Published in final edited form as:

Int Urogynecol J Pelvic Floor Dysfunct. 2009 December ; 20(12): 1423–1429. doi:10.1007/s00192-009-0905-y.

Is Lysyl Oxidase-Like Protein-1, Alpha-1 Antitrypsin, and Neutrophil Elastase Site- Specific in Pelvic Organ Prolapse?

Weng-Chi Man

Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California 94305

Jason Yen-Ping Ho

Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California 94305

Yan Wen

Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California 94305

Eric R. Sokol

Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California 94305

Mary L. Polan

Department of Obstetrics and Gynecology, Stanford University School of Medicine, Stanford, California 94305

Bertha Chen

Department of Obstetrics and Gynecology, Stanford University School of Medicine, 300 Pasteur Drive, HH-333, Stanford, CA 94305-5317. bchen@stanford.edu; Telephone: (650) 725-5986; Fax: (650) 723-7737

SUMMARY

The expression level of elastin metabolism-related proteins is variable in the vagina and does not correlate with the most prominent area of prolapse.

Introduction—We investigated whether the expression of alpha-1 antitrypsin (ATT), neutrophil elastase (NE) and lysyl oxidase-like protein 1 (LOXL-1) vary within the vagina in subjects with pelvic organ prolapse (POP).

Methods—Biopsies were obtained from the anterior and posterior vaginal wall of 22 women with POP ($\geq$ stage 2 by POP-Q). The subjects were grouped by the most prominent defect: cystocele, cystocele plus uterine prolapse, and rectocele. Comparative real-time PCR, Western blotting, and NE enzyme activity assay were performed.

Results—The ratio of anterior and posterior vaginal wall ATT, NE, and LOXL-1 expression varied between individuals within the same defect group.

Conclusions—ATT, NE, and LOXL-1 expression was variable among different biopsy sites in the vagina. No consistent pattern was present when the subjects were grouped by the most

prominent defect. We recommend careful consideration of biopsy sites in future studies on POP to enhance reproducibility of data.

Keywords

pelvic organ prolapse; lysyl oxidase-like protein 1; neutrophil elastase; alpha-1 antitrypsin

INTRODUCTION

Pelvic organ dysfunction is a major health issue affecting women throughout their reproductive and menopausal years [1]. It is well known that damage to the muscles, connective tissues and nerves in the pelvic floor during vaginal delivery plays a major role in the etiology of pelvic floor disorder. Other factors - including race, increased age, body mass index, smoking, estrogen deficiency, and familial predisposition - have also been implicated [2-6]. It has thus been hypothesized that the pathophysiology of pelvic floor disorder is a multifactorial process, where a genetically predisposed woman ultimately develops prolapse after experiencing a series of life events [7].

The female pelvic organs are supported by pelvic muscles, ligaments and the bony pelvis. The tensile strength of the pelvic floor connective tissues is crucial for supporting the pelvic organs which are constantly subjected to intra-abdominal pressure [8]. Collagen and elastin are the two major fibrillar components of the connective and supportive tissues of the vagina, with collagen being responsible for tensile strength and elastin for resilience and ability to recoil [9,10]. Recent studies have indicated that the metabolism of collagen and elastin is altered in the prolapsed tissue [9,11-13], implying that both the synthesis of the structural proteins of extracellular matrix and the degradation process, which involves a wide spectrum of proteases and their corresponding inhibitors, are important in the pathophysiology of pelvic organ disorder. A reduction in the expression of alpha-1 antitrypsin (ATT), a major inhibitor of neutrophil elastase (NE) and a relative increase of elastolytic activity in pelvic tissues from patients with pelvic floor dysfunction has been documented [14]. Metalloproteinases 2 (MMP-2) and 9 (MMP-9) are also increased in pelvic floor connective tissues of patients with pelvic organ prolapse [13,15].

Proteins responsible for the synthesis of the extracellular matrix are also important for tissue remodeling. Lysyl oxidases (LOX) are enzymes which catalyze the oxidative deamination of the ϵ -amino group of lysyl and hydroxylysyl residues of collagen as well as the lysyl residues of elastin [16]. Covalent cross-linkages in mature collagen and elastin are formed when spontaneous condensation occurs between the resulting aldehyde groups or with the ϵ -amino groups on the peptidyl lysine [17]. Normal cross-linking is important in providing protection against the degradation of elastin and collagen by nonspecific proteinases [18]. LOXL-1 is one of the five members in this LOX family [16]. Liu *et al.* showed that LOXL-1 is required for the synthesis of elastic fibers [17]. Targeted disruption of LOXL-1 in mice resulted in the inability of the reproductive tissues to repair the elastic fibers after parturition, thus causing pelvic organ prolapse in the animals [8].

The above studies suggest that the development of pelvic organ prolapse involves abnormalities in the degradation and/or synthesis of the extracellular matrix. If so, the expression of ECM proteins will be altered in individuals with pelvic floor dysfunction and should be homogeneous throughout the vagina. It is also possible that these observed differences result from the mechanical stresses imparted on the various surfaces of the vagina. In this case, areas with prolapse should differ in expression of these proteins compared with other less affected vaginal sites. In this descriptive study, we sought to examine whether the above changes in NE, ATT, and LOXL-1 in women with pelvic organ

prolapse is homogeneous throughout the vagina or if they correlate with the vaginal sites with higher degree of prolapse.

MATERIAL AND METHODS

Subject Selection

Approval from the Institutional Review Board of Stanford University Medical School was obtained prior to the commencement of the study. We screened women undergoing surgery for pelvic organ prolapse. All subjects underwent a pelvic examination with POP-Q [19]. We selected subjects with stage 2 or a more advanced stage of prolapse. Subjects were divided into 3 groups according to the most prominent type of prolapse: cystocele, rectocele, cystocele plus uterine prolapse. The cystocele group consisted of subjects with stage 3 cystocele and other defects above the hymen. The rectocele group consisted of subjects with stage 2-3 rectocele and other defects above the hymenal level. The cystocele plus uterine prolapse group consisted of subjects with stage 3 cystocele and uterine prolapse. Subjects with history of endometriosis, gynecologic malignancies, pelvic inflammatory conditions, connective tissue disorders, emphysema, and prior pelvic surgery were excluded. 31 potential subjects agreed to participate in the study. However, in order to ensure that the subjects in the same group (i.e. cystocele, rectocele or cystocele plus uterine prolapse) belong to the same menopausal status, only 22 subjects were retained in the study.

Tissue collection

After obtaining the subject's informed consent, biopsies of vaginal wall were excised from the anterior vaginal wall (at point Ba) and the posterior vaginal wall (at point Bp). Full-thickness vaginal wall tissue was resected after surgical dissection. The vaginal epithelium was first infiltrated with a dilute epinephrine solution and dissection was carried down to the loose connective tissue plane between the vaginal epithelium and the bladder or rectum. This produced a tissue sample approximately 4-5 mm in thickness, to include lamina propria and muscularis layer of the vaginal wall. The lamina propria is a layer rich in connective tissue. Roughly 1 cm² full-thickness vaginal wall biopsies were taken from each site. Hematoxylin and eosin stain was performed on sample tissue to ensure that a full thickness biopsy (including lamina propria and muscularis) was obtained. Specimens were then snap frozen with liquid nitrogen and were stored at -80°C. Right before protein and RNA extraction, tissues were thawed and washed in diethylpyrocarbonate (DEPC)-treated phosphate buffered saline (PBS), pH 7.4. The epithelial layer was scraped off thoroughly using a razor blade and hence the final specimen contained mostly lamina propria and muscularis.

Total tissue protein extraction

Vaginal tissues were dissected into small pieces and homogenized in the solubilization buffer (150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate, 0.04% SDS, 4 mM EDTA, 50 mM Tris-HCl, pH 7.4). The homogenate was rotated overnight at 4°C, followed by centrifugation at 10,000 g for 30 minutes. The supernatant was collected for Western blot analysis and enzyme activity assay. Protein concentration was determined using the Bradford method.

Immunoblot Analysis

Protein samples were loaded and separated on a 10% SDS-PAGE, after which the proteins were transferred to nitrocellulose membranes (Pierce, Rockford, IL). The membranes were blocked with 5% nonfat milk and were then subjected to immunoblot analysis. The following antibodies were used: rabbit anti human ATT (1:5000) and mouse anti goat

(1:5000) (Sigma, St Louis, MO), goat anti GAPDH (1:2000) (Abcam, Cambridge, MA), donkey anti-rabbit IgG (1:100000) (Sigma, St. Louis, MO). Visualization of the bound antibodies was performed using chemiluminescence. Pure human alpha anti-trypsin was used as a control in the immunoblot analysis to verify the specificity of the antibody. The protein samples were analyzed twice.

RNA extraction and Comparative Quantitative Real-Time PCR

Total RNA extraction, reverse transcription and comparative quantitative real-time PCR were performed as described [20]. The sequences of the primers for the comparative quantitative real-time PCR are shown in Table I. Total RNA was extracted from the vaginal wall specimens using the guanidium isothiocyanate method (RNAzol, Tel-test, Inc, Friendswood, TX). The concentration of RNA and its purity was determined by spectrophotometry in a Beckman Coulter DU 640 spectrophotometer (Beckman Coulter, Inc., Fullerton, CA). For downstream evaluation of a particular mRNA in the vaginal wall sample, 1 μ g of total RNA was reversely-transcribed into cDNA using the Gen Amp RNA PCR Kit (Applied Biosystems, Foster City, CA) and M-MLV reverse transcriptase (Invitrogen, Carlsbad, CA). We performed the comparative quantitative real-time PCR using Brilliant SYBR Green QPCR Master Kit (Stratagene, La Jolla, CA) on the Mx4000 Multiplex Quantitative PCR system with Mx4000 software (Stratagene, La Jolla, CA, USA). Following a hot start of 15 minutes at 95°C, the PCR product was amplified in a three-step fashion with denaturation at 94 °C for 30 seconds, annealing at 60 °C for 1 minute, followed by extension at 72 °C for 30 seconds. A total of forty cycles were performed. We also ran a dissociation profile to assess the specificity of the amplicon as well as the formation of primer-dimer. The specificity of the primers was evaluated by sequencing the PCR products. Relative quantitation of the gene expression was performed and hypoxanthine phosphoribosyl transferase 1 (HPRT1) was used as the endogenous reference for normalization. To generate the relative expression level of each experimental sample, we divided the normalized sample value by that of a common calibrator, which serves as a reference point for comparison between samples.

Neutrophil elastase activity assay

The assay was performed based on the method published by Yoshimura *et al* [21]. Briefly, the sample (40 μ l) was incubated with the substrate Ala-Ala-Pro-Val-p-nitroaniline at a final concentration of 4 mM (Sigma, St Louis, MO) and 0.1 M Tris-HCl (pH 8.0) containing 0.5 M NaCl in a final volume of 200 μ l at 37 °C for 4 hours. A corresponding reaction for each sample in which DMSO was substituted for the substrate was included to serve as the blank, so that calculation could be made to cancel out the absorbance due to the intrinsic color of the protein sample. Absorbance at 405 nm was measured. The neutrophil elastase activity was measured as duplicates.

Statistical analysis

Paired comparison between expression at anterior and posterior vaginal walls was performed using Wilcoxon signed-rank test. Paired-t test was not used because some data set did not show a normal distribution.

RESULTS

Full-thickness vaginal wall tissues from 22 subjects were included in this study. Based on the most prominent prolapse defect, the subjects were divided into three categories: cystocele group (Ba: 3.9 $\pm$ 1.8cm, Bp:-1.0 $\pm$ 2.2cm, C:-2.3 $\pm$ 2.1cm), rectocele group (Ba: -0.9 $\pm$ 1.5cm, Bp:1.9 $\pm$ 1.1cm, C:-6.3 $\pm$ 1.8cm) and cystocele plus uterine prolapse group (Ba: 2.8 $\pm$ 1.7cm, Bp:0.7 $\pm$ 1.8cm, C:4.3 $\pm$ 2.7cm) The numbers in paranthesis are the means $\pm$ SD of

the POP-Q measurements for each group. Age, body mass index (BMI), and parity of the three groups of subjects are summarized in Table II. All the subjects in the rectocele group were premenopausal while the subjects in the other 2 groups were postmenopausal. This may reflect the epidemiology of the specific defect.

The anterior and posterior vaginal walls were chosen as the sites of comparison. For each pair of anterior and posterior vaginal wall samples, the following assays were performed: Western blot analysis for ATT protein expression level (with normalization to loading control GAPDH), NE activity assay, comparative quantitative real-time PCR for ATT, NE and LOXL-1. To facilitate comparison between individuals, we expressed our data as ratio of the posterior wall to the anterior wall. For example, the NE activity data shown in the figures are actually $\text{NE activity}_{\text{posterior wall}} / \text{NE activity}_{\text{anterior wall}}$ from the same subject. A ratio greater than 1 indicates that the expression at the posterior wall is higher than that in the anterior wall in the subject. Figures 1 and 2 summarize these data. Within each group of subjects (cystocele, cystocele plus uterine prolapse or rectocele), the ratios of ATT, NE, and LOXL-1 expression in posterior-to-anterior vaginal wall varied significantly (up to 3.5 fold in several cases). This variability was observed at both the protein and mRNA levels. Moreover, no specific patterns or trends exist even when subjects were grouped by the most prominent type of defect. Paired comparisons using Wilcoxon signed-rank test showed high variability across the locations but no overall difference in the rank ordered data between the anterior and posterior wall biopsy sites for each of the three groups.

DISCUSSION

Pelvic organ prolapse affects negatively the lives of millions of women. Although the factors underlying the pathogenesis of female pelvic floor disorders are complex, it is believed that a major contributor is the loss of support from the connective tissues.

Previous studies have shown that a shift in the balance between the degradation and synthesis of extracellular matrix is involved in the development of pelvic organ prolapse [9]. It is known that in patients with pelvic organ prolapse, the expression of ATT decreases whereas the relative elastolytic activity is upregulated [14]. It has also been shown in mouse models that genetic disruption of LOXL-1 results in the development of pelvic organ prolapse postpartum owing to the failure to synthesize and assemble functional elastic fibers [17,22]. Data on LOXL-1 expression in human tissues are scant and inconsistent. Kobak *et al.* have found that the mRNA expression of LOX, a relative of LOXL-1, was significantly reduced in patients with severe pelvic organ prolapse [23]. Wieslander *et al.*, in an abstract, reported LOXL-1 mRNA expression decreased in premenopausal women with prolapse, but was upregulated in postmenopausal women with prolapse [24]. Prospective studies are necessary to determine whether these changes in ECM cause the development of POP or are seen as a result of altered mechanical loads on the prolapsed tissue.

In the current study, we sought to understand whether the altered expressions of NE, ATT, and LOXL-1 observed in women with pelvic organ prolapse is homogeneous or site-specific throughout the vagina and whether their expressions correlate with the most prominent site of prolapse. The grouping in this study reflects the most prominent site of prolapse since pelvic organ prolapse is usually associated with pelvic support defect in other segments of the vagina [25]. Our data show that the expression level of ATT, NE and LOXL-1 vary among different biopsy sites within the same individual and no statistically significant patterns exist even when the subjects are grouped by the most prominent type of defect. The variability in protein expression among various biopsy sites is reflective of the complex environment pelvic organs function in. Factors such as BMI, bony pelvic anatomy, previous pelvic injuries, chronic pelvic stresses (cough, exercise, parity) can all have different effects

on different sites in the vagina. In addition to the baseline ECM abnormalities in women with pelvic organ prolapse, the expression of ECM proteins can be further modulated by these external forces.

We also examined the P/A ratios in relation to the continuous POP-Q measurements in all the 3 groups of subjects. We did not observe any statistically significant correlation between P/A ratios and the POP-Q measurements (data not shown). However, P/A ratio tended to cluster around 1 in the cystocele-only and cystocele plus uterine prolapse groups, independent of POP-Q measurements, suggestive of similar ECM processes in different areas of the vagina. We acknowledge that this preliminary study is not powered to prove the null hypothesis (that no differences exist between anterior and posterior vaginal wall sites). A larger study is needed to confirm these findings. For LOXL-1, we only presented our data from the comparative quantitative real-time PCR rather than Western blot data as we had reservation on the specificity of the LOXL-1 antibody currently commercially available.

While our data hint that similar ECM processes exist between different site in the vagina, the findings of this descriptive study also raise concerns that consistency in biopsy site and larger sample size are critically important in study design due to the high variability in protein expression and large standard deviations. These factors should be taken into account when comparing data from different data sets. Given this, we recommend careful consideration of biopsy sites, in addition to the selection of a homogeneous study group in future studies on pelvic organ prolapse in order to enhance reproducibility of data.

Acknowledgments

We thank Lorna Groundwater at the Stanford University School of Medicine for editing the manuscript. The study is supported by National Institutes of Aging RO1 AG01790.

The study is supported by National Institutes of Aging RO1 AG01790

REFERENCES

1. Chen B, Wen Y, Yu X, Polan ML. Elastin metabolism in pelvic tissues: is it modulated by reproductive hormones? *Am J Obstet Gynecol.* 2005; 192:1605–1613. [PubMed: 15902165]
2. Nygaard I, Bradley C, Brandt D. Pelvic organ prolapse in older women: prevalence and risk factors. *Obstet Gynecol.* 2004; 104:489–497. [PubMed: 15339758]
3. Samuelsson EC, Victor FT, Tibblin G, Svardsudd KF. Signs of genital prolapse in a Swedish population of women 20 to 59 years of age and possible related factors. *Am J Obstet Gynecol.* 1999; 180:299–305. [PubMed: 9988790]
4. Swift SE, Pound T, Dias JK. Case-control study of etiologic factors in the development of severe pelvic organ prolapse. *Int Urogynecol J Pelvic Floor Dysfunct.* 2001; 12:187–192. [PubMed: 11451007]
5. Graham CA, Mallett VT. Race as a predictor of urinary incontinence and pelvic organ prolapse. *Am J Obstet Gynecol.* 2001; 185:116–120. [PubMed: 11483914]
6. Buchsbaum GM, Duecy EE, Kerr LA, Huang LS, Perevich M, Guzick DS. Pelvic organ prolapse in nulliparous women and their parous sisters. *Obstet Gynecol.* 2006; 108:1388–1393. [PubMed: 17138771]
7. Weber AM, Richter HE. Pelvic organ prolapse. *Obstet Gynecol.* 2005; 106:615–634. [PubMed: 16135597]
8. Liu X, Zhao Y, Pawlyk B, Damaser M, Li T. Failure of elastic fiber homeostasis leads to pelvic floor disorders. *Am J Pathol.* 2006; 168:519–528. [PubMed: 16436666]
9. Alperin M, Moalli PA. Remodeling of vaginal connective tissue in patients with prolapse. *Curr Opin Obstet Gynecol.* 2006; 18:544–550. [PubMed: 16932050]

10. Kielty CM, Sherratt MJ, Shuttleworth CA. Elastic fibres. *J Cell Sci.* 2002; 115:2817–2828. [PubMed: 12082143]
11. Chen B, Wen Y, Polan ML. Elastolytic activity in women with stress urinary incontinence and pelvic organ prolapse. *Neurourol Urodyn.* 2004; 23:119–126. [PubMed: 14983422]
12. Yamamoto K, Yamamoto M, Akazawa K, Tajima S, Wakimoto H, Aoyagi M. Decrease in elastin gene expression and protein synthesis in fibroblasts derived from cardinal ligaments of patients with prolapsus uteri. *Cell Biol Int.* 1997; 21:605–611. [PubMed: 9570896]
13. Jackson SR, Avery NC, Tarlton JF, Eckford SD, Abrams P, Bailey AJ. Changes in metabolism of collagen in genitourinary prolapse. *Lancet.* 1996; 347:1658–1661. [PubMed: 8642960]
14. Chen B, Wen Y, Yu X, Polan ML. The role of neutrophil elastase in elastin metabolism of pelvic tissues from women with stress urinary incontinence. *Neurourol Urodyn.* 2006; 26:274–279. [PubMed: 17089373]
15. Chen BH, Wen Y, Li H, Polan ML. Collagen metabolism and turnover in women with stress urinary incontinence and pelvic prolapse. *Int Urogynecol J Pelvic Floor Dysfunct.* 2002; 13:80–87. discussion 87. [PubMed: 12054187]
16. Kagan HM, Li W. Lysyl oxidase: properties, specificity, and biological roles inside and outside of the cell. *J Cell Biochem.* 2003; 88:660–672. [PubMed: 12577300]
17. Liu X, Zhao Y, Gao J, Pawlyk B, Starcher B, Spencer JA, Yanagisawa H, Zuo J, Li T. Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nat Genet.* 2004; 36:178–182. [PubMed: 14745449]
18. Romero N, Tinker D, Hyde D, Rucker RB. Role of plasma and serum proteases in the degradation of elastin. *Arch Biochem Biophys.* 1986; 244:161–168. [PubMed: 3511842]
19. Bump RC, Mattiasson A, Bo K, Brubaker LP, DeLancey JO, Klarskov P, Shull BL, Smith AR. The standardization of terminology of female pelvic organ prolapse and pelvic floor dysfunction. *Am J Obstet Gynecol.* 1996; 175:10–17. [PubMed: 8694033]
20. Wen Y, Polan ML, Chen B. Do extracellular matrix protein expressions change with cyclic reproductive hormones in pelvic connective tissue from women with stress urinary incontinence? *Hum Reprod.* 2006; 21:1266–1273. [PubMed: 16452154]
21. Yoshimura K, Nakagawa S, Koyama S, Kobayashi T, Homma T. Roles of neutrophil elastase and superoxide anion in leukotriene B4-induced lung injury in rabbit. *J Appl Physiol.* 1994; 76:91–96. [PubMed: 8175552]
22. Drewes PG, Yanagisawa H, Starcher B, Hornstra I, Csiszar K, Marinis SI, Keller P, Word RA. Pelvic organ prolapse in fibulin-5 knockout mice: pregnancy-induced changes in elastic fiber homeostasis in mouse vagina. *Am J Pathol.* 2007; 170:578–589. [PubMed: 17255326]
23. Kobak W, Lu J, Hardart A, Zhang C, Stanczyk FZ, Felix JC. Expression of lysyl oxidase and transforming growth factor beta2 in women with severe pelvic organ prolapse. *J Reprod Med.* 2005; 50:827–831. [PubMed: 16419630]
24. Wieslander CK, Marinis SI, Keller P, Boreham MK, Drewes PG, Schaffer JI, Word RA. Elastic fiber homeostasis in vaginal tissues from women with pelvic organ prolapse. *Int Urogynecol J.* 2006; 17:S407–S408.
25. Shull BL. Pelvic organ prolapse: anterior, superior, and posterior vaginal segment defects. *Am J Obstet Gynecol.* 1999; 181:6–11. [PubMed: 10411783]

Figure 1. Data from a) protein expression of ATT and b) neutrophil elastase activity in cystocele (C), rectocele (R) and cystocele plus uterine prolapse (CU) groups. Data are presented as the ratio of expression at posterior wall (P) to anterior wall (A).

Figure 2.

Data from quantitative real-time PCR showing mRNA expression of a) ATT, b) NE, and c) LOXL-1 in cystocele (C), rectocele (R) and cystocele plus uterine prolapse (CU) groups. Data are presented as ratio of expression at posterior wall (P) to anterior wall (A).

Table I

Sequences of primers used in quantitative real-time PCR

Primer Name	Primer Sequence
Human Neutrophil Elastase Forward	5'-CAGCAACGTCTGCACTCTC-3'
Human Neutrophil Elastase Reverse	5'-GAGGCAATTCGGTGGATTAGC-3'
Human Alpha-1 Antitrypsin Forward	5'-GGTCAACTGGGCATCACTAAG-3'
Human Alpha-1 Antitrypsin Reverse	5'-TCCCTTTCTCGTCGATGGTCA-3'
Human Lysyl Oxidase-Like 1 Forward	5'-CCAGGGCACAGCAGACTTC-3'
Human Lysyl Oxidase-Like 1 Reverse	5'-GTAGTGGCTGAACTCGTCCA-3'
Human hypoxanthine phosphoribosyl transferase 1 Forward	5'-TGACACTGGCAAAACAATGCA-3'
Human hypoxanthine phosphoribosyl transferase 1 Reverse	5'-GGTCCTTTTCACCAGCAAGCT-3'

Table II

Demographic data of subjects

	Cystocele (n=7)	Rectocele (n=8)	Uterine prolapse and cystocele (n=7)
Age	62.6 (53-72)	43.5 (35-57)	69.3 (56-82)
Parity	3.4 (1-7)	2.6 (1-4)	3.4 (2-9)
BMI	30.4 (26.3-36.0)	23.8 (18.5-33.5)	26.7 (17.6-32.4)
Menopausal status	postmenopausal	premenopausal	postmenopausal

Data are expressed as mean (range).