Published in final edited form as:

Fertil Steril. 2007 October ; 88(4): 1003–1005.

Differential Effects of Aging on Activin A and its Binding Protein, Follistatin, across the Menopause Transition

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NARRATIVE ABSTRACT

To assess the involvement of ovarian-derived regulatory proteins in FSH modulation, we compared FSH, inhibin A, inhibin B, activin A and follistatin in 79 women from the following five groups: young cycling (YC), older cycling (OC), perimenopause (PERI), spontaneous menopause (PM), and surgical menopause receiving estrogen (OVX+ET). Although inhibin B varied as expected by ovarian function, no group differences were observed in activin A, barring a tendency for an increase in PERI, while FS 288 was lower in the PERI, PM and OVX+ET group and negatively correlated with advancing age.

The monotropic rise in FSH in the late reproductive years prior to menopause is accompanied by declining inhibin B (1-3) and increasing activin A (2,3) in the presence of unchanged follistatin (FS) (2), suggesting the potential for a net stimulatory effect on FSH regulation. To what extent such changes reflect ovarian aging is unknown, because these peptides are also produced in multiple sites including the pituitary (4-7). To better assess the importance of ovarian aging in FSH modulation by these regulators, we compared circulating levels of inhibin A, inhibin B, activin A, and the neutralizing activin-binding protein, follistatin (FS), in women with functioning ovaries in varying pre and perimenopausal states to those in women of similar age after spontaneous menopause or ovariectomy while receiving estrogen replacement therapy.

Prior to initiation of the study, approval of the protocol was obtained from the University of Michigan Hospital's institutional review board for use of human subjects. All participants (n = 79) provided written consent. Sixty-three healthy volunteers ages 40–50 yrs were recruited into the following four study groups: older cycling (OC mean age = 45.9 ± 0.8 yrs, n=17), perimenopause (PERI mean age = 49.0 ± 0.6 yrs, n=21), spontaneous menopause (PM mean age = 49.4 ± 0.6 yrs, n = 10), and surgical menopause receiving estrogen (OVX+ET mean age = 49.0 ± 1.6 yrs, n=15). A fifth group of 16 young cycling women (YC mean age = 23.0 ± 0.9 yrs) served as controls. All participants had a body mass index of 20–25, and reported no current medical or psychiatric illness, no current use of sex steroid therapy, no pregnancy or breastfeeding in the past 6 months, no current history of dieting, excessive exercise or smoking. Plasma values for prolactin, testosterone and DHEAS, obtained during the screening visit, were

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within normal female ranges of the reference hospital laboratory. Criteria for the regular-cycling groups included a history of menses every 25–32 days with evidence of presumptive ovulation as determined by a midluteal serum progesterone value above 9.5 nmol/L during the study cycle. For the perimenopausal group, subjects included those with a menstrual cycle in the last 3 months and with at least one menopausal-related complaint. Postmenopausal subjects had experienced their final spontaneous menstrual period at least 12 months prior to study. Women in the ovariectomized group had experienced an elective total hysterectomy for benign causes in the last 5 years and were receiving estrogen replacement therapy. For study purposes, those women taking equine estrogens were placed on the estradiol patch (0.1 mg q 3 days) for 8 wks prior to study. Ovarian status was confirmed by transvaginal ultrasound.

Participants were admitted to the General Clinical Research Center of the University of Michigan Hospital for an overnight study to undergo intensive blood sampling every 10 min for 8 - 24 hrs as part of a study addressing changes in LH pulse characteristics (data not presented here). Cycling subjects were studied on cycle day 5 ± 1 of the follicular phase. Concentrations of FSH, estrogen and FSH regulatory proteins (inhibin A, inhibin B, activin A, FS 288) were measured from blood samples drawn at 0900, 1500 and 0100 and 0500 hrs.

Plasma FSH from the first 52 subjects studied were measured in duplicate by Delfia fluorometric immunoassay (IFMA) (Wallacoy, Turku, Finland, Perkin Elmer Life Sciences, Sorton, OH 44203). Plasma FSH from the remaining 27 subjects was measured using an automated Chemiluminiscent Immulite system (Diagnostic Products Corp, Los Angeles, CA 90045). The sensitivities of the Delfia and Immulite FSH assays were 0.05 IU/L and 1 IU/L, respectively. Overall there was excellent correlation of results measured by Delfia and Immulite for FSH ($r=0.976$, $p<0.001$). Estradiol (E_2) levels were measured using double antibody radioimmunoassay (RIA) kits (Diagnostic Products Corp., Los Angeles, CA, 92626). The sensitivity of the E_2 assay was 18 pmol/L. The intra- and interassay coefficients of variation (CV) were less than 10%. Two-site assays for inhibin A (8), inhibin B (9), activin A (10) and FS 288 (11) were used. The FS 288 assay cross-reacts 9.9% with FS 315, an alternate spliced variant of FS (11). The sensitivities of inhibin A, inhibin B, activin A and FS 288 assays were 3.9, 8.2, 40, and 20.1 pg/ml, respectively. The intraassay CV for all 4 assays averaged less than 10%, with interassay CVs averaging between 13.2 and 18.6%. Hormone values are expressed as international units per liter for FSH calibrated to the Delfia assay, picomoles per liter for E_2 (1 pg = 3.6 pmol), and pg/ml for inhibin A, inhibin B, activin A and FS 288. Group comparisons were conducted using two-tailed nonparametric tests for non-paired observations (Kruskal Wallis ANOVA; post hoc Mann Whitney U). Data are reported as mean + SEM.

Mean FSH and E_2 values were in the expected ranges for the two postmenopausal groups. For cycling women, despite similar estradiol concentrations, mean FSH was higher in the OC and PERI women vs the YC group (Fig 1, top left). Endometrial thickness on cycle day 5 was similar across YC, OC and PERI groups. In contrast, ovarian volumes differed by study group ($p<0.001$): volumes were larger in the YC vs the OC (1316 ± 132 mm vs 971 ± 130 mm, $p=0.02$), lowest in the PM group (514 ± 82 mm), and highest in the PERI group (mean $\pm$ SE = 1551 ± 149 mm) owing to the presence of numerous cysts in 7 of the 19 PERI subjects.

Follicular phase levels of Inhibin A were low and near the assay detection limit in all groups (data not shown). Inhibin B was highest in the YC and OC, reduced in the PERI and near or below detection in PM and OVX+ET groups (Fig 1, top right). In contrast, there were no differences in activin A between groups, barring a tendency for increased activin-A levels in PERI group (Fig 1, bottom left). Circulating FS 288 levels were significantly lower in the PERI, PM and OVX+ET groups vs the YC group ($p<0.03$), but did not differ from each other (Fig 1, bottom right). A significant inverse correlation (-0.31 ; $p<0.01$) was demonstrated between chronologic age and FS 288, but not for the other FSH regulators, or with estrogen.

These findings of similar levels of activin in PM, OVX+E and YC substantiate the view that the ovary, while serving as the major source of inhibin B, contributes very little to the circulating pool of activin. This premise has been suggested by earlier work (12), although not stringently tested across the menopause transition in tandem with its binding neutralizer, FS. In support of earlier findings of a modest increase in activin A across the menopausal transition (2,3), circulating levels of activin A tended to be higher in the PERI group. Taken together, these findings suggest an inverse relationship between inhibin B and activin A during the menopausal transition.

In contrast to the age-related decline in FS observed here using the ultrasensitive FS288 assay (11), we (2) and others (13) have found circulating levels of FS to increase across the menopause transition (2) and with advancing age (13), when measured with an assay for total FS. These discrepant findings are likely due to the much larger contribution of FS315, an alternate spliced variant of FS 288 of pituitary origin, to the overall total FS concentrations in human serum (14). However, as FS 288 levels were lower in PERI, PM and OVX+ET groups compared to YC, an ovarian contribution of FS 288 to the circulating pool cannot be ruled out. Until an FS 315 specific assay is developed, the role of ovarian-derived FS 288 in regulating FSH in relation to pituitary-derived FS 315 (14) will remain unclear.

Considering that both FS and inhibin regulation of FSH are mediated by altered activin signaling (15,16), and the importance of their relative equilibrium for FSH control (5), our findings of a decline in these negative regulators in concert with unchanging levels of activin A point to increased bioavailability of activin during reproductive aging. Although very little free activin has been demonstrated in the circulation (17,18), confirmation of this premise awaits availability of more sensitive assays.

In summary, this cross-sectional study, which for the first time examined all regulators of activin availability in unison, reports that the changes in FSH and inhibin B across the menopause transition are accompanied by a sustained presence of activin A in concert with an age-dependent reduction in its binding protein, follistatin. The differential patterns of secretion in these regulatory proteins, which occur prior to the loss of estradiol negative feedback, are consistent with enhanced activin bioavailability, and potentially a contributory role to the rise in FSH. Longitudinal studies of large cohorts of healthy women across the menopause transition, such as the Study of Women Across the Nation (SWAN) (19) should help shed light on the role of both ovarian and non-ovarian contributors to reproductive aging.

ACKNOWLEDGEMENTS

We wish to thank Alice Rolfes-Curl (hormone assays), Julie Chilimigras (data analysis), and the nursing staff of the General Clinical Research Center. We are especially grateful to the women who served as research participants. This manuscript is dedicated to the memory of Dr. Mei-Yu Yu, a dedicated women's health researcher and friend.

Supported by NIH grants U54 HD29184, NU 01373, 5MO RR00042, AG15083 and Blue Cross-Blue Shield of Michigan Foundation Award

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Figure 1.

FSH, inhibin B, activin A and Follistatin 288 by Study Group. Data are mean $\pm$ SEM. For inhibin B, mean concentrations in PM and OVX+ET groups were near the limits of assay detection, thus SE bars for these groups are not shown. Mean E₂ concentrations are indicated at the top of the FSH panel. Significant group differences (*, **) determined by Kruskal Wallis ANOVA and post hoc Mann Whitney U test.