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Full Length Article

Effect of hormone replacement therapy on atherogenic lipid profile in postmenopausal women

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ABSTRACT

Background: Women develop cardiovascular disease (CVD) approximately 7–10 years later than men, but progress with similar risk after menopause. Recent studies suggest that hormone replacement therapy (HRT) is cardioprotective when initiated early after menopause, but the mechanisms involved are still unclear.
Objective: In the current study, we aimed to examine the effects of HRT treatment on the plasma atherogenicity in postmenopausal women. We studied the total lipid profile in blood samples collected in a randomized, double-blinded, placebo controlled clinical trial of women with a history of venous thrombosis (VT), the EVTET study.
Methods: One-hundred and forty postmenopausal women < 70 years were included in EVTET and randomized either to active treatment (one tablet of 2 mg estradiol and 1 mg norethisterone acetate daily) ($n = 71$) or placebo ($n = 69$). Blood samples were taken at baseline and after 3 months and subjected to routine assessment of hemostatic factors and lipids.
Results: Our study show that HRT compared to placebo significantly reduced plasma levels of Lp(a), ApoA1, ApoB, total cholesterol (TC), HDL-C, LDL-C, TC/HDL-C and LDL-C/HDL-C ratio at 3 months. No effect was observed on ApoB/ApoA1 ratio or triglycerides. The change in Lp(a) was significantly and inversely correlated with the change in estradiol ($r = -0.32$; $P = 0.001$) and positively correlated to the change in lipids, tissue factor pathway inhibitor activity and antigen, protein C and fibrinogen (r between 0.26 and 0.45, $p < 0.01$).
Conclusion: In sum, this study confirms a strong effect of HRT on atherogenic lipids with a large reduction in the pro-thrombotic Lp(a), suggesting an overall favorable effect on thrombogenicity after HRT replacement therapy in post-menopausal women at risk of VT.

1. Introduction

Lipid metabolism is of major importance for development of atherosclerotic disorders, and it is well established that elevated serum low density lipoprotein cholesterol (LDL-C) levels, as well as low levels of high density lipoprotein cholesterol (HDL-C), are associated with increased cardiovascular risk [1,2]. Further, elevated levels of apolipoprotein (APO) B, the major apolipoprotein in LDL, lipoprotein (a) (Lp [a]) and circulating triglycerides (TG), are associated with increased risk of coronary heart disease (CHD) [3–9]. On the other hand, a cardioprotective role of APOA1, a constituent of plasma HDL, has been suggested [9,10].

In western societies, women develop CHD approximately 7–10 years later than men, but after the age of 75 years, women represent the majority of patients admitted with CHD [11]. The reason for these differences is not fully understood, but could partly be explained by clear differences in cardiovascular risk factors among women and men. A Dutch cohort study of over 130,000 individuals reported that men are

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Table 1
Characteristics and laboratory data of study population.

	Reference values[a]	Baseline	Placebo 3 months	Change	Baseline	HRT 3 months	Change
Age, years		55.0 (5.2)			55.5 (6.8)		
BMI, kg/m^2		27.4 (2.8)			26.7 (4.7)		
n = [b]		66–68	59–60	59–60	66–70	62–65	62–65
Lipids							
Total cholesterol, mmol/L	3.9–7.8[c]	6.61 (1.18)	6.55 (1.38)	0.04 (0.74)	6.51 (0.97)	5.46 (0.82)***,†††	−0.93 (0.66)†††
HDL cholesterol, mmol/L	1.0–2.7	1.63 (0.40)	1.69 (0.51)	0.03 (0.25)	1.75 (0.45)	1.57 (0.39)***	−0.16 (0.27)†††
LDL cholesterol, mmol/L	2.0–5.3[c]	4.21 (1.29)	4.06 (1.23)	−0.02 (0.65)	4.15 (0.95)	3.36 (0.77)***,†††	−0.72 (0.56)†††
Non-HDL cholesterol		4.98 (1.20)	4.86 (1.40)	0.01 (0.72)	4.76 (1.02)	3.9 (0.83)***,†††	−0.77 (0.59)†††
Triglycerides, mmol/L	0.45–2.6	1.36 (1.01, 1.86)	1.32 (1.00, 1.91)	0.03 (−0.17, 0.31)	1.24 (0.84, 1.66)	1.07 (0.88, 1.47)††	−0.05 (−0.31, 0.13)
ApoA1, g/L	1.1–2.3	1.42 (1.30, 1.59)	1.43 (1.29, 1.63)	0.02 (−0.06, 0.13)	1.44 (1.24, 1.60)	1.34 (1.22, 1.44)***,††	−0.09 (−0.18, 0.01)†††
ApoB, g/L	0.5–1.3	1.33 (1.16, 1.53)	1.27 (1.14, 1.50)	−0.02 (−0.09, 0.08)	1.27 (1.10, 1.45)	1.16 (1.03, 1.34)***,††	−0.11 (−0.2, 0.00)†††
Lipoprotein (a), pmol/L	< 250[d]	288 (148, 819)	382 (151, 878)*	−29 (−71, 14)	267 (169, 739)	221 (125, 518)***,†	−72 (−197, −24)††
apoB/apoA		0.91 (0.75, 1.16)	0.88 (0.72, 1.06)	−0.02 (−0.10, 0.05)	0.86 (0.74, 1.09)	0.85 (0.75, 1.02)	−0.02 (−0.11, 0.07)
TC/HDL-C		3.94 (3.29, 4.94)	3.83 (3.15, 4.92)	0.01 (−0.33, 0.28)	3.81 (3.03, 4.63)	3.53 (2.98, 4.17)**	−0.29 (−0.60, 0.10)†
LDL-C/HDL-C		2.55 (2.03, 3.33)	2.32 (1.73, 3.05)	−0.06 (−0.37, 0.20)	2.41 (1.73, 3.17)	2.22 (1.80, 2.57)***	−0.28 (−0.59, 0.12)†
Coagulation							
Fibrinogen, g/L	2.0–4.0	3.78 (0.67)	3.7 (0.50)	−0.11 (0.55)	3.73 (0.68)	3.8 (0.75)	0.05 (0.64)
Protein C, IU/dL	70–130	130 (20)	128 (21)	−2 (15)	124 (18)†	113 (19)***,†††	−12 (14)†††
TFPI activity		129 (26)	127 (24)	−1 (13)	134 (25)	117 (29)***,†	−17 (16)†††
TFPI antigen		29 (7)	27 (6)	−1 (5)	29 (7)	21 (5)***,†††	−8 (6)†††
Miscellaneous							
Estradiol, nmol/L		14 (10, 18)	16 (11, 22)	1 (−2, 6)	15 (10, 23)	166 (90, 223)***†††	129 (63, 206)†††
CRP, mg/L	< 4	1.85 (0.70, 3.56)	2.19 (0.61, 3.91)	−0.02 (−0.63, 0.64)	1.28 (0.73, 2.71)	2.59 (1.50, 6.12)***†	1.04 (0.08, 2.77)†††
NAMPT, pg/mL		456 (225, 1104)	428 (188, 1048)	−84 (−245, 237)	443 (224, 1006)	629 (398, 923)	89 (−280, 253)

Mean (SD) or median (25–75) or frequency (%). Apo, apolipoprotein; g/L, grams per litre; HDL, high density lipoprotein; kg, kilograms; LDL, low density lipoprotein; Lp(a), lipoprotein (a); m, meters; mg, milligram; mmol/L, millimoles per litre; nmol/, nanomoles per litre. Coagulation markers previously published [38].

[a] From the Norwegian "National user manual for medical biochemistry".
[b] Numbers vary due to variation in sample availability.
[c] > 49 years.
[d] Reference value according to method of analysis.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ vs. baseline.
† $p < 0.05$.
†† $p < 0.01$.
††† $p < 0.001$ vs. same time-point placebo.

characterized by a higher degree of dyslipidemia and display a different temporal profile in lipids with advancing age compared to women [12,13]. High levels of non-HDL cholesterol and TG seem to be more important risk factors for cardiovascular disease (CVD) in women than in men, whereas the high HDL-C levels may be more protective in both genders [14,15].

The transition phase when the women undergo a gradual loss of estrogen (menopause), usually within the age range from 40 to 60 years, is associated with cardiovascular complications [16,17]. Further, menopause is associated with modest proatherogenic changes in lipoprotein pattern, such as increased TC, LDL-C and APOB [18–20], small dense LDL particles [21], and increased levels of Lp(a) [22,23]. The cardioprotective effect of female sex-hormones has been widely discussed, but is still unclear [19,24]. However, observational data have consistently demonstrated a decreased risk of CHD with use of hormone replacement therapy (HRT) in postmenopausal women [25–30] and a meta-analysis covering > 100 randomized trials involving 39,049 postmenopausal women demonstrated that HRT reduced CHD events when administered to younger postmenopausal women [31].

Several studies have investigated isolated constituents of the lipid profile after HRT, in relation to CHD risk [18,31–36]. Sex steroids, both endogenously or given exogenously, are associated with changes of lipoprotein pattern [18–23], however the effect of HRT on total lipid profile, remains to be studied in order to better reflect the overall atherogenic profile [8,37]. In the present study we therefore investigated the effect of HRT on total lipid profile in plasma obtained during a randomized, double-blinded, placebo-controlled clinical trial (RCT) [38].

2. Methods

In the current study, we used serum or plasma samples from the Estrogen in Venous Thromboembolism Trial (EVTET) study; a randomized, double-blinded, and placebo-controlled clinical trial (RCT) with a double triangular sequential design [38]. A more detailed description about the methodological aspects of the EVTET study has previously been described [38,39]. Briefly, one-hundred and forty postmenopausal women < 70 years were included in the study. They were randomized either to active treatment (one tablet of 2 mg estradiol and 1 mg norethisterone acetate (NETA) daily) (n = 71) or placebo (n = 69). *Postmenopausal* was defined as not having had natural menstruation for at least one year. The aim of the main study was to determinate if HRT alters the risk of VT in high risk women defined as women with at least one prior VT event, i.e., either deep vein thrombosis or pulmonary embolism, before inclusion in the study. The primary outcome was

recurrent VT. The study was terminated prematurely as other studies were reporting increased risk of VT on HRT, during execution of the study [39,40]. The study was approved by the Norwegian Medicines Agency and the eastern Norway Regional Committee for Medical and Health Research Ethics, and all participants gave their written informed consent to participate. The study was executed during 1996–1998 before introduction of mandatory trial registration prior to trial execution.

2.1. Analyses

The EVTET study included follow-up visits at 3, 12 and 24 months. However, in the present study, only plasma and data from baseline and at three months were used, as lipids were our primary target of investigation and changes in lipid levels are usually stable 12 weeks after an intervention. The hemostatic parameters including fibrinogen, tissue factor protein inhibitor (TFPI) and protein C were assayed in batches at the Hematological Research Laboratory at Oslo University Hospital (formerly Ullevål University Hospital) during 1999–2001, as described earlier [38]. In detail, commercial kits were used and run essentially as described by the manufacturer. Fibrinogen was assayed on an ACL Futura coagulometer (Instrumentation Laboratories, Milan, Italy) with reagents from Instrumentation Laboratories. Protein C activity was assayed with the chromogenic assay Coamatic Protein C from Chromogenix AB (Mölndal, Sweden). Tissue factor pathway inhibitor (TFPI) activity and free TFPI antigen were assayed with in-house methods as described earlier [41,42]. Estradiol was measured using the Vidas Estradiol II enzyme linked fluorescent assay (Biomerieux, Marcy-l'Étoile, France) in the same laboratory. Routine measurements of HDL-C, TG, total cholesterol [TC], APOA1, APOB (total) and Lp(a) were measured consecutively at the Department of Clinical Biochemistry, Oslo University Hospital. LDL-C was calculated using Friedewald's formula for mmol/L given by: TC-HDL-C-(0.45*TG). For some of the participants, follow up samples were missing or available in limited amount, excluding some of the analysis. The analyzed number for the respective factors is indicated in Tables 1 and 2.

2.2. Statistics

All data at baseline and 3 months intervention were tested parametric or non-parametric according to their distribution. Treatment effects were calculated as the difference from baseline to 3 months, and presented as percentage change from baseline within each group. Data were presented as mean (SD or 95% confidence intervals) or median (25–75 percentiles). Independent samples test (*t*-test or Mann Whitney U) were used for testing for differences in change score between placebo and treatment group. The Wilcoxon or paired *t*-test was used to test for within group differences. Correlation analyses were calculated by the Spearman rank test. All statistical testing were conducted with the software IBM SPSS statistics 22 and GraphPad Prism 8, a probability value of ≤ 0.05 was set as significant.

3. Results

Data from 138 women were available, 68 from the placebo group and 70 from the HRT group. There was no difference between the two intervention groups at baseline in any of the markers except for protein C (Table 1). As expected, estradiol increased significantly in the HRT group. Moreover, three months of HRT treatment significantly reduced plasma levels of Lp(a), ApoA1, ApoB, TC, HDL-C, LDL-C, TC/HDL-C and LDL-C/HDL-C ratio, compared to placebo. In contrast, no significant differences were found in plasma levels of ApoB/ApoA1 ratio and TG between the groups (Fig. 1, Table 1).

The regulation of Lp(a) is not fully understood, but in the present study we found that the change in Lp(a) in the total group was significantly and inversely correlated with the change in estradiol ($r = -0.32$; $P < 0.001$), and positively correlated to the change in total cholesterol ($r = 0.31$; $P < 0.001$), apoB ($r = 0.38$; $P < 0.001$), LDL-C ($r = 0.29$; $P = 0.002$), LDL/HDL ratio ($r = 0.21$, $P = 0.027$), and non-HDL ($r = 0.30$, $P = 0.001$) (Fig. 2). The change in Lp(a) levels were also positively correlated with the changes in several hemostatic parameters; TFPI activity ($r = 0.45$; $P < 0.001$), TFPI antigen ($r = 0.37$; $P < 0.001$), protein C ($r = 0.36$; $P < 0.001$), and fibrinogen ($r = 0.26$; $P = 0.005$) (Fig. 2). No such correlations were found for the other lipid parameters which underscore the targeted impact HRT may have on the Lp(a) level.

3.1. Adverse events

During follow up, 8 women in the intervention group suffered from VT. In a post hoc analysis, these women had higher levels of ApoA1 and HDL at baseline than the women who did not develop VT (ApoA (median [min-max]): 2.0 (1.7–2.1) g/L versus 1.4 (1.2–1.6) g/L, $p < 0.01$; HDL (mean [SD]): 2.2 (0.5) mmol/L versus 1.7 (0.4), $p < 0.01$) (Table 2). The apoB/apoA ratio was also significant lower in the 0.7 (0.5–0.9) versus 0.9 (0.8–1.1), $p < 0.05$. Also, three months of HRT gave a larger reduction in both ApoA1 and HDL levels in these women compared to the rest of the group (Table 2).

4. Discussion

In the present study we found that HRT altered the lipid profile with significant reductions in the levels of Lp(a), ApoB, and LDL-C, non-HDL, TC/HDL-C- and LDL-C/HDL-C ratio as well as a reduction in HDL-C and ApoA1 in postmenopausal women, with no effect on TG. A similar response on the lipid profile has previously been found after use of the same HRT regime [32–36], and both LDL and HDL were reduced by HRT treatment supplemented with an androgenic progestin [18,31–36]. HRT is in general considered cardioprotective, but its connection with atherosclerosis has been widely debated due to its complex relationship. To start, the beneficial effect of HRT seems to be dependent on administration of HRT before the development of advanced atherosclerosis. Estrogen seems to differently affect the endothelial, vascular smooth muscle and inflammatory cells involved in the atherosclerotic process, depending on the stage of its development. Early in the atherosclerotic process, estrogen seems to exhibit anti-atherogenic effects whereas later it seems to have proatherogenic effects [43–46]. Of further importance for the atherogenic effect of HRT, aging is associated with a down regulation of ERs [47,48] and consequently, loss of response to estrogen and protective effects on the vascular endothelium [49]. Further, the administration route of HRT will also affect the atherogenecity, and transdermal HRT administration is suggested as safer than oral HRT for women with increased cardiovascular risk, due to increased levels of TG and CRP after oral, but not transdermal HRT administration [50,51]. On the other side, oral estrogen is shown to cause greater reduction in Lp(a) concentrations than transdermal estrogen [52]. In our study, reduction of the anti-atherogenic lipid particles HDL-C and ApoA1 may seem unfavorable, but importantly, TC/HDL-C and LDL-C/HDL-C ratios were also decreased during HRT, suggesting a net anti-atherogenic effect on the lipid profile. Atherosclerosis and VT share common risk factors, such as age and obesity, however in contrast to the well-established role of plasma lipids in atherogenesis, the role in development of VT needs further investigation [53].

Plasma levels of Lp(a) were significantly reduced after HRT which is in line with previous studies [6,31–36]. The independent role of Lp(a) in increasing CVD risk has recently been well documented. Moreover, Lp(a) has also been suggested to have pro-thrombotic properties. Indeed, its similarity to plasminogen, causing thrombosis through anti-fibrinolytic properties, and a variety of clinical studies support this Lp(a)-thrombus axis [54,55]. However, few factors (pharmaceutical or dietary) influences the plasma level of this lipid parameter; even though treatment with PCSK9 inhibitors seem to have a Lp(a) lowering effect of

Table 2
Characteristics and laboratory data of women randomized to HRT.

	Reference values[a]	No recurrence			Recurrence		
		Baseline	3 months	Change	Baseline	3 months	Change
Age, years		55.2 (6.9)			58.3 (5.6)		
BMI, kg/m^2		27.2 (4.7)			21.7 (0.2)		
n =[b]		61–62	55–59	55–59	7–8	5–7	5–7
Lipids							
Total cholesterol, mmol/L	3.9–7.8[c]	6.40 (0.90)	5.41 (0.74)***	−0.91 (0.65)	7.34 (1.12)††	6.08 (1.40)*	−1.18 (0.78)
HDL cholesterol, mmol/L	1.0–2.7	1.69 (0.40)	1.54 (0.39)	−0.15 (0.25)	2.23 (0.52)††	1.82 (0.40)	−0.31 (0.45)
LDL cholesterol, mmol/L	2.0–5.3[c]	4.10 (0.89)	3.32 (0.73)***	−0.71 (0.57)	4.52 (1.34)	3.81 (1.15)*	−0.81 (0.57)
Non-HDL cholesterol		4.71 (0.98)	3.87 (0.79)***	−0.77 (0.60)	5.10 (1.32)	4.26 (1.30)*	−0.87 (0.49)
Triglycerides, mmol/L	0.45–2.6	1.24 (0.84, 1.69)	1.08 (0.88, 1.58)	−0.03 (−0.29, 0.13)	1.23 (1.02, 1.55)	0.96 (0.72, 1.28)	−0.07 (−0.33, −0.06)
ApoA1, g/L	1.1–2.3	1.39 (1.23, 1.55)	1.34 (1.22, 1.42)***	−0.08 (−0.17, 0.01)	1.99 (1.65, 2.07)††	1.51 (1.33, 1.81)	−0.26 (−0.41, −0.15)
ApoB, g/L	0.5–1.3	1.28 (1.10, 1.44)	1.16 (1.03, 1.34)***	−0.12 (−0.20, 0.00)	1.24 (1.09, 1.66)	1.19 (1.08, 1.53)	−0.08 (−0.14, −0.08)
Lipoprotein (a), pmol/L	< 250[d]	264 (169, 739)	199 (125, 518)***	−86 (−214, −16)	433 (130, 777)	329 (156, 928)	−65 (−133, −46)
apoB/apoA		0.88 (0.76, 1.10)	0.85 (0.75, 1.02)	−0.03 (−0.11, 0.07)	0.68 (0.54, 0.90)†	0.80 (0.79, 0.86)	0.04 (0.02, 0.13)
TC/HDL-C		3.91 (3.22, 4.71)	3.55 (3.04, 4.17)**	−0.29 (−0.60, 0.10)	2.87 (2.66, 4.10)	3.17 (2.89, 3.52)	−0.04 (−0.38, 0.17)
LDL-C/HDL-C		2.54 (2.01, 3.17)	2.23 (1.80, 2.57)***	−0.29 (−0.59, 0.12)	1.72 (1.42, 2.82)	1.97 (1.67, 2.27)	−0.03 (−0.44, 0.24)
Coagulation							
Fibrinogen, g/L	2.0–4.0	3.74 (0.64)	3.79 (0.74)	0.05 (0.65)	3.65 (0.98)	3.96 (0.97)	0.07 (0.62)
Protein C, IU/dL	70–130	124 (19)	114 (20)***	−11 (14)	120 (17)	106 (12)*	−14 (11)
TFPI activity		132 (25)	114 (29)***	−17 (15)	149 (17)	149 (15)††	−2 (25)†
TFPI antigen		29 (7)	21 (5)***	−7 (6)	29 (7)	23 (3)	−7 (6)
Miscellaneous							
Estradiol, nmol/L		15 (10, 24)	167 (90, 235)***	129 (63, 210)	14 (11, 16)	166 (151, 168)**	152 (126, 153)
CRP, mg/L	< 4	1.28 (0.75, 2.71)	2.56 (1.48, 5.96)***	1.00 (0.08, 2.49)	1.22 (0.71, 3.62)	6.05 (2.48, 6.82)	5.32 (1.80, 6.04)
NAMPT, pg/mL		469 (224, 979)	663 (380, 925)	95 (−279, 322)	325 (217, 1827)	522 (435, 895)	86 (−730, 164)

Mean (SD) or median (25–75) or frequency (%). Apo, apolipoprotein; g/L, grams per litre; HDL, high density lipoprotein; kg, kilograms; LDL, low density lipoprotein; Lp(a), lipoprotein (a); m, meters; mg, milligram; mmol/L, millimoles per litre; nmol/, nanomoles per litre. Coagulation markers previously published [38].

* $p < 0.05$.

** $p < 0.01$.

*** $p < 0.001$ vs. baseline.

[a] From the Norwegian "National user manual for medical biochemistry".

[b] Numbers vary due to variation in sample availability.

[c] > 49 years.

[d] Reference value according to method of analysis.

† $p < 0.05$.

†† $p < 0.01$.

approximately 30% [56]. Notably, the change in Lp(a) was significantly and inversely correlated with the change in estradiol and positively correlated to the change in total cholesterol, apoB and LDL, TFPI, protein C and fibrinogen, suggesting interaction with other pro-atherogenic factors and also further suggest an interaction with hemostatic factors and risk of thrombosis. This observation supports the role of Lp(a) as an important risk factor that can be modulated by HRT.

An extensive meta-analysis was performed by Anagnostis et al. in 2017 analyzing 47 publications which had monitored the effect of HRT and tibolone on Lp(a) concentrations in postmenopausal women. The overall finding of this analysis was that HRT may significantly reduce Lp(a) concentrations compared with placebo or no treatment [52]. These studies were, however, performed in individuals with no previous cardiovascular disease and only a very few studies have studied the effect of HRT on Lp(a) in women with established disease [57]. This study is to our knowledge, the first to address this in a population of postmenopausal women with previous VT receiving HRT.

The degree of androgenicity seems to determine the estrogen opposing effect of the progestogen [35,36,58]. The progesterone used in EVTET was NETA which is considered one of the androgenic progestogens [58]. We found no effect of HRT on TG levels; however HDL-C levels were decreased which may be caused by the androgenic NETA [35,36,58]. In addition to finding an opposing effect of HRT (with NETA) on HDL-C and TG, Kwok et al. found NET-Ac to reduce Lp(a) more effectively than less androgenic progestogens, such as desogestrel, but less effective in opposing the estrogen related increase in CRP [18]. In support of the findings by Kwok et al. [18] and also Farish et al. [59], we found a decrease in serum Lp(a) after HRT accompanied by an increase in serum CRP and thrombotic markers, such as fibrinogen and protein C after HRT in the women as previously published [51]. These observations suggest that HRT affects the liver and cause a complex regulation of the overall metabolism.

In our cohort, six of the women, all in the HRT group, suffered recurrent VT during the three months follow-up period. These women had higher levels of ApoA and HDL at baseline, and despite marked decrease after three months HRT, HDL levels remained higher than the women not experiencing these events. HDL has been suggested to have anti-thrombotic effects, and our findings may apparently seem in conflict with previous data, showing no or a negative association between HDL levels and recurrent VT [60,61]. However, these studies did not include HRT and it is possible that it was the observed decrease in HDL-C levels after HRT, and not the baseline levels that predisposed these women for VT in our study. Moreover, recent studies suggest that the relationship between HDL and cardiovascular risk and thrombus formation is more complex. HDL functionality and composition, as well as HDL:ApoA1 ratio are probably important to determine the

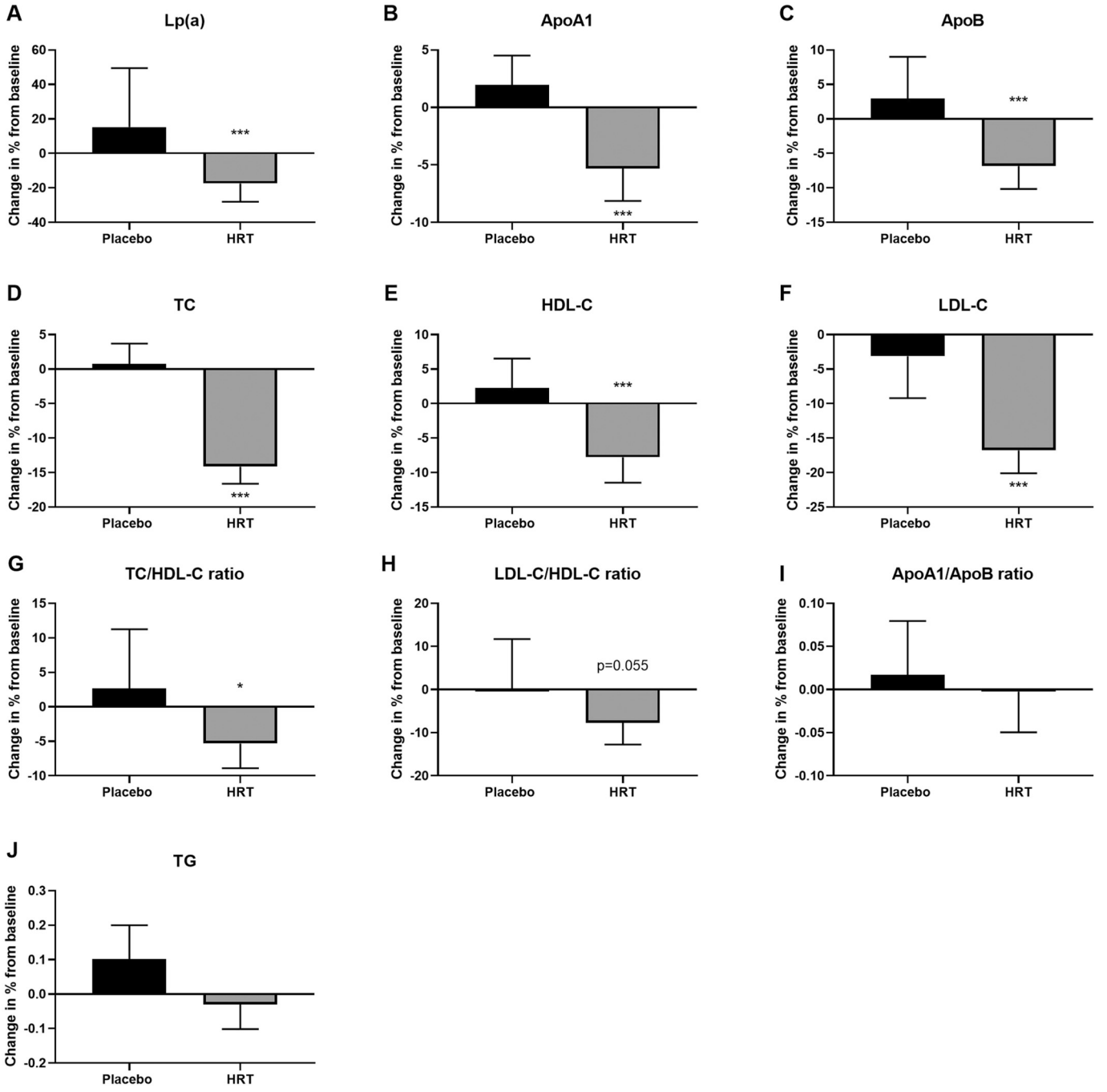

Fig. 1. Median percentage change in Lp(a) (A), ApoA1 (B), ApoB (C), total cholesterol (D), HDL-C (E), LDL-C (F), TC/HDL-C ratio (G), LDL-C/HDL-C ratio (H), ApoA1/ApoB ratio (I) and TG (J) after 3 months of HRT compared to placebo. Data presented as mean and 95% CI intervals. $*p < 0.05$, $***p < 0.001$.

cardiovascular risk [62], and potentially also its effects on thrombus formation. Further, it has been suggested that the protective effects of HDL is lost after menopause [63]. Nevertheless, our data suggest that HDL and ApoA levels should be addressed to evaluate cardiovascular risk in postmenopausal women before and during HRT, but these findings need to be validated in a larger cohort.

A major strength of the present study is the randomized controlled design. A weakness was that the EVTET study was restricted to patients with a history of VT, and may thus not necessarily be applied on other groups of postmenopausal women. Also, the number of women suffering VT was very modest restricting the significance of the findings related to VT. Nonetheless, our findings suggest that HRT reduced the amount of atherogenic lipoproteins including Lp(a) in plasma and had an overall favorable effect on lipid profile. Further studies should clarify the mechanisms and consequences of the decrease in HDL-C and ApoA1 during such therapy.

Author contributions

Høibraaten, Sandset: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing - review & editing.

Halvorsen, Aukrust: Conceptualization; Data curation; Project administration; Resources; Supervision; Validation; Visualization; Writing - original draft; Writing - review & editing.

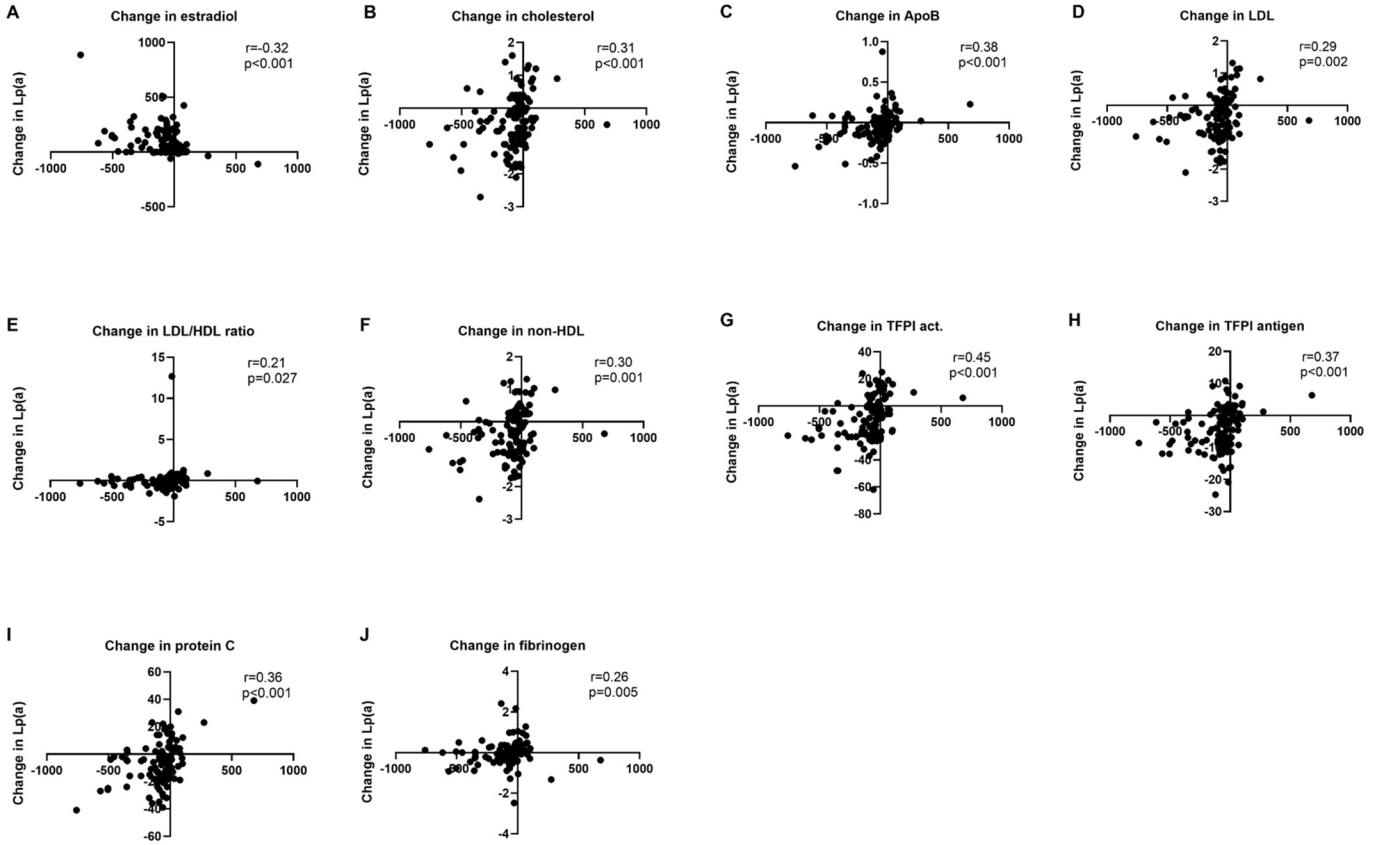

Fig. 2. Correlation plots between change in Lp(a) levels and changes in estradiol (A), total cholesterol (B), ApoB (C), LDL-C (D), LDL/HDL ratio (E), and non-HDL (F), TFPI activity (A), TFPI antigen (B), protein C (C), and fibrinogen (D). Correlations are presented by r and *p* values.

Løvdahl: Data curation; Formal analysis; Writing - original draft; Writing - review & editing.

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Gregersen: Investigation; Writing - original draft; Writing - review & editing.

Ueland, Mowinckel: Formal analysis; Validation; Writing - review & editing.

Data statement

Data will be made available upon request.

Declaration of competing interest

None.

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References

[1] B.A. Ference, H.N. Ginsberg, I. Graham, et al., Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel, Eur Heart J 38 (32) (2017) 2459–2472, https://doi.org/10.1093/eurheartj/ehx144.

[2] W.B. Kannel, W.P. Castelli, T. Gordon, et al., Serum cholesterol, lipoproteins, and the risk of coronary heart disease. The Framingham study, Ann Intern Med 74 (1) (1971) 1–12.

[3] B. Kurt, M. Soufi, A. Sattler, et al., Lipoprotein(a)-clinical aspects and future challenges, Clin Res Cardiol Suppl 10 (Supplement 1) (2015) 26–32, https://doi.org/10.1007/s11789-015-0075-z.

[4] R. Clarke, J.F. Peden, J.C. Hopewell, et al., Genetic variants associated with Lp(a) lipoprotein level and coronary disease, N Engl J Med 361 (26) (2009) 2518–2528, https://doi.org/10.1056/NEJMoa0902604.

[5] C. The Emerging Risk Factors, Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality, JAMA: The Journal of the American Medical Association 302 (4) (2009) 412–423, https://doi.org/10.1001/jama.2009.1063.

[6] J. Suk Danik, N. Rifai, J.E. Buring, et al., Lipoprotein(a), hormone replacement therapy, and risk of future cardiovascular events, J Am Coll Cardiol 52 (2) (2008) 124–131, https://doi.org/10.1016/j.jacc.2008.04.009.

[7] R.A. Hegele, Plasma lipoproteins: genetic influences and clinical implications, Nat Rev Genet 10 (2) (2009) 109–121, https://doi.org/10.1038/nrg2481.

[8] B.J. Arsenault, J.S. Rana, E.S. Stroes, et al., Beyond low-density lipoprotein cholesterol: respective contributions of non-high-density lipoprotein cholesterol levels, triglycerides, and the total cholesterol/high-density lipoprotein cholesterol ratio to coronary heart disease risk in apparently healthy men and women, J Am Coll Cardiol 55 (1) (2009) 35–41, https://doi.org/10.1016/j.jacc.2009.07.057.

[9] G. Walldius, I. Jungner, I. Holme, et al., High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study, Lancet 358 (9298) (2001) 2026–2033, https://doi.org/10.1016/s0140-6736(01)07098-2.

[10] P.J. Barter, K.A. Rye, The rationale for using apoA-I as a clinical marker of cardiovascular risk, J Intern Med 259 (5) (2006) 447–454, https://doi.org/10.1111/j.1365-2796.2006.01647.x.

[11] V. Regitz-Zagrosek, S. Oertelt-Prigione, E. Prescott, et al., Gender in cardiovascular diseases: impact on clinical manifestations, management, and outcomes, Eur Heart J 37 (1) (2016) 24–34, https://doi.org/10.1093/eurheartj/ehv598.

[12] J.W. Balder, J.K. de Vries, I.M. Nolte, et al., Lipid and lipoprotein reference values from 133,450 Dutch Lifelines participants: age- and gender-specific baseline lipid values and percentiles, J Clin Lipidol 11 (4) (2017) 1055–1064.e6, https://doi.org/10.1016/j.jacl.2017.05.007.

[13] J.K. de Vries, J.W. Balder, M.J. Pena, et al., Non-LDL dyslipidemia is prevalent in the young and determined by lifestyle factors and age: The LifeLines cohort, Atherosclerosis 274 (2018) 191–198, https://doi.org/10.1016/j.atherosclerosis.2018.05.016.

[14] W. Lu, H.E. Resnick, K.A. Jablonski, et al., Non-HDL cholesterol as a predictor of

cardiovascular disease in type 2 diabetes: the strong heart study, Diabetes Care 26 (1) (2003) 16–23.
[15] J.E. Hokanson, M.A. Austin, Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies, J Cardiovasc Risk 3 (2) (1996) 213–219.
[16] A.L. Dessapt, P. Gourdy, Menopause and cardiovascular risk, J Gynecol Obstet Biol Reprod (Paris) 41 (7 Suppl) (2012) F13–F19, https://doi.org/10.1016/j.jgyn.2012.09.003.
[17] M.E. Mendelsohn, R.H. Karas, Molecular and cellular basis of cardiovascular gender differences, Science 308 (5728) (2005) 1583–1587, https://doi.org/10.1126/science.1112062.
[18] S. Kwok, V. Charlton-Menys, P. Pemberton, et al., Effects of dydrogesterone and norethisterone, in combination with oestradiol, on lipoproteins and inflammatory markers in postmenopausal women, Maturitas 53 (4) (2006) 439–446, https://doi.org/10.1016/j.maturitas.2005.07.006.
[19] X. Wang, F. Magkos, B. Mittendorfer, Sex differences in lipid and lipoprotein metabolism: it's not just about sex hormones, J Clin Endocrinol Metab 96 (4) (2011) 885–893, https://doi.org/10.1210/jc.2010-2061.
[20] K.A. Matthews, S.L. Crawford, C.U. Chae, et al., Are changes in cardiovascular disease risk factors in midlife women due to chronological aging or to the menopausal transition? J Am Coll Cardiol 54 (25) (2009) 2366–2373, https://doi.org/10.1016/j.jacc.2009.10.009.
[21] H. Campos, J.R. McNamara, P.W. Wilson, et al., Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women, J Clin Endocrinol Metab 67 (1) (1988) 30–35, https://doi.org/10.1210/jcem-67-1-30.
[22] W.B. Kannel, Metabolic risk factors for coronary heart disease in women: perspective from the Framingham Study, Am Heart J 114 (2) (1987) 413–419.
[23] J.L. Jenner, J.M. Ordovas, S. Lamon-Fava, et al., Effects of age, sex, and menopausal status on plasma lipoprotein(a) levels. The Framingham Offspring Study, Circulation 87 (4) (1993) 1135–1141.
[24] V. Vaccarino, L. Badimon, R. Corti, et al., Ischaemic heart disease in women: are there sex differences in pathophysiology and risk factors? Position paper from the working group on coronary pathophysiology and microcirculation of the European Society of Cardiology, Cardiovasc Res 90 (1) (2011) 9–17, https://doi.org/10.1093/cvr/cvq394.
[25] E. Barrett-Connor, D. Grady, Hormone replacement therapy, heart disease, and other considerations, Annu Rev Public Health 19 (1998) 55–72, https://doi.org/10.1146/annurev.publhealth.19.1.55.
[26] M.J. Stampfer, G.A. Colditz, Estrogen replacement therapy and coronary heart disease: a quantitative assessment of the epidemiologic evidence, Prev Med 20 (1) (1991) 47–63.
[27] F. Grodstein, J.E. Manson, G.A. Colditz, et al., A prospective, observational study of postmenopausal hormone therapy and primary prevention of cardiovascular disease, Ann Intern Med 133 (12) (2000) 933–941.
[28] J.C. Stevenson, H.N. Hodis, J.H. Pickar, et al., Coronary heart disease and menopause management: the swinging pendulum of HRT, Atherosclerosis 207 (2) (2009) 336–340, https://doi.org/10.1016/j.atherosclerosis.2009.05.033.
[29] C.L. Shufelt, C.N. Merz, R.L. Prentice, et al., Hormone therapy dose, formulation, route of delivery, and risk of cardiovascular events in women: findings from the Women's Health Initiative Observational Study, Menopause 21 (3) (2014) 260–266, https://doi.org/10.1097/GME.0b013e31829a64f9.
[30] L.L. Schierbeck, L. Rejnmark, C.L. Tofteng, et al., Effect of Hormone Replacement Therapy on Cardiovascular Events in Recently Postmenopausal Women: Randomised Trial, 345 (2012).
[31] S.R. Salpeter, J.M. Walsh, T.M. Ormiston, et al., Meta-analysis: effect of hormone-replacement therapy on components of the metabolic syndrome in postmenopausal women, Diabetes Obes Metab 8 (5) (2006) 538–554, https://doi.org/10.1111/j.1463-1326.2005.00545.x.
[32] M. Jirapinyo, U. Theppisai, J. Manonai, et al., Effect of combined oral estrogen/progestogen preparation (Kliogest) on bone mineral density, plasma lipids and postmenopausal symptoms in HRT-naive Thai women, Acta Obstet Gynecol Scand 82 (9) (2003) 857–866.
[33] N. Munk-Jensen, L.G. Ulrich, E.B. Obel, et al., Continuous combined and sequential estradiol and norethindrone acetate treatment of postmenopausal women: effect of plasma lipoproteins in a two-year placebo-controlled trial, Am J Obstet Gynecol 171 (1) (1994) 132–138.
[34] F.H. Loh, L.H. Chen, S.L. Yu, et al., The efficacy of two dosages of a continuous combined hormone replacement regimen, Maturitas 41 (2) (2002) 123–131.
[35] F. Al-Azzawi, M. Wahab, S. Sami, et al., Randomized trial of effects of estradiol in combination with either norethisterone acetate or trimegestone on lipids and lipoproteins in postmenopausal women, Climacteric 7 (3) (2004) 292–300.
[36] O. Ylikorkala, P. Lim, P. Caubel, Effects on serum lipid profiles of continuous 17beta-estradiol, intermittent norgestimate regimens versus continuous combined 17beta-estradiol/norethisterone acetate hormone replacement therapy, Clin Ther 22 (5) (2000) 622–636, https://doi.org/10.1016/s0149-2918(00)80049-1.
[37] J. Millán, X. Pintó, A. Muñoz, et al., Lipoprotein ratios: physiological significance and clinical usefulness in cardiovascular prevention, Vascular Health and Risk Management 5 (2009) 757–765.
[38] E. Hoibraaten, E. Qvigstad, T.O. Andersen, et al., The effects of hormone replacement therapy (HRT) on hemostatic variables in women with previous venous thromboembolism—results from a randomized, double-blind, clinical trial, Thromb Haemost 85 (5) (2001) 775–781.
[39] E. Hoibraaten, E. Qvigstad, H. Arnesen, et al., Increased risk of recurrent venous thromboembolism during hormone replacement therapy—results of the randomized, double-blind, placebo-controlled estrogen in venous thromboembolism trial (EVTET), Thromb Haemost 84 (6) (2000) 961–967.
[40] S. Hulley, D. Grady, T. Bush, et al., Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group, Jama 280 (7) (1998) 605–613.
[41] B. Bendz, T.O. Andersen, P.M. Sandset, A new sensitive chromogenic substrate assay of tissue factor pathway inhibitor type 1, Thrombosis Research 97 (6) (2000) 463–472, https://doi.org/10.1016/S0049-3848(99)00183-8.
[42] P.B. Ostergaard, T.C. Beck, H. Orsted, et al., An enzyme linked immunosorption assay for tissue factor pathway inhibitor, Thromb Res 87 (5) (1997) 447–459, https://doi.org/10.1016/s0049-3848(97)00161-8.
[43] R.K. Dubey, S. Oparil, B. Imthurn, et al., Sex hormones and hypertension, Cardiovasc Res 53 (3) (2002) 688–708, https://doi.org/10.1016/s0008-6363(01)00527-2.
[44] D.P. Edwards, Regulation of signal transduction pathways by estrogen and progesterone, Annu Rev Physiol 67 (2005) 335–376, https://doi.org/10.1146/annurev.physiol.67.040403.120151.
[45] M.E. Mendelsohn, R.H. Karas, The protective effects of estrogen on the cardiovascular system, N Engl J Med 340 (23) (1999) 1801–1811, https://doi.org/10.1056/nejm199906103402306.
[46] J. Pitha, Lost in menopausal transition: the timing of atherosclerosis prevention in women, Physiol Res 66 (Supplementum 1) (2017) S39–s45.
[47] S. Diano, T.L. Horvath, G. Mor, et al., Aromatase and estrogen receptor immunoreactivity in the coronary arteries of monkeys and human subjects, Menopause 6 (1) (1999) 21–28.
[48] D.W. Losordo, M. Kearney, E.A. Kim, et al., Variable expression of the estrogen receptor in normal and atherosclerotic coronary arteries of premenopausal women, Circulation 89 (4) (1994) 1501–1510, https://doi.org/10.1161/01.cir.89.4.1501.
[49] T.S. Mikkola, T.B. Clarkson, M. Notelovitz, Postmenopausal hormone therapy before and after the women's health initiative study: what consequences? Ann Med 36 (6) (2004) 402–413, https://doi.org/10.1080/07853890410035430.
[50] N.W. Kopper, J. Gudeman, D.J. Thompson, Transdermal hormone therapy in postmenopausal women: a review of metabolic effects and drug delivery technologies, Drug Des Devel Ther 2 (2009) 193–202.
[51] A.L. Eilertsen, E. Hoibraaten, I. Os, et al., The effects of oral and transdermal hormone replacement therapy on C-reactive protein levels and other inflammatory markers in women with high risk of thrombosis, Maturitas 52 (2) (2005) 111–118, https://doi.org/10.1016/j.maturitas.2005.01.004.
[52] P. Anagnostis, P. Galanis, V. Chatzistergiou, et al., The effect of hormone replacement therapy and tibolone on lipoprotein (a) concentrations in postmenopausal women: a systematic review and meta-analysis, Maturitas 99 (2017) 27–36, https://doi.org/10.1016/j.maturitas.2017.02.009.
[53] M.-C. Andrei, A. Andercou, Is there a link between atherothrombosis and deep venous thrombosis? Maedica 9 (1) (2014) 94–97.
[54] F. Dentali, V. Gessi, R. Marcucci, et al., Lipoprotein(a) as a risk factor for venous thromboembolism: a systematic review and meta-analysis of the literature, Semin Thromb Hemost 43 (6) (2017) 614–620, https://doi.org/10.1055/s-0036-1598002.
[55] G. Ferretti, T. Bacchetti, T.P. Johnston, et al., Lipoprotein(a): a missing culprit in the management of athero-thrombosis? J Cell Physiol 233 (4) (2018) 2966–2981, https://doi.org/10.1002/jcp.26050.
[56] K.C. Ferdinand, S.A. Nasser, PCSK9 inhibition: discovery, current evidence, and potential effects on LDL-C and Lp(a), Cardiovasc Drugs Ther 29 (3) (2015) 295–308, https://doi.org/10.1007/s10557-015-6588-3.
[57] M.G. Shlipak, J.A. Simon, E. Vittinghoff, et al., Estrogen and progestin, lipoprotein (a), and the risk of recurrent coronary heart disease events after menopause, Jama 283 (14) (2000) 1845–1852, https://doi.org/10.1001/jama.283.14.1845.
[58] S. Kwok, P.L. Selby, P. McElduff, et al., Progestogens of varying androgenicity and cardiovascular risk factors in postmenopausal women receiving oestrogen replacement therapy, Clinical Endocrinology 61 (6) (2004) 760–767, https://doi.org/10.1111/j.1365-2265.2004.02166.x.
[59] E. Farish, H.A. Rolton, J.F. Barnes, et al., Lipoprotein (a) concentrations in postmenopausal women taking norethisterone, BMJ: British Medical Journal 303 (6804) (1991) 694.
[60] S. Eichinger, N.M. Pecheniuk, G. Hron, et al., High-density lipoprotein and the risk of recurrent venous thromboembolism, Circulation 115 (12) (2007) 1609–1614, https://doi.org/10.1161/circulationaha.106.649954.
[61] V.M. Morelli, W.M. Lijfering, F.R. Rosendaal, et al., Lipid levels and risk of recurrent venous thrombosis: results from the MEGA follow-up study, Journal of Thrombosis and Haemostasis 15 (4) (2017) 695–701, https://doi.org/10.1111/jth.13640.
[62] E.J. Rhee, C.D. Byrne, K.C. Sung, The HDL cholesterol/apolipoprotein A-I ratio: an indicator of cardiovascular disease, Curr Opin Endocrinol Diabetes Obes 24 (2) (2017) 148–153, https://doi.org/10.1097/med.0000000000000315.
[63] S.R. El Khoudary, HDL and the menopause, Curr Opin Lipidol 28 (4) (2017) 328–336, https://doi.org/10.1097/mol.0000000000000432.