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ORIGINAL ARTICLE

Inhibin A and B as markers of menopause: a five-year prospective longitudinal study of hormonal changes during the menopausal transition

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Objectives. A more direct and precise hormonal marker of the menopause has been required for some time. The aim of this study was to identify the most accurate marker of the menopause, based on analyses of inhibin A and B, FSH, LH and estradiol (E_2), among 59 healthy women without hormonal treatment during the perimenopause and early postmenopause.
Methods. Fifty-nine women, aged 46–56 years (mean age 51.2 years), were examined annually for 5 years during the menopausal transition and had venous blood drawn simultaneously for later analyses of the above-mentioned hormones.
Results. Inhibin A showed a steady decline from at least 4 years before the final menstrual period (FMP) until 1 year before menopause, whereas inhibin B had a shorter lasting decline from year 3 to year 2 before menopause, concomitant with a rise in FSH and LH.
Conclusion. The present study confirmed previous observations that inhibin A had a continuous decline starting before the decline of inhibin B, suggesting that an increasing part of the cycle was anovulatory. The fall in inhibin B and the increase in FSH constitute markers of ovarian aging. One year prior to menopause neither inhibin A nor inhibin B could be detected. The disappearance of these peptide hormones is an important predictor of the approaching menopause.

Key words: menopausal transition; inhibin A; inhibin B; FSH; LH; E_2

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Serum FSH is usually regarded as the endocrine marker of the menopause. Increasing values of FSH have been considered to be the first sign of the menopausal transition. The FSH levels in postmenopausal women are 10–15 times higher than levels found during the follicular phase in women of reproductive age, whereas LH levels are only three to five times higher (1). The increase in the gonadotropins has been attributed to a fall in follicular steroid production. Recent evidence suggests that changes in inhibin secretion are responsible for an increase in FSH, and may be a direct marker of a decline of ovarian reserve (2,3).

The hormonal events surrounding the transition from regular menstrual cycles to the postmenopausal amenorrhea have only partially been described. In particular, there are few reports on the changes in circulating immunoreactive inhibin concentrations measured longitudinally

in the same subject during the menopausal transition period.

There is current confusion regarding the nomenclature applied to female reproductive senescence, with vague starting points and terms that overlap. The term "perimenopause" is often used to describe the time interval dating from the onset of menstrual irregularity to the menopause itself (4)—this period is currently termed the "menopausal transition," ending with the final menstrual period (FMP). The term menopause is usually defined after 12 months of amenorrhea following the FMP, which reflects a near-complete but natural decrease in ovarian hormone secretion (5).

Both estradiol (E_2) and the peptide hormones inhibins are secretory products of the ovarian granulosa cells, the major cell type of the ovarian follicle. Inhibins are glucoprotein hormones made up of disulfide-linked subunits α linked to either a βA-subunit (inhibin A) or a βB-subunit (inhibin B), and secreted under the influence of FSH and LH (6–10).

While inhibin A appears to be primarily secreted by the mature follicle and corpus luteum (7,9), inhibin B is a product of smaller preovulatory follicles. Both inhibins may act as intraovarian paracrine as well as endocrine factors. As endocrine factors inhibins A and B appear capable of directly suppressing pituitary secretion of FSH. Inhibins are believed to contribute to the regulation of FSH secretion in the normal menstrual cycle, a process that eventually leads to the timely ovulation of a single mature follicle (2,11,12). FSH is postulated to drive the secretion of inhibin B, which in turn restrains FSH (10). In older women, during the early stage of the menopausal transition, inhibin B concentration is significantly lowered in the follicular phase but unchanged or even elevated levels of E_2 and inhibin A in both the follicular and luteal phase are seen (13). Recent studies by Welt et al. (9) concluded that increases in both FSH and E_2 in the early follicular phase resulted in increased inhibin B secretion at early stages of follicle development, whereas the selective LH rise in the late follicular phase favors inhibin A secretion from mature follicles. The pattern of inhibin B and inhibin A secretion in the follicular phase mirrored those of FSH and LH, respectively, suggesting that FSH and LH may differentially regulate inhibin B and inhibin A secretion from small antral and preovulatory follicles. Danforth et al. (3) suggested that in evaluating ovarian aging, correlation of inhibins A and B and FSH levels with time to FMP would provide the strongest test to identify the most accurate markers of the aging ovary. Longitudinal prospective studies that could identify more indicators of ovarian function and reserve have been demanded (14).

This paper reports a prospective longitudinal study of hormonal changes of inhibins A and B, FSH, LH and E_2 during the menopausal transition (and early postmenopause), and focuses on inhibins as predictors of menopause.

Materials and methods

The present study is based on 59 women, who participated in a longitudinal prospective study of menopausal transition among healthy Norwegian women, and has been described previously with respect to recruitment, inclusion and exclusion criteria (15). Venous blood samples were obtained annually for five consecutive years. At enrollment, all of the women were menstruating regularly. None received hormone therapy during the observation period. Blood samples were taken at random during the menstrual cycle between 1000 and 1500 h due to the logistic of the study. To relate the hormonal changes to the menstrual cycle, the serum progesterone was analyzed. The diurnal variations of FSH, LH and E_2 are considered small compared to the cyclic variation throughout the menstrual cycle, and accordingly the time of the day for venous blood sampling was not considered important. The samples were analyzed simultaneously to minimize interassay variations. FSH and E_2 were measured by well-characterized immunoassays as described previously (15).

Both inhibin A and B were analyzed according to standard procedures at the Department of Growth and Reproduction at Rigshospitalet in Copenhagen, Denmark. Inhibins A and B were determined using specific two-site enzyme immunometric assays from Oxford Bio-Innovation Ltd, Oxford, UK. These assays are essentially identical to the immunoassays developed by Groome et al. (16). The detection limit of the inhibin A assay was 5 pg/L and the intra- and interassay coefficients of variation were $< 11\%$ and $< 5\%$, respectively. In the inhibin B assay the detection limit was 14 pg/L and the intra- and interassay coefficients of variation were $< 12\%$ and $< 17\%$, respectively. The detection limits were defined as the value corresponding to the mean $\pm$ 2 SD OD of the 0-standard.

Statistics

The data for inhibins A and B were not normally distributed, mainly because the majority of postmenopausal values were below the level of detection. Hence data are given as medians and interquartile range (QD) and nonparametric statistical methods were adopted. Spearman rank correlation was applied for examining the relation between variables, and the Kruskal–Wallis test for multigroup comparison. The Wilcoxon signed rank test was applied to examine the changes in hormonal levels between adjacent years, and the *p*-values were adjusted by Bonferroni's rules (individual *p*-values were multiplied by number of comparisons). *p*-values < 0.05 were considered statistical significant. The variable "difference in years between sampling and menopause" was designated "time," and included $(-4, -3, \ldots, +3)$.

Results

This study focuses on the hormone values before the final menstrual period and the early

postmenopause. The age distribution was close to normal. Mean age at the start of the study was 51.2 years (SD 2.1, range 47.3–55.7), and at menopause 52.9 years (SD 2.1, range 48.9–58.7). The numbers of individuals participating each year were 18 (year − 4), 36 (year − 3), 51 (year − 2), 56 (year − 1), 59 (year 0), 41 (year + 1), 23 (year + 2) and 8 (year + 3). The corresponding numbers of individuals with luteal phase progesterone (> 20 nmol/L) were 1, 5, 2, 1, 0, 0 and 0. Inhibins A and B were examined in relation to FSH, LH, E_2 and "time." The median values and QD of FSH, LH, E_2 and inhibins A and B are shown in Table I.

Figure 1 demonstrates the median values of inhibins A and B, FSH, LH and E_2 during the menopausal transition and early postmenopause.

A statistically significant all-over group difference between the different years was found for both inhibin A and inhibin B (Kruskal–Wallis test, $p < 10^{-6}$). This was further illustrated by Spearman rank correlation between the variable "time" and the hormone values, which indicated a statistically significant increase in serum FSH and LH and a concomitant fall in E_2 and inhibins during the observation period before FMP. Disregarding the period from menopause to the last assessment (years 0, +1, +2 and +3), when the portion of nondetectable inhibin was substantial, we found $r = 0.4970$, 0.5203, −0.2911, −0.4063 and −0.3022 for FSH, LH, E_2, inhibin A and inhibin B, respectively ($p < 0001$).

When examining the changes between individual years, a significant decline in inhibins A and B and a significant increase in the levels of FSH and LH were seen from year −3 to year −2 before menopause (Wilcoxon test, Bonferroni corrected p-values < 0.01, $0.05, < 0.02$ and < 0.02, respectively). In the same period no significant decline in the hormonal levels of E_2 was found.

The median inhibin B value was below the level of detection from two years before menopause. The median inhibin A value, by contrast, was detectable until 1 year before menopause, and also showed a different pattern, a steady decline at least from four years before menopause (Table I). However, a certain but decreasing percentage of the women still had detectable values of inhibin even after menopause (Table II).

Table I. Serum concentrations, median and interquartile range (in parentheses) of FSH, LH, E_2, inhibin A and inhibin B from 59 women without hormone treatment during the menopausal transition and postmenopause. Data from 4 years before to 3 years after the final menstrual period (FMP) are shown. Year 0 = FMP. nd = not detectable

Year	No. of women	FSH (IU/L)	LH (IU/L)	E_2 (nmol/L)	Inhibin A (ng/L)	Inhibin B (ng/L)
−4	18	5.6 (10.5)	7.6 (9.0)	0.33 (0.4)	41 (66)	21 (67)
−3	36	11.6 (16.9)	10.2 (10.2)	0.30 (0.5)	26 (30)	23 (42)
−2	51	25.7 (30.5)	21.3 (18.8)	0.2 (0.4)	12 (27)	nd
−1	56	41.2 (40.2)	29.7 (15.2)	0.09 (0.3)	nd	nd
0	59	55.0 (41.4)	33.1 (15.3)	0.06 (0.1)	nd	nd
1	41	68.1 (36.5)	33.2 (18.4)	0.05 (0.03)	nd	nd
2	23	66.1 (42.1)	34.6 (17.6)	0.04 (0.03)	nd	nd
3	8	66.6 (38.4)	39.6 (14.5)	0.04 (0.03)	nd	nd

Discussion

The present study provides a longitudinal picture of the changes in inhibins A and B, FSH, LH and E_2 during the menopausal transition and early postmenopause in healthy women. The substantial decrease in inhibins in the last 2 years before menopause makes these peptides important predictors of the approaching menopause. Increasing values of FSH and LH were observed from 3 years before menopause (Fig. 1), and indicate that the women were in a late reproductive stage.

The observation that a percentage of women had inhibin A and B levels below the detection limit (13 and 37%, respectively) four years before menopause could partly be explained by limitations in assay sensitivities (16), or could indicate that the samples were taken late in the luteal phase or early in the follicle phase where levels normally are low or not detectable. The progesterone values were analyzed to determine whether women with luteal phase progesterone would contribute to the inhibin values. Only a few women (1, 5, 2, 1 and 0, corresponding to years −4, −3, −2, −1 and 0) had luteal phase progesterone. When calculating the values of progesterone and inhibin the values were almost identical, and accordingly did not alter the results.

Our data on inhibins A and B confirm those of Burger et al. (17), who also documented a substantial fall in the levels of inhibins A and B before the FMP. They further postulated that the falling levels of inhibin A as the final menses approaches are caused by a progressively larger proportion of menstrual cycles becoming anovulatory, with failure of development of the dominant follicle. Hence it is hypothesized that only those women who continue to ovulate close to the menopause would contribute significant inhibin A levels to the population mean. The marked decline in follicular number suggests that the most important explanation for the hormonal change lies within the ovary (18). Current interpretations of the sources of inhibins A and B are in accordance with the concept that inhibin A is primarily a secretory product of the dominant follicle and the corpus luteum, and inhibin B of small antral follicle in the recruited cohort (19,20). The most characteristic morphological

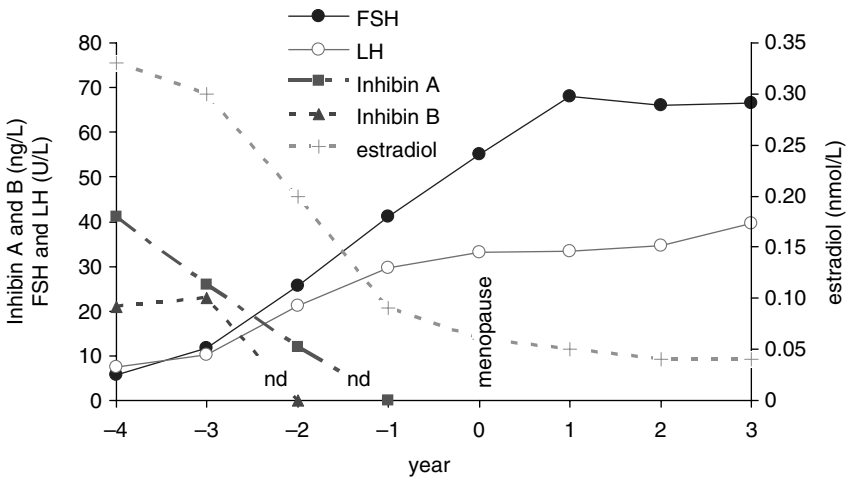

Fig. 1. FSH, LH, estradiol (E_2), inhibin A and B (median) values during the menopausal transition. Year 0 marks the final menstrual period (FMP). The values of inhibin A after year −2 and of inhibin B after year −3 were below the detection limits of 5 and 14 ng/L, respectively, as indicated by not detectable (nd) in the figure

change in the ovary with increasing age is a progressive decline in ovarian follicle number (21). By the time of menopause few, if any, primordial follicles can be found in the ovaries of normal women. The observation that estrogen levels did not show any significant decline between the third and second year before the FMP in our study while FSH had a significant increase indicates that inhibin in the early menopausal transition is the dominant hormone to suppress FSH. Later in the menopausal transition E_2 and also inhibin play a significant role in the ovarian hypothalamic feedback system. Examination of granulosa cells from the follicles of women with declining ovarian reserve who were undergoing assisted reproductive techniques has revealed a reduced number of cells per follicle, less steroid production per cell, less inhibin production per cell, decreased proliferation, and increased apoptosis compared with granulosa cells from the follicles of younger women (14). The proposition that inhibin B is produced by immature follicles and its level is increased following FSH stimulation has led to the hypothesis that inhibin B measurements during the early follicular phase may be a marker of the hormonally responsive follicle reserve in the ovary, which may be useful in a clinical context. This proposal has been supported by studies that showed a significant positive correlation between inhibin B levels early in *in vitro* fertilization (IVF) treatment and cycles and the number of oocytes obtained at oocyte retrieval (22). Thus, the ovarian production of inhibin is a direct marker of ovarian aging, whereas pituitary FSH secretion is an indirect marker, and inhibin could be an early indicator of declining ovarian reserve (14,21,23).

Table II. Percentage of detectable inhibin A and B levels during the menopausal transition and early postmenopause (see text)

Year	Percentage of women with detectable inhibin A	Percentage of women with detectable inhibin B
−4	87	63
−3	86	63
−2	26	38
−1	25	29
0	22	27
1	17	17
2	4	8

The present study confirmed previous observations that an increasing number of the cycles become anovulatory, resulting in a continuous decline of inhibin A. It is only those women who continue to ovulate close to the FMP who contribute a significant amount of inhibin A. Inhibin B, being a product of antral follicles, was not detectable later than 2 years before menopause, suggesting that the cohort of antral follicles was small and a substantial number of women had few, if any, follicles remaining at the time of menopause. With respect to evaluating ovarian aging, the decrease in inhibin A and B and the increase in FSH levels seem to provide the strongest test to identify the most accurate markers of the aging ovary, and this has clinical implications in evaluating the follicular reserve in the ovary. The significant correlation of inhibins A and B and FSH levels with "time" confirmed that these hormones are clinically important predictors of the preceding menopause.

Although hormone measurements were based on blood samples taken once a year, with little control over when in the menstrual cycle samples were taken and at what time of day, our results clearly demonstrate that there is a significant age-dependent decline starting at least 4 years before menopause.

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