Clinical Endocrinology (1997) **49,** 173–178

The effects of low-dose testosterone treatment on lipid metabolism, clotting factors and ultrasonographic ovarian morphology in women

H. M. Buckler*, K. McElhone*, P. N. Durrington†, M. I. Mackness†, C. A. Ludlam‡ and F. C. W. Wu†
**Department of Endocrinology, Hope Hospital, Salford,*
†*Department of Medicine, Manchester Royal Infirmary, Manchester and* ‡*Department of Haematology, Royal Infirmary, Edinburgh*

(Received 25 September 1997; returned for revision 28 January 1998; finally revised 20 February 1998; accepted 3 March 1998)

Summary

INTRODUCTION Low doses of androgen are used in women for the symptomatic treatment of sexual dysfunction and premenstrual syndrome (PMS). However, little is known about the long-term safety of androgen use in women. This study investigated the effects of low dose exogenous testosterone (T) on lipid metabolism, markers of activation of the coagulation system and ultrasonographic ovarian morphology in women.

PATIENTS Twenty-two patients with severe PMS (age 39·6 ± 3·1 years, mean ± SD) treated with subcutaneous T implants (100 mg six monthly) for at least two years (mean duration 3·3 (±0·9 years) were compared with 22 age-matched (age 37·7 ± 2·9 years) control patients with severe PMS who had not previously received T treatment. All women continued to have regular menses.

MEASUREMENTS Fasting blood samples were obtained for measurement of lipids and clotting factors and ovarian ultrasound examination carried out between days 1–4 of the menstrual cycle (2·3 ± 1·2 months after the T implant in T-treated group).

RESULTS Mean plasma T was 4·5 ± 2·2 nmol/l, and 1·9 ± 0·6 nmol/l in the treated and control groups, respectively. In the T-treated group apolipoprotein-A1 (Apo-A1) (treated 99·2 ± 12 *vs* controls 116·2 ± 27·7 g/l, $P < 0·01$) and high density lipoprotein cholesterol (HDL-C) (treated 1·3 ± 0·3 *vs* controls 1·5 ± 0·4 nmol/l, $P < 0·01$) were significantly decreased. In addition very low density lipoprotein cholesterol (VLDL-C) (treated 0·4 ± 0·3 *vs* controls 0·2 ± 0·1 nmol/l, $P < 0·05$) was increased in T-treated patients. There were no differences in total serum cholesterol and triglyceride or low density lipoprotein cholesterol (LDL-C), apolipoprotein B (Apo-B), lipoprotein(a), lecithin:cholesterol acyltransferase and cholesteryl ester transfer protein activity. There was no difference in clotting factors between the two groups which included prothrombin time, fibrinogen, antithrombin-III, protein-C, protein-S (total and free), tissue plasminogen activator, plasminogen activator inhibitor, beta-thromboglobulin and prothrombin fragments 1·2. Ultrasound showed normal ovarian architecture with no evidence of polycystic ovarian changes in any patients in the T-treated group.

No patient experienced adverse symptoms while on T treatment, in particular, there were no complaints of hirsutism or acne and no one requested termination of treatment.

CONCLUSION Low-dose testosterome administration to women for over two years did not induce changes in ovarian architecture but had small, potentially atherogenic effects on some parameters of lipid and lipoprotein metabolism. However, no differences were detected in markers of activation of the clotting system to indicate an actual increase in the risk of thrombosis. Overall, this study provides largely reassuring data about the safety of low-dose androgen treatment in women. However, caution should be exercised in women with existing or a familial predisposition to lipid abnormalities, because of the small but significant changes found in HDL-C, apo-A1 and VLDL-C.

Correspondence: Dr H M Buckler, Department of Endocrinology, Hope Hospital, Salford M6 8HD, UK. Fax: + 44 (0)161 787 5989

The role of androgens in normal female physiology is incompletely understood. Androgens, however, are produced in premenopausal women in higher molar quantities than oestrogens and are obligate precursors for ovarian oestrogen synthesis. In the female, androgens may have an effect on sexual behaviour, affect and cognitive functions (Sherwin & Gelfand, 1985; Sherwin, 1988; Kaplan & Owett, 1993) and low doses of androgen are being increasingly used in women for the

treatment of sexual dysfunction (Burger *et al*., 1987) and premenstrual syndrome (Buckler *et al*., 1989).

There is a fall in circulating androgens in postmenopausal women (Longcope 1986) and testosterone levels significantly decrease following oophorectomy (Vermeulen, 1976). The addition of testosterone implants to oestradiol implants enhances sexuality in postmenopausal women and can significantly benefit women who complain of low sexual interest despite adequate oestradiol replacement (Burger *et al*., 1987). Oestradiol and testosterone implant therapy has been shown to have an additive effect on bone density in postmenopausal women (Davis *et al*., 1995). Androgens may reverse inhibitory effects of oestrogen on bone formation (Raisz *et al*., 1996). This therefore suggests a potential therapeutic role for androgen therapy in osteoporosis prevention.

Little is known about the long-term safety of androgen treatment in women. It is, however, of great importance in view of the well established difference in the epidemiology of coronary heart disease and arterial thrombosis between men and women. Several lines of evidence suggest a protective role of oestrogens against coronary artery disease. Bilateral oophorectomy has been found to significantly increase the risk of coronary heart disease and postmenopausal oestrogen for patients with surgical or natural menopause may decrease this risk (Stampfer *et al*., 1985). Although women appear to be protected from coronary artery disease up until the time of the menopause, this protection may be reduced if androgens are administered to women even in low doses. It may be expected that androgens would reduce serum TG, VLDL-C and HDL and increase LDL-C (Furman *et al*., 1967). Anabolic steroid hormones have been reported to reduce lipoprotein(a) levels (Albers *et al*., 1984; Crook *et al*., 1992).

The polycystic ovarian syndrome (PCOS) is characterized by a heterogenous clinical picture including anovulation, menstrual disturbances, hirsutism, raised circulating androgens and typical polycystic ovarian morphology (Yen, 1980). Raised androgen levels may be important in the pathogenesis of PCOS. The effect of the adminisration of exogenous androgens on ovarian morphology has been examined.

In this study we have investigated the effects of exogenous testosterone on lipid metabolism, clotting factors and ultrasonographic ovarian morphology in premenopausal women receiving low dose testosterone therapeutically.

Materials and methods

Twenty-two women (age 39·6 ± 1·2 years, mean ± SD) who had received 100 mg testosterone implants six monthly for greater than two years for the treatment of severe pre–menstrual syndrome (PMS) were included. They were compared with a control group ($n = 22$) of age-matched women (mean age 37·7 ± 1·2 years) presenting with PMT who had not previously received T treatment. Body mass index was similar in both groups (T treated group 24·0 ± 2·1, control group 23·7 ± 2·3). The mean length of T treatment in the testosterone-treated group was 3·3 ± 0·9, range 2·2–5·1 years. All women continued to have regular menses and used effective nonhormonal contraception if sexually active.

Fasting blood samples for measurement of lipids (cholesterol, TC; triglycerides, TG; VLDL-C; HDL-C; LDL-C; lipoprotein(a), Lp(a); apolipoprotein A1, apo-A1; apolipoprotein B, Apo-B; lecithin:cholesterol acyltransferase, LCAT and cholesterol ester transfer protein activity, CETP) were performed between day 1–4 of the menstrual cycle. Clotting factors (prothrombin time; fibrinogen; antithrombin-III, AT-III; protein C; total protein S; free protein S; tissue plasminogen activator, tPA; plasminogen activator inhibitor, PAI-1; beta thromboglobulin, βTG; prothrombin fragments 1:2) were also performed on fasting blood samples between day 1–4 of the menstrual cycle. Transvaginal pelvic ultrasound scanning was performed blinded in the follicular phase by the same examiner. Serum for the measurement of testosterone levels by RIA was obtained weekly throughout one menstrual cycle. In the T treatment group the T levels were measured 2·3 ± 1·2 months after their last T implant. The mean of the four levels was used for comparison between the groups. Statistical comparisons were performed by paired Student's *t*-test for testosterone which was normally distributed and the Mann–Whitney *U*-test was used for the other non-normally distributed variables (all lipids and clotting factors).

Lipoproteins were isolated by sequential ultracentrifugation using the Beckman L8–55 M ultracentrifuge (Beckman, Palo Alto, California) (Mackness & Durrington, 1991). Total cholesterol was measured by the CHOD-PAP method (Biostat, Stockport, UK), free cholesterol was measured by the cholesterol oxidase method (BCL, Lewes, U.K.) and triglycerides were measured by the GPO-PAP method (BCL, Lewes, U.K.). Lipids in the lipoprotein subfractions were measured by adding 2,4,6-tribromo-3-hydroxybenzoic acid to the reagent to enhance the sensitivity of the colourimetric reagent (Trinder & Webster, 1984). Serum apo-A1 and B concentrations were measured by immunonephelometric assays using the Beckman assay with antisera and standard supplied by the manufacturers. Lipoprotein(a) was measured by a two-site IRMA (Pharmacia, Sweden). LCAT activity was measured by the modification of the Stokke and Norum method (Stokke & Norum, 1971) and CETP activity was determined by the rate of accumulation of radiolabelled cholesteryl esters on LDL and VLDL in an assay that uses endogenous lipoproteins (Channon *et al*., 1990). In brief, plasma was incubated at 37°C with a (3)H cholesterol: albumin emulsion for three hours and the appearance of radiolabelled cholesteryl esters on VLDL and LDL was

Table 1 Lipid and lipoprotein levels in testosterone treated and control groups.

Range	Treated	Control	Normal
TC nmol/l	5·8 ± 1·1	5·7 ± 1·1	< 6·5
TG nmol/l	0·9 (0·8–1·2)	0·8 (0·6–1·1)	< 2·0
Apo A1 g/l	99·2 ± 13·9	116·2 ± 27·7**	70·00–210·0
Apo B g/l	88·2 ± 29·4	82·6 ± 26·2	54·00–150·0
Lp (a) nmol/l	11·5 (5·9–40·6)	9·8 (5·1–12·8)	0·20–87·4
VLDL-C nmol/l	0·26 (0·16–0·49)	0·18 (0·12–0·29)**	0·10–1·2
LDL-C nmol/l	4·1 ± 1·1	3·9 ± 1·0	1·80–6·8
HDL-C nmol/l	1·3 ± 0·3	1·5 ± 0·4*	0·8–2·6
LCAT mmol/ml plasma/ h	53·1 ± 17·8	57·3 ± 12·4	41·0 ± 13·0
CETP mmol/ml plasma/ h	13·2 ± 6·5	13·6 ± 9·5	17·3 ± 7·0

** $P < 0{\cdot}01$; * $P < 0{\cdot}05$. mean ± SD shown except for TG, Lp(a) and VLDL-C when median and interquartile range are shown.

measured by precipitation of these lipoproteins from incubated serum by sodium phosphotungstate/magnesium chloride. The rate of cholesteryl ester transfer was estimated from the radioactivity in the HDL containing supernatant and the accumulation of radiolabelled cholesteryl esters in the precipitate containing VLDL and LDL. The coefficients of variation from the LCAT and CETP activity were 4 and 12%, respectively. The coagulation component assay methods were as follows. β-thromboglobulin (RIA kit, Amersham International), tissue plasminogen activator and plasminogen activator inhibitor (Spectrolyse, Biopool AB), antithrombin III (Immunochrom AT3, Immuno Limited), protein C (Coa test, Kabi Vitrum Limited), protein S (free and total) (in-house enzyme-linked immunosorbent assay), prothrombin fragments 1·2 (Enzygnotes F1 + 2, Behring). Protein C, AT-III, PAI-1 and tPA were measured by activity assays while the other haemostatic parameters were quantified by specific antigen assays. Fibrinogen was measured by the Clauss technique.

Plasma T concentration was measured by radioimmunoassay (Corker & Davidson, 1978). Sensitivity of the assay was 0·2 nmol/l, within and between assay coefficients of variations were 8 and 15%, respectively, cross-reactivity with 5α–dihydrotestosterone was 12·1%, normal range for adult male was 10–30 nmol/l and female 0·8–2·2 nmol/l.

Transvaginal ovarian ultrasonography was performed during the early follicular phase, blinded, by the same observer. The criteria of Adams *et al*. (1985) were used for the diagnosis of the appearances of polycystic ovaries.

Statistics

Testosterone, lipid and markers of activation of the coagulation system are displayed as mean and SD except for TG, Lp(a) and VLDL which are shown as mean and interquartilerange. Students *t* tests are used for analysis of data that is normally distributed and the Mann Whitney test for non-normally distributed data

Results

Testosterone levels

Testosterone levels in the testosterone-treated group were 4·5 ± 2·2 nmol/l as opposed to 1·9 ± 0·6 ($P < 0{\cdot}001$) in the control group.

Lipid levels

Lipid levels in both groups are shown in Table 1. HDL-C ($P < 0{\cdot}05$) and apo-A1 ($P < 0{\cdot}01$) levels were lower in the testosterone-treated group (HDL-C, 1·3 ±0·3 *vs* 1·5 ±0·4 nmol/l apo-A1 99·2 ±13·9 *vs* 116·2 ±27·7 g/l). The concentration of VLDL-C ($P < 0{\cdot}05$) was, however, higher in the testosterone-treated group. There was no change in any other lipid or lipoprotein levels including total cholesterol, triglyceride, LDL-C, apoB, Lp(a), LCAT and CETP activity.

There was no correlation between testosterone levels and triglycerides, VLDL, HDL, lp(a) or apo-A1.

Levels of haemostatic measurements

The haemostatic components in both groups are shown in Table 2. These all remained within the normal range, there was no change in any of the clotting factor levels measured between the control and testosterone-treated group.

Table 2 Clotting factor levels.

	Treated	Control	Normal range
Prothrombin time (s)	12·1 ± 0·7	12·3 ± 1·3	10·5–14·5
Fibrinogen (g/l)	2·8 ± 0·6	2·7 ± 1·1	1·5–4·0
AT III (iu/ml)	1·1 ± 0·2	1·1 ± 0·2	0·8–1·2
Protein C (iu/ml)	1·21 ± 0·27	1·22 ± 0·30	0·67–1·38
Protein S total	1·04 ± 0·26	0·98 ± 0·30	0·64–1·54
Protein S free	1.00 ± 0·40	1·08 ± 0·43	0·61–1·54
t-PA (iu/l)	0·5 ± 0·3	0·5 ± 0·2	0·2–2·0
PAI (au/ml)	11·0 ± 9·2	7·9 ± 8·1	< 15·0
B-TG (ng/ml)	45·8 ± 33·6	51·3 ± 50·2	< 52·0
Proth frag 1 & 2 (nmol/l)	1·4 ± 0·6	1·6 ± 1·9	0·4–2·1

Mean ± SD shown.

Ultrasonographic ovarian morphology

Testosterone-treated group.–Uterine appearances were normal except in two women where the following were found: (1) a 1·3 cm uterine fibroid, (2) a 3·8 cm haemorrhagic cyst. Ovarian morphology was normal and no patient had any findings typical of PCOS (Adams *et al*., 1985). Ovarian volumes were: right 6·8 ± 1·3 cm^3, left 7·4 ± 1·3 cm^3.

Control group.–Uterine appearances were normal. Four patients (18%) had typical findings of PCOS. Ovarian volumes were: right 10·6 ± 2·3 cm^3, left 7·4 ± 2·1 cm^3.

Discussion

This present study confirmed that T implant 100 mg six monthly raised plasma T levels in women by 2–3 fold compared to controls (Dewis *et al*., 1986). As reported previously, these patients continue to have regular menstrual cycles and seldom display clinical evidence of hyperandrogenism (e.g. hirsutism, acne) (Dewis *et al*., 1986).

Gender has been regarded as one of the major determinants of cardiovascular disease risks. Premenopausal women have a lower risk of cardiovascular disease than men who have higher triglyceride and VLDL-C, and lower HDL-C concentrations than women (Heiss *et al*., 1980). In postmenopausal women serum lipids and lipoproteins appear to show a pattern of increased serum concentrations of triglycerides, LDL and lipoprotein(a) (Seed & Crook, 1994) but there is little change in HDL cholesterol (Stevenson *et al*., 1993). Oestrogen treatment counters the increase in LDL-C and Lp(a) and increases HDL-C levels (Lobo, 1991).

In normal men and women a strong negative association between levels of testosterone and of HDL has been reported (Semmens *et al*., 1983). In general, the effect of androgens is thought to reduce serum HDL cholesterol, raise LDL and to decrease triglycerides and VLDL (Bagatell & Bremner, 1996). Anabolic steroid hormones (Alpers *et al*. 1984; Crook *et al*., 1992) and norethisterone (Farish *et al*., 1991) appear to decrease the Lp(a) level.

Little is known about the effects of exogenous androgens in the female (pre and postmenopausally). In view of the increasingly prevalent use of androgens in hormone replacement therapy (HRT). It is therefore important to investigate whether the addition of T would mitigate against the beneficial effects of oestrogen on lipids if the two were administered together and whether the use of low-dose T in women can induce changes in lipoprotein profile similar to men and therefore potentially increase the risk of cardiovascular disease. Although this study has shown a decrease in serum HDL-C and apoAI, and an increase in VLDL in premenopausal women receiving T for greater than 2 years the magnitude of the changes are small.

This contrasts with the study of Burger *et al.*, 1984) where no changes in the concentration of cholesterol, its subfractions or triglyceride over six months in postmenopausal women treated with either estradiol implants alone or with T implants, was found. Reductions in total cholesterol and LDL-C have been found in patients treated with oestradiol and T implants (Davis *et al*., 1995; Farish *et al*., 1984) and withan oral oestrogen/androgen preparation but this also reduced HDL-C (Watts *et al.*, 1995) and produced a potential detrimental increase in the HDL-C/total cholesterol ratio (Hickok *et al.*, 1993). Intramuscular administration of an oestrogen and androgen containing formulation did not induce an increased atherogenic lipid profile (Sherwin *et al*., 1987). A large cross-sectional study of the longer term effects of adding T to various regimes of HRT did not reveal any significant effects of the added T on lipoproteins (Gambrell & Teran, 1991).

Increased T levels have been associated with increased triglyceride and LDL levels and reduced HDL levels (Furman *et al*., 1967; Haffner *et al*., 1989; Wild *et al*., 1990). Patients with polycystic ovarian syndrome and androgen excess have increased triglycerides and decreased HDL levels (Wild *et al*., 1990). Therefore we may have expected to have found a more marked decrease in HDL and apo-A1 in our study. The route of administration may modulate the response to androgens: it may be that oral administration has more effect on lipoproteins than the parenteral route (Thompson *et al*., 1989). This suggests that excessive androgen exposure of the liver following oral androgen ingestion increases the likelihood of alterations in lipoproteins.

The effect of T on the haemostatic system is largely unknown. There are reports of thromboembolism following the abuse of anabolic steroids (Lowe *et al*., 1979; Ferenchick, 1990; Robinson & Wise, 1993). However, an increase in fibrinolysis has been described with stanazolol (Kluft *et al*.,

1984; Greer *et al*., 1985) and with T (Mirand *et al*., 1965). In a study to examine the effects of exogenous T on the haemostatic system in men with supraphysiological levels of T (Anderson *et al*., 1995), there appeared to be a mild activation of the haemostatic system during initial treatment. After several months the raised activation markers had returned to pretreatment levels. There was a fall in plasma fibrinogen during T treatment. Levels of AT-III and prothrombin fragments 1·2 rose initially and levels of protein-C and protein-S (free) and PAI-1 fell, but concentrations of all these factors returned to pretreatment levels during continued treatment. There was no change in βTG, TPA and protein-S (Anderson *et al*., 1995). Our female subjects have been on treatment for over 2 years so that effects, if any, on clotting factors would be expected to have been stabilized and any initial changes on starting T will have been missed.

Increased blood levels of fibrinogen and PAI-1, a primary inhibitor of fibrinolysis, have been associated with coronary artery disease in women (Olofsson *et al*., 1989; Meilahn *et al*., 1992) but fibrinogen and PAI levels have not been found to be related to endogenous T levels in women (Meilahn *et al*., 1996).

There is, however, very little information on the relationships of these clotting factors with sex hormones, particularly in women. In this study low dose T treatment given to women for at least 2 years appeared to have no effect on clotting factors and, by inference no effect on the haemostatic system.

Plasma T concentrations obtained in this study ($< 5{\cdot}0$ nmol/l) are similar to those seen in PCOS (Franks, 1995; Yen, 1980) but they appeared to have no effect on ovarian morphology in women not previously documented to have PCOS. Treatment of premenopausal women with low doses of exogenous T for 2 or more years has no adverse effects on ovarian morphology and does not seem to predispose them to features of PCOS.

The data from our study confirm that exogenous androgens exert significant though moderate effects on lipid metabolism but no detectable changes were observed in haemostasis and ovarian morphology. This data was obtained in premenopausal women and we cannot therefore assume that the same results would be found in postmenopausal women. However, when androgens are used in HRT they are only given to women already receiving oestrogen. We think we can therefore be confident that the results would apply to postmenopausal women receiving combined androgen and oestrogen HRT.

Whether the small changes found in HDL-C, Apo-A1 and VLDL-C translate to actual increased risk or incidence of cardiovascular disease is uncertain. However, until further information is available, caution should be exercised in women with existing or familial predisposition to lipid abnormalities when considering starting androgen treatment and lipid profiles should be monitored during therapy.

From the increasing body of information on the effectiveness of testosterone treatment for sexual dysfunction (Burger *et al*., 1987) reduced well being (Sherwin, 1988) and possible prevention of osteoporosis (Davis *et al*., 1995) in postmenopausal women or for PMS (Buckler *et al*., 1989), there is enough persuasive evidence that androgen replacement therapy in women merits further investigation.

Acknowledgements

We are grateful to Ethical Pharmaceutical Ltd (UK) for financial support for this study.

References

Adams, J., Polson, D.W., Abdulwahid, N., Morris, D.V., Frank, S., Mason, H.D., Tucker, M., Price, J. & Jacobs, H.S. (1985) Multifollicular ovaries: clinical and endocrine features and response to pulsatile gonadotrophin releasing hormone. *Lancet*, **2**, 1375–1378.

Albers, J.J., Taggart, H.M., Applebown-Bowden, D., Haffner, S.M., Chestnut, C.H. & Hazard, W.R. (1984) Reduction of lecithin cholesterol acyltransferase, apolipoprotein D and the lipoprotein-a lipoprotein with the anabolic steroid Stanazolol. *Biochemical Biophysical Acta*, **795**, 293–296.

Anderson, R.A., Ludlam, C.A. & Wu, F.C.W. (1995) Haemastatic effects of supraphysiological levels of testosterone in normal men. *Thrombosis and Haemostasis*, **74**, 693–697.

Bagatell, C.J. & Bremner, W.J. (1996) Androgens in men uses and abuses. *New England Journal of Medicine*, **334**, 707–714.

Buckler, H.M., Sheffield, B., Misra, R. & Anderson, D.C. (1989) The use of low dose testosterone implants in premenstrual syndrome. *Journal of Endocrinology*, **123**, 34.

Burger, H., Hailes, J., Menelaus, M., Nelson, J., Hudson, B. & Balaz, S.N. (1984) The management of persistent menopausal symptoms with oestradiol testosterone implants. Clinical lipid and hormone results. *Maturitus*, **6**, 351–358.

Burger, H., Hailes, J., Nelson, J. & Menelaus, M. (1987) Effects of combined implants of oestradiol and testosterone on libido in post menopausal women. *British Medical Journal*, **294**, 936–937.

Channon, K.M., Clegg, R.J., Bhatnagar, D., Ishola, M., Arrol, S. & Durrington, P.N. (1990) Investigation of lipid transfer in human serum leading to the development of an isotopic method for the determination of endogenous cholesterol esterification and transfer. *Atherosclerosis*, **80**, 217–276.

Corker, C.S. & Davidson, D.W. (1978) Radioimmunoassay of testosterone in various biological fluids without chromotography. *Journal of Steroid Biochemistry*, **9**, 373–374.

Crook, D., Fidhu, M., Seed, M., O'Donnell, M. & Stevenson, J.C. (1992) Lipoprotein(a) levels in women given Danazol and impeded androgen. *Atherosclerosis*, **92**, 41–47.

Davis, S.R., McLoud, P., Strauss, B.J.G. & Burger, H. (1995) Testosterone enhances oestradiol's effect on postmenopausal bone density and sexuality. *Maturitus*, **21**, 227–236.

Dewis, P., Newman, M., Ratcliffe, W.A. & Anderson, D.C. (1986) Does testosterone affect the normal menstrual cycle. *Clinical Endocrinology*, **24**, 515–521.

Farish, E., Fletcher, C.D., Hart, D.M., Alazzwi, F., Abdalla, H.I. & Grey, C.E. (1984) The effects of hormone implants on serum lipoproteins and steroid hormones in bilateral oophorectomised women. *Acta Endocrinology*, **106**, 116–120.

Farish, E., Rolton, H.A., Barnes, J.F. & Hart, D.M. (1991) Lipoprotein(a) concentrations in postmenopausal women taking norethisterone. *British Medical Journal*, **303**, 694.

Ferenchick, G.S. (1990) Are androgenic steroids thrombogenic? *New England Journal of Medicine*, **322**, 476.

Franks, S. (1995) Medical progress: Polycystic ovarian syndrome. *New England Journal of Medicine*, **333**, 853–861.

Furman, R.H., Alaupobic, P. & Howard, R.P. (1967) Hormones and lipoproteins. *Progress in Biochemical Pharmacology*, **2**, 215–258.

Gambrell, R.D. Jr. & & Teran, A.Z. (1991) Changes in lipids and lipoproteins with long term oestrogen deficiency and hormone replacement therapy. *American Journal of Obstetrics and Gynecology*, **165**, 307–315.

Greer, I.A., Greaves, M., Madhock, R., McLoughlin, K., Porter, N., Lowe, G.D.O., Preston, F.E. & Forbes, C.D. (1985) Effect of stanazolol on factors 8 and 9 and serum aminotransferases in haemophilia. *Thrombosis and Haemostasis*, **53**, 386–389.

Haffner, S.M., Katz, M.S., Stern, M.P. & Done, J.F. (1989) Association of decreased sex hormone by d-globulin and cardiovascular risk factors. *Arteriosclerosis*, **9**, 136–143.

Heiss, G., Tamir, I., Davies, C.E., Tyrolen, H.S., Rifkand, B.M. & Schonfeld, Y. (1980) Lipoprotein cholesterol distributions in selected North American populations. *The Lipid Research Clinics Program Prevalence Study Circulation*, **61**, 302–315.

Hickok, L.R., Toomey, C., & Speroff, L. (1993) A comparison of esterified estrogens with and without methyl testosterone: effects of endometrial histology ans serum lipoproteins in post-menopausal women. *Obstetrics and Gynecology*, **82**, 919–924.

Kaplan, H.S. & Owett, T. (1993) The female androgen deficiency syndrome. *Journal of Sexual Marital Therapy*, **19**, 3–24.

Kluft, C., Preston, F.E., Malia, R.G., Bertina, R.M., Wijngaards, G., Greaves, N., Berheijen, J.H. & Dooijewaard, G. (1984) Stanazolol induced changes in fibrinolysis and coagulation in healthy adults. *Thrombosis and Haemostasis*, **51**, 157–164.

Lobo, R.A. (1991) Effects of hormonal replacement on lipids and lipoproteins in postmenopausal women. *Journal of Clinical Endocrinology and Metabolism*, **73**, 925–930.

Longcope, C. (1986) Adrenal and gonadal androgen secretion in normal females. *Clinical Endocrinology and Metabolism*, **15**, 213–228.

Lowe, G.D.O., Thompson, J.E., Reavey, M.M., Forbes, C.D. & Prentice, C.R.M. (1979) Mesterolone: Thrombosis during treatment and a study of its prothrombotic effects. *British Journal of Clinical Pharmacology*, **7**, 107–109.

Mackness, M.I. & Durrington, P.N. (1991) Lipoprotein analysis for clinical studies. In: *Lipoprotein Analysis, A Practical Approach* (eds. C.A. Converse & E.R. Skinner), pp. 1–42. Oxford University Press, Oxford.

Meilahn, E.N., Kuller, L.A., Matthews, K.A. & Kiss, J.E. (1992) Haemastatic factors according to menopausal status and use of hormone replacement therapy. *The Annals of Epidemiology*, **2**, 445–455.

Meilahn, E.N., Cauley, J.A., Tracey, R.P., Macey, E.O., Gutay, J.P. & Kuller, L.H. (1996) Association of sex hormones in adiposity with plasma levels of fibrinogen and PAI-1 in postmenopausal women. *American Journal of Epidemiology*, **143**, 159–156.

Mirand, E.A., Gordon, A.W. & Wenwick, J. (1965) Mechanism of testosterone action in iatropoeisis. *Nature*, **206**, 270.

Olofsson, B.O., Dahlen, G. & Nilsson, T.K. (1989) Evidence for increased levels of plasminogen activator inhibitor and tissue plasminogen activator in plasma of patients with angiographically verified coronary artery disease. *European Heart Journal*, **10**, 77–82.

Raisz, L.G., Wiita, B., Artis, A., Bowen, A., Schwartz, S., Trahiotis, M., Shoukri, K. & Smith, J. (1996) Comparison of the effects of oestrogen alone and oestrogen plus androgen on biochemical markers of bone formation and resorption in menopausal women. *Journal of Clinical Endocrinology and Metabolism*, **81**, 37–43.

Robinson, R.J. & Wise, F. (1993) Misuse of anabolic drugs. *British Medical Journal*, **306**, 61.

Seed, M. & Cook, D. (1994) Hormonal replacement therapies, cardiovascular disease and plasma lipoproteins. *Current Opinion in Lipidology*, **5**, 48–58.

Semmens, J., Rouse, I., Beilen, L.J. & Masarei, J.R.L. (1983) Relationship of plasma HDL cholesterol to testosterone, estradiol and sex hormone binding globulin levels in men and women. *Metabolism*, **32**, 428–431.

Sherwin, B.B. (1988) Affective changes with oestrogen and androgen replacement therapy in surgically menopausal women. *Journal of Affective Disorders*, **14**, 177–187.

Sherwin, B.B. & Gelfand, M.M. (1985) Sex steroids and affect in the surgical menopause. A double blind crossover study. *Psychoneuroendocrinology*, **10**, 325–335.

Sherwin, B.B., Gelfand, M.M., Schucher, R. & Gabor, J. (1987) Postmenopausal oestrogen and androgen replacement and lipoprotein lipid concentrations. *American Journal of Obstetrics and Gynecology*, **156**, 414–419.

Stampfer, M.J., Willett, W.C., Colditz, G.A., Rosner, B., Speizer, F.E. & Hennekens, C.H. (1985) A prospective study of postmenopausal oestrogen therapy in coronary heart disease. *New England Journal of Medicine*, **313**, 1044.

Stevenson, J.C., Cook, D. & Godsland, I.F. (1993) Influence of age and menopause on serum lipids and lipoproteins in healthy women. *Atherosclerosis*, **98**, 83–90.

Stokke, K.T. & Norum, K.R. (1971) Determination of lecithin cholesterol acyltransferase in human blood plasma. *Scandinavian Journal of Clinical Investigation*, **27**, 21–27.

Thompson, P.D., Cullinane, E.M., Sady, S.P., Chenevert, C., Saritelli, A.L., Sady, M.A. & Herbert, P.W. (1989) Contrasting effects of testosterone and stanazolol on serum lipoproteins. *Journal of the American Medical Association*, **261**, 1165–1168.

Trinder, P. & Webster, D. (1984) Determination of HDL cholesterol using 2,4,6-tribromo-3-hydroxybenzoic acid with commercial CHOD-PAP reagent. *Annals of Clinical Biochemistry* **21**, 430–433.

Watts, N.B., Notelovitz, M., & Timmons, M.C., (1995) Comparison of oral estrogens and estrogens plus androgen on bone mineral density, menopausal symptons and lipid-lipoprotein profiles in surgical menopausal women. *Obstetrics and Gynecology*, **85**, 529–537.

Vermeulen, A. (1976) The hormonal activity of the postmenopausal ovary. *Journal of Clinical Endocrinology and Metabolism*, **42**, 247–252.

Wild, R.A., Applebown-Bowden, D., Demers, L.M., Hazzard, W. & Santen, W.J. (1990) Lipoprotein lipids in women with androgen excess. Independent associations with increased insulin and androgen. *Clinical Chemistry*, **36**, 283–289.

Yen, S.S.C. (1980) The polycystic ovary syndrome. *Clinical Endocrinology*, **12**, 177–208.