Effects of Estrogens and Progestins on High Density Lipoproteins

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ABSTRACT

High density lipoprotein (HDL) levels are known to be higher in women than in men, and to increase with estrogen use. To assess the effects of estrogens on HDL subspecies, analytic ultracentrifuge measurements of HDL were compared in 11 menopausal estrogen users and 16 controls. The difference in mean schlieren patterns between the groups showed a significantly higher level of HDL with flotation rate ($F^{\circ}_{1.20}$) > 1.5 (predominantly HDL_2) in the users. This was similar to the difference in HDL seen between nonusers of hormones and age-matched males. A previous study had shown that users of combination oral contraceptives had increased levels of HDL with $F^{\circ}_{1.20} \leq 3.5$ (primarily HDL_3) suggesting that the estrogen effect on HDL is altered by the presence of added progestin. The progestin effect was studied here in more detail in two women with type V hyperlipoproteinemia treated with norethindrone acetate. Reduction in serum triglyceride was accompanied by a reduction in HDL, predominantly in the less dense species (HDL_2). Among groups of oral contraceptive and noncontraceptive estrogen and progestin users whose HDL-cholesterol levels have been reported recently, there was a direct correlation ($r = 0.86$, $p < .001$) between mean HDL-cholesterol and triglyceride levels. Endogenous hormonal influences on HDL were assessed by serum hormone and lipoprotein measurements at weekly intervals during two consecutive menstrual cycles in four healthy females. An increase in HDL of highest flotation rate ($F^{\circ}_{1.20}$ 5-9) was seen, which corresponded with the time of ovulation, raising the possibility of pituitary as well as gonadal hormone effects on HDL.

INTRODUCTION

It has long been recognized that premenopausal women have higher levels of high density lipoprotein (HDL) than men (1-6), and that administration of estrogens to either sex may increase total HDL (6-8). The effects on HDL of another of the major female gonadal hormones, progesterone, have not been identified. There has been renewed interest in the influence of these hormones and of combination estrogen-progestin oral contraceptives on HDL because of the increased incidence of coronary disease reported in "pill" users (7-11), and the strong inverse relationships between levels of HDL and coronary risk (12,13).

Data are presented and reviewed here which indicate that exogenous estrogens, progestins and combinations have differing effects on specific subfractions of HDL and on HDL-cholesterol. Also, fluctuations of HDL during the normal menstrual cycle are shown in relation to endogenous estrogen, progestin, and luteinizing hormone levels.

METHODS

Subjects

Several groups of subjects were studied. Estrogen effects on HDL were analyzed in 11 menopausal women aged 44-66 who were using conjugated estrogens (0.625-1.25 milligrams per day) and in 16 menopausal women of comparable age who had not used estrogens or contraceptives for at least six months. The measurements were performed previously as part of a study of lipids and lipoproteins in a sample of the population of Modesto, California (14). For calculation of male-female differences in HDL, analytical ultracentrifuge data from the Modesto study population were also used. In the age group 27-46 years, there were 40 men and 29 women who were not using estrogens or oral contraceptives, and in the age group 47-66 years, there were 40 men and 25 women who were not using hormones. Blood samples were obtained within 8 hr of a light, fat-free breakfast or breakfast and lunch.

The influence of oral contraceptives and noncontraceptive estrogen and progestin use on serum HDL-cholesterol and triglyceride were studied in 4,978 healthy female volunteers enlisted in the Walnut Creek Contraceptive Drug Study. Details of this study and a description of the cohort have been published (15,16). In the present analysis, two "miscellaneous" treatment groups were omitted, leaving 17 groups of hormone users (n = 1382) and a group of 3422 nonusers.

HDL were also analyzed in two women ages 58 and 62 with type V hyperlipoproteinemia being treated with the progestin, norethindrone acetate. Blood samples were taken after overnight fast at intervals indicated in Results.

Finally, weekly measurements of HDL were

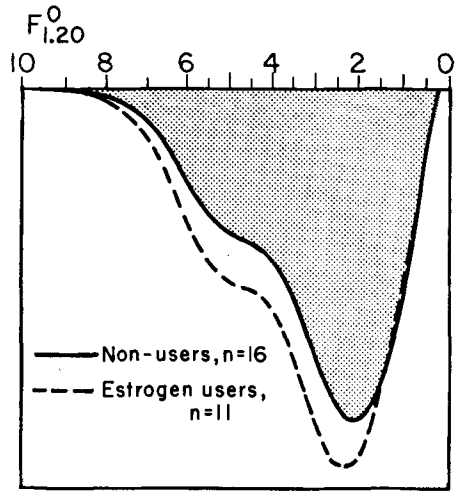

FIG. 1. HDL as measured by analytic ultracentrifugation in estrogen users and nonusers. The curves are the computer-derived means of individual curves in each group.

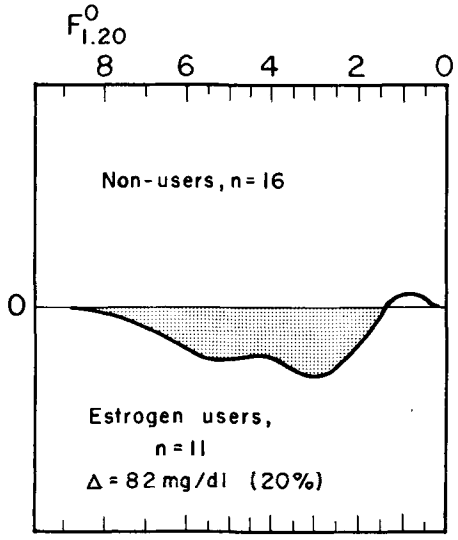

FIG. 2. Differences in HDL between estrogen user and nonuser groups. The curve represents the computer-derived difference between the mean HDL curves for the two groups shown in Fig. 1. The area under the baseline (shaded) represents the increase in lipoproteins in the user group.

carried out on blood samples obtained after an overnight fast during the course of the two sequential menstrual cycles in each of four healthy volunteers ages 22-26. The subjects were asked not to vary their diet or exercise levels during the course of the study.

Methods of Measurement

Analytical ultracentrifuge measurements of serum HDL were performed as described previously (17). Computer techniques were employed to generate individual and mean corrected schlieren patterns, to plot curves representing differences between pairs of schlieren patterns, and to calculate concentrations of total HDL and HDL of specified flotation rates (17).

HDL-cholesterol was measured by a modification of the heparin-manganese chloride method (18,19).

Total serum cholesterol and triglyceride concentrations were measured using either the Technicon Autoanalyzer (AA II) (18), or enzymatic methods (20,21).

Concentrations of serum estradiol, progesterone and luteinizing hormone (LH) were measured by radioimmunoassay techniques.

RESULTS

Estrogen Effects on HDL

The mean corrected schlieren patterns of HDL measured in the analytic ultracentrifuge in estrogen users and controls are shown in Figure 1. Estrogen users had higher levels of HDL with flotation rate ($F^0_{1.20}$) greater than 1.5. The differences are shown more precisely by subtracting the curve of the nonusers from that of the users (Fig. 2). This yields a "difference curve" with three peaks: two which are higher in the faster floating region, and one which is lower in the estrogen users in the slower floating region. The positive difference, 82 mg/dl or 20% of the control level, was significant at $p < 0.05$, while the negative difference was not statistically significant.

It is possible to compare these differences in HDL with those between men and women (nonusers of hormones) in the same population (14,22) (Fig. 3). The same three flotation peaks are present, the two higher in women corresponding to those higher in the estrogen users, and the third with slower peak flotation rate lower in both groups. These three peaks recently have been shown by Anderson et al. to be due to the presence of three subfractions of HDL separable by equilibrium density gradient ultracentrifugation (22) and have been designated as HDL_{2b}, HDL_{2a}, and HDL_3. Thus, estrogen use appears to be associated with higher levels of HDL_{2b} and HDL_{2a}, but not HDL_3 and the difference in HDL between users

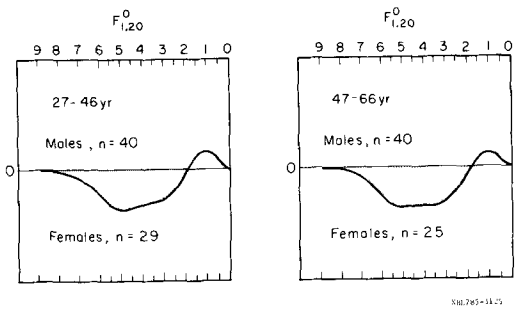

FIG. 3. Differences in HDL between men and women (nonusers of hormones) in age groups 27-46 and 47-66 in the Modesto population study (14). Mean HDL difference curves were computer-plotted as in Fig. 2. The shaded areas represent the lipoproteins higher in women than men.

and nonusers resembles the difference in HDL between women and men.

Norethindrone Acetate Effects on HDL and Triglyceride

The effect of estrogen on HDL (Fig. 2) is in contrast to the findings previously reported in users of oral contraceptives, namely an increase in HDL of $F^{0}_{1.20}$ 0-3.5, but not in faster floating HDL (23). On the basis of the HDL subclassification described above, the increase appears to include predominantly HDL_3 and to a lesser extent HDL_{2a}, but not HDL_{2b}.

The most likely cause of the different results in estrogen and contraceptive users was an effect of the added progestin on HDL. The effect on HDL of one of the progestins, norethindrone acetate, was studied in detail in two female subjects with type V hyperlipoproteinemia. Figure 4 shows serum total HDL as measured in the analytic ultracentrifuge and serum triglyceride in one of the subjects before, during, and following treatment with norethindrone acetate. The initial values were obtained when she was using conjugated estrogens. Upon withdrawal of estrogen, there was a reduction in serum triglyceride and total HDL, predominantly in the HDL_2 region. Within one week of introduction of norethindrone acetate, there was a further reduction in these measurements, progressing slightly with time and increased dosage to involve a reduction in HDL_3 as well. Drug withdrawal resulted in a return in all measurements towards baseline levels.

In the second subject, serum triglyceride fell from 4994 mg/dl to 2772, 2344, and 2073 mg/dl after one, two and three weeks of treatment with norethindrone acetate, 5 mg per day. Serum total HDL fell from 299 mg/dl to 229, 195, and 168 mg/dl at the same three points. As in the first subject, the reduction in HDL was predominantly in less dense HDL ($F^{0}_{1.20}$ 2-9), and in this case there was also a slight increase in HDL of $F^{0}_{1.20}$ 0-2.

Since norethindrone acetate treatment resulted in simultaneous lowering of triglyceride and HDL in these patients, the relationship of serum triglyceride and HDL-cholesterol was further examined using the Walnut Creek Oral Contraceptive Drug Study population (Fig. 5) (see Methods). A strong positive correlation is seen among the user groups, suggesting that use of contraceptive steroids, estrogens, and progestins results in parallel changes in serum HDL-cholesterol and triglyceride.

HDL and the Menstrual Cycle

Having investigated the effects of exogenous sex hormones on HDL, we turned to the study of changes in HDL as influenced by endogenous hormones in the normal menstrual cycle. Due to variation in cycle length and timing of hormonal peaks, it is not possible to group the data

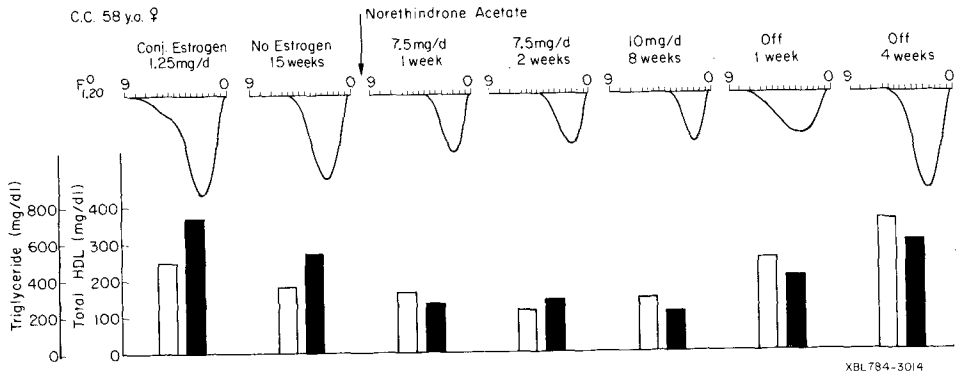

FIG. 4. Serum triglyceride and total HDL as measured by analytic ultracentrifugation in a subject with type V hyperlipoproteinemia and taking estrogen or norethindrone acetate.

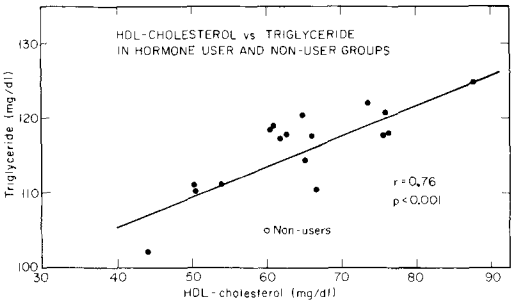

FIG. 5. Mean serum triglyceride plotted against mean HDL-cholesterol for each of the 17 hormone user groups (•) and the nonuser group (○) in the Walnut Creek Contraceptive Drug Study population (16).

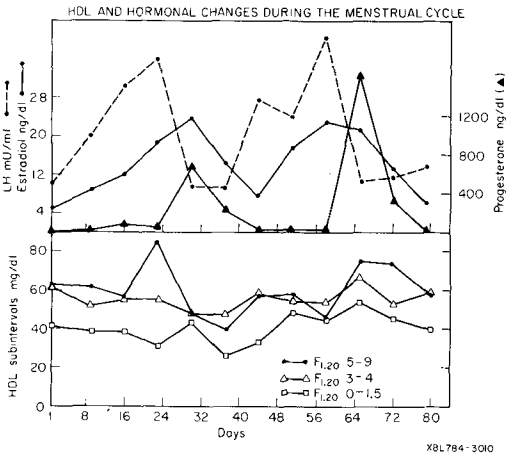

FIG. 6. Measurements of serum estradiol, progesterone and LH (upper panel), and HDL (lower panel) during two consecutive menstrual cycles in a healthy female. Cycles begin with the first day of menstruation at days 1 and 35. HDL concentrations, measured by analytic ultracentrifugation, are shown in three flotation intervals: $F^{\circ}_{1.20}$ 0-1.5 (contained within the HDL_3 subgroup), $F^{\circ}_{1.20}$ 3-4 (contained within HDL_{2a}), and $F^{\circ}_{1.20}$ 5-9 (contained within HDL_{2b}) (cf. Ref. 22).

for the four subjects studied. Fig. 6 shows the HDL (lower panel) and gonadal hormone levels (upper panel) in one subject during two sequential menstrual cycles, the results being representative of those in the other subjects. Ovulation occurs at the time of the midcycle peak in level of LH, after which progesterone concentration increases markedly. Estrogen levels before and after ovulation are comparable. Concentration of HDL of $F^{\circ}_{1.20}$ 0-1.5 and 3-4 showed no systematic changes during the cycle, while levels of HDL of $F^{\circ}_{1.20}$ 5-9, representative of HDL_{2b}, increased at or just after the time of ovulation and then declined rapidly. Due to the complexity of the various hormone patterns and the fact that other gonadal and pituitary hormones were not measured, it is not possible to link the midcycle increase in HDL_2 to any specific hormone change, but the temporal association with LH is suggestive.

DISCUSSION

Exogenous gonadal hormones (6-8) and derivatives (16, 23, 24-26) may exert major effects on serum HDL. Interest in these effects derives not only from the possible consequences of altered HDL in the large number of women using such preparations, but also from the insights that may be gained regarding the influence of endogenous gonadal hormones on the control of HDL levels.

Estrogens are known to increase HDL, and in the present paper this increase has been shown to involve primarily the less dense HDL_2 subfractions. Synthetic progestins may lower HDL, at least in women with hypertriglyceridemia. Again the major effect is on HDL_2. In combination with estrogen, progestins tend to shift the increase of HDL towards the more dense HDL_3 subspecies (23), and specific progestins may increase or decrease HDL-cholesterol (16). That these effects are pharmacologic is self-evident, but it is instructive to identify the possible differences between pharmacologic and physiologic hormone actions that might be involved in influencing HDL.

Estrogens, commonly administered as synthetic estrogen derivatives, or as conjugated "natural" estrogens (primarily estrone and equilin), result in supra-normal HDL levels. This may be due to specific drug effects, to unphysiologically high serum estrogen levels, or possibly to alterations in the normal pituitary-gonadal feedback relationships. It has not been determined whether elevated endogenous estradiol or estrone levels, as seen with estrogen-producing ovarian tumors, have a similar effect on HDL.

In the case of progestins, the situation is even more complex due to the number of pharmacological actions associated with these drugs, namely, progestational, androgenic, estrogenic, anabolic, and anti-estrogenic (16, 27). In a previous publication (16), we have suggested that the effects on HDL-cholesterol of progestins in oral contraceptives appear to bear some relation to the progestins' relative anti-estrogenic or estrogenic effects, but other properties such as androgenicity cannot be dismissed, particularly since androgens are

known to lower HDL levels (6,8). Furthermore, as with other cross-sectional data, it is not possible to rule out patient selection factors which might have influenced HDL levels in the treatment group.

A final aspect of the pharmacology of exogenous hormone effects on HDL is the direct correlation with effects on serum triglyceride and presumably VLDL. Although preliminary analyses (Wingerd and Krauss, unpublished) indicate that within groups of hormone users the expected inverse relation between HDL and triglyceride (28) is generally seen, the present results and those of others (8) suggest that the overall metabolic relationships between HDL and VLDL are influenced by hormone use, possibly by parallel effects on synthesis and/or catabolism of these lipoproteins.

Studies of lipoproteins in the normal menstrual cycle, while confirming an increase in HDL in mid-cycle (29,30), have not helped to define the hormonal determinants of this increase except to suggest that factors other than estrogen level are likely to be involved. It is not known what effects endogenous progesterone (as opposed to synthetic progestins) may have on HDL, but it may be that the postovulatory surge in progesterone has a role in reducing HDL toward baseline levels. A possible role of pituitary gonadotropin in contributing to the ovulatory peak in HDL must also be considered.

Since HDL levels are known to increase with age in women (16,29) it is difficult to sort out effects due to menopause per se. It may be, however, that loss of progesterone effect or enhanced gonadotropin levels might contribute to the increase in HDL in older women.

Since the inverse relationship in HDL and coronary risk has recently received renewed interest (12,12), it would seem appropriate to comment on the possible role that hormone-induced changes in HDL might have in relation to this risk. An argument has recently been brought forth that HDL_2, by virtue of its correlation with total HDL and HDL-cholesterol, represents the HDL components most likely to correlate with coronary disease incidence (31). Thus, in terms of HDL alone, estrogens would theoretically have an ameliorating effect on coronary risk, and the majority of progestins and contraceptives a neutral or negative effect. It has been shown, however, that estrogen use increases the incidence of coronary events in either sex (32-35), and recently high endogenous estradiol/testosterone ratios have been identified in men with accelerated coronary disease (36). Any putative "protective" role of enhanced HDL in estrogen users might well be reversed by other estrogen effects, such as increases in VLDL or changes in other lipoprotein fractions (8), or in blood pressure (37). Similar considerations hold true for the effects of oral contraceptives, although here it is tempting to suggest that specific preparations associated with reduced HDL-cholesterol might contribute to increased coronary disease in pill users.

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