphatidylethanolamine conjugate (LC3B-II), and Beclin 1. LC3B-II localizes to autophagosomes and is one of the best-characterized markers of autophagy. Beclin 1 is an essential protein for the initiation of autophagosome formation. As autophagy marker protein [11-12], LC3 was expressed in glandular and stromal cells throughout the menstrual cycle and was localized within the cytoplasm. As shown in Fig 1, in the early (Fig. 1A) and late proliferative phases (Fig, 1B), LC3B-II was negative or very weakly positive. However, the levels of LC3B-II in the secretory endometrium was significantly higher than that in the proliferative endometrium, peaking during the late secretory phase (Fig. 1D). Similarly, the beclin-1 level also increased significantly during the secretory phase (Fig. 1 G,H). Subsequently, we also evaluated the level of the apoptosis marker cleaved caspase-3 [13]. Endometrial cell autophagy is associated with apoptosis during the menstrual

cycle, and the level of cleaved caspase-3 also increased significantly during the secretory phase (Fig. 1K,L).

Both western blotting (Fig. 1M) and semi-quantitative western blotting analysis (Fig. 1N-P) also revealed that autophagy and apoptosis were upregulated in the human endometrium during the secretory phase.

2. Autophagy-related factors are decreased in obese patients during the secretory phase

We investigated whether autophagy was upregulated during the secretory phase in obese patients. On the same gel, we compared the levels of LC3B-II and Beclin 1 directly at the early proliferative phase and the late secretory phase in the endometria of lean and obese patients. In the obese patients, as shown in Fig. 2 D and H, immunofluorescent staining showed that LC3B-II and Beclin 1 levels were lower than those in lean patients during the late secretory phase (Fig. 2 C and G). Western blotting (Fig. 2I,K) and semi-quantitative (Fig. 2J,L) western blotting analysis also revealed that autophagy might be impaired in obese patients during the menstrual cycle.

3. Downregulation of autophagy-related genes expression in endometrial tissue during the late secretory phase from obesity patients

Autophagy-related (ATG) proteins are eukaryotic factors involved in autophagosome formation and in various stages of the autophagic process. Autophagy is initiated by post-translational modifications of ATG proteins. FOXO1 is involved in activation of various transcription factors, the regulation of cell cycle, apoptosis, autophagy. We determined the expression levels of mRNA encoding 3 important autophagy-related proteins, ATG5, ATG16L1, ATG7, as well as transcription factor forkhead box protein O1 (FOXO1) in the endometrium during the late secretory phase from obesity patients and control subjects. Research demonstrated that these proteins have a positive correlation with endometrium decidualization [14-16]. As shown in Fig. 3, the expression levels of ATG5, ATG16L1 and FOXO1 were significantly decreased in the endometrial tissue of from obesity patients. The expression levels of ATG7 did not significantly differ between the two groups, however, there was a trend towards a decrease in obesity patients. We demonstrate that the downregulation of autophagy-related gene expression lead to altered autophagy in obesity patients.

4. Autophagy-related Inflammatory cytokines are increased in the endometrium of obese patients

There is considerable evidence that defective autophagy induces inflammation: ATG5 deficiency stimulated an increase in tumor necrosis factor alpha (TNF-α) production [26]; high amounts of the proinflammatory cytokine Interleukin (IL)-1β and IL-18 were released from Atg16L1-deficient macrophage [28]; elevations in IL-1β were also observed in the ATG7 deficient macrophages [26]. Besides, ATG5 is also involved in the immune system, regulating innate and adaptive immune responses, including the activation of interferon (IFN)-I [25-26]. Therefore the expression levels of above related inflammatory cytokines in the endometria of lean and obese patients were assessed. We observed marked increases in the mRNA expression levels of inflammatory-related factors, such as TNF-α, IL-1β, IL-18 and IFN -I,

monocyte chemoattractant protein-1 (MCP-1), and accompanied by a significant decrease in the IL10 mRNA level. As shown in Fig. 4, IL -1b, MCP-, and TNF- a as pro-inflammatory cytokines, were produced by M1 macrophages. M2 macrophage produce the anti-inflammatory cytokine IL-10 [17]. Our results showed that proinflammatory factors were induced in the uterine cavity of obese patients associated with impaired autophagy.

5. Decidualization is impaired in patients with obesity

To investigate whether decidualization is impaired in the endometrium of obese patients, insulin-like growth factor binding protein 1 (IGFBP1) and prolactin (PRL) [18], two well-established markers of decidualization, mRNA expression levels at the late secretory phase of the menstrual cycle were detected. We found that these markers of decidualization were expressed at lower levels in endometrial tissue obtained from obese women compared with that from normal-weight women (Fig. 5A,C). Meanwhile, endometrial epithelial (ECC-1) cells were treated with ‘lean’ (2000 nmol/mol) or ‘obese’ (8000 nmol/mol) levels of lysine according to a previously established method of inducing decidualization [19]. After decidualization, cells treated with ‘obese’ levels of lysine expressed significantly lower mRNA levels of IGFBP1 and PRL (Fig. 5B and D) than control cells. From these data, we concluded that exposure to high levels of lysine and obesity could impair decidualization.

6. Obesity disrupts endometrial stromal cell autophagic flux

Next, we assayed the autophagic flux of decidualization of ‘lean’ - vs. ‘obese’ - treated ECC-1 cells. BafA1 can be used to block autophagy to lysosomal degradation; therefore, an accumulation of LC3B-II in these conditions indicates an increase in autophagic flux. We found that levels of LC3B-II tended to increase while p62 (also known as sequestosome 1) decreased in lean decidualized cells compared with non-decidualized cells (Fig.6A). However, we observed a significantly lower level of LC3B-II and a higher level of p62 in obese-treated non-decidualized and decidualized ECC-1 cells compare with that in lean-treated ECC-1 cells (Fig. 6B). The amount of LC3B-II significantly increased upon BafA1 treatment in lean-treated decidual ECC-1 cells, suggesting that lysosomal degradation of LC3B-II was inhibited. However, this trend was not obvious in the obese-treated decidual ECC-1 cells (Fig. 6B). Western blotting analysis comparing autophagic flux in obese- vs. lean-treated cells from matched patients revealed impaired autophagic flux in obese patients compared with that in cells from lean women.

Discussion

Autophagy is generally thought of as a survival mechanism to maintain the homeostatic balance between synthesis and degradation of life. Autophagy plays a housekeeping role in the turnover of cellular components, removing misfolded or aggregated proteins, clearing damaged organelles, eliminating intracellular pathogens, being equipped with several degradation systems. Autophagy is a major intracellular degradation system that derives its degradative abilities from the lysosome and participates in cellular and tissue remodeling, and cellular defense against extracellular insults and pathogens.

Autophagy also contributes to the maintenance of homeostatic balance in the regulation of endometrial cell apoptosis during the human endometrial cycle [20]. The amount of LC3B-II correlates with the number of autophagosomes [14,15, 21,22], serving as a valuable molecular biomarker for the detection and assessment of autophagic activity. Beclin 1 was not only the first-described mammalian autophagy protein, but also is one of the most widely characterized players in autophagy regulation [23,24]. The levels of LC3 and beclin-1 increased as the menstrual cycle progressed, reaching a maximum level during the late secretory phase, suggesting a role for the autophagic machinery in the highly regenerating epithelium operating under hormonal control. Furthermore, we also showed that the cyclic change in cleaved caspase-3 levels coincided with that of LC3 and beclin-1. Indeed, autophagy and apoptosis share common stimuli and signaling pathways; therefore, cell life or death depends on the cell response and which process is dominant. Indeed, caspase-mediated cleavage of Beclin 1 promotes crosstalk between apoptosis and autophagy. Although apoptosis-associated cleavage of Beclin 1 inactivates autophagy, the cleavage of autophagy-related 4D cysteine peptidase (ATG4D) by caspase-3 generates a fragment with increased autophagic activity. Further investigation of these cleavage events will be important to gain a deeper understanding of the interrelationship between autophagy and apoptosis [24]. However, our study showed that in endometrial tissues from the late secretory phase, the levels of LC3B-II and Beclin 1 are higher in normal patients than in obese patients. LC3B-II and Beclin 1 are present in the normal endometrium but were decreased in the endometria of obese patients throughout the menstrual cycle. This suggested that autophagy was impaired in the obese patients during the menstrual cycle and might reduce uterine receptivity.

There is considerable evidence that autophagy has important effects on the induction and modulation of the inflammatory reaction and defective autophagy induces inflammation [25-28]. Autophagy operates downstream of TLR signaling. In addition, ATG5 and ATG7 are required for LC3 localization on the phagosome following TLR stimulation [26]. Beclin-1 or ATG5 deficiency stimulated an increase in TNF-α production in primary human keratinocytes [27]. Atg16L1 deficiency induce the Toll/IL-1 receptor in lipopolysaccharide-stimulated macrophages, eventually leading to active IL-1β and caspase-1 levels [28]. Inflammasomes are relevant for the pathogenesis of many human diseases, including obesity-induced inflammation, atherosclerosis, and type 2 diabetes [8,9,29]. Our study showed that endometrial levels of pro-inflammatory cytokines produced by M1 macrophages were induced which might associated with impaired autophagy. Furthermore, Autophagy Gene Atg16L1, Atg5, Atg7 and FOXO1 are necessary for endometrial decidualization and uterine epithelial integrity[13-15]. However, the function of autophagy in controlling the inflammatory responses in the endometrium of obese patients need further exploration.

To confirm the *in vivo* results, ECC-1 cells were treated with 2000 nmol/mol or 8000 nmol/mol levels of lysine in the presence of estrogen and progesterone, which mimic secretory phases of the lean and obese patients, respectively. By measuring markers of endometrial decidualization we established that obese women have a reduced ability to undergo normal decidualization. Decidualization is essential, not only for implantation, but also for placentation, fetal health, and maintenance of pregnancy [30]. When this key process of pregnancy is impaired, it can have both negative short-term effects on implantation and long-term fetal effects. This is also supported by evidence that obese women have recurrent early

pregnancy loss, despite euploid embryos, indicting the endometrial environment as a contributor to pregnancy loss [2-4,31]. Obesity after menopause is directly related to endometrial cancer, and it is possible that mechanisms implicated in the development of uterine cancer might provide alternative pathways of reproductive disorders and infertility [32]. The present study showed that in endometrial tissues from the late secretory phase, the levels of LC3B-II, Beclin 1 and autophagy-related genes were higher in normal-weight patients compared with those in obese patients. The low expression of autophagy-related factors lead to a defective decidualization, furthermore, compromising pregnancy.

Autophagy is a dynamic process in which the end-point is fusion of the autophagosome with a lysosome, resulting in protein degradation. Although the amount of LC3B-II correlates with the number of autophagosomes, its amount at a certain time point does not necessarily indicate the degree of autophagic flux, a term used to indicate overall autophagic degradation rather than autophagosome formation. Some populations of LC3B-II might be ectopically generated in an autophagy-independent manner. LC3 can also be recruited directly to bacteria-containing phagosome membranes in a process termed LC3-associated phagocytosis. Thus, it is important to measure the amount of LC3B-II delivered to the lysosomes by comparing LC3B-II levels in the presence and absence of bafilomycin A1 (a vacuolar H+-ATPase inhibitor), and lysosomal protease inhibitors [33] to inhibit lysosomal degradation of LC3B-II. Another widely used autophagy marker, p62, also called sequestosome 1 (SQSTM1), binds directly to LC3 and GABARAP (ATG8 orthologs) family proteins via a short LC3 interaction region (LIR). This may serve as a mechanism to deliver selective autophagic cargoes for degradation by autophagy. The p62 protein is itself degraded by autophagy and serves as a marker to study autophagic flux [34-36], especially when autophagy is suppressed. When autophagy is inhibited, p62 accumulates, whereas when autophagy is induced, p62 levels decrease. Western blotting analysis comparing autophagic flux in obese- *vs*. noemal-weight treated ECC-1 matched patients revealed that autophagy is upregulated during decidualization in lean patients compared with that in obese patients, as shown by a decrease in p62 and an increase in LC3B II, which might explain the impaired autophagic flux in obese patients compared with normal-weight patients.

Autophagy is induced in decidualization under physiological hypoxia during the early phases of pregnancy and is believed to be a principal element for implantation [37-39]. Increased autophagic flux was also observed in decidualized human embryonic stem cells (ESCs), confirming a role for autophagy in decidualization [38,39]. Autophagy flux and decidualization were both impaired in ‘obese’- treated endometrial epithelial (ECC-1) cells. These altered autophagic flux and increased activation of pro-inflammatory cytokines might play an underlying role in the decreased decidualization and altered uterine receptivity in obese women.

The present study provides strong scientific evidence to support clinical reports of an adverse impact of obesity on uterine receptivity. If our hypothesis that altered autophagic flux and hyper-inflammation impact of obesity on uterine receptivity borne out by further studies, the clinical implications could be important. For example, autophagic activators and immunomodulators might be a potential therapeutic

regimen for obese women by alleviating poor decidualization and ultimately poor pregnancy outcomes in this population [40].

Conclusion

In conclusion, in this work, we describe a connection previously unknown between autophagy and proinflammatory cytokines in endometrial decidualization. Obesity downregulates the expression of autophagy-related genes and impairs endometrial stromal cell autophagic flux, and induces inflammation. These alterations in autophagy are associated with increased activation of autophagy-related proinflammatory cytokines might decrease basic uterine receptivity.

Abbreviations

BMI: body mass index; RT-qPCR: Quantitative real-time reverse transcription PCR; TBS-T: Tris-Buffered Saline and Tween; cDNA: complementary DNA; PVDF: polyvinylidene fluoride; LC3: Microtubule-associated protein 1 light chain 3; LC3B-II: protein 1 light chain 3 beta; ATG: Autophagy-related proteins; ATG3: Autophagy-related 3; ATG5: Autophagy-related 5; ATG7: Autophagy-related 7; FOXO1: transcription factor Forkhead Box; TNF-α:Tumor necrosis factor alpha; IL: Interleukin; IFN: interferon; MCP-1: monocyte chemoattractant protein-1; IGFBP1:insulin-like growth factor binding protein 1; PRL: prolactin; SPSS: statistical package for the social sciences; SEM: standard error of the mean.

Declarations

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Conflicts of interest

The authors declare they have no competing interests.

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Availability of data and materials

The primary data for this study is available from the authors on direct request.

Consent for publication

Not applicable.

Ethical approval and consent to participate

This study was approved by the Institutional Ethics Committees at Beijing Chaoyang Hospital.

Authors' contributions

Ya-su Lv was responsible for the study design, laboratory operation and manuscript drafting. Chen Wang, Ling-xiu Li and Shuo Han were responsible for the tissue collection and critical discussion. Yuan Li was responsible for the study design, data analysis and manuscript writing. All authors read and approved the final manuscript.

References

1. Obesity and Overweight, World Health Organization, 2018.
2. Antoniotti G, Coughlan M, Salamonsen L, et al. Obesity associated advanced glycation end products within the human uterine cavity adversely impact endometrial function and embryo implantation competence. Human Reproduction. 2018;33(4):654–665.
3. Boots C, Bernardi L, M. Stephenson. Frequency of euploid miscarriage is increased in obese women with recurrent early pregnancy loss. Fertility and Sterility. 2014;102 (2):455–459.
4. K. Luzzo, Q. Wang, S. Purcell, et al., High fat diet induced developmental defects in the mouse: oocyte meiotic aneuploidy and fetal growth retardation/brain defects. PLoS One. 2012;7(11): e49217.
5. Rimm A. Prevalence of Childhood and Adult obesity in the United States. 2011-2012. JAMA. 2014;311(8):806-814.
6. Bellver J, Martinez-Conejero J, Labarta E, et al. Endometrial gene expression in the window of implantation is altered in obese women especially in association with polycystic ovary syndrome. Fertility and Sterility. 2011; 95(7): 2335–2341.
7. Broughton D, Moley K, Obesity and female infertility: potential mediators of obesity's impact, Fertility and Sterility. 2017;107(4): 840-847.
8. Rhee J, Saben J, Mayer S, et al. Diet-induced obesity impairs endometrial stromal cell decidualization: a potential role for impaired autophagy. Human Reproduction. 2016;31(4):1315–1326.
9. Higgins L, Mills T, Greenwood S, et al. Maternal obesity and its effect on placental cell turnover, Matern Fetal Neonatal Med. 2013; 26(8): 783–788,.
10. Hayes E, Tessier D,Percival M, et al. Trophoblast invasion and blood vessel remodeling are altered in a rat model of lifelong maternal obesity. Reproductive Sciences. 2014;21(5):648–657.
11. T. Isei, M. Naoko, U. Takashi, et al. Lysosomal Turnover, but Not a Cellular Level, of Endogenous LC3 is a Marker for Autophagy, Autophagy. 2005;2(1):84–91.
12. Cao Y, Klionsky D. Physiological functions of Atg6/Beclin 1: a unique autophagy- related protein. Cell Research. 2007;17(10): 839–849,.

13. Choi J, M. Jo, Lee E, et al. The Role of Autophagy in Human Endometrium. Biology of Reproduction. 2012;83(6):70–71.
14. Vasquez YM, Wang X, Wetendorf M, et al. FOXO1 regulates uterine epithelial integrity and progesterone receptor expression critical for embryo implantation. PLoS Genet. 2018;14(11):e1007787.
15. Zhu H, Hou C, Luo L, et al. Endometrial stromal cells and decidualized stromal cells: origins, transformation and functions. Gene. 2014;551(1):1–14.
16. Oestreich AK, Chadchan SB, Popli P, et al. The Autophagy Gene Atg16L1 is Necessary for Endometrial Decidualization. Endocrinology. 2020;161 (1): bqz039.
17. Carey N, Bodzin J, Saltiel A. Obesity Induces a Phenotypic Switch in Adipose Tissue Macrophage Polarisation. Journal of Clinical Investigation. 2007;117 (1):175–184.
18. Zhu H, Hou C, Luo L, et al. Endometrial stromal cells and decidualized stromal cells: origins, transformation and functions. Gene. 2014;551(1):1–14.
19. Antoniotti G, Coughlan M, Salamonsen L, et al. Obesity associated advanced glycation end products within the human uterine cavity adversely impact endometrial function and embryo implantation competence. Human Reproduction. 2018;33(4):654–665.
20. Mei J, Zhu X, Jin L, et al. Estrogen promotes the survival of human secretory phase endometrial stromal cells via CXCL12/CXCR4 up-regulation-mediated autophagy inhibition, Human Reproduction. 2015;30(7):1677–1689.
21. Mizushima N, Yoshimori T, Levine B, Methods in Mammalian Autophagy Research. Cell. 2010;140(3):313–326.
22. Klionsky D, Abdalla F, Abeliovich H, et al. Guidelines for the use and interpretatoin of assays for monitoring Autophagy. Autophagy. 2016;12(1):1–222.
23. Gaytan M, Morales C, Jose E, et al. Immunolocalization of beclin 1, a bcl-2-binding, autophagy-related protein, in the human ovary: possible relation to life span of corpus luteum. Cell and Tissue Research. 2008;331(2):509–517,.
24. Kang R, Zeh H, Lotze M, et al. The Beclin 1 network regulates autophagy and apoptosis, Cell death and differentiation. 2011;18(4): 571–580.
25. Harris J. Autophagy and cytokines, Cytokine. 2011;56(2): 140–144.
26. Franco LH, Fleuri AKA, Pellison NC, et al. Autophagy downstream of endosomal Toll-like receptor signaling in macrophages is a key mechanism for resistance to Leishmania major infection[J]. J Biol Chem, 2017, 292(32): 13087-13096..
27. Meng Q, Cai D. Defective hypothalamic autophagy directs the central pathogenesis of obesity via the IkappaB kinase beta IKKbetapp/NF-kappaB pathway. Journal of Biological Chemistry. 2011;286(37):32324–32332.
28. Plantinga T, Joosten L, Meer J, et al. Modulation of inflammation by autophagy: consequences for Crohn's disease. Curr Opin Pharmacol. 2012;12(4): 497–502.

29. Salminen A, Kaarniranta K, Kauppinen A. Inflammaging:disturbed interplay between autophagy and inflammasomes. Aging. 2012; 4(3): 166–175.
30. Galliano D, Bellver J. Female obesity: short- and long-term consequences on the offspring, Gynecol Endocrinol. 2013;29(7):626–631,.
31. Boots C, Bernardi L, Stephenson M. Frequency of euploid miscarriage is increased in obese women with recurrent early pregnancy loss. Fertility and Sterility. 2014;102(2):455–459.
32. Moley K, Colditz G. Effects of obesity on hormonally driven cancer in women. Science Translational Medicine. 2016;8(323):323–323.
33. Sanjuan M, Dillon C, Tait SW, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. Nature. 2007; 450(7173):1253–1257.
34. Komatsu M, Waguri S, Koike M, et al. Homeostatic Levels of p62 Control Cytoplasmic Inclusion Body Formation in Autophagy-Deficient Mice. Cell. 2007;131(6): 1149–1163.
35. M. Komatsu, S. Kageyama, Y. Ichimura, p62/SQSTM1/A170: physiology and pathology, pharmacological research. 2012;66(6):457–462.
36. Pankiv S, Clausen T, Lamark T, et al. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, Biol Chem. 2007;282(33):24131–24145.
37. Armant D. Autophagy expanding role in development: implantation is next, Endocrinology. 2011;152 (5): 1739–1741.
38. Rhee J, Saben J, Mayer A, et al. Diet-induced obesity impairs endometrial stromal cell decidualization: a potential role for impaired autophagy. Human Reproduction. 2016;31(6):1315-1326.
39. Yang L, Li P, Fu S. Defective hepatic autophagy in obesity promotes ER stress and causes insulin resistance. Cell Metab. 2010;11(6):467–478.
40. Rubinsztein D, Codogno P, Levine B, Autophagy modulation as a potential therapeutic target for diverse diseases. Nature Reviews Drug Discovery. 2012;11(9):709-730.

Figures

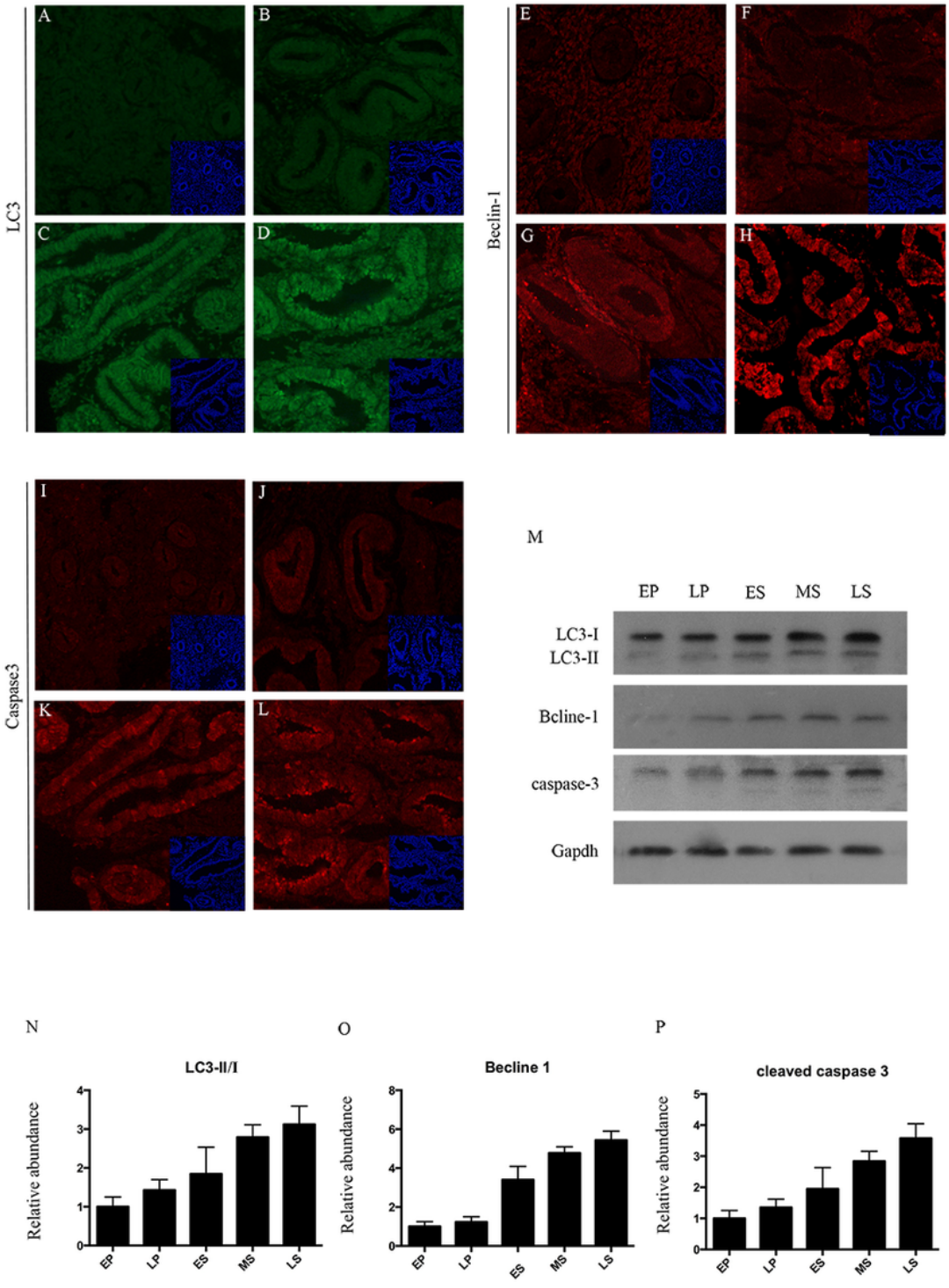

Figure 1

Autophagy and Apoptosis are upregulated in the human endometrium during the menstrual cycle. Immunofluorescence staining showing the expression of LC3 (A-D), beclin-1 (E-H), and cleaved caspase 3 (I-L) in human endometrial tissues during the menstrual cycle: (A, E, I) Early proliferative, (B, F, J) late proliferative, (C, G, K) early secretory, and (D, H, L) late secretory endometrium. Densitometric quantification (M) and representative immunoblots (N, O, P) of each marker. Experiments were repeated

three times, and data are expressed as the mean ±SEM. LC3, microtubule associated protein 1 light chain 3 alpha.

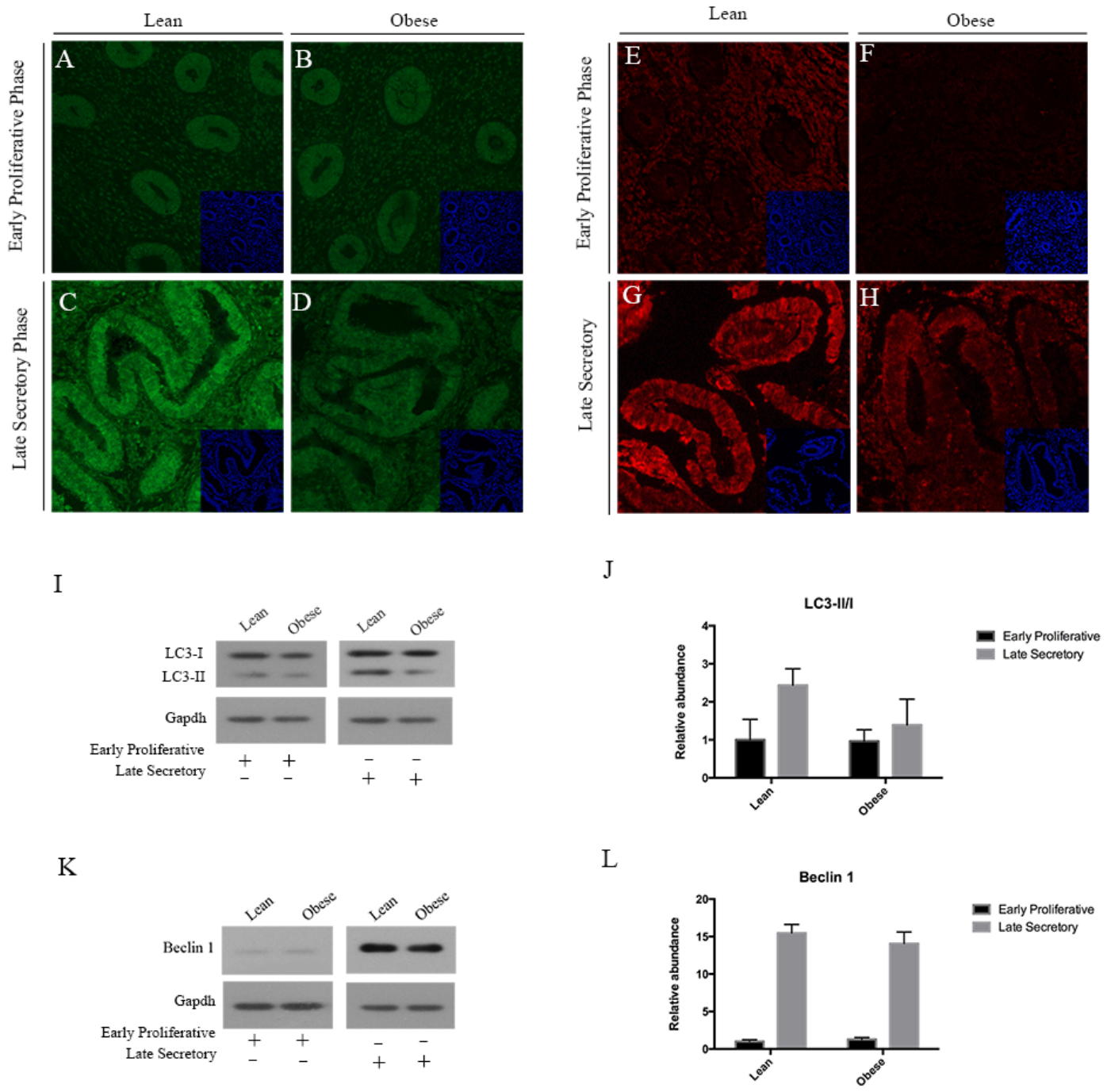

Figure 2

Autophagy-related proteins are decreased in obese patients during the secretory phase. Immunofluorescent staining showing the levels of LC3B-II in human endometrial tissues at the early proliferative phase (A, B) and the late secretory phase (C, D) in the endometrium of lean and obese patients. The levels of beclin 1 in human endometrial tissues at the early proliferative phase (E, F) and the late secretory phase (G, H) in the endometrium of lean and obese patients. Densitometric quantification (J, L) and representative immunoblots (I, K) of each marker. Experiments were repeated three times, and

data are expressed as the mean ± SEM. LC3B-II, microtubule associated protein 1 light chain 3 beta phosphatidylethanolamine conjugate.

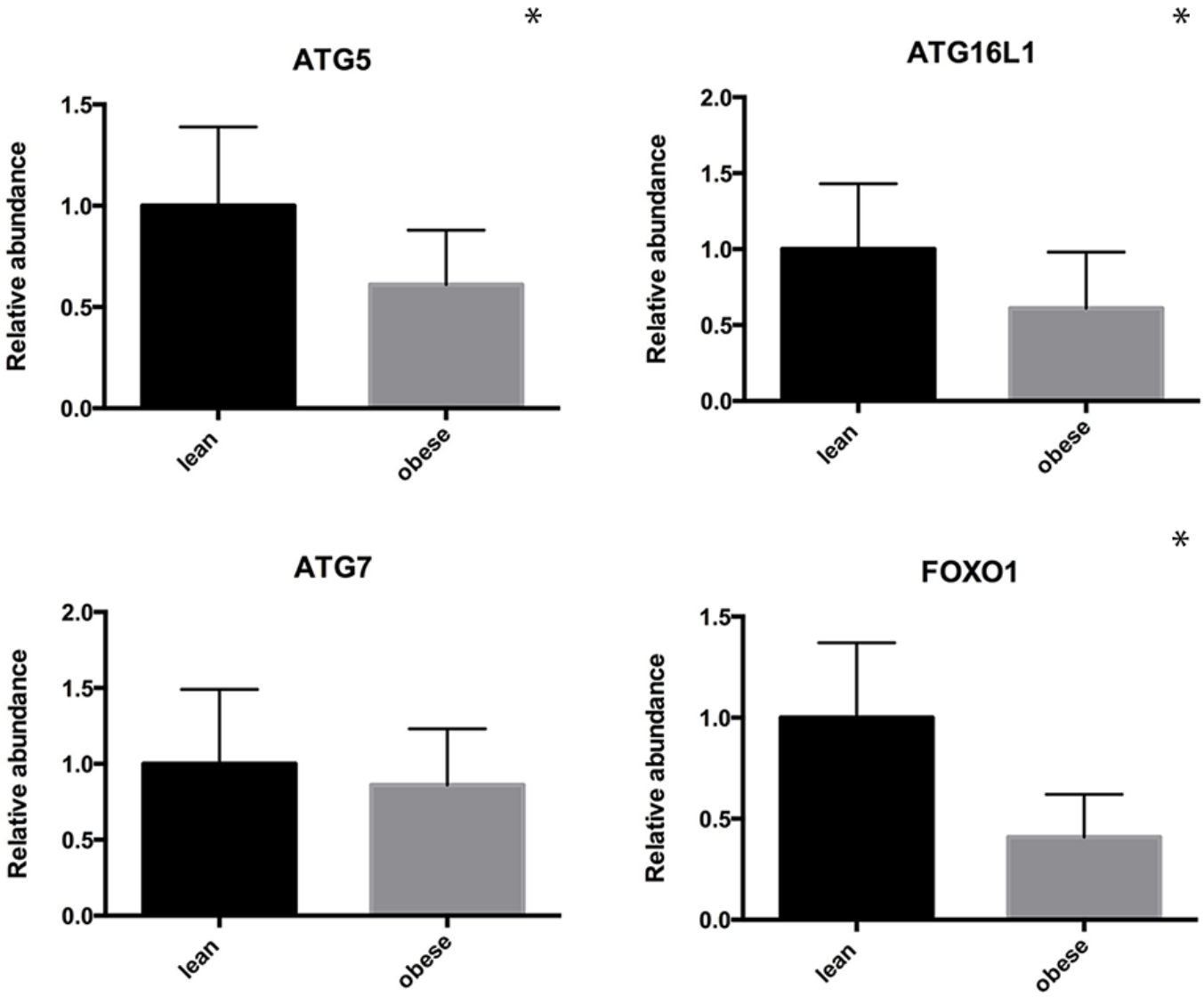

Figure 3

Downregulation of autophagy-related genes expression in endometrial tissue during the late secretory phase from obesity patients. The mRNA levels of ATG5, ATG16L1 and FOXO1 expression were significant decreases in in the endometrial tissues of obesity patients (n = 7) compared in the control subjects (n 11, p< 0.05). The levels of ATG7 expression did not significantly differ between the two groups. Data are presented as the mean ± SEM. *P < 0.05.

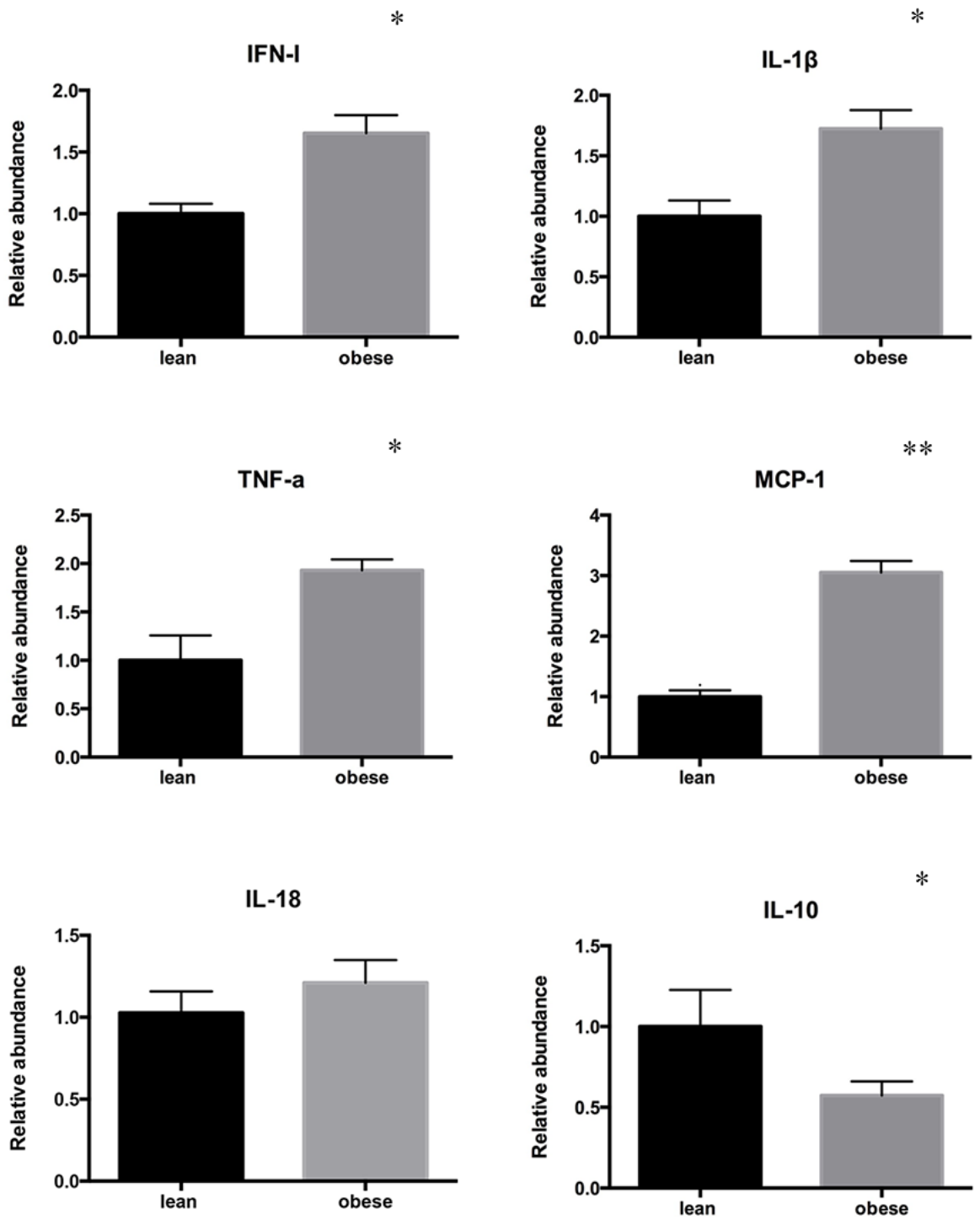

Figure 4

Inflammatory chemokines and cytokines are elevated within the uterine cavity of obese patients. RT-qPCR demonstrated significant increases in IFNA1 (IFN α), MCP1 , IL1B (IL-1β), TNF (TFN-α) expression and no significant increase in IL18(IL-18) within the obese (n = 7) versus lean (n =11) uterine cavity and a significant decrease in IL10 (IL-10). Data are presented as the mean ± SEM. *P < 0.05, **P < 0.01. RT-qPCR, quantitative real-time reverse transcription PCR; IFN-α, interferon alpha; MCP-1, monocyte

chemotactic protein 1; IL-1β, interleukin 1 beta; TFN-α, tumor necrosis factor alpha; IL-18, interleukin 18; IL-10, interleukin 10.

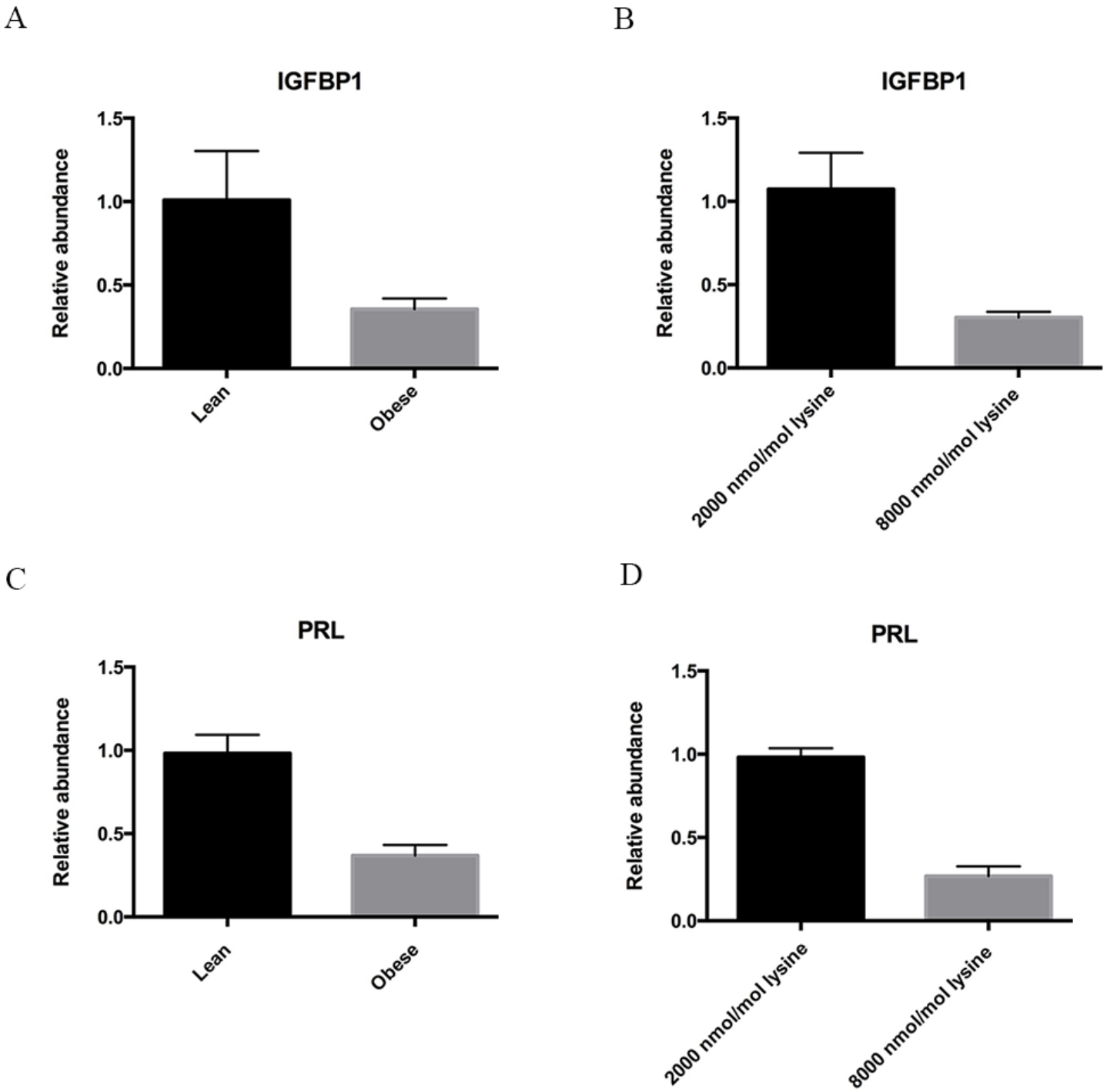

Figure 5

Obesity impairs endometrial stromal cell decidualization Expression levels of IGFBP1 (A) and PRL (B) mRNA in decidualized endometrial cells from obese and lean women (obese, n = 7; lean, n = 11). Expression levels of IGFBP1 (C) and PRL (D) mRNA in ECC-1 cells treated with 'lean' (2000 nmol/mol) or 'obese' (8000 nmol/mol) levels of lysine after decidualization. Data are presented as mean ± SEM. IGFBP1, insulin like growth factor binding protein 1; PRL, prolactin.

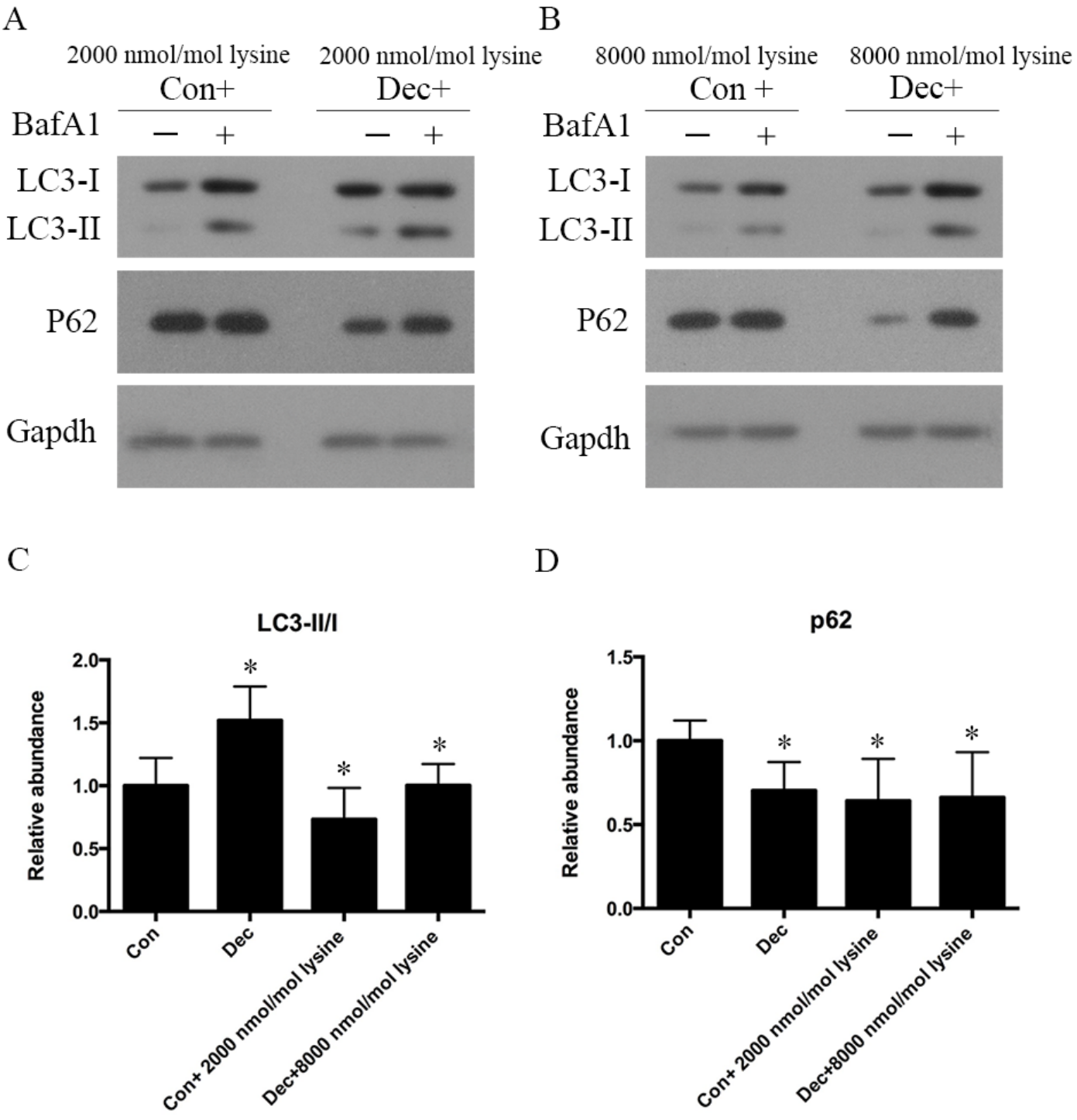

Figure 6

Obesity impairs endometrial stromal cell autophagic flux Western blot of LC3B-II and p62 (also known as sequestosome 1) in control (Con) and decidualized (Dec) human ESCs in the presence or absence of Bafilomycin A1 (BafA1) with 'lean' (A) or 'obese' (B) treatment. Quantification of LC3B-II levels relative to GAPDH in BafA1-treated cells (C). Quantification of the p62 level relative to GAPDH in BafA1-treated cells (D). Values are expressed as the mean + SEM. *P < 0.05. This experiment was performed in triplicate.

LC3B-II, microtubule associated protein 1 light chain 3 beta phosphatidylethanolamine conjugate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ESCs, embryonic stem cells.