Metabolic Effect of Conjugated Oestrogens (USP) on Lipids and Lipoproteins

M. NOTELOVITZ, B. SOUTHWOOD

SUMMARY

A study was undertaken to investigate the effect of conjugated oestrogens (USP) on the lipid profile of postmenopausal women. Twenty unselected menopausal women were treated for one year with cyclically administered oral conjugated oestrogens. Lipid studies were performed before, during and after treatment. The results showed that conjugated oestrogens had a slightly depressant effect on plasma cholesterol, beta-lipoproteins, and pre-beta-lipoproteins, in both normal and hyperlipidaemic subjects. Plasma triglycerides were slightly raised during treatment in normal women, but depressed in those with baseline hypertriglyceridaemia values. None of these changes was of statistical significance.

The apparent protective effect of endogenous oestrogen in the premenopause is probably related to its ability to maintain a normal lipid balance, rather than to reduce an abnormal one. This may be mediated through the known depressant effect that oestrogens have on the release of lipoprotein lipase. As a result, the breakdown of large lipid molecules to smaller particles, which could be more easily absorbed by the intima of the larger blood vessels, will be prevented.

S. Afr. Med. J., **48**, 2552 (1974).

When assessing the significance of coronary heart disease (CHD) in postmenopausal women, two facts are clearly established. There is a definite correlation between atherosclerotic CHD, hyperlipaemia and diet; and secondly, there is a definite increase in the incidence of CHD in postmenopausal women when compared with their younger cohorts.

This leads to the hypothesis that the oestrogen-deficient menopause was primarily responsible for the increased incidence of atherosclerotic CHD, and that substitution with the lacking steroid would protect against the development of this disease. The association between lipid metabolism, atherosclerosis and oestrogen was further confirmed by the classic experiments of Pick *et al.*,[1] who demonstrated that oestrogen was effective in chickens in preventing the development, and aided regression, of atherosclerosis. Although this experimental evidence is not strictly applicable to human subjects, extensive experience with oestrogen substitution therapy in postmenopausal women suggested that the retardation of atherosclerosis by this means is highly significant.[2]

More recently, experience with the steroid contraceptives has shown that they are frequently associated with an increase in the plasma cholesterol and triglycerides, while an increasing body of opinion feels that the role of oestrogen in the prevention of coronary heart disease is no more than a minor one.[3,4] If oestrogen therapy is to be of any value in the prevention of postmenopausal CHD, it is obvious that the treatment would have to be prolonged. Since most of the investigations involving the natural oestrogens have been conducted over a relatively short period,[3] it was decided to initiate a long-term prospective study to investigate the effect of conjugated oestrogens on the lipid profile of postmenopausal women on a year's therapy.

Chelmsford Medical Centre, Durban
M. NOTELOVITZ, M.B. B.CH., M.D., M.R.C.O.G., *Gynaecologist*

Addington Hospital, Durban
B. SOUTHWOOD, *Principal Laboratory Technologist*

Date received: 21 August 1974.

PATIENTS AND METHODS

A group of 20 unselected postmenopausal White women attending the Climacteric Clinic at Addington Hospital was chosen for study. The average postmenopausal period was 7,0 years (range 6 months-21 years). Their mean age was 46,6 years (range 21 - 68 years) and their mean weight 67 kg (range 52,5 - 113 kg). The objectives of the trial were explained to them and their permission obtained. After ensuring that all subjects had been off any form of hormonal therapy for at least a month, samples of venous blood were taken after an overnight fast. The specimens were collected between 0800 and 0900. Since one of the objectives of the study was to test the effect of conjugated oestrogens under everyday living conditions, no specific instructions were given to the patients regarding the dietary control of fat and/or carbohydrates.

The patients were then put onto Premarin—1,25 mg daily at cyclical intervals of 3 weeks—and the tests repeated at the end of the 3 and 9 months. After a year, therapy was suspended for one month and the lipid profile assessed once more.

The following lipids and lipoproteins were studied: cholesterol, beta-lipoprotein, pre-beta-lipoprotein, and chylomicrons. The lipoproteins were measured by the estimation of light scattering in a Thorp micronephlometer; cholesterol on a Technicon AutoAnalyzer II (clinical method number 16a), and triglycerides using a Dade Tri-25 reagent kit.

TABLE I. VARIATIONS IN MEAN LIPID AND LIPOPROTEIN VALUES IN 20 POSTMENOPAUSAL WOMEN BEFORE, DURING AND AFTER TREATMENT
(measured in mg/100 ml)

Variable		Baseline	3 months	9 months	Post-treatment
Chylomicrons	**Mean**	**9,17**	**7,53**	**8,11**	**7,53**
	± SEM	**0,99**	**1,21**	**1,14**	**1,21**
Triglyceride	**Mean**	**173,72**	**168,75**	**173,70**	**179,85**
	± SEM	**28,27**	**15,67**	**13,17**	**22,84**
Cholesterol	**Mean**	**284,30**	**265,50**	**294,15**	**295,45**
	± SEM	**12,48**	**9,38**	**14,91**	**13,01**
Pre-beta-lipoprotein	**Mean**	**274,63**	**247,70**	**263,20**	**270,16**
	± SEM	**28,85**	**34,25**	**51,55**	**51,81**
Beta-lipoprotein	**Mean**	**555,58**	**510,65**	**567,55**	**573,16**
	± SEM	**26,43**	**18,62**	**30,32**	**30,10**

Statistical Analysis

The effect of treatment (and its withdrawal) was determined by calculating the mean value of each variable (cholesterol; triglycerides; pre-beta-lipoproteins; chylomicrons) at each of the 4 time intervals noted previously. These are referred to as 'baseline'; '3-month'; '9-month'; and 'after-treatment' (Table I). The statistical significance of the observed changes in the variables was determined by calculating the main difference between the 2 treatment intervals (3-month and 9-month) and the baseline value (Table II); and the mean difference between the 'after-treatment' level and the '9-month' treatment and baseline observations (Table III). As the study was designed on a longitudinal basis, the data were analysed by the matched-pairs test and the results interpreted by the Bonferonni inequality procedure. A *t*-value of 3,2 was found to be significant at the 0,05 level, and values above 3,7 at the 0,01 level.

TABLE II. STATISTICAL ASSESSMENT OF THE EFFECT OF CONJUGATED OESTROGEN (USP) ON LIPIDS AND LIPOPROTEINS BY CALCULATING THE MEAN CHANGE IN VALUES AFTER 3 AND 9 MONTHS' TREATMENT

Variable		3 mo. - baseline	9 mo. - baseline
Chylomicrons	**Mean diff.**	**− 1,22**	**− 0,65**
(mg/100 ml)	***t***	**− 0,81**	**− 0,44**
	P	**NS**	**NS**
Triglyceride	**Mean diff.**	**− 2,72**	**1,33**
(mg/100 ml)	***t***	**− 0,10**	**0,07**
	P	**NS**	**NS**
Cholesterol	**Mean diff.**	**−18,80**	**9,85**
(mg/100 ml)	***t***	**− 2,31**	**1,09**
	P	**NS**	**NS**
Pre-beta-lipo-	**Mean diff.**	**−20,32**	**− 2,95**
proteins	***t***	**− 0,75**	**− 0,07**
(mg/100 ml)	***P***	**NS**	**NS**
Beta-lipo-	**Mean diff.**	**−44,00**	**14,37**
proteins	***t***	**− 2,91**	**0,06**
(mg/100 ml)	***P***	**NS**	**NS**

TABLE III. STATISTICAL ASSESSMENT OF THE EFFECT OF CONJUGATED OESTROGEN (USP) ON LIPIDS AND LIPOPROTEINS BY NOTING THE MEAN CHANGE IN THEIR VALUES AFTER THE WITHDRAWAL OF TREATMENT (see text)

Variable		Post-treatment baseline	Post-treatment - 9 months
Chylomicrons	**Mean diff.**	**−0,65**	**−0,58**
(mg/100 ml)	***t***	**−1,00**	**−0,37**
	P	**NS**	**NS**
Triglycerides	**Mean diff.**	**5,94**	**6,15**
(mg/100 ml)	***t***	**0,31**	**0,45**
	P	**NS**	**NS**
Cholesterol	**Mean diff.**	**11,15**	**1,30**
(mg/100 ml)	***t***	**1,38**	**0,12**
	P	**NS**	**NS**
Pre-beta-lipo-	**Mean diff.**	**14,94**	**11,63**
proteins	***t***	**0,31**	**0,24**
(mg/100 ml)	***P***	**NS**	**NS**
Beta-lipo-	**Mean diff.**	**16,22**	**4,84**
proteins	***t***	**0,84**	**0,21**
(mg/100 ml)	***P***	**NS**	**NS**

RESULTS

Most of the patients studied were between 40 and 60 years of age. Taking into consideration the changes which occur with ageing, the normal values (for our laboratory) of the various lipid and lipoprotein fractions for this age group are: triglycerides 5 - 175 mg/100 ml; cholesterol 150 - 300 mg/100 ml; pre-beta-lipoproteins <240; beta lipoproteins <550; and chylomicrons <28 mg/100 ml. If the initial baseline value exceeded these limits, the patient was regarded as being 'abnormal'. They were not classified according to Frederickson, since the object was to study the effect of oestrogen on the individual components of the lipid profile. There were no abnormal clinical features relating to their treatment with conjugated oestrogens.

Cholesterol

After 3 months' treatment with conjugated oestrogens a distinct fall in the cholesterol level was noted, from a baseline of 284,30 ± 12,48 mg/100 ml, to 256,50 ± 9,38 mg/100 ml (Table I, Fig. 1). This fell just short of statistical significance at the 0,05 level (Table II).

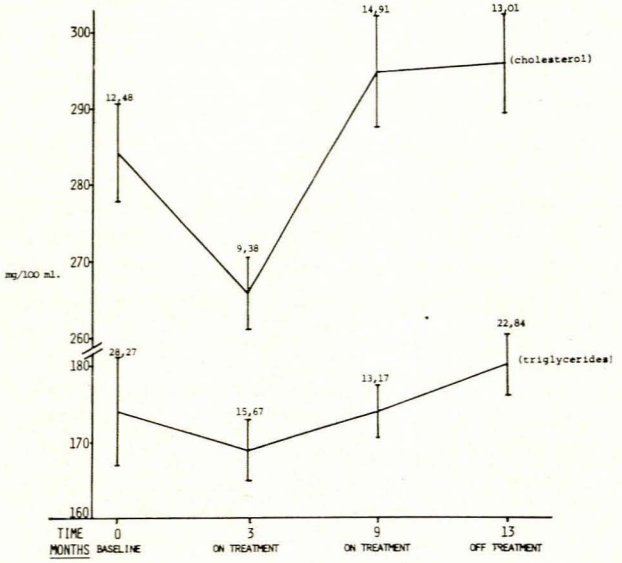

Fig. 1. Variation in mean lipid values before, during and after treatment with Premarin.

After a further 6 months' treatment, the mean value for cholesterol rose above the base'ine level—295,15 ± 14,91 mg/100 ml. The 'one month off treatment' level was almost identical, viz. 295,45 ± 13,01 mg/100 ml. Neither of these changes was of statistical significance (Tables II and III).

Triglycerides

A similar response was noted when the triglyceride response was analysed, although the mean changes were less marked. Thus the respective baseline, 3-month, 9-month and after-treatment values were 173,72 (± 28,27); 168,75 (± 15,67); 173,70 (±13,17), and 179,85 (± 22,84) mg/100 ml. None of the observed changes was of statistical significance (Tables II and III).

Lipoproteins

As anticipated, the change in the lipoprotein fractions mirrored those of the lipids, the changes in the beta-lipoprotein fraction being more marked than in the pre-beta-lipoproteins. Once again, none of the observed changes was of statistical significance. The results are summarised in Fig. 2 and Table I.

Chylomicrons

They responded to conjugated oestrogen treatment in a less predictable fashion—thus the baseline value of 9,17 (± 0,99) fell to 7,53 (± 1,21) at 3 months, followed by an increase to 8,11 (± 1,14) at the 9-month time interval and a subsequent fall to 7,53 (± 1,21) one month after treatment. None of these observed differences was of statistical significance.

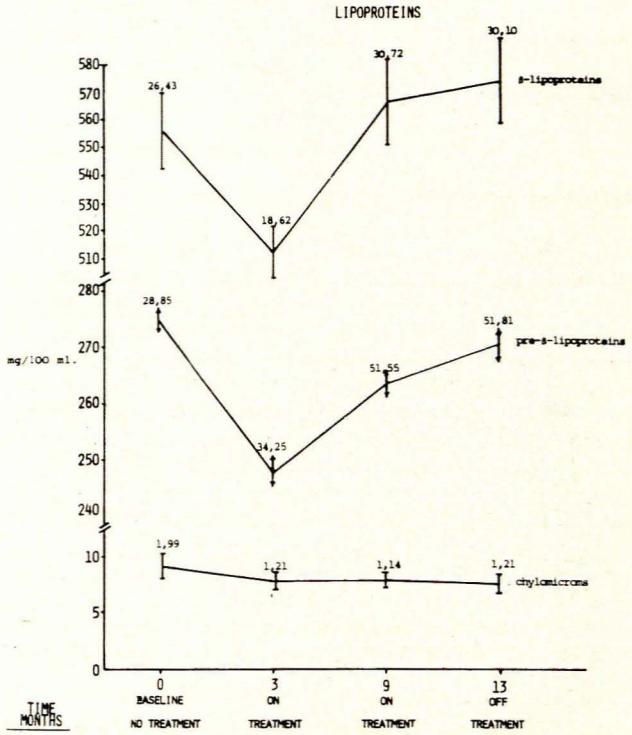

Fig. 2. Variation in mean lipoprotein values before, during and after treatment with Premarin.

DISCUSSION

There is much evidence to link total blood cholesterol with the development of atherosclerosis and coronary heart disease (CHD). The lipid elements incriminated include cholesterol, triglyceride, phospholipid and esterified fatty acids. In addition, the lipoproteins and the proteins to which the lipids are linked for transport, have also been blamed. They include the cholesterol-rich beta-lipoproteins and the triglyceride-rich pre-beta-lipoproteins. The alpha-lipoproteins of the albumin fractions are said to be less important.[5]

Because of the sudden increase in CHD after the menopause, lack of oestrogen has also been invoked as a provocative factor. There is much epidemiological evidence to support this concept. Thus, Kagen *et al.*[6] have shown that coronary artery disease is twenty times more common among males than among females in the 30 - 39-year age group, but that the frequency in females increases during the later years to a 2 to 1 ratio.

Similarly, the incidence of cardiovascular complications was 7 times greater in a group of women who stopped menstruating before the age of 40 years, in comparison with a healthy normal population,[7] while castration without oestrogen replacement therapy was shown to have a similar effect.[8] The association between lipid metabolism, atherosclerosis and oestrogen was further confirmed by the classic experiments of Pick and colleagues[1] and the clinical experience of Davis, alluded to previously.

There is, however, no consensus on this issue, and there are many authors who feel that oestrogen substitution therapy plays an insignificant role in the retardation of atherogenesis.[3,4] The issue has been further clouded by the observation that the oestrogen used in the contraceptive pill substantially increases the blood lipid content in many women and has a tendency to impair their carbohydrate tolerance and raise their blood pressure.[9-12] This combination of side-effects has rather ominous atherogenic implications. Therefore, rather than decrease the potential for CHD, Inman *et al.*[13] have noted a positive correlation between the dose of oestrogen (as used in the contraceptive pill) and the risk of coronary thrombosis. In this regard it is only fair to add that whereas oral contraceptives may increase the risk of myocardial infarction, it is more likely to occur in those women who would be otherwise prone to ischaemic heart disease because of a family history, hypertension or excessive cigarette smoking.[14] It should also be noted that in pregnancy there is a rise in cholesterol, total phospholipids and triglycerides, without an associated increase in CHD.

A more rational understanding of the association between lipid metabolism, atherosclerosis and oestrogen will be obtained by considering the following facts:

1. Atherosclerotic disease is not the product of a single aetiological agent.

2. Epidemiological studies have shown that persons with high cholesterol values develop CHD with greater frequency than those with low values,[15,16] but that this is only true in women before the age of 55 years. Pre-beta-lipoproteins are more atherogenic in women after the age of 55 years.[17]

3. It has not been conclusively demonstrated in man that correction of a lipid abnormality in the general asymptomatic population will result in a better prognosis of the atherosclerotic lesion.

4. Not all hyperlipidaemias will respond to the same form of treatment—e.g. endogenous hypertriglyceridaemia is treated by reducing the carbohydrate intake, while a reduction of exogenous hypertriglyceridaemia would depend upon a restriction of the fat intake.[5]

5. Whereas coronary artery disease rarely occurs before the menopause, the frequency subsequent to the climacteric increases so that it approximates closely with the incidence in males.[7] From this one can conclude that menopausal females with hyperlipidaemia and hyperlipoproteinaemia are more liable to the development of coronary heart disease, and that by reduction of the cholesterol level before the age of 55 years, and triglycerides after this age, one might be able to prevent or decrease the incidence of CHD due to atherosclerosis, provided the predisposing factors are treated simultaneously.

For reasons which are at present not clear, the use of synthetic oestrogens in postmenopausal women tends to decrease circulating cholesterol and low density beta lipoproteins, but increases triglyceride-rich alpha lipoproteins and total triglycerides.[18] In the present study, conjugated oestrogens were found to have little effect on the lipid profile of menopausal women when treated for one year. With the exception of the triglyceride fraction, the response was similar in both hyperlipaemic and normal women. In short, the response usually observed was a slight decrease in the lipid and lipoprotein level at 3 months, followed by a moderate elevation at 9 months. On withdrawal of conjugated oestrogen a further slight elevation was noted. None of these changes was found to be of statistical significance. The same pattern was noted in women with hypertriglyceridaemia. In normal women, however, conjugated oestrogens induced an increase in the triglyceride levels which persisted until the 9-month treatment interval. On withdrawal of treatment, a distinct drop in the triglyceride level was noted. Once again, these changes were not of statistical significance. One may therefore conclude that conjugated oestrogens, when administered over a prolonged period in a constant dosage schedule, have neither a lipolytic nor an atherogenic potential.

Robinson *et al.*[19] reported a significant lowering of serum cholesterol over a period of 12 months when administering conjugated oestrogens to oöphorectomised females. Utian[4] could not confirm these findings and questioned the empirical use of oestrogenic hormones for the prevention of ischaemic heart disease in postmenopausal women. However, his conclusions are based on only a 6-month follow-up. The answer to this problem will only result from controlled prospective longitudinal studies based on the prophylactic treatment of healthy women in early menopause (before evidence of hyperlipidaemia has developed). It may also be that the dose of oestrogen needs to be titrated according to the degree of lipaemia, in very much the same way that diabetic control is governed by adjusting therapy according to regular blood checks. Hyperlipaemic and hyperlipoproteinaemic patients require additional therapy such as dietary restriction and/or chemotherapy, if their endogenous biochemical disorder is to be reversed.

It is interesting to note that whereas prolonged use of the contraceptive pill produces an atherogenic environment, there have been few reports of an increase in CHD attributable to atherosclerosis. This apparent paradox may be explained by the study of Hazzard *et al.*[10] They showed that the elevated triglyceride level subsequent to oral contraception may arise from a decreased removal of triglyceride, as much as by an increase in the endogenous synthesis of this fraction. Oestrogens (ethinyl oestradiol) significantly decrease post-heparin lipolytic activity—a measure of heparin releasable lipoprotein lipase. Lipoprotein lipase is the enzyme responsible for the breakdown of lipids and lipoproteins into smaller molecules of fatty acids. This so-called 'clearing factor' is

also located near the capillary endothelium, and is associated with the known uptake of free fatty acids by the intima of the blood vessels. The protective effect of the oestrogens may therefore lie in their ability to prevent the breakdown of larger lipid molecules into smaller free fatty acid molecules, which can presumably gain easier access to the intima of the blood vessels, there to be synthesised into the characteristic atherosclerotic plaque.[20] Others have ascribed the increase in the lipid fraction subsequent to the taking of oestrogen to be secondary to an elevation of cortisone, thyroxin or growth hormone.[18]

CONCLUSION

It would be unrealistic to expect oestrogens *per se* (synthetic or natural) to be effective in the treatment of the hyperlipidaemias and hyperlipoproteinaemias. These conditions require specific therapy. Since atherosclerotic coronary heart disease is only liable to develop in individuals with elevated lipid levels, the oestrogen-deficient menopause *per se* is not likely to play a major role in the development of atherosclerosis. However, it is essential that the use of oestrogens in the treatment of the climacteric (for other reasons), should not provide an environment that could be conducive to the development of a hyperlipidaemic state. In this regard conjugated oestrogens, when used for a period of one year, have been found to have little effect on blood lipids in the normal individual.

It may be that the apparent protective effect of endogenous oestrogen in the premenopause is related to its ability to maintain a normal lipid balance (rather than to reduce an abnormal one). This effect may be mediated through the depressant effect that oestrogens have on the release of lipoprotein lipase. As a result, the breakdown of large lipid molecules to smaller particles, which could be more easily absorbed by the intima of the larger blood vessels, will be prevented.

We wish to thank the staff of the Climacteric Clinic and the Biochemistry Laboratory for their co-operation, and Professor D. Hawkins, Department of Statistics, University of the Witwatersrand, for the statistical analysis. Conjugated oestrogen (USP), viz. Premarin, was provided by courtesy of Ayerst Laboratories (Pty) Ltd.

REFERENCES

1. Pick, R., Stander, J., Robard, S. and Katz, L. N. (1952): Circulation, **6**, 276.
2. Davis, M. E. *in* Greenhill, J. D. ed. (1965): *Year Book of Obstetrics and Gynaecology*, p. 339. Chicago: Year Book Medical Publishers.
3. Manchester, J. H., Herman, M. V. and Gorlin, R. (1971): Amer. J. Cardiol., **28**, 33.
4. Utian, W. H. (1971): S. Afr. Med. J., **45**, 359.
5. Lehmann, H. and Lines, J. G. (1972): Lancet, **1**, 557.
6. Kagen, A., Dawber, T. R., Kamnel, W. B. and Revotskie, N. (1962): Fed. Proc., **21**, 4.
7. Szanjderman, M. and Oliver, M. F. (1963): Lancet, **1**, 962.
8. Higamo, N., Robinson, R. W. and Cohen, W. D. (1963): New Engl. J. Med., **268**, 1123.
9. Chidell, M. P. (1970): Practitioner, **205**, 58.
10. Hazzard, W. R., Spiger, M. J., Bagdade, J. D. and Bierman, E. L. (1969): New Engl. J. Med., **280**, 471.
11. Spellacy, W. N. (1969): Amer. J. Obstet. Gynec., **104**, 448.
12. Wynn, V., Doar, J. W. H., Mills, G. L. and Stokes, T. (1969): Lancet, **2**, 756.
13. Inman, W. H. W., Vessey, M. P., Westerholm, B. and Engelund, A. (1970): Brit. Med. J., **2**, 203.
14. Oliver, M. F. (1970): *Ibid.*, **2**, 210.
15. Co-operative Study (1956): Circulation, **14**, 691.
16. Kannel, W. B., Dawber, T. R., Friedman, G. D., Glennon, W. E. and McNamara, P. M. (1964): Ann. Intern. Med., **61**, 888.
17. Kannel, W. B. (1971): Amer. J. Clin. Nutr., **24**, 1074.
18. MacDonald, R. R. *in* MacDonald, R. R. ed. (1971): *Scientific Basis of Obstetrics and Gynaecology*, p. 372. London: J. & A. Churchill.
19. Robinson, R. W., Higano, N. and Cohen, W. D. (1960): New Engl. J. Med., **263**, 828.
20. Notelovitz, M. (1967): S. Afr. J. Obstet. Gynaec., **5**, 9.