Journal of Internal Medicine 2002; **251**: 148–155

The effect of tibolone on the lipoprotein profile of postmenopausal women with type III hyperlipoproteinemia

F. DE BEER[1,2], A. H. M. SMELT[2], L. C. VAN VARK[1,2], N. HOOGERBRUGGE[3], L. M. HAVEKES[1,2] & J. A. GEVERS LEUVEN[1,2,4]

From the [1]TNO Prevention and Health, Gaubius Laboratory, [2]The Department of Internal Medicine, Leiden University Medical Center, Leiden; and the Departments of [3]Internal Medicine and [4]Cardiology, Thoraxcentre, University Hospital Dijkzigt, Rotterdam, The Netherlands

Abstract. de Beer F, Smelt AHM, van Vark LC, Hoogerbrugge N, Havekes LM, Gevers Leuven JA (Gaubius Laboratory, Leiden; Leiden University Medical Center, Leiden; and University Hospital Dijkzigt, Rotterdam, The Netherlands). The effect of tibolone on the lipoprotein profile of postmenopausal women with type III hyperlipoproteinemia. *J Intern Med* 2002; **251:** 148–155.

Objective. To investigate the short-term effect of treatment with tibolone on plasma lipid and lipoprotein levels in postmenopausal women with type III hyperlipoproteinemia (HLP).

Design and intervention. Patients were randomized to receive, in a double-blind cross-over fashion, a fixed dose of tibolone, 2.5 mg once daily or placebo for 8 weeks. The two treatment periods were separated by a wash-out period of 6 weeks. At each visit body weight and blood pressure were determined. Before and after each treatment period, fasting venous blood samples were obtained from the patients for biochemical measurements.

Setting. The Leiden University Medical Center.

Subjects. Postmenopausal women with type III HLP (aged ≤65 years) were recruited from the Lipid Clinics of the Leiden University Medical Center, the Amsterdam Medical Center, the Utrecht Medical Center and the University Hospital Rotterdam. Five out of 25 women with type III HLP were eligible to be included in the study. Four of the five included patients completed the study according to the protocol. One patient was excluded from blinded therapy because total cholesterol levels increased above 20 mmol L^{-1}.

Main outcome measures. A significant reduction of plasma triglyceride, total cholesterol, VLDL cholesterol and VLDL triglyceride levels.

Results. Plasma triglyceride and total cholesterol levels decreased from 6.82 ± 3.58 to 2.45 ± 1.36 mmol L^{-1} and from 13.53 ± 3.64 to 6.61 ± 2.03 mmol L^{-1}, respectively (both $P < 0.05$). The body mass index remained unchanged. The glycated haemoglobin percentage decreased significantly from 5.8 to 5.3%. Treatment with tibolone resulted in a profound reduction in plasma apolipoprotein E, VLDL cholesterol and VLDL triglyceride levels (mean reductions of 66, 77 and 70%, respectively, $P < 0.05$).

Conclusions. Tibolone is a valuable adjuvant to current therapy in postmenopausal women with type III HLP.

Keywords: hormone replacement therapy, lipoproteins, tibolone, type III hyperlipoproteinemia.

Introduction

Familial dysbetalipoproteinemia (FD) is an autosomal recessive disorder of the lipoprotein metabolism caused by a dysfunctional apolipoprotein (apo) E [1, 2]. ApoE is the primary ligand for the receptor-mediated clearance of chylomicron- and VLDL-remnants by the liver [3, 4] and its functional defect results in accumulation of these remnants in the circulation. Thus, FD is also called 'remnant removal disease'.

More than 90% of all FD subjects are homozygous carriers of a specific isoform of apoE: apoE2 (Arg158→Cys) [1, 5]. In comparison with the other

two common apoE isoforms, apoE3 (Cys112; Arg158) and apoE4 (Cys112→Arg), the binding capacity of apoE2 for the hepatic low-density lipoprotein receptor (LDLR) is <1% [3, 6, 7].

Despite the accumulation of remnants in the circulation, the majority of the apoE2 homozygous subjects (>95%) are normolipidemic or even hypolipidemic [5, 8]. However, apoE2 homozygosity with additional environmental and genetic factors that interfere with normal lipoprotein metabolism may lead to the expression of overt hyperlipidemia known as type III hyperlipoproteinemia (HLP) [9], a highly atherogenic condition requiring dietary therapy and often life-long medication.

Type III HLP is more prevalent in men than in women. In men the disorder is normally expressed between 30 and 40 years of age, whereas in women type III HLP is primarily expressed after the menopause, suggesting that estrogens play a role in the phenotypic expression [2]. In line with this, it has been demonstrated that ovariectomy of female apoE2 transgenic rabbits results in hyperlipidemia [10].

Estrogens are prescribed to postmenopausal women to reduce climacteric symptoms and to prevent osteoporosis. In addition, estrogens have a cholesterol-lowering effect (for reviews see Refs [11, 12]). An important disadvantage of estrogens is an increased risk on endometrial hyperplasia [13]. In hormone replacement therapy (HRT), the estrogen substitution is being combined with progestogens which prevents endometrial hyperplasia [14], but most preparations cause a cyclic monthly vaginal bleeding.

Tibolone [(7α,17α)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one] (OrgOD14, Livial®, Organon Nederland BV, Oss, The Netherlands) is a synthetic steroid structurally related to norethynodrel. Tibolone displays weak estrogenic, progestogenic and androgenic properties [15]. The compound is registered for the alleviation of postmenopausal complaints. Clinical studies amongst postmenopausal women have shown that tibolone prevents bone loss and improves plasma cholesterol and triglyceride levels, without stimulating endometrial growth and without causing withdrawal bleeding (for reviews see Refs [16, 17]).

VLDL metabolism is strongly influenced by estrogens and androgens, the former enhancing the VLDL triglyceride synthesis in the liver [18, 19] and the latter decreasing it [20, 21]. Estrogens inhibit hepatic lipase (HL) activity [22, 23], whereas androgens have a stimulating effect [23, 24]. Furthermore, estrogens increase LDL clearance, most likely via the induction of hepatic LDLRs [18, 25].

In type III HLP patients, administration of estrogens has been found to cause a profound decrease in plasma lipid levels [26–28]. To determine whether tibolone has the same beneficial effects on lipid metabolism as estrogens, we investigated the effect of tibolone on lipid and lipoprotein parameters in postmenopausal women with type III HLP.

Materials and methods

Patients

Postmenopausal women with type III HLP (aged ≤65 years) were recruited from the Lipid Clinics of the Leiden University Medical Center, the Amsterdam Medical Center, the Utrecht Medical Center and the University Hospital Rotterdam. Menopause was assumed to be present if the menstrual cycle had stopped for more than 1 year and was confirmed biochemically by follicle-stimulating hormone levels >20 IU L^{-1} in plasma. The diagnosis type III HLP was based on the means of two fasting blood samples obtained after a dietary period of at least 8 weeks. The diagnostic criteria for type III HLP were: total plasma cholesterol >6.5 mmol L^{-1}, plasma triglycerides >2.0 mmol L^{-1} and homozygosity for apoE2 (Arg158→Cys) as determined by isoelectric focusing [29] and apoE genotyping [30].

Exclusion criteria were: vaginal bleeding within 6 months prior to study entrance, treatment with any sex hormones in a depot within 5 years or otherwise within 3 months prior to study entrance, medical history of estrogen-dependent malignancy, thromboembolic events in the past, liver disease, blood pressure >170/100 mmHg, diabetes mellitus using insulin or medical history of cardiovascular disease.

The study was approved by the local committee of medical ethics of the Leiden University Medical Center. All patients gave their informed consent.

Study design and blood sampling

Before study entrance, lipid-lowering medication was discontinued for 1 month. Patients were randomized to receive, in a double-blind cross-over

fashion, a fixed dose of tibolone, 2.5 mg once daily or placebo for 8 weeks. The two treatment periods were separated by a wash-out period of 6 weeks. At each visit body weight and blood pressure were determined. Tibolone intake during the trial was monitored by counting unused pills. A rising of plasma cholesterol ≥20 mmol L^{-1} during the study had been convened to exclude the patient from further blinded therapy. During the study, patients were instructed to adhere to their normal dietary habits.

Before and after each treatment period, fasting venous blood samples were obtained from the patients for biochemical measurements. Blood was collected in ethylenediaminetetraacetic acid containing evacuated tubes (1 mg mL^{-1}) and immediately placed on ice. Plasma was obtained after centrifugation at 1500*g* for 15 min at 4°C. Freshly prepared plasma was used for measurements of total cholesterol, total triglycerides, free fatty acids (FFA) and glycated haemoglobin (HbA1c). Furthermore, plasma samples for lipoprotein analyses were stored in the following way: plasma samples were brought to a final concentration of 10% (v/v) sucrose, capped under nitrogen, snap-frozen in liquid nitrogen and stored at −80°C. Under these conditions, lipoprotein size and biological properties have been shown to remain intact for months [31, 32].

Lipid and lipoprotein analysis

Plasma triglyceride, total cholesterol and FFA levels were measured enzymatically, using commercially available kits (337-B: Sigma Chemical Co., St Louis, MO, USA; 236691: Boehringer–Mannheim, Mannheim, Germany; and 994-75409: Wako Chemicals, Neuss, Germany, respectively). Plasma apoE levels were determined using an enzyme-linked immunosorbent assay as described previously [33]. Plasma apoAI and apoB100 levels were assessed by rate immunonephelometry using an automated Beckman Array analyser (Beckman Instruments Brea, Fullerton, CA, USA) as described previously [34].

Separation of lipoproteins was performed by density gradient ultracentrifugation according to Zhao *et al.* [35] with some slight modifications. Briefly, the gradient consisted of 2 mL plasma (adjusted to $d = 1.21$ g mL^{-1} by adding 0.65 g KBr), overlayered by 5 mL of $d = 1.03$ g mL^{-1} and 3.5 mL of $d = 1.006$ g mL^{-1} NaCl solutions and 1.5 mL water. The gradient was centrifuged at 285 000*g* in a SW40 swing out rotor (Beckman, Geneva, Switzerland) for 18 h at 4°C. The gradient was then fractionated in fractions of 0.5 mL. In each fraction, cholesterol and triglyceride levels were measured with enzymatic assay kits (236691 and 701904: Boehringer–Mannheim, Mannheim, Germany).

HbA1c measurements

HbA1c was determined by high-performance liquid chromatography (Bio-Rad Laboratories, Kyoto, Japan).

Statistical methods

Statistical analyses were performed with SPSSWIN 6.1.3 (SPSS, Chicago, IL, USA). The results are presented as mean values ± standard deviation (SD). Differences between the patients before and after tibolone therapy were evaluated pairwise using the Wilcoxon paired signed-ranks test. *P*-values lower than 0.05 were considered as indicative of significant differences.

Results

Baseline clinical and biochemical characteristics

Twenty out of 25 postmenopausal women with type III HLP had to be excluded because of: manifest atherosclerotic cardiovascular disease ($n = 5$), diabetes mellitus ($n = 3$), vaginal bleeding within 6 months before sampling ($n = 2$), presence of an apoE variant not being apoE2 (Arg158→Cys, $n = 1$), nephrotic syndrome ($n = 1$), mamma carcinoma ($n = 1$), address unfindable ($n = 1$) and not willing to participate ($n = 6$).

Clinical and biochemical characteristics of the five eligible patients before study entrance are summarized in Table 1. Mean age of the patients was 60 ± 5 years and mean body mass index (BMI) was 26.9 ± 2.7 kg m^{-2}. Plasma total cholesterol and triglyceride levels were markedly elevated.

Compliance and side-effects

Four of the five included patients completed the study according to the protocol. One patient (P03)

Table 1 Baseline clinical and biochemical characteristics

	Patient				
	P01	P02	P03	P04	P05
Age (years)	54	61	65	55	63
Weight (kg)	65	65	61	81	59
BMI (kg m^{-2})	26.7	23.9	26.1	31.2	26.4
WHR	0.89	0.92	1.06	1.02	0.91
SBP (mmHg)	155	160	170	175*	140
DBP (mmHg)	85	95	90	90	85
Xanth	Yes	No	Yes	Yes	No
Smoking	No	Yes	No	Yes	Yes
Alcohol use	No	<3 u day^{-1}	No	<3 u day^{-1}	No
Co-medication	No	DI	No	AC, NS	No
TC (mmol L^{-1})	14.60	11.67	18.95	13.23	9.19
TG (mmol L^{-1})	8.28	3.42	10.90	8.82	2.70
HDL-C (mmol L^{-1})	1.30	1.34	1.33	0.96	1.93

AC, angiotensin converting enzyme inhibitor; BMI, body mass index; DBP, diastolic blood pressure; DI, diuretics; NS, nonsteroidal anti-inflammatory drug; SBP, systolic blood pressure; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein-cholesterol; u, units; WHR, waist-to-hip ratio; Xanth, the presence of xanthomata, i.e. yellow palmar creases (xanthochromia striata palmaris) and/or tuberous xanthomas at both elbows. Smoking was defined as the consumption of at least 10 cigarettes per day. The nonsmokers also included ex-smokers, who stopped smoking for at least 1 year preceding the study. *Later the systolic blood pressure was found to be <170 mmHg, allowing inclusion.

was excluded from blinded therapy because total cholesterol levels increased above 20 mmol L^{-1}. Before study entrance total cholesterol and triglyceride levels were 18.95 and 10.90 mmol L^{-1}, respectively (Table 1). After 1 month on study medication, her total cholesterol level was 23.10 mmol L^{-1}. Disclosure of the study code revealed that she had received placebo. The patient was then administered tibolone for 8 weeks under close monitoring every week, resulting in a gradual decrease of total cholesterol and triglyceride levels to 9.02 and 4.68 mmol L^{-1}, respectively.

Counting of the pills revealed that the mean compliance of the patients was 99 ± 2%. Treatment with tibolone was well tolerated in three patients. Two patients reported side-effects that were possibly related to tibolone treatment. One patient suffered from a headache, increased heart beat, abdominal pain and agitation. Another patient reported a few days nausea. None of the patients had vaginal bleedings.

Effect of tibolone on weight, blood pressure, glycated haemoglobin and plasma lipid levels

Treatment with placebo had no effect on plasma lipid levels and other parameters (data not shown). The effects of tibolone therapy are shown in Table 2. Patient P03 was included in the statistical analyses. Tibolone had no significant effect on BMI and blood pressure compared with values before treatment. HbA1c showed a significant reduction from 5.8 to 5.3% during tibolone treatment.

All five patients showed a decrease in total cholesterol levels during tibolone treatment (mean reduction of 51%, $P < 0.05$). The most profound effect of tibolone was observed on plasma triglycerides, showing a significant reduction of 64%. HDL cholesterol levels tended to decrease and plasma FFA levels to increase.

In all patients, treatment with tibolone resulted in a profound decrease in VLDL cholesterol and VLDL triglyceride levels (Fig. 1, mean reductions of 77 and 70%, respectively, $P < 0.05$). Concomitant to the decrease in VLDL levels, plasma apoE levels decreased by 66% during treatment with tibolone. IDL cholesterol and IDL triglyceride levels tended to decrease (mean reduction of 47 and 23%, respectively, $P > 0.05$). LDL cholesterol levels remained unchanged (data not shown). ApoB100 levels showed a decrease in all patients (mean reduction of 26%, $P < 0.05$), most likely as a result of the profound decrease in VLDL levels. Plasma apoAI levels did not change (data not shown).

Table 2 Effect of tibolone on weight, blood pressure, glycated haemoglobin and plasma lipid levels

	Tibolone treatment			
	Before ($n = 5$)	After ($n = 5$)	Difference (%)	*P*-value
BMI (kg m^{-2})	26.9 ± 2.7	27.3 ± 2.6	+2	0.07
WHR	0.96 ± 0.08	1.01 ± 0.02	+5	0.14
SBP (mmHg)	160 ± 14	154 ± 21	−4	0.28
DBP (mmHg)	89 ± 4	82 ± 7	−8	0.07
HbA1c (%)	5.8 ± 0.6	5.3 ± 0.5	−9	0.04
TC (mmol L^{-1})	13.53 ± 3.64	6.61 ± 2.03	−51	0.04
TG (mmol L^{-1})	6.82 ± 3.58	2.45 ± 1.36	−64	0.04
HDL-C (mmol L^{-1})	1.37 ± 0.35	1.16 ± 0.21	−16	0.18
FFA (mmol L^{-1})	0.49 ± 0.25	0.60 ± 0.07	+23	0.22

BMI, body mass index; DBP, diastolic blood pressure; FFA, free fatty acids; HbA1c, lycated haemoglobin; SBP, systolic blood pressure; TC, total cholesterol; TG, total triglycerides; HDL-C, high-density lipoprotein-cholesterol; WHR, waist-to-hip ratio. Values are presented as mean ± SD.

Discussion

This study demonstrates for the first time the effect of tibolone on total cholesterol, triglyceride, VLDL cholesterol, VLDL triglyceride, apoE and apoB100 levels in postmenopausal women with type III HLP.

Most studies concerning the effect of tibolone on plasma lipids have been performed in healthy postmenopausal women (for reviews see Refs [16, 17, 36]). In these studies, tibolone did not affect plasma LDL levels, but lowered both triglycerides and HDL. The decrease in HDL cholesterol levels appears to be transient as reported in a 36-month monitoring [37], but in another study it failed to return to baseline after 2 years [38]. Studies performed in postmenopausal women with secondary hyperlipidemia (e.g. noninsulin dependent diabetes mellitus [39, 40]) showed similar effects of tibolone on plasma lipid levels as compared with those observed in healthy subjects. In line with this, we found a triglyceride-lowering effect of tibolone in postmenopausal women with type III HLP. In contrast to the majority of studies performed in healthy postmenopausal women [16, 17, 36], we did not find a significant reduction in HDL cholesterol and apoAI levels. We observed no change in LDL cholesterol, whereas apoB100 levels were decreased. The latter is probably the result of the profound reduction in VLDL levels.

Estrogens affect VLDL/LDL metabolism in at least three ways: (i) by enhancing hepatic VLDL triglyceride and VLDL apoB synthesis [18, 19], (ii) by increasing hepatic LDL clearance [18, 25] and (iii) by stimulating lipoprotein lipase (LPL) activity [10]. Estrogens increase the plasma concentration of HDL particles as they increase apoAI synthesis [41] and they inhibit HL activity [22, 23]. In addition, estrogen administration in rats reduces the expression of the HDL receptor, scavenger receptor class B type I (SR-B1) on liver cells (for a review see Ref [42]). In contrast to estrogens, androgens are known to inhibit the VLDL triglyceride synthesis [20, 21], whereas they increase HL activity [23, 24] and consequently lower HDL cholesterol levels [22, 43].

Applying these facts on the effect of tibolone in our patients, the mechanism can only partly be understood. Both estrogenic and androgenic properties of tibolone are probably involved. Although previously reported data [26–28] suggest the opposite, theoretically the estrogenic effects of tibolone on the triglyceride synthesis should result in an increase of the plasma VLDL concentration. Although the induction of LDLRs in the liver by estrogens might be of relevance for binding apoB, this mechanism does not seem to be a likely explanation as the accumulated remnants in the plasma are primarily cleared via an apoE-dependent process which is impaired in type III HLP patients. There is evidence supporting a role for HL in the clearance of remnants [44, 45]. However, this mechanism would be inconsistent with the estrogenic properties of tibolone. A stimulating effect of estrogens on LPL activity that has been observed in rabbits [10] could be invoked to help us to understand our results. Taking the androgenic effects of tibolone into account a much more plausible explanation of our observations appears. The decreased total cholesterol, total triglyceride, VLDL, IDL and apoE levels can be readily explained by a

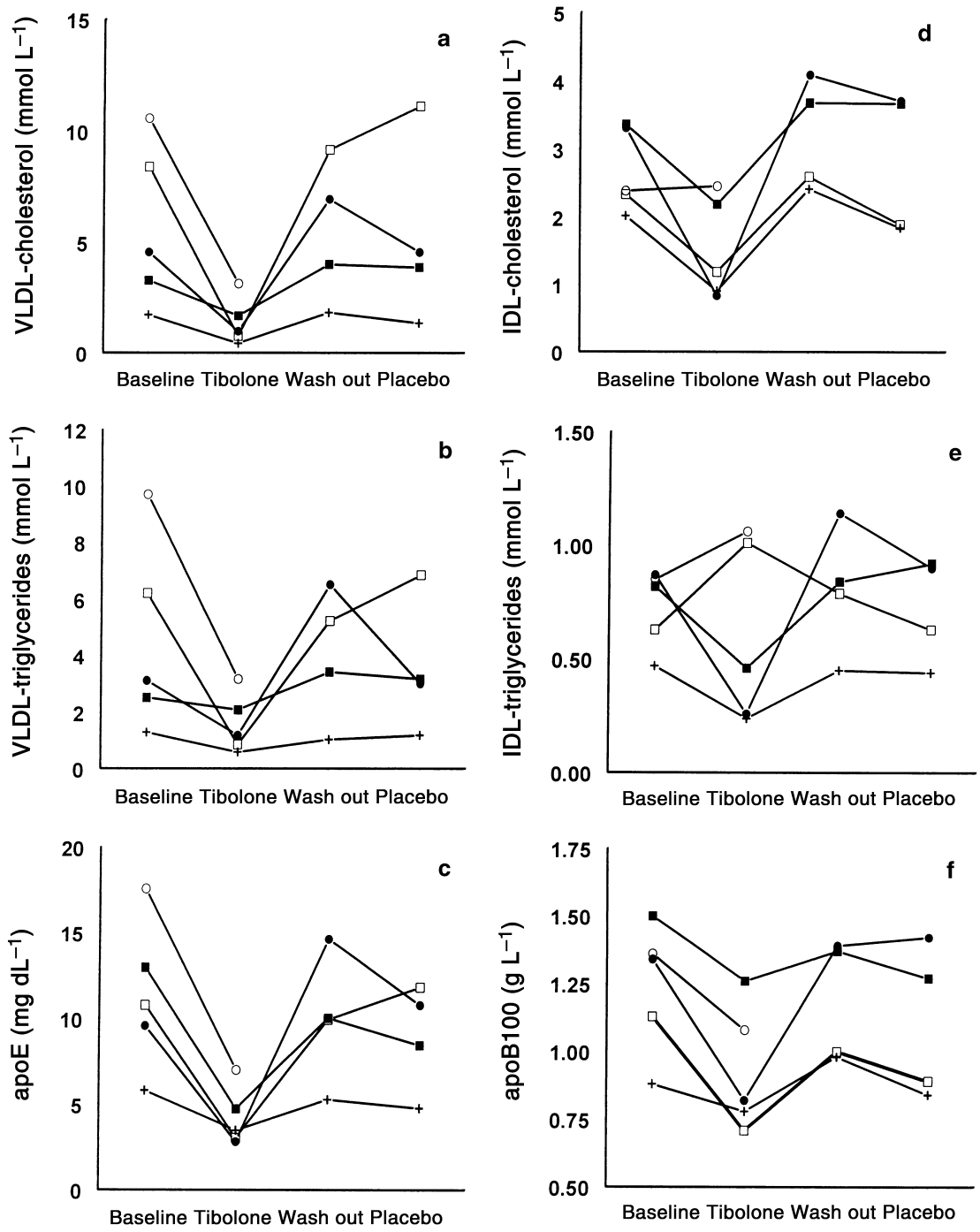

Fig. 1 The effect of tibolone on VLDL cholesterol (a), VLDL triglycerides (b), apoE (c), IDL cholesterol (d), IDL triglycerides (e) and apoB100 (f) levels in patients P01 (●), P02 (■), P03 (○), P04 (□) and P05 (+). In patient P03, measurements were only performed before and after treatment with tibolone.

decrease in VLDL triglyceride synthesis, and possibly by an increased lipolysis of IDL by HL. An androgenic preponderance of tibolone is suggested by the slight decrease in HDL cholesterol levels.

Interestingly, we found decreased levels of HbA1c upon tibolone treatment. Recently, our group has demonstrated that during short-term oral estrogen replacement therapy, HbA1c levels

decreased in patients with noninsulin-dependent diabetes mellitus [46]. In addition, data from that same study [46] indicated that the major site of improvement of diabetic control during short-term estrogen treatment was the liver. Thus, the lowering of HbA1c in the present study may be the result of the estrogenic effect of tibolone on liver metabolism.

In conclusion, tibolone has a profound beneficial effect on the lipoprotein profile of postmenopausal women with type III HLP. The compound is well tolerated in most patients and causes less monthly vaginal bleedings than many other HRTs. Tibolone may be used as an alternative or an adjuvant to fibrates and statins in the lipid-lowering therapy of postmenopausal women with type III HLP.

Acknowledgements

This study was financially supported by the Netherlands Heart Foundation (project no. 94.114). Organon Nederland BV is thanked for an Educational Grant and the study medication. We are grateful to Bep Ladan for collecting blood samples. Marijke Frölich is thanked for the HbA1c measurements. Hans Princen, Rien Buytenhek and Anneke Kramp are acknowledged for performing the apoB100 and apoAI analyses. Finally, we wish to express our gratitude to the patients who agreed to participate in this study.

References

1 Mahley RW, Innerarity TL, Rall SC Jr, Weisgraber KH, Taylor JM. Apolipoprotein E: genetic variants provide insights into it structure and function. *Curr Opin Lipidol* 1990; **1:** 87–95.

2 Mahley RW, Rall SC. Type III hyperlipoproteinemia (dysbetalipoproteinemia). The role of apolipoprotein E in normal and abnormal lipoprotein metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic and Molecular Bases of Inherited Disease*. New York: McGraw-Hill, 1995; 1953–80.

3 Havel RJ, Chao Y, Windler EE, Kotite L, Guo LS. Isoprotein specificity in the hepatic uptake of apolipoprotein E and the pathogenesis of familial dysbetalipoproteinemia. *Proc Natl Acad Sci USA* 1980; **77:** 4349–53.

4 Sherill BC, Innerarity TL, Mahley RW. Rapid hepatic clearance of the canine lipoproteins containing only the E apoprotein by a high affinity receptor. *J Biol Chem* 1980; **255:** 1804–7.

5 Utermann G, Vogelberg KH, Steinmetz A, *et al.* Polymorphism of apolipoprotein E. II. Genetics of hyperlipoproteinemia type III. *Clin Genet* 1979; **15:** 37–62.

6 Innerarity TL, Weisgraber KH, Arnold KS, Rall SC Jr, Mahley RW. Normalization of receptor binding of apolipoprotein E2. Evidence for modulation of the binding site conformation. *J Biol Chem* 1984; **259:** 7261–7.

7 Weisgraber KH, Innerarity TL, Mahley RW. Abnormal lipoprotein receptor-binding activity of the human E apoprotein due to cysteine–arginine interchange at a single site. *J Biol Chem* 1982; **257:** 2518–21.

8 Utermann G, Hees M, Steinmetz A. Polymorphism of apolipoprotein E and occurrence of dysbetalipoproteinemia in man. *Nature* 1977; **269:** 604–7.

9 Utermann G. Morgagni lecture: genetic polymorphism of apolipoprotein E – impact on plasma lipoprotein metabolism. In: Crepaldi G, Tiengo A, Baggio G, eds. *Diabetes, Obesity and Hyperlipidemias*. Amsterdam: Elsevier, 1985; 1–28.

10 Huang Y, Schwendner SW, Rall SC, Sanan DA, Mahley RW. Apolipoprotein E2 transgenic rabbits. Modulation of the type III hyperlipoproteinemic phenotype by estrogen and occurrence of spontaneous atherosclerosis. *J Biol Chem* 1997; **272:** 22685–94.

11 Gevers Leuven JA. Sex steroids and lipoprotein metabolism. *Pharmac Ther* 1994; **64:** 99–126.

12 Sacks FM, Walsh BW. Sex hormones and lipoprotein metabolism. *Curr Opin Lipidol* 1994; **5:** 236–40.

13 Woodruff JD, Pickar JH. Incidence of endometrial hyperplasia in postmenopausal women taking conjugated estrogens (Premarin) with medroxyprogesterone acetate or conjugated estrogens alone. The Menopause Study Group. *Am J Obstet Gynecol* 1994; **170:** 1213–23.

14 Gambrell RDJ. The prevention of endometrial cancer in postmenopausal women with progestogens. *Maturitas* 1978; **1:** 107–12.

15 de Visser J, Coert A, Feenstra H, van der Vies J. Endocrinological studies with (7α,17α)-17-hydroxy-7-methyl-19-norpregn-5(10)-en-20-yn-3-one (Org OD 14). *Arzneimittelforschung* 1984; **34:** 1010–7.

16 Albertazzi P, Di Micco R, Zanardi E. Tibolone: a review. *Maturitas* 1998; **30:** 295–305.

17 Rymer JM. The effects of tibolone. *Gynecol Endocrinol* 1998; **12:** 213–20.

18 Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. *N Engl J Med* 1991; **325:** 1196–204.

19 Walsh BW, Sacks FM. Effects of low dose oral contraceptives on very low-density and low-density lipoprotein metabolism. *J Clin Invest* 1993; **91:** 2126–32.

20 Wolfe BM, Huff MW. Effects of combined estrogen and progestin administration on plasma lipoprotein metabolism in postmenopausal women. *J Clin Invest* 1989; **83:** 40–5.

21 Wolfe BM, Huff MW. Effects of low dosage progestin-only administration upon plasma triglycerides and lipoprotein metabolism in postmenopausal women. *J Clin Invest* 1993; **92:** 456–61.

22 Applebaum-Bowden D, McLean P, Steinmetz A *et al.* Lipoprotein, apolipoprotein, and lipolytic enzyme changes following estrogen administration in postmenopausal women. *J Lipid Res* 1989; **30:** 1895–906.

23 Tikkanen MJ, Nikkila EA, Kuusi T, Sipinen SU. High density lipoprotein-2 and hepatic lipase: reciprocal changes produced by estrogen and norgestrel. *J Clin Endocrinol Metab* 1982; **54:** 1113–7.

24 Tikkanen MJ, Nikkila EA. Regulation of hepatic lipase and serum lipoproteins by sex steroids. *Am Heart J* 1987; **113:** 562–7.
25 Campos H, Walsh BW, Judge H, Sacks FM. Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. *J Clin Endocrinol Metab* 1997; **82:** 3955–63.
26 Kushwaha RS, Hazzard WR, Gagne C, Chait A, Albers JJ. Type III hyperlipoproteinemia: paradoxical hypolipidemic response to estrogen. *Ann Intern Med* 1977; **87:** 517–25.
27 Falko JM, Schonfeld G, Witzum JL, Kolar J, Weidman SW. Effects of estrogen therapy on apolipoprotein E in type III hyperlipoproteinemia. *Metabolism* 1979; **28:** 1171–7.
28 Stuyt PM, Demacker PN, van't Laar A. A study of the hypolipidemic effect of estrogen in type III hyperlipoproteinemia. *Horm Metab Res* 1986; **18:** 607–10.
29 Havekes LM, de Knijff P, Beisiegel U, Havinga JR, Smit M, Klasen E. A rapid micromethod for apolipoprotein E phenotyping directly in serum. *J Lipid Res* 1987; **28:** 455–63.
30 Reymer PWA, Groenemeyer BE, van de Burg R, Kastelein JJP. Apolipoprotein E genotyping on agarose gels. *Clin Chem* 1995; **41:** 1046–7.
31 Rumsey SC, Galeano NF, Arad Y, Deckelbaum RJ. Cryopreservation with sucrose maintains normal physical and biological properties of human plasma low density lipoproteins. *J Lipid Res* 1992; **33:** 1551–61.
32 Princen HM, van Duyvenvoorde W, Buytenhek R, *et al.* No effect of consumption of green and black tea on plasma lipid and antioxidant levels and on LDL oxidation in smokers. *Arterioscler Thromb Vasc Biol* 1998; **18:** 833–41.
33 Jong MC, Dahlmans VEH, Hofker MH, Havekes LM. Nascent very-low-density lipoprotein triacylglycerol hydrolysis by lipoprotein lipase is inhibited by apolipoprotein E in a dose-dependent manner. *Biochem J* 1997; **328:** 745–50.
34 van Poppel G, Hospers J, Buytenhek R, Princen HM. No effect of β-carotene supplementation on plasma lipoproteins in healthy smokers. *Am J Clin Nutr* 1994; **60:** 730–4.
35 Zhao SP, Smelt AH, Leuven JA, van den Maagdenberg AM, van der Laarse A, van't Hooft FM. Lipoproteins in familial dysbeta-lipoproteinemia. Variation of serum cholesterol level associated with VLDL concentration. *Arterioscler Thromb* 1993; **13:** 316–23.
36 Ginsburg J, Prelevic GM. Tibolone and the serum lipid/lipoprotein profile: does this have a role in cardiovascular protection in postmenopausal women? *Menopause* 1999; **6:** 87–9.
37 Kloosterboer HJ, Benedek Jaszmann LJ, Kicovic PM. Long-term effects of OrgOD14 on lipid metabolism in post-menopausal women. *Maturitas* 1990; **12:** 37–42.
38 Hannover Bjarnson N, Bjarnson K, Haarbo J, Coelingh Benink HJT, Christiansen C. Tibolone: influence on markers of cardiovascular disease. *J Clin Endocrinol Metab* 1997; **82:** 1752–6.
39 Feher MD, Cox A, Levy A, Mayne P, Lant AF. Short term blood pressure and metabolic effects of tibolone in postmenopausal women with non-insulin dependent diabetes. *Br J Obstet Gynaecol* 1996; **103:** 281–3.
40 Prelevic GM, Beljic T, Balint-Peric L, Ginsburg J. Metabolic effects of tibolone in postmenopausal women with non-insulin dependent diabetes mellitus. *Maturitas* 1998; **28:** 271–6.
41 Walsh BW, Li H, Sacks FM. Effects of postmenopausal hormone replacement with oral and transdermal estrogen on high density lipoprotein metabolism. *J Lipid Res* 1994; **35:** 2083–93.
42 Trigatti B, Rigotti A, Krieger M. The role of the high-density lipoprotein receptor SR-BI in cholesterol metabolism. *Curr Opin Lipidol* 2000; **11:** 123–31.
43 Gilmer MDG. Mechanism of action/effects of androgens on lipid metabolism. *Int J Fertil* 1992; **37** (Suppl. 2): 83–92.
44 Santamarina-Fojo S, Haudenschild C, Amar M. The role of hepatic lipase in lipoprotein metabolism and atherosclerosis. *Curr Opin Lipidol* 1998; **9:** 211–9.
45 Mahley RW, Ji ZS. Remnant lipoprotein metabolism: key pathways involving cell-surface heparan sulfate proteoglycans and apolipoprotein E. *J Lipid Res* 1999; **40:** 1–16.
46 Brussaard HE, Gevers Leuven JA, Frölich M, Kluft C, Krans HMJ. Short-term oestrogen replacement therapy improves insulin resistance, lipids and fibrinolysis in postmenopausal women with NIDDM. *Diabetologia* 1997; **40:** 843–9.

Received 18 June 2001; revision received 13 November 2001; accepted 22 November 2001.

Correspondence: Dr J.A. Gevers Leuven, TNO-Prevention and Health, Gaubius Laboratory, Zernikedreef 9, 2333 CK Leiden/2301 CE Leiden, PO Box 2215, The Netherlands (fax: +31 71 5181904; e-mail: ja.geversleuven@pg.tno.nl).