Influence of age and sex on serum concentrations of total dimeric activin A

Paola Loria1, Felice Petraglia2, Mauro Concari1, Marco Bertolotti1, Stefano Luisi1, Chiarina Grisolia1, Carlo Forresta4, Annibale Volpe2, Andrea R Genazzani5 and Nicola Carulli1

1Department of Internal Medicine, 2Department of Obstetrics and Gynecology, 3Service of Endocrinology, Università di Modena, Modena, Italy, 4Department of Internal Medicine, Università di Padova, Padova, Italy and 5Department of Obstetrics and Gynecology, Università di Pisa, Pisa, Italy

(Correspondence should be addressed to P Loria, Dipartimento di Medicina Interna, Università degli Studi di Modena, Policlinico, Via del Pozzo, 71, 41100 Modena, Italy)

(F Petraglia is now at Università di Udine, Udine, Italy)

Abstract
Several studies have shown that activin A is secreted in substantial amounts into the systemic circulation. The changes that occur during menstrual cycle and pregnancy suggest a correlation with reproductive function. At present, however, no definitive evidence has confirmed this pattern throughout adult life; moreover, neither the origin nor the physiological implications of this circulating growth factor have been clearly defined. The aim of the present study was to evaluate whether circulating concentrations of activin A change in adult men and women according to age and sex, and to examine the possible correlation with serum concentrations of FSH. Total dimeric activin A was measured using a specific two-site enzyme immunoassay in serum specimens collected from a cohort of normal individuals enrolled in an epidemiological survey. A group of men (n = 106) and one of women (n = 151) were subdivided into six age groups (20–30, 30–40, 40–50, 50–60, 60–70 and 70–90 years). In a small group of 8 men and 11 women, serum concentrations of activin A were evaluated twice, in specimens collected at an interval of 10 years. Serum FSH concentrations were also measured in all specimens.

Serum concentrations of activin A were not significantly different in men and women and showed an age-related progressive increase between 20 and 50 years of age (P < 0.01, those aged 40–50 compared with those aged 20–30 years). After the age of 50 years, activin A concentrations remained in the same range of values in women, whereas they increased significantly in men, reaching peak values between 70 and 90 years (P < 0.01 compared with the group aged between 20 and 50 years). From the age of 50 years, activin A concentrations were significantly greater in men compared with those in women in the corresponding age groups (P < 0.001). Activin A concentrations correlated with age in men, but not in women. No significant correlation between concentrations of activin A and FSH was found in either sex. Activin A concentrations in specimens collected 10 years apart showed an increase in seven of eight men, but not in women. Finally, no significant variations of activin A concentrations were observed when fertile and postmenopausal women were compared.

The present data indicate that circulating concentrations of activin A vary according to age; furthermore, men older than 50 years have greater concentrations than women. These changes, which occur irrespectively of FSH concentrations, indicate that circulating activin A is not a hormone of the reproductive axis.

European Journal of Endocrinology 139 487–492

Introduction
Activin A is a dimeric protein that was initially isolated and cloned in ovarian follicular fluid (1, 2). Because its major bioactivity was its capacity to increase the release of follicle-stimulating hormone (FSH) from cultured pituitary cells, it was considered to be a member of the hypothalamus–pituitary–gonadal axis (3–5). This hypothesis was in part modified when it was proven that activin A belonged to the transforming growth factor β (TGF-β) family and showed some biological properties of growth factors (6). Indeed, expression of activin A β-subunit mRNA was found in several organs other than gonads. Organs such as brain, liver, thymus, kidney, adrenal and pituitary gland, placenta and bone marrow produce activin A, and it has been shown to exert a biological effect in them (7, 8).

Specific assays for detecting activin A in biological fluids have recently been developed (9, 10) and early studies have shown that serum concentrations of dimeric activin A vary slightly throughout the menstrual cycle (11), and that pregnant women have the greatest serum concentrations (12, 13). However, while substantial amounts of data indicate that the major
source of activin A in pregnancy is the placenta (14), no clear evidence exists as to the source of activin A in non-pregnant subjects.

The findings of Harada et al. (15) indicated a significant increase in serum activin A in some pathological conditions – hyperthyroidism, chronic renal failure, liver cirrhosis and advanced hard cancers – but this does not help in clarifying the origin of circulating activin A. Furthermore, this study showed that serum concentrations of activin A increase with age, but any relationship with FSH concentrations was not investigated (15).

The aim of the present study was to evaluate serum concentrations of activin A in a large number of healthy adult men and women over a wide age range, and to investigate possible correlations with serum FSH concentrations.

Patients and methods

Serum samples were collected from two sources. Samples for the age group 30–70 years were obtained from a cohort of individuals enrolled in an epidemiological survey investigating the prevalence and incidence of gallstone disease in Italy (Multicentric Italiana Coléitiiasi – MICOL) (16). Briefly, the enrolled population completed a questionnaire and underwent physical examination and ultrasonographic screening for the presence of gallstones. This screening was performed in 1984 and repeated in 1993. On both occasions, a blood sample was collected. Among the cohort studied in 1993, we selected 256 serum samples from healthy volunteers (151 women and 106 men), selected on the basis of the following criteria: no clinical history of chronic inflammatory, neoplastic or metabolic diseases; normal biochemical tests and physical examination. None of the subjects was taking medication, and the women were non-pregnant nor using hormonal contraception or hormone replacement therapy. At the 1984 screening, serum samples from only 8 men and 11 women were available and sufficient for analysis.

Serum specimens for the age groups 20–30 and 80–90 years were obtained from a serum bank available in our department. These blood samples were collected between 1993 and 1994 from normal individuals: clinical history, biochemical parameters and physical examination were negative and comparable to those of the MICOL study.

All blood samples, taken after an overnight fast, were left to clot and after centrifugation serum was collected and stored immediately at −80°C.

Table 1 shows the distribution of the population studied among the different age groups.

**Activin A assay**

Total concentration of dimeric activin A was measured using a specific two-site enzyme immunoassay as described previously (10, 11) (purchased from Serotec, Oxford, UK). Briefly, in each assay, standard and samples were diluted as appropriate and mixed with an equal volume of distilled water containing 10% SDS. After 3 min at 100°C the tubes were cooled before the addition of a freshly prepared solution of hydrogen peroxide. After an additional period of incubation at room temperature, duplicate aliquots of denatured and oxidized samples/standards were transferred to antibody-coated microtitre plates. These plates were incubated at room temperature, overnight. After washing with a phosphate buffer, 50 µl alkaline phosphatase-conjugated extravidin was added, and the plates were incubated for 2 h. The plates were then washed, and bound alkaline phosphatase was quantified by means of a commercially available enzyme immunoassay amplification kit (Immuno Select ELISA Amplification System, Dako, Milan, Italy) which was used in accordance with the supplier’s instructions. The limit of detection for activin A was 10 pg and the intra- and interassay coefficients of variation (CVs) were 5.0% and 9.0% respectively. The assay crossreacts with activin B, inhibin A, inhibin B, follistatin and α2 macroglobulin by less than 0.5%.

### Table 1: Serum concentrations of activin A in the patients studied.

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Activin A (ng/ml)</td>
<td>n</td>
<td>Activin A (ng/ml)</td>
<td>p</td>
</tr>
<tr>
<td>20–30</td>
<td>18</td>
<td>0.50 ± 0.17</td>
<td>10</td>
<td>0.52 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>30–40</td>
<td>20</td>
<td>0.55 ± 0.28</td>
<td>26</td>
<td>0.63 ± 0.10</td>
<td>NS</td>
</tr>
<tr>
<td>40–50</td>
<td>50</td>
<td>0.77 ± 0.21</td>
<td>25</td>
<td>0.75 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>50–60</td>
<td>31</td>
<td>0.58 ± 0.13</td>
<td>15</td>
<td>1.05 ± 0.17</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>60–70</td>
<td>32</td>
<td>0.59 ± 0.10</td>
<td>14</td>
<td>1.11 ± 0.20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>70–90</td>
<td>20</td>
<td>0.67 ± 0.10</td>
<td>16</td>
<td>1.09 ± 0.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total</td>
<td>151</td>
<td>0.64 ± 0.21</td>
<td>106</td>
<td>0.84 ± 0.26</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are expressed as means ± s.d. n, number of patients studied. Statistical analysis was performed by Student’s t-test for unpaired data compared with the same age group of different sex.
FSH assay

Serum concentrations of FSH were determined using the Auto Delphia h-FSH kit (Wallak, Turku, Finland). The assay had a limit of detection of 0.05 U/l; mean intra- and interassay CVs were 3% and 1.5% respectively.

Statistical analysis

The data are expressed as the mean ± S.D., with n indicating the number of samples. Differences between the means in different age groups were analysed by analysis of variance (ANOVA). Student’s t-test for unpaired or paired data was used where appropriate. The relationship between activin A concentration and age, and activin A and FSH concentrations was calculated by linear regression analysis. A P value <0.05 was regarded as being statistically significant.

Results

Values of serum activin A concentrations in the different age intervals in the total population of men and women studied are illustrated in Table 1.

As shown in Fig. 1, in both sexes mean activin A values progressively increased in the first three decades studied (20–30, 30–40 and 40–50 years); furthermore, the mean value of the age group 40–50 years was significantly greater than that found in the age group 20–30 years (P<0.05). After age 50 years, activin A concentrations in women remained in the same range up to the latest decade (70–90 years), whereas in men the concentrations showed a further increase with age, reaching greatest values at 50–60 years (P<0.05). Table 1 shows that there were no differences in the mean values of serum concentration of activin A between the two sexes from age 20 to age 50 years. After age 50 up to age 90 years, activin A concentration in men was greater than that in women, when the same age groups were compared (P<0.01).

When groups were distinguished on the basis of the presence of a regular ovulatory function (data collected from the epidemiological survey questionnaire), serum concentrations of activin A did not appear to differ significantly between fertile (0.57 ± 0.19 ng/ml; n = 38) and postmenopausal women (0.67 ± 0.23 ng/ml; n = 47, from 3 to 10 years of amenorrhea).

A direct relationship between age and activin A concentrations was found in men, but not in women (Fig. 2), whereas no correlation between serum activin A and FSH concentrations was found in either men (Fig. 3a) or women (Fig. 3b).

Longitudinal evaluation on serum samples collected ten years apart in 19 individuals showed that activin A concentrations increased with advancing age in men (n = 8; 0.65 ± 0.14 compared with 0.98 ± 0.19; P<0.02), but not in women (n = 11; 0.66 ± 0.31 compared with 0.62 ± 0.10; NS) (Fig. 4).

Discussion

This study has demonstrated for the first time that normal men from the age of 50 years onwards have greater serum concentrations of activin A than have
age-matched women. From this age, serum concentrations of activin A in men remain significantly greater than those in women until the age of 90 years. Mean values in men during the last three decades are two- to threefold greater than in the 20–30-year age group. Our findings are partly in accordance with those presented by Harada et al. (15), who demonstrated an age-related increase in serum activin A concentrations in both men and women. However, whereas in this study no difference was found between men and women in the age-related variation in activin A concentration, we have found that serum activin A concentrations were greater in men than in women, reaching a statistically significant difference after the age of 50 years. These variations may be due to the different assay adopted, or to the different number of subjects included in each age group in the two studies. In this regard, it may be hypothesized that the lower number of samples per age group examined in the study by Harada et al. (15), could have concealed the age-related variation, because of the high variability of serum activin A concentrations in humans in different age groups.

The question that remains unanswered concerns the possible source(s) of circulating activin A. Even though it is generally accepted that inhibin A and inhibin B have a testicular origin in men (17, 18) and an ovarian origin in women (17, 19–21), the present findings seem to exclude this hypothesis for activin A. Our study did, indeed, show that serum concentrations of activin A in men and women are not related to FSH concentration throughout adult life; furthermore, no differences exist between fertile and postmenopausal women. It has been proven that serum inhibin concentrations become undetectable in postmenopausal women (22). These findings clearly suggest that gonads are therefore not the major source of circulating activin A in both sexes. This conclusion does not contradict the finding by Muttukrishna et al. (11) of biphasic changes in serum activin A concentrations at midcycle and early follicular and late luteal phases, thus suggesting that activin A has an important role in the recruitment of follicles for...
suggest that the secretion of these proteins is most probably part of a systemic homeostatic reaction, and thus reflects a series of local paracrine/autocrine events that occur in the various organs.

The evidence that activin A circulates with a sex- and age-related concentration profile is clearly the major outcome of the present study: however, its origin and the physiological implications remain to be elucidated in further studies.

References


11 Muttukrishna S, Fowler PA, George L, Groome NP & Knight PG. Changes in peripheral serum levels of total activin A during the human menstrual cycle and pregnancy. Journal of Clinical Endocrinology and Metabolism 1996 81 3328–3334.


17 Lambert-Messerlian GM, Hall Jr, Sluss PM, Taylor AE, Martin KA, Groome NP et al. Relatively low levels of dimeric inhibin circulate


Received 25 June 1998
Accepted 6 August 1998