Published in final edited form as:

Lipids. 2012 January ; 47(1): . doi:10.1007/s11745-011-3600-y.

Serum 2-Methoxyestradiol, an Estrogen Metabolite, is Positively Associated with Serum HDL-C in a Population-Based Sample

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Abstract

Serum HDL cholesterol (HDL-C) is inversely associated with coronary artery disease, ischemic stroke, and atherosclerosis in men and women. Among postmenopausal women, oral conjugated equine estrogen (CEE) increases serum HDL-C. This is due to activation of hepatic nuclear estrogen receptors, resulting in increased HDL-C expression, as well as modulation of proteins which metabolize HDL-C. 2-methoxyestradiol (2-MeOE2), an estrogen metabolite, has several vasculoprotective effects and may play a role in HDL-C production. 2-MeOE2 inhibits HMG-CoA reductase in vitro but no study has examined the relationship between serum 2-MeOE2 and serum HDL-C. A population-based sample provided information regarding demographic characteristics and use of antihyperlipidemic medications. Serum was analyzed for 17 β -estradiol (E2), estrogen metabolites (EMs), and lipoproteins. Results included serum EM data from 51 men and 47 postmenopausal women. Preliminary analysis revealed no correlation between 2-MeOE2 and serum HDL-C in men so the current analysis includes only women ($N = 40$) with no missing demographic, medication, EM, or lipoprotein data. Linear regression revealed that serum 2-MeOE2 and antihyperlipidemic medications were positively associated with serum HDL-C ($\beta = 0.276$, $P = 0.043$, and $\beta = 0.307$, $P = 0.047$, respectively) when age, race/ethnicity, and body mass index were held constant. Prospective studies are needed to determine if 2-MeOE2 is causally related to HDL-C in women.

Keywords

HDL; Lipoprotein metabolism; Mass spectrometry; Atherosclerosis; Coronary artery disease; HMG-CoA reductase

Introduction

Serum HDL-C is inversely associated with coronary artery disease [1], ischemic stroke [2], and atherosclerosis in men and women [3]. HDL-C provides atheroprotection by promoting cholesterol efflux from peripheral tissues, transporting cholesterol to the liver for excretion as free cholesterol or bile acids, serving as an anti-oxidant, and suppressing inflammation [3, 4]. Serum HDL-C levels are higher [5] and cardiovascular disease rates are lower among

pre-menopausal women compared to similarly aged men [6]. Recent analysis of WHI data indicate that women in the 50–59 year age group with prior hysterectomy who took CEE without progesterone had a significantly lower risk of MI (HR = 0.54, 95% CI, 0.34–0.86) compared to controls [7]. This effect may have been due to atheroprotection from HDL-C, which is known to increase with oral estrogen supplementation [8].

But how does oral CEE increase serum HDL-C levels? A hepatic first pass effect may be important [9] because transdermal estrogen does not lead to increased serum HDL-C [10]. CEE contains E2 [11] and in the liver, E2 activates nuclear estrogen receptors (ERs) and increases the expression of HDL-C and apo A-I, the major protein component of HDL-C [9, 12]. E2 also modulates the expression of proteins involved in the metabolism of HDL-C, such as HL and SR-BI [13, 14]. Human studies demonstrate a correlation between HL activity and HDL catabolism [15]. Estrogen reduces HL mRNA levels in rats, suggesting transcriptional inhibition of this enzyme [16]. In vitro, E2 increases SR-BI promoter activity via nuclear ERs [13]. SR-BI has several effects, including enhanced HDL cholesterol uptake by the liver (reverse cholesterol transport) [17] and increased efflux to lipoproteins from atheromatous arteries [18].

Increasing evidence suggests that 2-MeOE2, a major endogenous metabolite of E2, also has beneficial effects on serum cholesterol and vascular integrity [19]. In ovariectomized rats, oral administration of 2-MeOE2 reduced total cholesterol levels by 34% compared to controls [20]. Subcutaneous infusion over 24 weeks of 2-hydroxyestradiol, the precursor of 2-MeOE2, into obese ZSF1 rats, a genetic model of obesity and the metabolic syndrome, led to significant reductions in total cholesterol, body weight, fasting plasma glucose, and glycated hemoglobin compared to controls [21]. More recently, subcutaneous infusion of 2-MeOE2 into apolipoprotein E-deficient mice reduced both total serum cholesterol and aortic atherosclerotic plaque size compared to controls [22]. However, the mechanism of these effects is not known [20–22].

A recent study found that 2-MeOE2 inhibits HMG-CoA reductase in vitro [23]. Because HMG-CoA reductase inhibitors reduce LDL-C and raise HDL-C in vivo [24], a mechanism exists by which endogenous 2-MeOE2 may influence circulating cholesterol levels. In addition, the ability of oral CEE to increase serum HDL-C may be augmented by the conversion of E2 in CEE to 2-MeOE2 by hepatic estrogen metabolizing enzymes, such as CYP3A4 [25]. Given its inhibition of HMG-CoA reductase, we hypothesized that serum 2-MeOE2 would be positively associated with serum HDL-C in a population-based sample of adults.

Materials and Methods

Data for this study were gathered in the fifth year of the Chicago Health, Aging, and Social Relations Study (CHASRS), which is a population-based longitudinal study designed to examine the relationships between psychosocial characteristics and health outcomes among middle-aged and older adults. Written, informed consent was obtained from the subjects prior to their participation in this study (protocol # 11143B), which was approved by the University of Chicago's Institutional Review Board.

Subjects completed surveys regarding their demographic, dietary, and exercise characteristics, as well as their medication usage. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. The following medications were classified as antihyperlipidemic agents: HMG-CoA reductase inhibitors (statins), niacin, bile acid sequestrants, and cholesterol absorption inhibitors. Serum was analyzed for E2 and 14 EMs using LC–MS/MS [26]. Mean values for all EMs are reported separately for men and

women in Masi et al. [27]. EM values exhibited a positively skewed distribution and were therefore subjected to natural log (ln) transformation. Non-fasting HDL-C, total cholesterol, and triglycerides were measured using the Cholestech LDX kit (Cholestech Corporation, USA), a system that meets CDC reference standards for accuracy and reproducibility. LDL-C was calculated using the Friedewald equation. In year 5, we had serum EM data from 51 men and 51 women; all of the women were postmenopausal. Four women were excluded because they were taking hormone replacement therapy. Preliminary analysis revealed no correlation between 2-MeOE2 and serum HDL-C in men so the current analysis includes only women who had no missing demographic, medication, EM, or cholesterol data ($N = 40$).

Ordinary linear regression analysis was used to evaluate the relationship between serum HDL-C and predictor variables, including age, race/ethnicity, BMI, use of anti-hyperlipidemic medications, and serum 2-MeOE2. Given the directional nature of the hypothesis, a one-tailed t test of the significance of the regression coefficient for 2-MeOE2 was used to evaluate our hypothesis that 2-MeOE2 would be positively associated with HDL-C.

Results and Discussion

Table 1 provides descriptive statistics of the sample of post-menopausal women in the Chicago Health, Aging, and Social Relations Study. Correlational analysis revealed a sizeable positive relationship between serum ln 2-MeOE2 and serum HDL-C ($r = 0.31$; $P = 0.05$). Neither serum ln E2 ($r = 0.08$; $P = 0.59$) nor any other serum ln EM was significantly associated with serum HDL-C. Multivariate regression analysis showed that 2-MeOE2 retained a positive association ($\beta = 0.28$, $P < 0.05$) with serum HDL-C when age, BMI, race/ethnicity, and antihyperlipidemic medications were held constant (see Table 2). In the same model, use of antihyperlipidemic medications was also positively associated with serum HDL-C. To test whether the positive relationship between serum ln 2-MeOE2 and serum HDL-C reflected an effect of E2, we added serum ln E2 to the model shown in Table 2. In this ancillary analysis, serum ln E2 was not associated serum HDL-C. In addition, the positive association between serum ln 2-MeOE2 and HDL-C remained unchanged.

Consistent with our hypothesis, we found a positive association between serum 2-MeOE2 and serum HDL-C. This was true among postmenopausal women but not among similarly aged men. Animal studies indicate that 2-MeOE2 lowers serum total cholesterol [20–22] and shrinks atherosclerotic plaque size [22]. A key factor in cholesterol efflux from peripheral tissues, including atherosclerotic plaques, is HDL-C [3]. It is possible, therefore, that the reduction of atherosclerotic plaque size among mice receiving subcutaneous 2-MeOE2 is due to induction of HDL-C by 2-MeOE2. This is plausible given the demonstrated inhibition of HMG-CoA reductase by 2-MeOE2 [23] and that HMG-CoA reductase inhibition increases serum HDL-C in humans [24].

ER-dependent mechanisms of oral E2-induced HDL-C production include increased hepatic expression of HDL-C and apo A-I [9, 12], as well as modulation of proteins involved in the metabolism of HDL-C [14, 15]. Although the ability of oral E2 to raise serum HDL-C in postmenopausal women is well-recognized [8, 28], research has not established a relationship between endogenous E2 and HDL-C, at least in premenopausal women [8, 29]. The lack of correlation we found between serum E2 and HDL-C is therefore consistent with previous studies. In addition, because E2 was not correlated with HDL-C in univariate or multivariate analysis, we are confident that the association between 2-MeOE2 and HDL-C does not reflect an association between E2 and HDL-C.

The validity of our results is supported by our finding of an expected positive association between use of antihyperlipidemic medications and serum HDL-C. The effect size of these medications ($\beta = 0.31$) is only slightly larger than the effect size of 2-MeOE2 ($\beta = 0.28$), suggesting that 2-MeOE2 may be a potent inducer of HDL-C. However, given the cross-sectional nature of the study design, causal inferences cannot be made. For example, our results are also consistent with induction of 2-MeOE2 by HDL-C or with induction of both by a third variable. Prospective studies are therefore needed to determine the causal direction of this association.

Whereas EMs were once thought to be biologically insignificant, an increasing number of studies have demonstrated the metabolic importance of these molecules [25], particularly with respect to breast cancer risk [30]. We previously demonstrated an inverse relationship between serum 16 α -hydroxyestrone and systolic blood pressure among postmenopausal women [27]. In addition, Dubey et al. have documented several vasculoprotective effects of 2-MeOE2, including inhibition of abnormal growth of vascular smooth muscle cells, cardiac fibroblasts, and glomerular mesangial cells, as well as suppression of inflammation, and enhanced endothelium-dependent arterial relaxation (reviewed in [31]). Despite progress in this area, ours is the first study to document a positive association between serum 2-MeOE2 and serum HDL-C, an essential component of athero- and cardioprotection. Further studies are needed to determine whether 2-MeOE2 can indeed increase HDL-C and what behavioral and genetic factors lead to higher levels of this EM. If 2-MeOE2 is causally related to HDL-C, this association may point to new strategies for reducing cardiovascular disease risk among postmenopausal women.

Acknowledgments

This work was supported by a National Institute on Aging Career Development K08 Award (AG027200, C.M. Masi, PI), a National Institute on Aging R01 Award (AG036433, L.C. Hawkey, PI), and a National Institute on Aging R01 Award (AG034052, J.T. Cacioppo, PI).

Abbreviations

Apo A-I	Apolipoprotein A-I
CEE	Conjugated equine estrogen
E2	17 β -Estradiol
EM	Estrogen metabolites
ER	Estrogen receptor
HDL-C	High density lipoprotein cholesterol
HL	Hepatic lipase
HMG-CoA	3-Hydroxy-3methylglutaryl-CoA
HRT	Hormone replacement therapy
LC-MS/MS	Liquid chromatography–tandem mass spectrometry
LDL-C	Low density lipoprotein cholesterol In Natural log
2-MeOE2	2-Methoxyestradiol
MI	Myocardial infarction
SR-BI	Scavenger receptor class B type I
WHI	Women's Health Initiative

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Table 1

Demographic characteristics, mean 2-MeOE2 concentration, and mean serum lipid profile of study participants

Variable	Women not on HRT (N = 47)
Age (years)	57.3 (3.9) ^a
White (%)	48.9
Black (%)	23.4
Latino (%)	27.7
Body mass index	32.0 (7.3)
Use of antihyperlipidemic medications (%)	32.6
2-Methoxyestradiol (2-MeOE2, pg/ml)	19.0 (17.6)
Serum cholesterol & triglycerides (mg/dl)	
Total cholesterol	205.6 (38.2)
HDL-C	56.2 (18.4)
LDL-C	117.8 (36.9)
Triglycerides	163.0 (84.3)

^aNumbers in parentheses represent standard deviations

Table 2Model predicting serum HDL-C in postmenopausal women ($N = 40$)

Predictor	Standardized coefficient (β)	<i>P</i> value
Constant		0.309
Age	0.098	0.289
Black	-0.119	0.250
Latino	-0.258	0.080
Body mass index	-0.121	0.253
Use of antihyperlipidemic medications	0.307	0.047
ln 2-MeOE2	0.276	0.043