Control of estradiol secretion in reproductive ageing

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BACKGROUND: Estradiol (E\(_2\)) concentration is preserved in older reproductive-aged women despite a decrease in follicle number and androstenedione (AD) levels. We hypothesized that increased aromatase activity accounts for E\(_2\) preservation in older women. METHODS: Older (36–46 years; \(n = 11\)) and younger (21–35 years; \(n = 10\)) women with 25- to 35-day menstrual cycles participated in a parallel design study. Daily blood samples were drawn starting at menses, and recombinant human FSH (rhFSH), 150 IU, was administered when the dominant follicle’s diameter was ≥16 mm. FSH, LH, E\(_2\), estrone (E\(_1\)), AD and the AD/E\(_1\) ratio were compared. RESULTS: E\(_2\) and E\(_1\) concentrations and the E\(_1\)/E\(_2\) ratio were similar across the follicular phase in older compared with younger women, whereas AD and the AD/E\(_1\) ratio were lower. Older women had higher FSH concentrations in the early follicular phase and fewer small follicles. RhFSH-stimulated changes in E\(_1\) were similar between older and younger subjects despite the smaller number of follicles. CONCLUSIONS: These findings suggest that E\(_2\) secretion is maintained by increased aromatase function in older compared with younger reproductive-aged women, whereas there is no apparent difference in 17β-hydroxysteroid dehydrogenase activity. The increased aromatase is probably driven by increased FSH in the early follicular phase and compensates for the decreased follicle number in older reproductive-aged women.

Key words: androstenedione/aromatase/FSH/ovary

Introduction

Although reproductive ageing was previously thought of as a state of estrogen deficiency, more recent studies have shown that estradiol (E\(_2\)) concentrations in the follicular phase are the same or higher in women over 35 years of age with regular menstrual cycles compared with their younger counterparts (Reyes et al., 1977; Lee et al., 1988; Klein et al., 1996a; Santoro et al., 1996; Burger et al., 1998; Reame et al., 1998; Welt et al., 1999b). Reproductive ageing is also associated with an early follicular phase increase in FSH that is accounted for by a decrease in negative feedback from inhibin B (Klein et al., 1996b; Reame et al., 1998; Santoro et al., 1999; Welt et al., 1999b). The reciprocal changes in FSH and inhibin B mark the decrease in follicle number that also occurs at approximately 35 years of age (Block, 1952; Richardson et al., 1987; Gougeon et al., 1994). Thus, early in reproductive ageing, E\(_2\) secretion is spared and even increased in the face of decreased follicle number as reflected by decreased inhibin B and increased FSH.

Reproductive ageing is also associated with lower levels of androstenedione (AD) and testosterone. Approximately, half of circulating AD, the precursor of testosterone, derives from the ovary and half from the adrenal gland (Judd, 1976). Although there is a known decrease in adrenal androgen secretion that begins in women aged >20 years (Orentreich et al., 1984; Labrie et al., 1997), there is also evidence that ovarian androgen secretion is decreased in older reproductive-aged women. AD peaks at the midcycle in normal women (Mushayandebvu et al., 1996; Adams et al., 2004), and this midcycle peak remains in the face of dexamethasone suppression of adrenal androgen secretion (Abraham, 1974). Previous studies have reported that AD levels are lower in the early- and mid-follicular phases of older compared with younger reproductive-aged women and fail to rise at midcycle (Mushayandebvu et al., 1996; Piltonen et al., 2003; Davison et al., 2005). Thus, the contribution of ovarian AD appears to decrease during reproductive ageing.

AD is converted to estrone (E\(_1\)) by aromatase and is subsequently converted to E\(_2\) by 17β-hydroxysteroid dehydrogenase (HSD) (Brally et al., 1981). Thus, the relative concentration of E\(_1\) and AD can be used to approximate aromatase function. Decreased AD in association with unchanged or increased E\(_2\) concentrations in older compared with younger reproductive-aged women suggests that ovarian aromatase activity increases during reproductive ageing. However, previous studies have not concomitantly examined E\(_1\), E\(_2\) and AD concentrations across ageing in women with regular menstrual cycles.

To further investigate the changes in ovarian steroidogenesis and aromatase function in older compared with younger reproductive-aged women, we measured AD, E\(_2\) and E\(_1\) in the early and late follicular phases. Recombinant human FSH (rhFSH) was administered in the presence of a single, preovulatory-sized follicle, and recombinant human FSH (rhFSH), 150 IU, was administered when the dominant follicle’s diameter was ≥16 mm. FSH, LH, E\(_2\), estrone (E\(_1\)), AD and the AD/E\(_1\) ratio were compared. RESULTS: E\(_2\) and E\(_1\) concentrations and the E\(_1\)/E\(_2\) ratio were similar across the follicular phase in older compared with younger women, whereas AD and the AD/E\(_1\) ratio were lower. Older women had higher FSH concentrations in the early follicular phase and fewer small follicles. RhFSH-stimulated changes in E\(_1\) were similar between older and younger subjects despite the smaller number of follicles. CONCLUSIONS: These findings suggest that E\(_2\) secretion is maintained by increased aromatase function in older compared with younger reproductive-aged women, whereas there is no apparent difference in 17β-hydroxysteroid dehydrogenase activity. The increased aromatase is probably driven by increased FSH in the early follicular phase and compensates for the decreased follicle number in older reproductive-aged women.

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follicle to determine the ovarian AD, E₂, and E₁ responses to an acute and standardized FSH stimulus. The resulting change in the AD/E₁ ratio was examined as an indirect indicator of aromatase function in older and younger women.

**Methods**

**Subjects**

Subjects included ten younger (21–35 years) and eleven older (36–45 years) reproductive-aged women, with documentation of regular menstrual cycles between 25 and 35 days in length and an ovulatory luteal phase progesterone >19.1 nmol/l. Subjects were in good general health with normal thyroid-stimulating hormone (TSH) and prolactin levels, on no medications for at least 2 months before the study and with no evidence of androgen excess. The study protocol was approved by the Partners Human Research Committee, and all subjects gave written informed consent before participation.

**Protocol**

Starting on the day of menses, blood was sampled daily for measurement of FSH, LH, AD, E₂, and E₁. Ultrasound examinations were performed at baseline, on day 7 and then every 2–3 days until the maximum follicle diameter was ≥16 mm. A single dose of rhFSH (150 IU) was then administered subcutaneously. Blood samples were drawn immediately before administration of rhFSH and then every 6 h for 24 h for measurement of FSH, E₂, E₁ and AD. A final ultrasound and blood draw were performed at 48 h to document follicle size and to determine whether ovulation had occurred.

**Assays**

Serum LH, FSH and E₂ were measured using a two-site monoclonal non-isotopic system according to the manufacturer’s directions (AxSYM, Abbott Laboratories, Abbott Park, IL, USA), as previously described (Welt et al., 1999a, 2003). LH and FSH levels are expressed in IU per litre as equivalents of the Second International Pituitary Standard 80/552 for LH and the Second International Pituitary Reference preparation 78/549 for FSH. The interassay coefficients of variation (CVs) for the LH assay were 5.3, 5.5 and 7.4% for quality control sera containing 5.6, 26.2 and 69.0 IU/l, respectively. The interassay CVs for the FSH assay were 6.9, 7.1 and 6.3% for quality control sera containing 4.3, 35.4 and 79.5 IU/l, respectively. The interassay CVs for the E₂ assay were 9.2, 5.4 and 9.6% at E₂ concentrations of 312.0, 1101 and 2570 pmol/l, respectively. Serum E₁ was measured using a non-isotopic system according to the manufacturer's directions (Welt et al., 1999a, 2003). LH and FSH levels are expressed in IU per litre as equivalents of the Second International Pituitary Standard 80/552 for LH and the Second International Pituitary Reference preparation 78/549 for FSH. The interassay coefficients of variation (CVs) for the LH assay were 5.3, 5.5 and 7.4% for quality control sera containing 4.3, 35.4 and 79.5 IU/l, respectively. The interassay CVs for the FSH assay were 6.9, 7.1 and 6.3% for quality control sera containing 4.3, 35.4 and 79.5 IU/l, respectively. The interassay CVs for the E₂ assay were 9.2, 5.4 and 9.6% at E₂ concentrations of 312.0, 1101 and 2570 pmol/l, respectively. Serum E₁ was measured using a RIA (Diagnostic Systems Laboratories, Webster, TX, USA). The interassay CVs were 9.6 and 4.1% for quality control sera containing 126 and 1084 pmol/l. Serum AD was measured using a RIA (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA, USA), as previously described (Adams et al., 2004). The interassay CVs were 8.7 and 6.2% for quality control sera containing 3.63 and 16.8 nmol/l.

**Data analysis**

The greatest number of 2–10 mm follicles in a single plane and the number of follicles >10 mm on ultrasound were documented in both ovaries on the day of FSH administration. Mean FSH, LH, AD, E₂, and AD concentrations from days 1–5 of the follicular phase as well as the FSH, LH, AD, E₂, and AD concentrations, the molar AD/E₁ ratio, follicle number and the number of follicles >10 mm on the day of rhFSH administration were compared in older and younger reproductive-aged women using unpaired t-tests. The E₁/E₂ ratio was used as a marker of 17β-HSD activity and also compared between older and younger women using unpaired t-tests. FSH, E₂, AD and the AD/E₁ and E₁/E₂ ratios on days 1–5 and on the day of rhFSH administration were also compared within groups using one-way repeated measures analysis of variance (ANOVA). One younger and three older subjects had an LH surge within the 24 h after rhFSH, whereas four younger and five older subjects had an LH surge within the 48 h after rhFSH administration. For those with an LH surge within the first 24 h, results during and after the LH surge were removed from the analysis of the response to FSH. Responsiveness to rhFSH was expressed as the difference between peak and baseline for FSH, E₂, and E₁ and the difference between the nadir and the baseline for AD and the AD/E₁ ratio in the 24 h following administration of rhFSH. Results were compared between younger and older women using unpaired t-tests. The E₁, E₂ and AD concentrations were also divided by the total follicle number in a single plane on ultrasound and compared between the two groups using unpaired t-tests. Finally, the percent change in FSH, E₂, E₁, AD and the AD/E₁ ratio after rhFSH administration was examined using a two-way, repeated measures ANOVA.

All data are expressed as mean ± SEM and a P level of <0.05 is considered significant.

**Results**

BMI in the older reproductive-aged women tended to be higher than in the younger women but was not significantly different (P = 0.052). Cycle length was not different between older and younger women (Table I). As expected, mean FSH concentrations on days 1–5 in the follicular phase were higher in older compared with younger reproductive-aged women (Table I and Figure 1). There was no difference in mean LH, E₁ or E₂ on days 1–5 between the two groups, and there was no difference in the E₁/E₂ ratio. However, AD levels and the AD/E₁ ratio were lower in the older group (P < 0.05).

The pattern of changes across the follicular phase was similar between the two groups for FSH, which decreased from days 1–5 to the day of FSH administration, and for LH, E₁ and E₂, all of which increased over that time period. The E₁/E₂ ratio in the early follicular phase was not different between the younger and older women (1.24 ± 0.1 and 1.39 ± 0.17, respectively). The E₁/E₂ ratio decreased from the early to the late follicular phase (P < 0.001), compatible with increased 17β-HSD activity across the follicular phase, but this response was not different between the young and older women (0.61 ± 0.07 and 0.56 ± 0.06, respectively). In contrast, AD increased from the early to late follicular phase in the younger but not in the older women, whereas the AD/E₁ ratio decreased in the older but not in the younger women (Figure 1).

| Table 1. Patient characteristics and mean hormone concentrations on days 1–5 in the early follicular phase |
|------------------|------------------|
| **Younger**     | **Older**        |
| Age (years)     | 25.7 ± 1.5       | 40.2 ± 1.0*   |
| BMI (kg/m²)     | 22.7 ± 0.7       | 25.7 ± 1.3    |
| Cycle length (days) | 26.1 ± 0.9     | 27.8 ± 0.9    |
| FSH (IU/l)      | 6.5 ± 0.1        | 8.6 ± 0.5*    |
| LH (IU/l)       | 4.9 ± 0.3        | 5.1 ± 0.2     |
| E₁ (pmol/l)     | 212.9 ± 24.7     | 190.9 ± 8.1   |
| E₂ (pmol/l)     | 180.5 ± 19.1     | 159.0 ± 21.9  |
| AD (mol/l)      | 8.7 ± 1.0        | 5.5 ± 0.6*    |
| AD/E₁           | 44.8 ± 3.9       | 30.6 ± 3.1*   |

*P < 0.05 older versus younger reproductive-aged women.
Hormone concentrations and follicle size before and after recombinant human FSH administration to women. Of note, FSH decreased, and LH, E1 and E2 increased between the early and late follicular phases within younger and older groups. In contrast, AD increased only within the younger group, and the AD/E1 ratio decreased only within the older group.

On the day of FSH administration, there was no difference in LH, FSH, E1 or E2 between older and younger reproductive-aged women, but AD and the AD/E1 ratio were lower in older women (Table II and Figure 1). The size of the dominant follicle was matched between the two groups (17.3 ± 0.5 mm versus 17.5 ± 0.6 mm) on the day of FSH administration. There were fewer follicles on ultrasound in older compared with younger subjects; however, there was no difference in the number of follicles with diameter >10 mm.

Although the FSH and E1 responses to rhFSH administration were virtually identical in the older and younger subjects (Table II and Figure 2), the increase in E2 (P < 0.05) and the decreases in AD (P < 0.001) and the AD/E1 ratio (P < 0.01) were attenuated in the older group. When expressed as a function of the number of follicles, the increases in E2 (42.8 ± 10.1 versus 38.0 ± 4.1 pmol/l/follicle for older and younger, respectively; P = 0.3) and E1 (15.3 ± 4.3 versus 7.6 ± 1.4 pmol/l/follicle; P = 0.1) in response to rhFSH and the decrease in AD (0.2 ± 0.05 versus 0.3 ± 0.07 pmol/l/follicle; P = 0.1) were not different between the two groups (Figure 3).

Within the 48 h surrounding the administration of rhFSH, five older and four younger subjects ovulated as indicated by a peak LH level that reached >36.2 IU/l (Adams et al., 1994). This late follicular phase increase in LH was evident during the 24 h after rhFSH administration and was greater in older compared with younger women (P < 0.05) in conjunction with increased AD and the AD/E1 ratio in older compared with younger women (Figure 2).

**Figure 1.** Mean FSH, estrone (E1), estradiol (E2), LH androstenedione (AD) and the AD/E1 ratio on days 1–5 and on the day of rhFSH stimulation (150 IU) in younger (black bars) and older (white bars) reproductive-aged women. *P < 0.05 between older and younger women. Of note, FSH decreased, and LH, E1 and E2 increased between the early and late follicular phases within younger and older groups. In contrast, AD increased only within the younger group, and the AD/E1 ratio decreased only within the older group.

**Table II.** Hormone concentrations and follicle size before and after recombinant human FSH administration

<table>
<thead>
<tr>
<th></th>
<th>Baseline pre-FSH</th>
<th>Maximum or minimum post-FSH</th>
<th>Δpost-FSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>LH (IU/l)</td>
<td>Older</td>
<td>7.2 ± 1.1</td>
<td>10.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>6.9 ± 1.0</td>
<td>9.0 ± 1.9</td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>Older</td>
<td>5.4 ± 0.5</td>
<td>7.9 ± 0.4</td>
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<tr>
<td></td>
<td>Younger</td>
<td>5.0 ± 0.2</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>E1 (pmol/l)</td>
<td>Older</td>
<td>341.3 ± 36.0</td>
<td>438.3 ± 34.5</td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>299.3 ± 30.6</td>
<td>391.1 ± 42.2</td>
</tr>
<tr>
<td>E2 (pmol/l)</td>
<td>Older</td>
<td>679.8 ± 114.5</td>
<td>905.3 ± 91.8</td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>578.9 ± 103.1</td>
<td>1066.8 ± 127.8</td>
</tr>
<tr>
<td>AD (nmol/l)</td>
<td>Older</td>
<td>6.6 ± 0.7*</td>
<td>5.6 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>11.2 ± 1.0</td>
<td>7.0 ± 1.0</td>
</tr>
<tr>
<td>AD/E1</td>
<td>Older</td>
<td>22.8 ± 3.2*</td>
<td>15.2 ± 2.4*</td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>40.1 ± 3.6</td>
<td>22.0 ± 1.9</td>
</tr>
<tr>
<td>Follicle number</td>
<td>Older</td>
<td>6.5 ± 0.7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>13.5 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Follicles with diameter &gt;10 mm</td>
<td>Older</td>
<td>1.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Younger</td>
<td>1.6 ± 0.3</td>
<td></td>
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</table>

*P < 0.01 older versus younger reproductive-aged women.

Discussion

Although it is now recognized that follicular phase E2 levels are maintained or increased during reproductive age, few studies have investigated the mechanism by which this occurs in the face of a decrease in the number of follicles contributing to total E2 output as demonstrated by anatomical studies (Block, 1952; Richardson et al., 1987; Gougeon et al., 1994) and by ultrasound in the current and previous studies (Scheffer et al., 1999). E2 secretion is also preserved despite decreased ovarian AD production, as suggested by the lower AD levels in the early follicular phase in this study and others and by the failure of AD to rise in the midcycle of older reproductive-aged women in this study and others (Mushayandebvu et al., 1996; Piltonen et al., 2003; Davison et al., 2005). Finally, we now provide evidence for a decrease in the AD/E1 ratio with reproductive age. Taken together, the preserved E2 and E1 concentrations in the face of decreased follicle number and AD concentrations along with the low AD/E1 ratio suggest that aromatase function is more active in older compared with younger reproductive-aged women, thus explaining the maintenance of E2 production in the older group.
In this study, aromatase activity appears to be increased in older women in both the early follicular phase, as indicated by the lower AD concentrations, similar E_2 and E_1 concentrations and a lower AD/E_1 ratio on days 1–5 and in the late follicular phase. It is unlikely that these findings are related to the slightly higher BMI in older women, because peripheral aromatization contributes little to circulating serum estrogen concentrations in premenopausal women (Kopelman et al., 1980; Zumoff et al., 1981; Leenen et al., 1994). Increased ovarian aromatase activity is also suggested by the more marked rise in E_2 after hCG stimulation in older women despite decreased AD precursor secretion (Piltonen et al., 2003) and by the increased urinary E_1 conjugate excretion in older compared with younger reproductive-aged women (Santoro et al., 1996). Finally, the E_2/E_1 ratio was not different in older compared with younger reproductive-aged women, suggesting that increased 17β-HSD activity does not account for the maintenance of E_2 levels with reproductive ageing. However, future studies that address the quantity and activity of aromatase are needed to confirm this hypothesis and to determine whether changes in aromatase function are evident in the dominant follicle or are contributed primarily by small antral follicles both early and later in the follicular phase.

The control of aromatase is both complex and tissue specific (Bulun et al., 2005). Although growth factors and cytokines have been implicated in the control of aromatase in other tissues, this appears not to be the case for ovarian aromatase, which is exquisitely sensitive to FSH stimulation of the cyclic AMP signalling pathway (Bulun et al., 2005). Thus, the increase in aromatase activity in the early follicular phase is most likely because of the higher FSH concentration in older women as there was no difference in LH to drive substrate availability (Falck, 1959). FSH also stimulates follicle growth with an exponentially increasing number of granulosa cells. With follicle growth, E_2 production increases FSH receptor number with the potential of further augmenting aromatase activity. We hypothesize that the early follicular phase increase in FSH in older women sets the stage for the continued increase in aromatase activity in the late follicular phase that was observed in this study. As in the early follicular phase, LH was not different between the two groups in the late follicular phase; however, we cannot rule out the potential contribution of other factors to aromatase function in the late follicular phase that have yet to be identified (Mendelson et al., 2005).

The question of whether the increase in FSH in older women is necessary to maintain E_2 levels in older cycling women has been addressed in a previous study in which endogenous E_2 secretion was suppressed using a GnRH agonist and a fixed dose of exogenous FSH was added back (Hansen et al., 2005). E_2 concentrations were similar in older and younger reproductive-aged women during identical FSH stimulation leading the authors to conclude that higher FSH levels are not required to maintain E_2 secretion at normal levels in older women. Although not statistically significant, FSH appeared to be less suppressed by the GnRH agonist at baseline in the older group (Hansen et al., 2005), leaving the possibility that older women had greater baseline FSH stimulation. Furthermore, there was a trend towards an increased E_2 response to FSH in the older group when expressed in relation to the number of follicles with diameter >10 mm. Unfortunately, AD levels were not measured in this study and an estimate of aromatase activity was not possible.

Despite the potentially greater aromatase activity across the follicular phase in older women, the response to an identical exogenous FSH stimulation was not consistent with greater aromatase function. However, the interpretation of these results is confounded by the greater increase in LH during the 24 h after rhFSH administration in the older compared with the younger women. Although a similar number of older and younger subjects had a documented midcycle LH surge during the 48 h after rhFSH administration, the LH surges in older
Subjects occurred slightly earlier within the 48 h time frame and were associated with higher LH levels leading up to the surge. The initial increase in the AD/E1 ratio is compatible with LH stimulation of AD precursor in older women. The decreases in AD and the AD/E1 ratio and the rise in E1 24 h after rhFSH suggest that the additional AD was then converted to E1; however, a longer follow-up is needed to make this determination. Further studies in which both FSH and LH are controlled will be required to address the question of whether aromatase function is more responsive to FSH in older compared with younger women.

In summary, E2 secretion is preserved in older reproductive-aged women in the face of decreased AD concentrations and decreased follicle number. Although there is no evidence of increased 17β-HSD activity in older cycling women, the lower AD/E1 ratio across the follicular phase is consistent with increased aromatase activity. It is likely that increased early follicular phase FSH drives the increase in aromatase activity across the follicular phase in older cycling women, preserving E2 levels, but further studies will be required to confirm this hypothesis.

Acknowledgements
We thank Christopher Lui, BS for his help in recruiting subjects for this study. We also thank the members of the Reproductive Endocrine Unit Laboratory for their assay expertise. This work was supported by the National Institutes of Health RO1HD042708 and National Center for Research Resources General Clinical Research Centers Program grant M01-RR-01066.

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Submitted on December 4, 2005; resubmitted on March 7, 2006; accepted on April 4, 2006.

Estradiol secretion in ageing