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# Lipoprotein(a) changes during natural menstrual cycle and ovarian stimulation with recombinant and highly purified urinary FSH

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**This prospective, randomized, controlled study compared the effects of recombinant human FSH (r-hFSH) and highly purified urinary FSH (u-hFSH HP) on lipoprotein(a) [Lp(a)] concentrations in women undergoing ovarian stimulation. Fifty infertile women were randomly allocated into two equally sized treatment groups ($n = 25$ per group). Thirty normal ovulation women were recruited as controls. The infertile women received u-hFSH or r-hFSH 150 IU/day starting on cycle day 2. From cycle day 6 the dose was adjusted according to ovarian response. Human chorionic gonadotrophin 10 000 IU was administered once there was at least one follicle ≥18 mm in diameter. The luteal phase was supported with progesterone 50 mg/day for at least 15 days. Repeated measurements of Lp(a) concentrations were performed during both stimulated and natural cycles. A significant increase in luteal phase Lp(a) concentrations was detected in the stimulated cycles, whereas no significant changes in serum Lp(a) concentrations were observed during natural cycles. There were no significant differences between the urinary and recombinant FSH effects on serum Lp(a). The luteal Lp(a) increase was transitory because after 1 month Lp(a) concentrations returned to baseline values if pregnancy failed to occur; in pregnant women persistent increased Lp(a) concentrations were found at the 8th week. The percentage changes in serum Lp(a) were positively correlated with the luteal progesterone increase ($r = 0.40$, $P < 0.05$), but not with follicular or luteal oestradiol increase. The women with low baseline Lp(a) (≤5 mg/dl) had a greater increase of the Lp(a) concentrations at midluteal phase than women with baseline Lp(a) >5 mg/dl. In conclusion, the recombinant or urinary hFSH administration does not directly influence Lp(a) concentrations. The luteal Lp(a) increase in stimulated cycles is not related to gonadotrophin treatment *per se*, but appears to be related to the high luteal progesterone concentrations, physiologically or pharmacologically determined. Our results also suggest that the sensitivity to the progesterone changes could be related to apolipoprotein(a) phenotype.**

*Key words:* highly purified urinary FSH/lipoprotein(a)/ovarian stimulation/progesterone/recombinant FSH

## Introduction

Lipoprotein(a) [Lp(a)], which was first described by Berg (1963), differs from low density lipoprotein by an additional large protein, apoliprotein(a) [apo(a)], disulphide-linked to an apo B-100 apoprotein (Murai *et al*., 1986; Marcovina and Morrisett, 1995; White and Lanford, 1995). Several studies have suggested that Lp(a) is a strong independent risk factor both for ischaemic heart disease and for cerebrovascular disease (Uterman, 1989; Heinrich *et al*., 1991; Scanu, 1992; Cremer *et al*., 1994; Schaefer *et al*., 1994; Dahlen and Stenlund, 1997; Schwartzman *et al*., 1998). Other prospective data have found no relation between Lp(a) and coronary heart disease, but these studies comprised only male subjects (Ridker *et al*., 1993; Cantin *et al*., 1998). In women, case-control studies (Dahlen *et al*., 1986; Labeur *et al*., 1992; Solymoss *et al*., 1993; Farrer *et al*., 1994; Wang *et al*., 1994; Bolibar *et al*., 1995; Sunayama *et al*., 1996; Orth-Gomer *et al*., 1997) and a prospective study (Bostom *et al*., 1994) have shown that Lp(a) is associated with coronary heart disease. Lp(a) concentrations are largely genetically determined, primarily by sequence polymorphisms in the apo(a) gene lipoprotein (Boerwinkle *et al*., 1992; Lackner *et al*., 1993; Mooser *et al*., 1995). However, there is evidence to suggest that previous genetic studies may have underestimated the contribution of non-genetic factors to the variation in Lp(a) concentrations in the population (Maeda *et al*., 1989; Von Eckardstein *et al*., 1997).

Premenopausal women have lower Lp(a) concentrations than postmenopausal women in cross-sectional studies (Heinrich *et al.*, 1991; Jenner *et al.*, 1993; Kim *et al.*, 1994). Lp(a) serum concentrations increase after a natural or surgical menopause (Heinrich *et al.*, 1991; Meilahn, *et al.*, 1991; Kim *et al.*, 1994).

The administration of oral oestrogens in postmenopausal women has been shown to reduce Lp(a) concentrations (Farish *et al.*, 1996; Haines *et al.*, 1996; Kim *et al.*, 1996; Hänggi *et al.*, 1997; Espeland *et al.*, 1998; Spencer *et al.*, 1999). No significant changes have been reported with the use of transdermal oestradiol, suggesting that the effects of orally administered oestrogens may stem from their capacity to influence hepatic metabolic processes (Hänggi *et al.*, 1997; Spencer *et al.*, 1999). Few studies are available on Lp(a) changes in women undergoing ovarian stimulation (Seed *et al.*, 1990, 1991; Haines *et al.*, 1997); these studies evaluated the effects of urinary human menopausal gonadotrophin (HMG) and conflicting results were reported. No data concerning the effects of more recent gonadotrophin preparations on Lp(a) serum concentrations have been published.

The aim of this study was to evaluate the effects of highly purified urinary FSH (u-hFSH HP) and recombinant human FSH (r-hFSH) treatment on Lp(a) concentrations in a prospective randomized study.

## Materials and methods

### *Patients*

Fifty infertile Caucasian women undergoing ovarian stimulation for assisted reproduction were enrolled in the study between January and December 1999. Inclusion criteria were: aged between 28 and 38 years; normal ovulatory cycles; FSH, LH, prolactin, testosterone-free and dehydroepiandrosterone sulphate within normal range on cycle day 3; primary infertility due to unexplained factor or male factor; no previous treatment with clomiphene citrate, gonadotrophins, sexual steroids or drugs known to influence lipoprotein metabolism.

Exclusion criteria included: chronic diseases, body mass index $<18$ or $>27$ kg/m$^2$; past or current abuse of alcohol; $>5$ cigarettes per day; regular intake of drugs.

Thirty comparable volunteer Caucasian nulliparous women were concurrently recruited as controls. Inclusion criteria were: aged between 28 and 38 years; regular menstrual cycles lasting from 26 to 30 days; previous three cycles of regular length; no administration of sex steroids or drugs known to affect lipid metabolism within the previous 12 months. The exclusion criteria were the same as for infertile subjects.

### *Study design*

This was an open, randomized, controlled study comparing the effect of u-hFSH and r-hFSH on Lp(a) serum concentrations. After confirming eligibility for the study, the infertile women were randomly allocated into two equally sized treatment groups ($n = 25$ per group) using a random number generator on a personal computer. Thirty non-randomized, comparable women were included in the untreated control group. Informed consent was obtained from each subject before the start of the study. The study was conducted in accordance with the guidelines proposed in The Declaration of Helsinki.

### *Study protocol*

Starting on cycle day 2, infertile patients received u-hFSH (Metrodin HP; Serono, Rome, Italy) or r-hFSH (Gonal F; Serono, Rome, Italy) 150 IU/day. From cycle day 6, dose adjustment was performed, according to follicular development, as assessed by ultrasound scanning. Human chorionic gonadotrophin (HCG) 10 000 IU was administered to induce ovulation when at least one follicle $\geqslant 18$ mm in diameter was present. The luteal phase was supported with 50 mg progesterone in oil (Prontogest, Amsa, Italy) daily i.m. beginning 3 days after HCG administration and continuing for at least 15 days. On the 18th day after HCG administration, a pregnancy test was performed. If positive, progesterone was continued for 14 days.

Blood samples for measurement of serum Lp(a), oestradiol and progesterone, were taken from all subjects on cycle day 2 before starting gonadotrophin administration, on cycle day 6, on the day of HCG administration, and 10 days after HCG administration. In patients who failed to achieve pregnancy, Lp(a) serum concentration measurements were repeated on day 2 of the following menstruation, providing one month of wash-out. In patients who achieved pregnancy measurements of Lp(a), oestradiol and progesterone serum concentrations were repeated after a comparable period at the 8th week. In the control group, blood was collected on cycle day 2. To standardize the different cycle lengths the timing of the successive blood samples was calculated personally. The presumed first day of the next menstruation, estimated on the basis of the length of the previous three cycles, was taken as day 0. The second, third and fourth blood samples were taken on days –21, –14, –7. The fifth sample was collected on cycle day 2 of the second successive menstrual cycle.

Each blood sample was collected between 0800 and 0900 h after a 12 h fast.

### *Laboratory analyses*

Blood was taken from an antecubital vein with minimal stasis using standard venepunture techniques into serum tubes. Serum samples were separated by centrifugation at 3000 *g* for 10 min and stored at –80°C until analysis. Each subject's sample was analysed in the same assay to minimize interassay variation. All samples were tested for Lp(a), 17β-oestradiol and progesterone concentrations. Lp(a) measurements were determined by means of a commercial enzyme-linked immunosorbent assay kit (Macra® Lp(a) manufactured by Strategic Diagnostics Inc. for Trinity Biotech USA, Jamestown, NY, USA); the test is a sandwich assay which utilizes both a monoclonal antibody and polyclonal antibodies which specifically bind to the apolipoprotein (a) moiety of Lp(a). All samples were run in duplicate and the results were expressed in mg/dl; the intra- and interassay coefficients of variation for this method were $<5\%$ and $<10\%$ respectively. Serum 17β-oestradiol and progesterone were determined by means of a solid-phase, ligand-labelled, competitive chemiluminescent immunoassay with an Immulite Analyzer (Immulite® Estradiol and Immulite® Progesterone, Diagnostic Products Corporation, Los Angeles, CA, USA) and results were expressed in pg/ml and ng/ml respectively.

### *Statistical analyses*

Given the skewed Lp(a) distribution, non-parametric statistics were used to protect against non-normal distribution of data. Statistical analyses of all baseline parameters for between-group differences were performed by means of Kruskal-Wallis analysis of variance (ANOVA) test. The $\chi^2$-test was used to compare categorized measures when appropriate. Multiple comparisons of paired data during natural and stimulated cycles were performed with Friedman's repeated measures test followed by Dunn's multiple comparisons post-hoc test. Statistically significant differences between the stimulated and control groups were examined by the Mann-Whitney *U*-test. Spearman's rank correlation coefficient was used to assess correlations between parameters. Statistical significance was assigned when $P < 0.05$.

**Table I.** Anthropometric data and baseline lipoprotein(a) [Lp(a)] concentrations

| Characteristics | Controls ($n$ = 30) | r-hFSH (Gonal-F) ($n$ = 25) | u-hFSH HP (Metrodin HP) ($n$ = 25) | $P$-value[a] |
|---|---|---|---|---|
| Age (years) | 33.3 ± 4.1 | 34.6 ± 3.3 | 35 ± 3 | NS |
| Weight (kg) | 59.7 ± 8.1 | 58.5 ± 7.5 | 58.7 ± 7.8 | NS |
| Height (cm) | 165 ± 5 | 163 ± 6 | 166 ± 6 | NS |
| Body mass index ($kg/m^2$) | 21.7 ± 2.5 | 21.7 ± 2.3 | 21.4 ± 2.5 | NS |
| Baseline Lp(a) (mg/dl) | 10.3 ± 9.1 | 8.2 ± 11.6 | 13.4 ± 16.3 | NS |

Values are means ± SD.
[a]Statistics for between-group differences were determined by Kruskal-Wallis analysis of variance.
r-hFSH = recombinant human follicle stimulating hormone; u-hFSH HP = urinary human FSH; NS = not significant.

**Table II.** Infertility characteristics of patients enrolled and randomized to treatment with human FSH

| | r-hFSH (Gonal-F) ($n$ = 25) | u-hFSH HP (Metrodin HP) ($n$ = 25) | $P$-value[a] |
|---|---|---|---|
| Duration of infertility (years, mean ± SD) | 3.6 ± 1.7 | 4.1 ± 1.8 | NS |
| Causes of infertility (%) | | | |
| Unexplained factor | 56 | 48 | NS |
| Male factor | 44 | 52 | NS |

[a]Statistics for between-group differences were determined by Mann-Whitney test and $\chi^2$-test.

r-hFSH = recombinant human follicle stimulating hormone; u-hFSH HP = urinary human FSH; NS = not significant.

## Results

No significant differences were seen between the three groups with respect to age, body mass indices, or mean baseline Lp(a) (Table I). The infertility characteristics of the two treatment groups were not significantly different (Table II). There were no significant differences in the stimulation characteristics of the patients receiving HCG (Table III). The pregnancy rate cycle was 24% for r-hFSH and 20% for u-hFSH (not significantly different). In the control group two subjects had an anovulatory cycle and were excluded from the analysis. No significant changes in serum Lp(a) concentrations during the different phases of the menstrual cycle were observed in the control group, whereas a significant increase in luteal phase Lp(a) concentrations was detected in the stimulated women (Table IV, Figure 1). There were no significant differences between the urinary and recombinant FSH effects on serum Lp(a) (Figure 2). The luteal Lp(a) increase can be considered transitory because after 1 month Lp(a) concentrations returned to baseline values if pregnancy failed to occur; in pregnant women ($n$ = 11) persistently increased Lp(a) concentrations were found at the 8th week (Figure 3). The percentage changes (versus baseline) in serum Lp(a) were not significantly correlated with the increase in serum oestradiol on HCG day

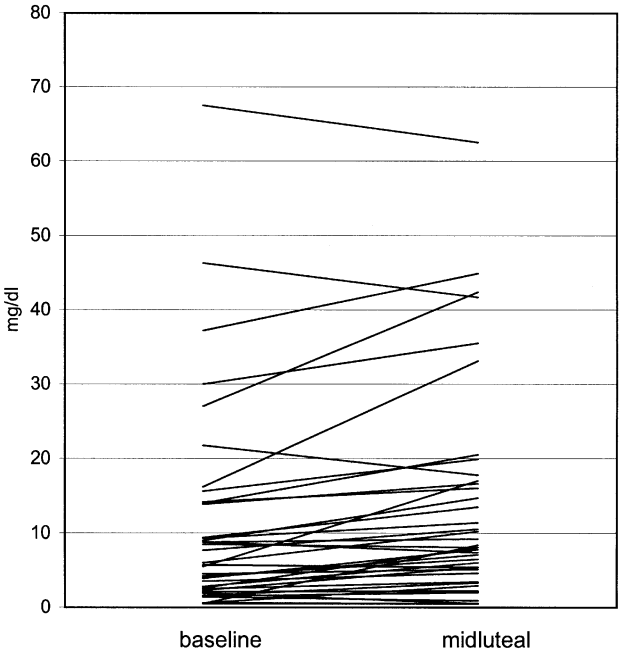


**Figure 1.** Differences in concentrations of lipoprotein (a) in the baseline and midluteal phase of stimulated cycles.

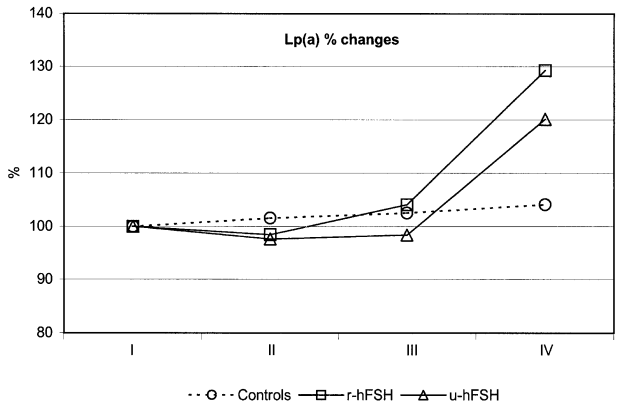


**Figure 2.** Percentage changes in serum lipoprotein [Lp(a)] during natural and stimulated cycles. Blood samples were taken at different time points. I = baseline; II = cylce day 6; III = human chorionic gonadotrophin day; IV = midluteal. Friedman repeated measures test: controls, not significant; recombinant human (r-h)FSH, $P < 0.001$; urinary human (u-h)FSH, $P < 0.01$.

($r$ = -0.28; $P > 0.05$) or at midluteal phase ($r$ = 0.07; $P > 0.05$). There was a positive correlation between the percentage changes (versus baseline) in serum Lp(a) concentrations and the increase in serum progesterone at midluteal phase ($r$ = 0.40, $P < 0.05$) (Figure 4). To evaluate the sensitivity of Lp(a) to progesterone increase, we divided the treatment group into two subgroups classified according to a baseline serum Lp(a) lower ($n$ = 24) or higher ($n$ = 24) than 5 mg/dl. There was a significant correlation between the percentage changes of the Lp(a) concentrations (versus baseline) and the midluteal progesterone increase in women with baseline Lp(a) <5 mg/dl ($r$ = 0.60, $P < 0.05$) (Figure 5), but not in women with baseline Lp(a) >5 mg/dl ($r$ = 0.25, $P > 0.05$) (Figure 6). Furthermore, although the midluteal progesterone mean concentrations were not statistically different, the percentage

**Table III.** Stimulation characteristics of patients receiving human chorionic gonadotrophin (HCG)

| | r-hFSH (Gonal-F) ($n$ = 24) | u-hFSH HP (Metrodin HP) ($n$ = 24) | $P$-value[a] |
|---|---|---|---|
| Days of FSH stimulation required | 7.7 ± 2 | 6.6 ± 1 | NS |
| Total dose of FSH required (no. of FSH 75 IU ampoules) | 13.4 ± 4.3 | 12.5 ± 3.3 | NS |
| No. of follicles ⩾16 mm diameter on day of HCG | 3.0 ± 2.3 | 3.6 ± 2.5 | NS |
| Oestradiol on day of HCG (pg/ml) | 745 ± 467.9 | 796.6 ± 377.6 | NS |
| Oestradiol at midluteal phase (pg/ml) | 397.9 ± 307.7 | 412.9 ± 272.8 | NS |
| Progesterone at midluteal phase (ng/ml) | 38.4 ± 18.1 | 38.1 ± 17.7 | NS |

Values are means ± SD.
[a]Statistics for between-group differences were determined by Mann-Whitney test.
r-hFSH = recombinant human follicle stimulating hormone; u-hFSH HP = urinary human FSH; NS = not significant.

**Table IV.** Lipoprotein(a) changes (mg/dl; mean ± SD) during natural menstrual cycle and ovarian stimulation with recombinant (r-hFSH) or highly purified urinary (u-hFSH) human FSH

| Group | I Baseline | II Cycle day 6 | III HCG day | IV Midluteal |
|---|---|---|---|---|
| r-hFSH ($n$ = 24) | 8.2 ± 11.6[a] | 8.1 ± 11.0[b] | 8.5 ± 10.3[c] | 10.6 ± 12.7[abc] |
| u-hFSH ($n$ = 24) | 13.4 ± 16.3[d] | 13.1 ± 16.1[e] | 13.2 ± 14.2[f] | 16.2 ± 16.3[def] |
| Controls ($n$ = 28) | 10.3 ± 9.1 | 10.4 ± 9.6 | 10.5 ± 9.6 | 10.6 ± 9.6 |

[a]$P < 0.05$; [b]$P < 0.001$; [c]$P < 0.05$; [d]$P < 0.05$; [e]$P < 0.01$; [f]$P < 0.05$ (by Dunn's multiple comparison test).
HCG = human chorionic gonadotrophin.

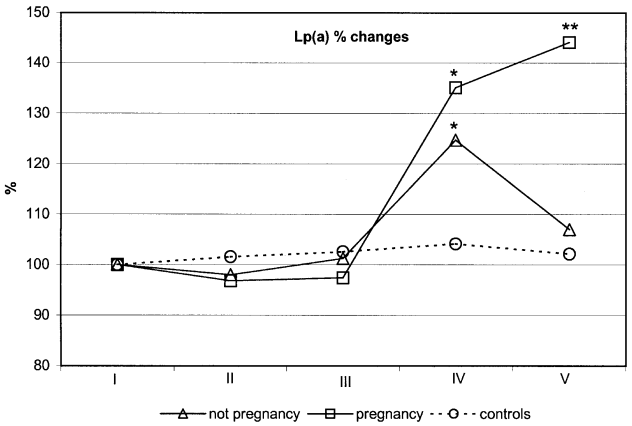


**Figure 3.** Percentage lipoprotein [Lp(a)] changes in pregnant and non-pregnant women who underwent ovarian stimulation. Blood samples were taken at different time points. I = baseline; II = cylce day 6; III = human chorionic gonadotrophin day; IV = midluteal. *I versus IV, $P < 0.05$; **I versus V, $P < 0.01$ (Dunn's multiple comparisons test).

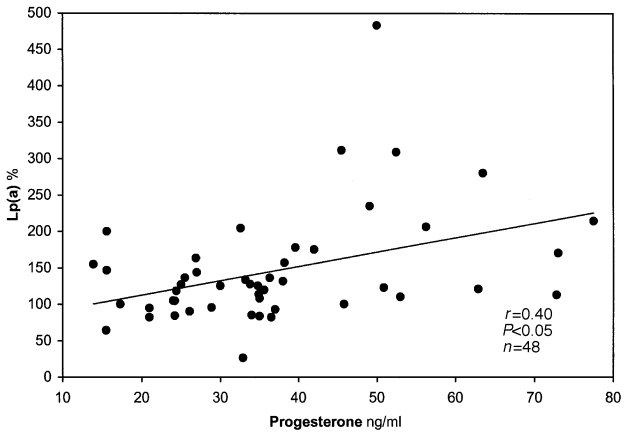


**Figure 4.** Correlation between midluteal progesterone and lipoprotein [Lp(a)] percentage change in total samples.

changes (versus baseline) in serum Lp(a) concentrations at midluteal phase were greater in women with baseline Lp(a) <5 mg/dl than in women with baseline Lp(a) >5 mg/dl (Figure 7).

## Discussion

Although the number of women who undergo ovarian stimulation with gonadotrophins has grown rapidly in the last 20 years, the long-term effects of these treatments have not been fully addressed. Furthermore, during these years, different gonadotrophin preparations have been developed: until the late 1970s, urinary-derived gonadotrophin preparations contained FSH in combination with LH whereas later purified FSH (with <1% LH contamination) or highly purified FSH (with <0.1% LH contamination) were introduced. Recently, 99% pure FSH preparations produced by recombinant DNA technology have become available. Recombinant technology ensures there is no LH activity and no contaminating urinary proteins of undetermined origin (Olijve *et al.*, 1996). Previous studies, aimed at assessing whether gonadotrophin administration can adversely affect cardiovascular risk, evaluated changes in Lp(a) serum concentrations during ovarian stimulation (Seed *et al.*, 1990, 1991; Haines *et al.*, 1997). Lp(a), in fact, has been

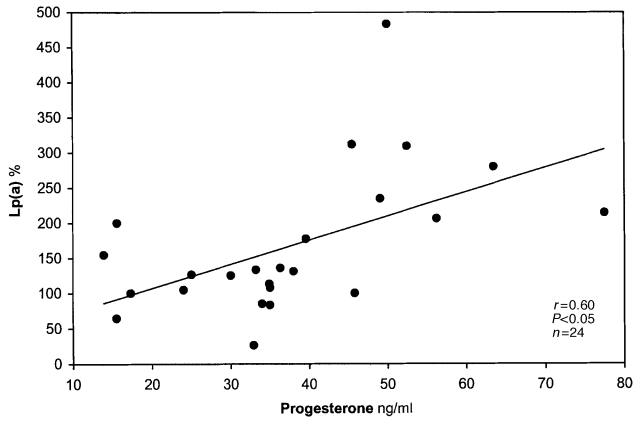


**Figure 5.** Correlation between midluteal progesterone and lipoprotein [Lp(a)] percentage change in the subgroup of women with baseline Lp(a) <5 mg/dl.

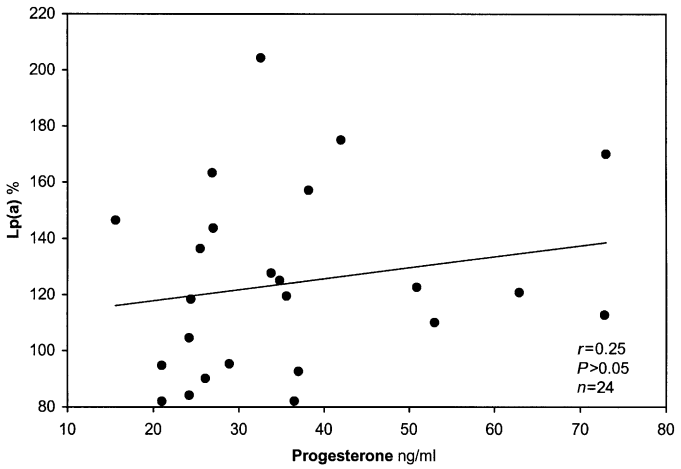


**Figure 6.** Correlation between midluteal progesterone and lipoprotein [Lp(a)] percentage change in the subgroup of women with baseline Lp(a) >5 mg/dl.

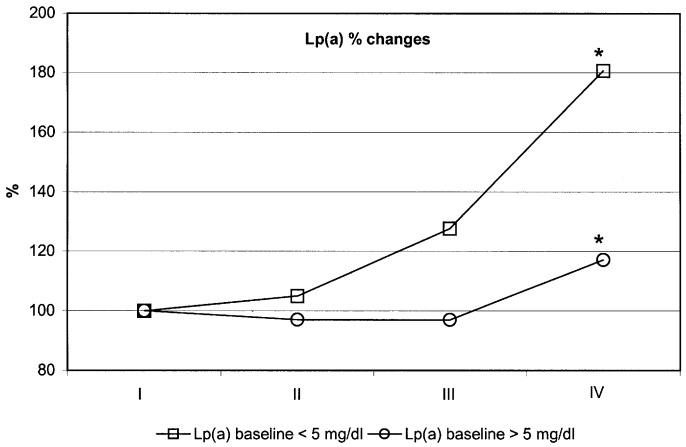


**Figure 7.** Percentage changes in lipoprotein [Lp(a)] at midluteal phase in stimulated women with baseline Lp(a) >5 or <5 mg/dl. Blood samples were taken at different time points. I = baseline; II = cylce day 6; III = human chorionic gonadotrophin day; IV = midluteal. *$P < 0.05$ (Mann-Whitney *U*-test).

reported to be an independent risk factor both for ischaemic heart disease and for peripheral vascular disease.

In two small series, respectively 22 and 20 women undergoing ovarian stimulation, the effects of HMG administration (Seed *et al*., 1990, 1991) were evaluated. An increase, albeit not statistically significant, probably because of the low number of subjects investigated, in Lp(a) median concentration from 3 to 9 mg/dl and from 6.4 to 7.9 mg/dl at the end of the HMG administration was observed. Unfortunately, luteal Lp(a) changes were not considered, thus the whole effect of HMG administration could not be evaluated.

In this study, we have shown that FSH administration does not directly influence serum Lp(a) concentrations. No increase in Lp(a) concentrations was observed during the follicular phase of r-hFSH or u-hFSH stimulated cycles. The different effects of HMG and uFSH on Lp(a) might be due to the LH content of HMG. In hypogonadotrophic women, HMG significantly increases follicular phase androgens when compared with u-hFSH HP (Couzinet *et al*., 1988). However, no conclusive data regarding androgen influence on Lp(a) concentrations are available. No significant correlations were found between Lp(a) and testosterone concentrations in myocardial infarction patients and healthy controls (Marques-Vidal *et al*., 1995). In normal men, parenteral testosterone reduced Lp(a) concentrations (Zmuda *et al*., 1996), whereas, in males suffering from prostatic carcinoma, parenteral testosterone administration lowered Lp(a) concentrations by 20%, while orchidectomy increased Lp(a) concentrations by 20% (Henriksson *et al*., 1992; Berglund *et al*., 1996). In patients with hypogonadism Lp(a) concentrations did not change significantly following parenteral testosterone or gonadotrophin treatment (Ozata *et al*., 1996). No data have been reported on the relationship between Lp(a) and androgens in women. Alternatively, gonadotrophin-releasing hormone (GnRH) analogue administration before starting HMG treatment might influence Lp(a) concentrations. In young volunteer males, pituitary suppression by the GnRH antagonist Cetrorelix was associated with a pronounced increase in Lp(a) concentrations (Von Eckardstein *et al*., 1997), although buserelin reduced concentrations of Lp(a) by 48% in elderly males suffering from cancer of the prostate (Arrer *et al*., 1996). Unfortunately, in Seed's studies (Seed *et al*., 1990, 1991) Lp(a) serum concentrations prior to GnRH analogue administration were not evaluated.

Haines *et al*. (1997) reported an increased concentration of Lp(a) in the luteal phase of natural and HMG-stimulated cycles, suggesting that progesterone may cause an increase in Lp(a) serum concentrations. This conclusion, however, was speculative, as the design of this study did not allow for evaluation of the separate effects of increased progesterone and oestrogen concentrations on Lp(a) concentrations. Since only two blood samplings, in the late midfollicular and midluteal phases, were performed, and no base data were available, the results could not demonstrate whether the higher Lp(a) concentrations were the outcome of the raised progesterone or oestrogen luteal concentrations.

Our data on stimulated cycles demonstrated that Lp(a) increases only when progesterone is raised. There were no significant changes in Lp(a) concentrations at the end of the follicular phase, even when very high concentrations of oestrogens were present.

Furthermore, the luteal increase in Lp(a) concentrations in stimulated cycles was statistically correlated with luteal progesterone increase, but not with follicular or luteal oestradiol increase. We found that Lp(a) concentrations did not significantly change during the natural cycle. This finding is consistent with a previous study (Owens *et al*., 1993) but not with Haines' data (Haines *et al*., 1997). Haines' study, however, has many limitations, such as a low number of blood samples during the menstrual cycle, or the lack of blood sample timing standardization. Therefore, we are led to conclude that progesterone only at supraphysiological concentrations significantly influences Lp(a) concentrations. However, we cannot rule out that some phenotypes have different responses to menstrual cycle progesterone changes. Alternatively, the luteal Lp(a) increase could be related to a pharmacological effect of the parenteral progesterone administration. Tonolo *et al*. (1995) in a small but well-designed study, considering only women with an ovulatory cycle, showed a significant luteal apo(a) increase in four cases, whereas no significant changes were detected in the remaining 11 cases. The four women with an increase of apo(a) had only S4 bands on sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Furthermore, these women had lower plasma mean concentrations (8 U/dl) compared to the 11 women who showed no significant apo(a) change (54 U/dl). Indeed, an inverse correlation between apparent molecular weight and plasma concentrations of apo(a) has previously been reported, i.e. small apo(a) phenotypes are associated with high concentrations of Lp(a), and large apo(a) phenotypes are associated with low concentrations of Lp(a) (Scanu and Fless, 1990; Boerwinkle *et al*., 1992; Lackner *et al*., 1993; Rader *et al*., 1993; Rader *et al*., 1994; Querfeld *et al*., 1999). Thus Tonolo's data (Tonolo *et al*., 1995) suggested that in women with alleles coding high molecular weight apo(a), the synthesis of apo(a) could be stimulated by progesterone. In our study we found that women with low baseline Lp(a) ($<$5 mg/dl) had a greater increase in Lp(a) concentrations at midluteal phase than women with baseline Lp(a) $>$5 mg/dl, providing further evidence that there is a different sensitivity of the apo(a) phenotypes to progesterone changes.

We are unable to explain why progesterone should increase Lp(a) concentrations. Previous studies suggest that the effects of progestogens on Lp(a) concentrations are dependent on the dose and degree of androgenicity of the preparation employed. Consistent reductions in Lp(a) concentrations were observed in patients treated with norethisterone alone at doses of 10 mg/day (Farish *et al*., 1991), and, to a lesser extent, when administered at 1 mg/day in combination with oestradiol (Farish *et al*., 1996; Spencer *et al*., 1999). Tibolone, a synthetic steroid structurally related to norethynodrel and norethisterone, and effective in the relief of climacteric symptoms, has also been reported to lower Lp(a) concentrations (Hänggi *et al*., 1997). Cyclical administration of dydrogesterone at different dosages has been reported not to modify significantly the favourable effects on Lp(a) induced by oestradiol (van der Mooren *et al*., 1993; Mijatovic *et al*., 1997). A large study regarding the effects of various progestogens combined with conjugated equine oestrogen (CEE) on Lp(a) concentrations has shown that medroxyprogesterone acetate (MAP) and norgestrel attenuated the Lp(a)-lowering effect of oestrogen (Kim *et al*., 1996). On the contrary, in the PEPI trial, no significant differences were apparent between CEE only and each combination regimen with MAP or micronized progesterone (Espeland *et al*., 1998).

The results of this study demonstrated that urinary and recombinant FSH administration had similar effects on Lp(a) concentrations. If no pregnancy occurred, we found that after 1 month Lp(a) concentrations fell to pre-treatment values, suggesting that the effect of ovarian stimulation with u-hFSH or *r*-hFSH was transitory and the 4-week wash-out period was probably sufficient to eliminate any residual drug influence. In women who have conceived, Lp(a) concentrations remained elevated. We cannot demonstrate whether an Lp(a) increase normally occurs during pregnancy or whether it was related to ovarian stimulation and/or parenteral progesterone administration. Few data are currently available on Lp(a) changes during spontaneous pregnancy and no study has evaluated Lp(a) concentrations before and after conceiving. Zechner *et al*. (1986) showed that Lp(a) concentrations rose steadily during the first trimester of pregnancy. However, this study did not evaluate Lp(a) concentrations before the eighth week; furthermore, Lp(a) was measured by the Laurell technique, a method with unacceptable analytical sensitivity. Recently, Sattar *et al*. (2000) reported a significant increase in Lp(a) values in normal pregnancy between 10 and 35 weeks. Other authors (Panteghini and Pagani, 1991) observed no significant differences between Lp(a) concentrations in non-pregnant controls and pregnant women before the eighth week of gestation. On the other hand, in stimulated cycles progesterone concentrations far exceed those in the natural cycle because of the presence of multiple corpora lutea and the luteal phase progesterone supplementation.

In conclusion, the administration of recombinant or urinary h-FSH does not directly influence Lp(a) concentrations. The total effects of ovarian stimulation with urinary and recombinant FSH on Lp(a) are comparable and are not related to gonadotrophin preparations, but appear to be related to the high luteal progesterone concentrations, physiologically or pharmacologically induced. The luteal Lp(a) increase in stimulated cycles is transitory. Therefore in women undergoing ovarian stimulation using the currently available FSH preparations, the cardiovascular risk as assessed by Lp(a) is not significantly affected.

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