

## Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Ladenson PW, Kristensen JD, Ridgway EC, et al. Use of the thyroid hormone analogue eprotirome in statin-treated dyslipidemia. *N Engl J Med* 2010;362:906-16.

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Supplement to: Ladenson PW, Kristensen JD, Ridgway EC, Olsson AG, Carlsson B, Klein I, Baxter JD, and Angelin B. Use of the Thyroid Hormone Analog Eprotirome in Statin-Treated Dyslipidemia

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## Analytical Methods

Direct enzymatic photometric methods were used to determine serum concentrations of LDL-cholesterol (Synchron LX/Beckman), triglyceride (GPO Trinder) and free fatty acids (Thermo T20xti, Wako, TX, USA). Total Cholesterol and HDL-Cholesterol were quantified with standard enzymatic techniques (cholesteroxidase and homogenous; Synchron LX/Beckman). Apolipoproteins A-I and B were measured by immunoturbidimetric assays (Synchron LX/Beckman).

Lp(a) was measured by an immunoturbidimetric assay from Roche (Tinaquant Lp(a), cat. No. 11660390) employing a polyclonal rabbit anti-human Lp(a) antibody, according to the manufacturer's instructions. Controls were run twice daily using low control (DAKO cat.no. X0961, set value 23 mg/dL) and high control (DAKO cat.no. X0962, set value 95 mg/dL). The total CV% at these levels for the period of the study was 8.0% and 4.7%, respectively. Our assay has been validated against WHO/IFCC SRM2B calibrated assays based on monoclonal antibodies (Marcovina SM, et al, Clin Chem 2000; 46: 1956-67; Dati F, et al, Clin Chem Lab Med 2004; 42: 60-6). The polyclonal antibodies do not recognize apo B or plasminogen, but do tend to overestimate Lp(a) values in samples with large apo(a) isoforms and underestimate samples with small apo(a) size.

Thyroid stimulating hormone concentration was determined by immunoenzymatic assay (Dxl/Beckman). Free T3 and free T4 were determined by chemiluminiscent immunoassays (Dxl/Beckman). Total T3 and total T4 were determined with immunofluorometric assays (Wallac/AutoDelfia). Thyroxine binding globulin was determined by competitive immunoassay (Immulite 2000/Siemens). Sex hormone binding globulin was determined by chemiluminiscent immunoassay (Modular E/Roche-reagents).

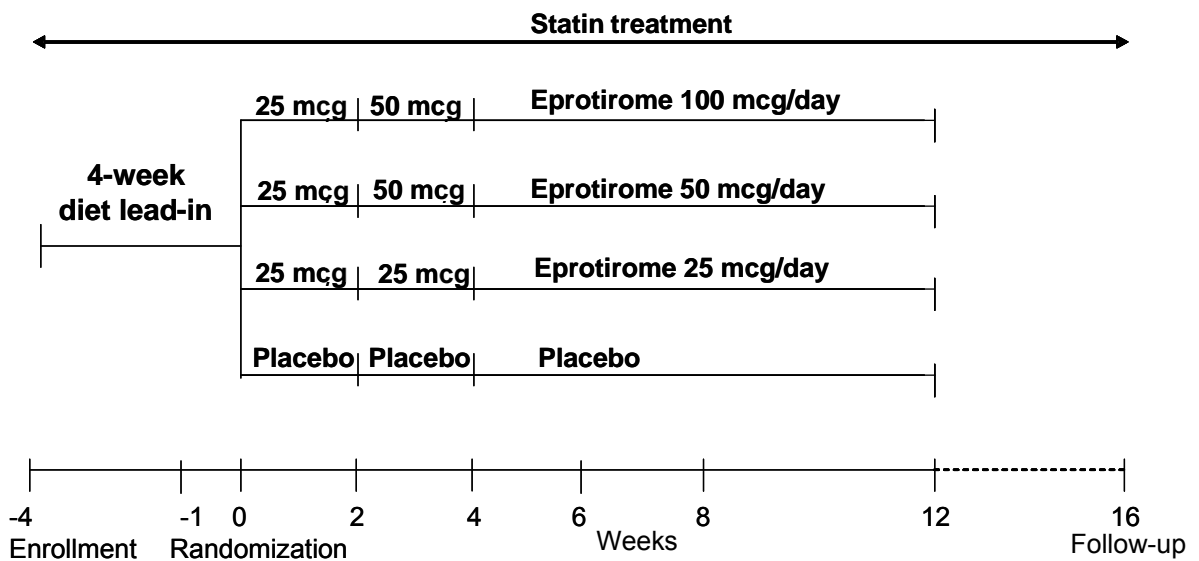
All other clinical chemistry tests not specified above were performed with current standard techniques at the central laboratory.

All analysis were performed at Karolinska University Laboratory, Stockholm, Sweden, except for TBG, which was analyzed at the department of Clinical chemistry, Malmö Hospital, Sweden.

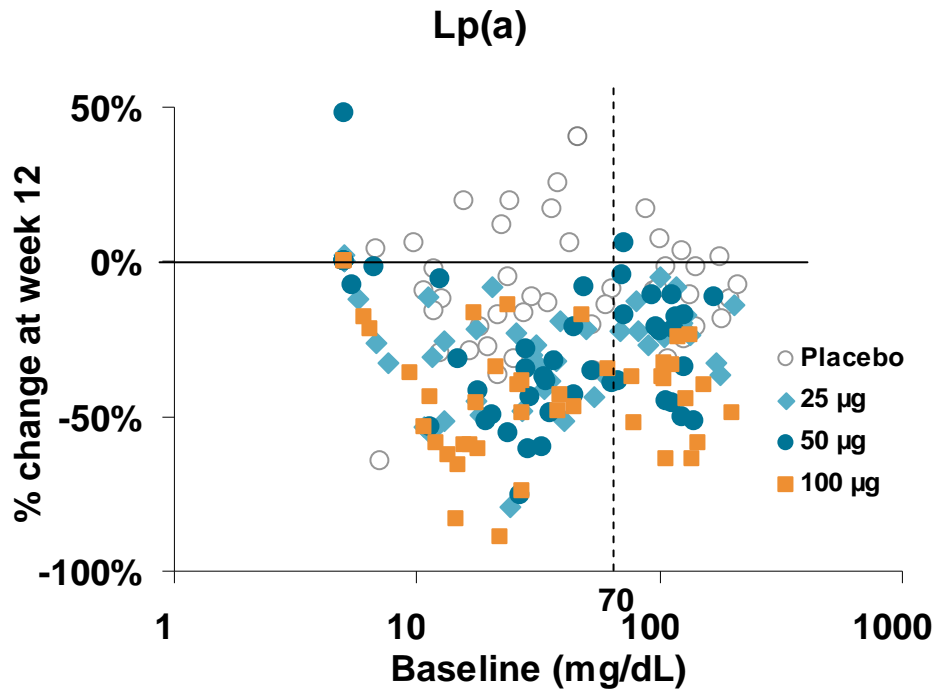
Statistical analyses were performed with SAS software (SAS Institute, Inc., Cary, NC USA)

## Study Design

Figure 1: Study Design



**Figure 2: Percentage changes in serum Lp(a) lipoprotein concentration as a function of the baseline LP(a) level for individual patients. Individuals with baseline values above 70 mg/dL represented 34% of the study patients.**



## **Serious Adverse Events and Study Patient Discontinuations**

In all, there were 8 severe adverse events reported: 1 in the placebo group (salmonella food poisoning); 3 in the 25 microgram group (erysipelas, ankle fracture, and duodenal ulcer); one in the 50 microgram group (dizziness), and 3 in the 100 microgram group (fibula fracture, loss of consciousness, and worsening of arthritis). All of these were categorized as severe adverse events by virtue of associated hospitalization. None of these severe adverse events were judged by the site investigator to have been related to the study medications, either eprotirome or statin.

Twenty-one patients withdrew from the study. Nine of these patient withdrawals were related to adverse events: 2 in the placebo group (abnormal thyroid function tests in 1 and neck pain in 1); 2 in the 25 microgram eprotirome group (stomach pain, nausea, pruritus and urticaria in 1; and change in skin touch sensitivity in 1); 4 in the 50 microgram eprotirome group (palpitation in 1, headache, tiredness, dizziness, and nausea in 1; abnormal liver function tests in 1; and abdominal pain in 1); and 1 in the 100 microgram group (worsening of pre-existing rosacea). Other reasons for withdrawal included withdrawal of consent (2 in the placebo and 2 in the 100 microgram eprotirome group; inappropriate enrollment (1 in the placebo group and 2 in the 50 microgram eprotirome group); protocol violation in (1 in the 50 microgram eprotirome group); and other reasons (1 in the placebo group, 2 in the 25 microgram eprotirome group and 1 in the 100 microgram eprotirome group).

## Overview of Adverse Events

**Table 1: Number of patients with adverse events**

Number of Patients	Treatment group							
	Placebo (N=49)		25 mcg (N=48)		50 mcg (N=48)		100 mcg (N=44)	
	n	%	n	%	n	%	n	%
Any adverse event	28	57	30	63	34	71	26	59
Serious adverse event	1	2.0	3	6	1	2	3	7
Discontinuation of study treatment due to adverse event	2	4.1	2	4	4	8	1	2

**Table 2: Number of adverse events given by intensity and relationship to study medication and statin**

Number of Adverse Events	Treatment group			
	Placebo (N=49)	25 mcg (N=48)	50 mcg (N=48)	100 mcg (N=44)
Total number of reported adverse events	82	97	115	68
<b>Intensity</b>				
Mild	66	75	82	54
Moderate	15	19	31	12
Severe	1	3	2	2
<b>Relationship to Eprotirome/ Placebo</b>				
Not likely / Unlikely	63	62	97	55
Possible	16	33	16	13
Probable	3	2	2	0
<b>Relationship to Statin</b>				
Not likely / Unlikely	79	81	105	60
Possible	3	14	9	8
Probable	0	2	1	0
Total number of Serious adverse events	1	3	1	3
related to Eprotirome/ Placebo	0	0	0	0
related to Statin	0	0	0	0

## Study Patients' Vital Signs

**Table 3: Vital signs**

		Baseline	Week 12	Change, Week 12 - Pretreatment	Week 16
Systolic blood pressure (mmHg) Overall p=0.11	Placebo	135 (15)	134 (14)	-1 (13)	135 (16)
	25 mcg	135 (14)	133 (16)	-2 (12)	134 (20)
	50 mcg	137 (13)	130 (14)	-8 (14)	135 (12)
	100 mcg	135 (14)	132 (14)	-3 (12)	135 (16)
Diastolic blood pressure (mmHg) Overall p=0.29	Placebo	81 (8)	81 (8)	-0. (8)	82 (9)
	25 mcg	83 (8)	80 (9)	-3(8)	81 (9)
	50 mcg	83 (7)	79 (9)	-4 (8)	81 (7)
	100 mcg	83 (8)	80 (10)	-3 (7)	81 (9)
Pulse (beats/min) Overall p=0.26	Placebo	63 (9)	65 (10)	3 (10)	65 (12)
	25 mcg	65 (9)	64 (9)	-0 (9)	63 (10)
	50 mcg	63 (8)	64 (9)	2 (8)	63 (8)
	100 mcg	63 (8)	62 (8)	-0 (6)	62 (9)
Body Temperature (°C) Overall p=0.21	Placebo	36.3 (0.4)	36.3 (0.5)	-0.0 (0.6)	36.3 (0.3)
	25 mcg	36.2 (0.5)	36.4 (0.4)	0.2 (0.6)	36.3 (0.4)
	50 mcg	36.2 (0.4)	36.3 (0.5)	0.1 (0.5)	36.3 (0.4)
	100 mcg	36.2 (0.4)	36.3 (0.4)	0.0 (0.5)	36.4 (0.4)
Body weight (kg) Overall p=0.12	Placebo	79.6 (11.7)	78.7 (10.6)	0.0 (1.5)	79.6 (11.1)
	25 mcg	81.0 (14.0)	81.5 (13.7)	-0.2 (1.3)	81.8 (13.5)
	50 mcg	87.9 (15.0)	86.1 (15.7)	-0.6 (1.6)	86.0 (15.6)
	100 mcg	83.1 (12.5)	83.7 (11.4)	-0.7 (1.3)	83.4 (11.4)

Mean (SD)

## **Sex Hormone Binding Globulin Data**

In eprotirome-treated men, serum follicle stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone concentrations were increased, but there were no changes in the calculated free testosterone or bioactive testosterone levels. These findings would be consistent with an appropriate increase in testosterone production to load the increased SHBG binding capacity. In treated women, all of whom were 50 years or older, the SHBG increase with eprotirome therapy was not associated with any serum estradiol, FSH, or LH change. No adverse effects related to sexual dysfunction or libido were observed with eprotirome treatment.

**Table 4: Serum SHBG, FSH, LH and Estradiol Levels in Female Study Patients**

		Baseline Mean(SD)	Week 12 Mean(SD)	Absolute Change, Week 12-Baseline Mean(SD)
<b>SHBG (nmol/L)</b>	Placebo	41.0 (19.4)	41.3 (19.9)	1.2 (6.3)
Overall p<0.0006	25 mcg	46.5 (15.8)	98.7 (31.7)	53.1 (19.2)***
Normal range: 29-95 (fertile), 25-100 (postmenopausal)	50 mcg	52.1 (25.1)	120.3 (26.1)	83.2 (23.8)***
	100 mcg	34.3 (15.5)	127.3 (16.9)	100.8 (8.8)***
<b>FSH (U/L)</b>	Placebo	66.1 (19.6)	66.4 (20.5)	-3.2 (6.3)
Overall p=0.97	25 mcg	59.1 (23.7)	56.5 (22.6)	-1.6 (5.6)
Normal range: 2.5-10 (follicular phase), 4-14 (mid phase), 0.7-8.5 (luteal phase), 25-150 (postmenopausal)	50 mcg	68.9 (22.9)	68.9 (26.2)	1.6 (5.7)
	100 mcg	63.2 (20.4)	55.3 (18.0)	-2.1 (4.1)
<b>LH (U/L)</b>	Placebo	32.6 (12.0)	30.6 (11.3)	-3.6 (4.2)
Overall p=0.48	25 mcg	31.4 (14.6)	29.1 (12.8)	-1.6 (4.7)
Normal range: 1.8-12 (follicular phase), 18-90 (mid phase), 0.6-15 (luteal phase), 18-78 (postmenopausal)	50 mcg	32.6 (11.7)	32.1 (10.9)	1.1 (4.6)
	100 mcg	32.6 (8.7)	29.1 (11.4)	-0.8 (5.5)
<b>Estradiol (pmol/L)</b>	Placebo	34.7 (11.5)	31.7 (11.7)	-4.3 (7.5)
Overall p=1.0	25 mcg	31.1 (13.7)	29.4 (10.7)	-2.3 (15.2)
Normal range: 100-200 (follicular phase), 500-1500 (mid phase), 200-800 (luteal phase), <50 (postmenopausal)	50 mcg	33.9 (9.6)	27.7 (9.1)	-4.3 (11.3)
	100 mcg	41.4 (22.6)	35.1 (9.9)	-8.0 (25.00)

Overall p-value from overall F-test of differences between the treatment groups [ANCOVA with Baseline value as covariate and adjusted with the Bonferroni method for multiplicity (four tests)];

\* (p<0.05), \*\* (p<0.01) or \*\*\* (p<0.001) indicates a statistically significant difference from Placebo in change from Baseline to Week 12.

**Table 5: Serum SHBG, FSH, LH and Testosterone Levels in Male Study Patients**

		Baseline Mean(SD)	Week 12 Mean(SD)	Absolute Change, Week 12-Baseline Mean(SD)
<b>SHBG (nmol/L)</b>	Placebo	37.9 (10.9)	38.9 (11.3)	0.8 (4.3)
Overall p<0.0006	25 mcg	33.9 (10.7)	66.9 (23.2)	32.8 (16.1)***
Normal range:	50 mcg	32.4 (13.2)	76.6 (27.9)	44.0 (21.6)***
15-56 (<60 years);	100 mcg	31.9 (12.8)	104.1 (36.9)	74.1 (31.6)***
20-70 (≥60 years)				
<b>FSH (U/L)</b>	Placebo	5.0 (2.7)	5.4 (2.7)	0.3 (0.7)
Overall p=0.0036	25 mcg	6.8 (9.3)	7.5 (8.9)	0.6 (1.3)
Normal range: 1-12.5	50 mcg	5.4 (4.1)	5.5 (3.6)	0.6 (0.7)
	100 mcg	6.7 (5.7)	8.2 (6.5)	1.5 (1.4)***
<b>LH (U/L)</b>	Placebo	3.6 (2.1)	4.3 (2.2)	0.5 (1.4)
Overall p=0.022	25 mcg	4.4 (3.6)	5.9 (3.1)	1.4 (2.2)*
Normal range: 1.2-9.6	50 mcg	3.8 (2.2)	5.2 (2.8)	1.6 (1.9)*
	100 mcg	4.7 (3.2)	7.2 (5.0)	2.5 (2.5)***
<b>Total Testosterone (nmol/L)</b>	Placebo	14.4 (3.5)	13.5 (3.5)	-0.7 (1.9)
Overall p<0.0001	25 mcg	13.7 (6.0)	18.7 (7.0)	5.6 (3.5)***
Normal range:	50 mcg	12.4 (4.1)	19.1 (6.8)	6.9 (4.6)***
10-30 (20-49 years),	100 mcg	13.7 (4.2)	25.4 (8.0)	12.00 (5.7)***
6-29 (≥50 years)				
<b>Free Testosterone (nmol/L)</b>	Placebo	0.28 (0.06)	0.26 (0.06)	-0.02 (0.05)
Overall p=0.27	25 mcg	0.29 (0.13)	0.27 (0.10)	-0.01 (0.09)
	50 mcg	0.27 (0.08)	0.23 (0.05)	-0.03 (0.07)
	100 mcg	0.30 (0.08)	0.24 (0.07)	-0.06 (0.06)

Overall p-value from overall F-test of differences between the treatment groups [ANCOVA with Baseline value as covariate and adjusted with the Bonferroni method for multiplicity (six tests)];

\* (p<0.05), \*\* (p<0.01) or \*\*\* (p<0.001) indicates a statistically significant difference from Placebo in change from Baseline to Week 12.