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ABBREVIATIONS: CHD: coronary heart disease; TC: total cholesterol; EPT: estrogen plus progestogen therapy; ET: estrogen therapy; HDL-C: high density lipoprotein cholesterol; HT: hormone therapy; LDL-C: low density lipoprotein cholesterol; LLA: lipid-lowering agent; NCEP: National Cholesterol Education Program; TG: triglyceride.

ABSTRACT

Objective: To evaluate plasmatic lipid levels in elderly women in the general population as a function of use of lipid lowering agents (LLA) and hormone therapy (HT)

Methods: 4271 women over 65 were recruited from three French cities. Analyses were performed after stratification by LLA treatment and HT and adjusting for a large range of socio-demographic and clinical factors.

Results: Fifteen percent of women currently used HT (78% transdermal estradiol), and 30% were taking LLA. In this population, 4.6% of women were taking both HT and LLA (fibrate for 2.4% and statin for 2.2%). In non-LLA treated women, current HT was associated with lower total cholesterol, LDL-C, and non-HDL-C compared to never users. Women treated with LLA, also had lower total cholesterol, LDL-C, and non-HDL-C compared to non-LLA users, whereas triglyceride levels were the highest in statin users and lowest in fibrate users. Fibrate was associated with a more favorable lipid pattern than statin independently of HT use. In women without coronary heart disease or diabetes HT, statin, or fibrate were associated with lower LDL-C level risk based on NCEP guidelines (adjusted OR=0.67 [IC95=0.53;0.85], 0.38 [0.29;0.47], and 0.32 [0.25;0.42], respectively) with a possible interaction between fibrate and HT (0.18 [0.10;0.30]).

Conclusions: Estradiol-based HT may lower atherogenic lipoproteins in post-menopausal women. In primary prevention of coronary heart disease, combining HT and fibrate may provide additional benefits compared to fibrate use.

INTRODUCTION

During the reproductive period, women generally have lower low-density lipoprotein cholesterol (LDL-C) and higher high-density lipoprotein cholesterol (HDL-C) than age-matched men. However, this is changed to a potentially atherogenic profile after menopause, *e.g.* increased LDL-C and decreased HDL-C[1,2], a pattern which has been reported to be corrected by hormone therapy (HT). Generally, estrogen are reported to lower total cholesterol (TC) and LDL-C and raise HDL-C, whereas progestogens are either neutral or oppose estrogen effects, notably depending on androgenicity[3,4]. However, although it is widely admitted that normalizing TC, especially LDL-C, allows the elimination of a cardiovascular risk factor, the cardio-protective effect of HT remains controversial. Observational studies have suggested possible beneficial effects of HT, notably on coronary heart disease (CHD) mortality (see for meta-analysis[5]), but the most recent randomized controlled trial (RCT) of the women's health initiative (WHI) study observed a significant increase in CHD risk related to estrogen plus progestogen therapy (EPT) only[6-8]. The inconsistency between studies has been attributed to differences in population, time from HT initiation[9] and HT formulation[10-17]. In the WHI, women were older than 65, less healthy, with vascular risk at randomization, and used conjugated equine estrogens (CEE) opposed or not with medroxyprogesterone acetate (MPA), which confers higher vascular risk than transdermal 17β-estradiol and micronized progesterone, regarding a number of markers, such as blood coagulation, C Reactive Protein, or LDL-C particle size[12-16].

The potential benefic effect of transdermal estradiol-based HT on vascular functioning has only been evaluated in a small secondary prevention RCT (PHASE study)[18], which did not show any modification in the incidence of acute coronary events in HT users compared to nonusers. This study was limited by the small number of women treated with transdermal estradiol, and also by failure to take into account statin treatment (used by almost half of the women) in the analyses. Hence, while it is now clear that oral estrogen shows no cardio-protective effects, this has not been established with transdermal based-HT. However, while differences in vulnerability to cardiovascular disease due to type of HT remains an important question to be addressed, large-scale RCTs are unlikely to be conducted in the near future. It is, however, currently feasible to examine biological intermediary factors associated with different types

of HT using existing large population-based studies with adequate data on hormone exposure. Such preliminary analyses would be of particular value in determining whether new RCTs with transdermal estradiol-based formulations may be worthwhile. The aim of the present study thus was to evaluate the levels of plasma lipids and cardiovascular risk in a large sample of elderly French women who commonly use transdermal estradiol and to compare the lipid levels as a function of HT or of lipid-lowering agents (LLA) (statin or fibrate) as well as possible interaction effects.

SUBJECTS AND METHODS

The 3C study is a population-based prospective study been carried out in three French cities: Bordeaux, Dijon, and Montpellier[19]. A sample of non-institutionalized subjects aged 65 years and over was randomly selected from the electoral rolls of each city. The acceptance rate was 37%. Refusers were replaced by another subject drawn at random from the same sector. Between January 1999 and March 2001, 9686 subjects meeting the inclusion criteria agreed to participate. Following recruitment, 392 subjects refused to participate in the baseline medical interview. Only women (n=5644) were considered in the present analysis. A further 1371 (24.3%) women had missing interview data and/or missing biochemical data. Of the 4273 remaining women, two taking both statin and fibrate, were excluded for the analysis.

Subject examinations included a standardized health interview, a standardized neuropsychiatric examination based on ICD-10 criteria[20], cognitive examination, and a health interview covering present state of health, medical history, and medication use. Other information concerning demographic characteristics, exposure to adverse environmental factors, food, drinking and smoking habits were also obtained. Blood pressure, body mass index (BMI) [weight (kg)/height $(m)^2$] and functional status were also assessed. The study protocol has been approved by the Ethical Committee of the University Hospital of Kremlin-Bicêtre and all subjects signed legal consent forms.

Medication

The questionnaire included an inventory of all drugs used during the preceding month, including LLA and HT. Participants interviewed at home were asked to show medical prescriptions, drug packages and any other relevant information; those interviewed at the study centre were asked to come with their prescription forms. Drug names were automatically coded using the World Health Organization ATC (Anatomical Therapeutic Chemical) classification. For 4535 of the 3C study participants, we obtained individual data on drug purchase by extraction from the database of the French National Health Insurance System. A cross-tabulation of self-reported LLA use with data extracted indicates a high concordance rate (92.2%).

Lipid measurement

Biological parameters were centralized and performed by the Biochemistry Laboratory of the University Hospital of Dijon (France). Venous blood samples were taken from subjects after fasting for 12h. TC, HDL-C and TG levels were measured in serum by routine enzymatic methods. LDL-C was determined by Friedwald formula[21] and non-HDL-C was computed as the difference between TC and HDL-C.

Other covariates

Education level was classified as low (5 years of schooling or less), medium low (6-9 years), medium high (10-12 years), and high (more than 12 years). History of CHD was defined as self-reported history of myocardial infarcts, angina, carotid artery stenosis, or coronary dilatation. Apolipoprotein E (ApoE) genotyping was performed as described previously[22]. Information on tobacco use (classified as past, present or never users) and usual alcohol intake were self-reported and quantified in glass number/day, categorized as: 0 for non drinkers, 1 for moderate drinkers (≤2), 2 for heavier drinkers. Physical activity was categorized as 0 (less than 1h/day), 1 (1-2h/day), 2 (>2h/day). According to BMI, subjects were classified as normal (<25 kg/m^2), overweight (25≤ BMI <30) and obese (≥30). Diabetes was defined as glucose ≥7.2 mmol/l or treated, and hypertension as systolic blood pressure ≥160 or diastolic blood pressure ≥95 mm Hg or intake of antihypertensive drugs.

Statistical analyses

Univariate analyses were carried out using Chi-square tests or analysis of variance for qualitative and quantitative data, respectively. For TG, the distribution of the variable was skewed. Logarithmically transformed values were thus used in statistical computations and the results were expressed as geometric means and SE ([Confidence interval: $m/SE^{1.96}$; $m \times SE^{1.96}$]). Multivariate covariance analyses were used to compare the lipid means with LLA treatment and HT after adjustment for potential confounders, *e.g.* age, education level, study centre, BMI, alcohol consumption, history of CHD,

physical activity and ApoE genotype. If an overall significant difference was found between the treatment groups, 2 by 2 comparisons were performed with Bonferroni's correction for multiple comparisons. For CHD-free subjects, the National Cholesterol Educational Program (NCEP) criteria, *i.e.* current smoking, hypertension (blood pressure ≥14/90 mmHg or treated), HDL-C<1.04 mmol/l, family history of death by heart attack before 65 years in women, and HDL-C≥1.55 mmol/l (as a negative risk factor) were used to define LDL-C cut-offs (>3.37 mmol/l for multiple risk factors [16.8% of subjects], and >4.1 for zero to one risk factor [23.4% of subjects])[23]. Subjects above these LDL-C goals were classified in a group at risk for CHD. The association of LLA treatment and/or HT with being above these LDL-C thresholds was assessed using logistic regression adjusted for the potential confounders mentioned above except CHD history. Analyses were carried out using SAS (release 9.01; SAS Statistical Institute, Cary, NC).

RESULTS

CHARACTERISTICS OF STUDY SUBJECTS

The median age (min, max) of the 4271 women included for the present analysis was 73 (65, 95). Women not included were significantly older, with a lower education level, more frequently with CHD, hypertriglycidemia, diabetes and hypertension, than the sample population retained for the present study. They also had significantly lower physical activity levels, used less LLA and were more alcohol abstinent compared with the sample population (**Table 1**). In our sample, 30.3% of women were taking LLA, either statin (15.5%, mainly simvastatin and pravastatin) or fibrate (14.8%, mainly fenofibrate). Hypercholesterolemia was frequent, irrespective of whether women were treated or not by LLA (24% and 46.8%, respectively). The lowest hypercholesterolemia prevalence was observed in the fibrate group (20.1% compared to 27.8% in the statin group). Hypertriglyceridemia was more frequent in the statin group (21.6% compared to 8.1%, in the fibrate group, and 14% in non-treated women, respectively) (data not shown).

HT FORMULATION AND LLA USE IN WOMEN

In our population, 14.6% of the menopausal women currently used HT and 16.9% have reported past HT use. Among current HT users, 31.6% of women were also treated with LLA (15.2% statin, 16.4% fibrate). Current HT users were significantly younger, less frequently living alone, less obese, with a higher education level, less frequently with CHD, diabetes or HTA, less frequently and more moderately alcohol consumers than never HT users ($p<0.0001$) and had higher physical activity levels ($p=0.02$).

Transdermal estradiol was used by the majority of current HT users (77.7%) either unopposed (14.6%) or associated with oral progesterone (31.5%) or synthetic progestagens (31.6%) (**Table 2**). Oral estradiol was used by only 18.5% of women, unopposed for 1.6%, associated with progesterone for 3.7%, and associated with synthetic progestagens for 13.2%. Other estrogen derivatives (ethinylestradiol, CEE…) were not used by this French elderly population. A few women used progestogen alone (1.8%) or other HT (tibolone or cyproterone, 2.1%). There was no significant difference in the type of LLA used (statin, fibrate, or none) regarding the type of HT ($p=0.95$).

LIPID LEVELS AS A FUNCTION OF LLA, OR/AND HT

Table 3 shows that women not treated by either LLA or HT (group 1) had significantly higher adjusted means of TC (by +3.9%), LDL-C (+6.2%), and non-HDL-C (+5.6%) compared to current HT users (group 4). The lipid profile in women taking only LLA was globally more advantageous compared to those taking neither LLA nor HT, regarding significantly lower TC (by –6.8% for statin [group 2] and –10.7% for fibrate [group 3]), LDL-C (-12.2 and –14.2%, respectively), and non-HDL-C (-9.3 and –14.5%, respectively). TC and non-HDL-C levels were significantly lower in fibrate than in statin users. TG level was also significantly lower in fibrate users (–20%), but higher in statin users (+8.8%) compared to women taking neither LLA nor HT. Neither HT nor LLA use was associated with a significant effect on HDL-C.

Compared to women treated by statin, the lipoprotein levels of current HT users (group 4) were lower for TG (-10.3%), comparable for non-HDL-C, but slightly higher for TC (+3.3%) and LDL-C (+7.2%). Fibrate was associated with lower TC (–7.2%), LDL-C (-8.9%), non-HDL-C (–9%), and TG (-18%). Lipid pattern of women combining HT use and LLA treatment was not significantly different from that of women only treated with LLA, whether statin (group 5 vs. 2) or fibrate (group 6 vs. 3). Again, a more favorable lipid pattern was observed with fibrate than with statin although this only reached significance for TG (group 6 vs. 5). The same pattern was observed when specifically dealing with the largest group of women currently using opposed transdermal estradiol (63% of the women) (data not shown).

LIPID LEVELS AS A FUNCTION OF HT FORMULATION

In non-LLA treated women, there was no significant difference in lipid levels whether women were using unopposed transdermal estradiol, or transdermal or oral estradiol associated with progestogen, except TG ($p=0.07$) and TC ($p=0.09$) which both tended to be higher in the unopposed transdermal estradiol group (data not shown). Only 10 women used unopposed oral estradiol and they were thus not considered in this analysis. Lipid levels were evaluated in LLA-treated women, but in this case, results were not adjusted on history of CHD (due to the absence of subjects in the fibrate categories). In statin-

treated women, transdermal administration of opposed estradiol was associated with significantly higher adjusted means for TC (+12.3%, p=0.02), LDL-C (+26.4%, p=0.002), non-HDL-C (+17.1%, p=0.02), and lower TG (-21.2%, p=0.01), compared to oral opposed estradiol (data not shown). Conversely for fibrate, only HDL-C level was significantly higher in women using transdermal compared to oral opposed estradiol (+14.2%, p=0.04).

IMPACT OF LLA TREATMENT OR/AND HT ON LDL-C LEVEL RISK

Treatment type was examined in relation to CHD risk, based on LDL-C levels, defined according to the NCEP criteria (see Methods). The analyses were performed in subjects without CHD or diabetes, *i.e.* 88% of women, of whom 40.5% were in the group at risk. Each treatment was associated with an odds ratio (95%CI) to be in the group at risk of elevated LDL-C levels, significantly lower than 1: 0.67 (0.53;0.85) for HT and less than 0.4 for LLA (**Table 4**). Combining statin with HT was not associated with a further decrease of the adjusted OR compared to statin alone. On the other hand, combining fibrate and HT was associated with the highest protective effect (OR=0.18 [0.10;0.32]), being even more important than fibrate alone. Taking into account TG or non-HDL-C in addition to LDL-C levels in the CHD risk profile did not modify this pattern, except for statin which revealed slightly less benefit in women currently using HT (OR=0.43 [0.27;0.69]) or not (OR=0.48 [0.38;0.61]) (data not shown).

DISCUSSION

Only few RCTs have compared in the same trial the effects of LLA, HT, or their associations, on lipid and lipoprotein profiles of postmenopausal women; three used CEE (alone or opposed with MPA) and statin[24-26], two oral estradiol and norethisterone, one in combination with statin[27] and the other with fibrate[28]. They were all performed in hyperlipidemic postmenopausal women, generally for a short period; none used transdermal estradiol, although this is considered to be less harmful.

Our population study including 4271 elderly French women is we believe the first study to take into account not only LLA use but also French practices in relation to HT use (predominantly transdermal estradiol) as well as the many clinical and socio-demographic factors which may independently contribute to differences in lipid levels. We observed a more favorable lipid pattern regarding total TC, LDL-C, and non-HDL-C, in women who were treated with HT or LLA, compared to non-treated women. Interestingly, the lowest lipid levels were observed in women treated with fibrate, which appeared to be better in relation to TC, non-HDL-C, and TG than statin. This strongly contrasts with the results observed in the elderly men from the same study for whom statin appeared more favorable than fibrate (regarding TC, LDL-C, and non-HDL-C but not for TG) (data not shown).

A greater lowering effect of LLA compared to HT has been reported in some RCTs, although of higher amplitude. Our results are compatible with the observation of a greater HT impact on TC and LDL-C on hyper- than in normo- lipidemic women[29]. We observed no modification in HDL-C levels, with LLA or HT (except for transdermal opposed estradiol, see below) which is in agreement with previous RCTs showing no modification[26,27], or of low amplitude, either positive[25,27,30], or negative[28]. Modulating effects may be less evident when HDL-C concentrations are already high (2/3 of women in our study had HDL-C higher than 1.55 mmol/l) or for women with lower clinical risk[30,31]. The use of some progestogens could also prevent the raising effect of estrogen on HDL-C. We did not find significant differences as a function of HT formulation, except that TC and TG tended to be higher in the group of women using unopposed compared to opposed transdermal estradiol which is consistent with previous

observations[4].

When taken together, HT and LLA globally were not associated with major additional effects compared to LLA alone, as observed in small RCTs with hyperlipidemic women[24-26,28]. However, in women using EPT and LLA, we observed significant differences when comparing transdermal to oral estradiol, with higher HDL-C level for women also treated with fibrate, but lower TG and higher LDL-C levels, for those treated with statin. This may indicate that transdermal estradiol could have less of a "normalizing effect" than oral estradiol on LDL-C in statin users but a more "normalizing effect" on TG in statin users as well as on HDL-C in fibrate users. However, the low number of subjects in the oral group treated with LLA does not allow drawing any definite conclusions.

When specifically dealing with the main group of CHD- or diabetes-free women, a significant protective effect of HT in relation to elevated LDL-C levels was observed (adjusted OR=0.67 compared to non-treated women), as well as an interaction with fibrate (OR=0.18, compared to 0.32 with fibrate alone) but not with statin. Combining HT and fibrate could thus provide additional benefits compared to fibrate use alone in primary prevention, an interesting possibility which has never been explored in a RCT[32].

Observational studies of postmenopausal women suffer from specific biases, HT users being younger, healthier, with a higher education level and socioeconomic status; in themselves protective factors against atherogenic lipid profile and cardiovascular diseases. The healthier profile of women included in the analysis compared to the non-included women could also be a cause of concern. However, this would have less impact in the context of potential primary prevention than secondary prevention. Women with a history of vascular pathology may also be less frequently prescribed HT. However, in our study, the same proportion of LLA intake was found in current or never HT users (data not shown). Bias of indication could probably not be invoked concerning the higher protective effect against elevated LDL-C levels, of the combination HT and fibrate compared to HT or fibrate alone, in "healthy" women. Lastly, lipids levels were evaluated after adjustment for a large range of potential confounding factors which allows to evidence specific effects of LLA or HT (with $p<0.0001$, even after multi-adjustment).

Presently, strategies to delay or prevent CHD among the elderly are of great clinical importance. Our results suggest that current estradiol-based HT may lower atherogenic lipoproteins independently of potential cofounders and may represent a therapeutic option in slightly dyslipidemic women. Combining fibrate and estradiol-based therapy could have the most protective effect in primary prevention regarding CHD risk in postmenopausal women. It is now increasingly acknowledged that the global risks associated with natural formulations (transdermal 17-β-estradiol, micronized progesterone) are very low compared for instance to oral preparations such as CEE+MPA[33-38]. For this reason, and because of the other possible benefits of postmenopausal HT –such as prevention of osteoporosis, urogenital aging and possibly depression– RCTs with transdermal estradiol and micronized progesterone, associated or not with fibrate are needed to confirm the broader applicability of our results to women in this rapidly expanding age group as well as their clinical significance.

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Table 1: Comparisons between the 3C Study source and the present study sample

*

	WOMEN				
	Non included n=1373		Included n=4271		*p*
		%	n	%	(Chi^2)
Age					
65-69	222	16.17	1064	24.91	
70-74	365	26.58	1394	32.64	
75-80	419	30.52	1183	27.70	
80+	367	26.73	630	14.75	<0.0001
Education level					
Low	473	34.75	1095	25.64	
Medium-low	489	35.93	1708	39.99	
Medium-high	254	18.66	896	20.98	
higher	145	10.65	572	13.39	<0.0001
BMI					
Normal	657	51.45	2330	54.55	
Overweight	428	33.52	1371	32.10	
Obese	192	15.04	570	13.35	0.11
Physical activity					
0	179	31.57	1103	25.83	
1	239	42.15	1845	43.20	
2	149	26.28	1323	30.98	0.007
Alcohol					
None	421	35.23	1180	27.63	
Moderate	680	56.90	2629	61.55	
Heavier	94	7.87	462	10.82	<0.0001
Smoking					
Never	1120	81.63	3446	80.80	
Former	189	13.78	641	15.03	
Current	63	4.59	178	4.17	0.44
Diabetes	118	8.59	227	5.31	<0.0001
CHD	249	18.23	514	12.03	<0.0001
ApoE4	201	20.76	835	19.55	0.39
Hypertension	573	41.73	1592	37.27	0.003
LLA					
Statin	175	12.76	663	15.52	
Fibrate	200	14.59	633	14.82	
None	996	72.65	2975	69.66	0.04
Hyper TC*	382	38.51	1704	39.90	0.42
Hyper TG*	184	18.57	610	14.28	0.0007

*Hypercholesterolemia and hypertriglyceridemia is defined by the NCEP [23] as a fasting level of > 6.20 mmol/l for TC and > 1.7 mmol/l for TG.

Table 2: Current HT according to LLA treatment

Current HT	LLA treatment			
	Statin	**Fibrate**	**None**	**Total**
	n %	n %	n %	n %
Unopposed transdermal estradiol	11 11.58	12 11.76	68 15.96	91 14.61
Transdermal estradiol + progesterone	33 34.74	33 32.35	130 30.52	196 31.46
Transdermal estradiol + synthetic progestagen	29 30.53	33 32.35	135 31.69	197 31.62
Unopposed oral estradiol	0 0.00	3 2.94	7 1.64	10 1.61
Oral estradiol + progesterone	4 4.21	6 5.88	13 3.05	23 3.69
Associated or combined oral estradiol + synthetic progestagen	13 13.68	11 10.78	58 13.62	82 13.16
Natural or synthetic progestagen	2 2.11	3 2.94	6 1.41	11 1.77
Others	3 3.16	1 0.98	9 2.11	13 2.09
Total n	95	102	426	623
%	15.25	16.37	68.38	100.00

Table 3: Comparison of lipid levels in never or current HT users as function of LLA treatment

WOMEN	NEVER HT USERS			CURRENT HT USERS				
	Group 1 No LLA n=2059	**Group 2 + Statin** n=442	**Group 3 + Fibrate** n=425	**Group 4 No LLA** n=426	**Group 5 + Statin** n=95	**Group 6 + Fibrate** n=102	*p*	**Significant 2x2 comparisons**
TC	6.19 (0.04)	5.77 (0.05)	5.53 (0.05)	5.96 (0.06)	5.64 (0.10)	5.50 (0.10)	< 0.0001	1 vs. (2,3,4,5,6) 2 vs. (3,4) 3 vs. 4 4 vs. (5,6)
LDL-C	3.93 (0.04)	3.45 (0.05)	3.37 (0.05)	3.70 (0.05)	3.35 (0.09)	3.40 (0.09)	< 0.0001	1 vs. (2,3,4,5,6) 2 vs. 4 3 vs. 4 4 vs. (5,6)
HDL-C	1.64 (0.02)	1.65 (0.02)	1.65 (0.02)	1.67 (0.02)	1.66 (0.04)	1.60 (0.04)	NS	
TG*	1.25 (1.02)	1.36 (1.02)	1.00 (1.02)	1.22 (1.02)	1.32 (1.04)	0.99 (1.04)	< 0.0001	1 vs. (2,3,6) 2 vs. (3,4,6) 3 vs. (4,5) 4 vs. 6 5 vs. 6
non-HDL-C	4.54 (0.04)	4.12 (0.05)	3.88 (0.05)	4.30 (0.06)	3.99 (0.10)	3.89 (0.10)	< 0.0001	1 vs. (2,3,4,5,6) 2 vs. 3 3 vs. 4 4 vs. (5,6)

Results are expressed as mean (SE) in mmol/l, *except for TG where geometric mean and SE are reported. Means are adjusted for age, BMI, educational level, centre, daily alcohol consumption, history of CHD, physical activity, and ApoE.

Table 4: Adjusted associations of LLA treatment and/or HT with having LDL-C values higher than the NCEP goals in CHD- and diabetes- free women.

	Women N=1258/3110			
	n	%	OR* (95%CI)	p
None	1798	49.0	1	
Statin	365	27.4	0.38 (0.29;0.47)	< 0.0001
Fibrate	350	24.6	0.32 (0.25;0.42)	< 0.0001
HT	413	37.5	0.67 (0.53;0.85)	0.001
Statin + HT	88	23.9	0.34 (0.20;0.56)	< 0.0001
Fibrate + HT	96	14.6	0.18 (0.10;0.32)	< 0.0001
Total	*3110*	*40.5*		

* adjusted on age, BMI, educational level, centre, daily alcohol consumption, physical activity and ApoE4.