Association between lipid profile and circulating concentrations of estrogens in young men

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Abstract

Objectives: Men show higher rates of cardiovascular morbidity and mortality than pre-menopausal women and this sexual dimorphism may be related to sex-specific effects of sex steroids on cardiovascular risk factors. Unlike androgens, estrogens were not extensively investigated in relation to cardiovascular phenotypes in men.

Methods: We examined associations of estradiol and estrone and their precursors (total testosterone and androstenedione) with traditional cardiovascular risk factors (lipids, blood pressure, body mass) in 933 young (median age – 19 years), apparently healthy Polish men.

Results: Total estradiol was associated with total cholesterol ($p=0.006$) and HDL-cholesterol ($p<0.001$) and estrone showed the strongest associations with both total cholesterol ($p<0.001$) and LDL-cholesterol ($p<0.001$) in the unadjusted ANOVA analysis. In the multivariable adjusted models in which other independent variables were held as constant one standard deviation increase in estradiol level was associated with 6%-standard deviation increase in total cholesterol (standardized $B=0.06$, $p=0.038$) and 6%-standard deviation decrease in HDL-cholesterol (standardized $B=-0.06$, $p=0.036$). An increase in estrone levels by one standard deviation was associated with respective 12%- and 13%-standard deviation increases in total cholesterol (standardized $B=0.12$, $p<0.001$) and LDL-cholesterol levels (standardized $B=0.12$, $p<0.001$) after controlling for other predictors of lipids. Estrone correlated linearly with androstenedione ($r=0.28$, $p<0.001$) but there was no correlation between estradiol and testosterone. Estrogens retained their independent associations

with lipids after adjustment for their biochemical precursors in the multivariable analysis.

Conclusions: Increased levels of estrogens are associated with unfavourable lipid profile in men and that this association is apparent early in life, before cardiovascular disease manifestations.

Key words: lipids, estrogens, sex steroids, association, risk factors

Introduction

Compared to age-related pre-menopausal women, men exhibit higher prevalence of cardiovascular disorders, most apparently - coronary artery disease (1,2). Consistently, the rates of death from cardiovascular diseases have been significantly higher among men than women in most age categories and across cohorts of different ethnicity (2). Genetic variation within the male-specific region of the Y chromosome (3-5) and dominance of androgens over estrogens (4, 6-7) have been proposed as the major factors contributing to the male predisposition to cardiovascular disorders. However, several investigations have shown protective rather than detrimental effects of endogenous testosterone on cardiovascular system, at least in elderly men (8-10). Consistently, in the majority of studies low circulating concentrations of testosterone have been associated with terminal manifestations of atherosclerosis such as peripheral arterial disease (PAD) (11), ischaemic stroke (12) and coronary artery disease (7). A large body of evidence suggests the opposite effect of estrogens on cardiovascular system in men – increased plasma levels of estradiol have been linked to coronary artery disease (13), higher risk of atherosclerosis (14), PAD (11), and stroke (15).

The observed associations between sex hormones and cardiovascular morbidity are most likely driven by traditional cardiovascular risk factors (7) including blood pressure, body weight and lipid profile. However, the majority of available data come from studies of middle-aged or elderly men (16) in whom associations among sex steroids and intermediate cardiovascular phenotypes may be difficult to dissect due to a confounding effect of aging, concomitant

medications and co-existent illnesses. In addition, biologically meaningful relationships between sex steroids and markers of cardiovascular disease are likely to have their roots early in life, prior to the onset of typical clinical manifestations of atherosclerosis. Indeed, the previous observations provided evidence for an independent correlation between circulating concentrations of testosterone and high-density lipoprotein cholesterol (HDL-C) in young men from the YMCA study (4).

Given that endogenous estrogens and androgens may exert differential effects on male cardiovascular system, we hypothesised that increased circulating concentrations of estrogens were associated with unfavourable cardiovascular risk profile in men and that this association was apparent early in life, prior to any overt manifestations of cardiovascular disease.

Material and Methods

Subjects

A sample of 933 men recruited in the Young Men Cardiovascular Association (YMCA) study (4) was included in the current analysis. In brief, the YMCA cohort consists of young, apparently healthy men recruited from randomly selected secondary schools of Silesia (in the south of Poland) (4) and phenotyped for several cardiovascular risk factors, as reported previously (4). Of 1157 subjects included in the Young Men Cardiovascular Association (YMCA) study (4), all men with available clinical, demographic data and plasma sample for hormonal analysis (933 - 80.6% of the original YMCA cohort/) were used in the current investigation.

The studies were approved by local Bioethical Committee and the subjects gave informed consent for participation.

Biochemical analysis

All samples were collected in the morning hours (9.00-12.00 am) after overnight fasting and stored in -70ºC until the biochemical analysis.

Plasma levels of estrogens (total estradiol and estrone) and their precursors (total testosterone and androstenedione) were measured using a 1470 Wallac Wizard Gamma Counter and commercially available radioimmunoassays (E2-RIA-CT, ESTRONE-RIA-CT, TESTO-RIA-CT, ANDROSTENEDIONE-RIA-CT, Biosorce). Each assay was highly specific – the cross-reactivity of a measured hormone with other sex steroids was minimal (generally below 1.0%). Consistent with the previous studies that examined estradiol concentrations in male population (14,17) we used an ultra-sensitive RIA designed specifically to measure estradiol in men and children. The sensitivity of other assays was also sufficient to measure circulating concentrations of sex steroids in men. The thresholds of detection of the assay used to evaluate estradiol, estrone, total testosterone and androstenedione were 7.34 pmol/L, 11.84 pmol/L, 0.17 nmol/L, 0.03 ng/mL, respectively. On average, intra- and inter-assay coefficients of variation were: 4.1% and 5.5% (total testosterone), 5.4% and 8.2% (total estradiol), 6.3% and 8.6% (estrone), 3.9% and 7.5% (androstenedione), respectively. These coefficients were comparable with indicators of intra- and inter-assay variation in other large-scale population studies that used RIA assays to measure sex steroids in men (17-19).

Lipid fractions (total cholesterol /TC/, HDL-C and triglycerides) were assessed under fasting conditions on a Cobas Integra automatic Bio-Autoanalyzer (Roche Diagnostics) with the use of enzymatic-colourimetric methods and reagents/calibrators recommended by the manufacturer (Roche Diagnostics). Intra- and inter-assay coefficients of variation were < 9%. Low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (20). The measurements were conducted once using the samples arranged in a random order.

Statistical analysis

Distribution of continuous variables was verified using the Kolmogorov-Smirnov test.

Crude comparisons of quantitative phenotypes across quartile distribution of sex hormones were performed by one-way ANOVA with weighted partitioning of the between-groups variation into linear trends and the Welch test (if the variances were not homogenous among the groups). Linear correlation between log concentrations of estrogens and their precursors was examined using Pearson's method. Multivariable adjusted analysis was based on linear regressions with the most significant parameters implicated in the crude analysis as dependent variables and other demographic, hormonal and cardiovascular phenotypes as covariates. Forward selection of the independent variables into the full model was based on the criterion of probability-of-F-to-enter ≤ 0.05.

Means and standard deviations or medians along with 25%-75% percentiles were used as general descriptives of variables with normal and non-normal distributions, respectively. The parameters that did not pass the normality test underwent log-transformation before further statistical analysis. Data from log-transformed variables in ANOVA were presented as geometric means (anti-logs of the mean of the logged data) and respective 95% confidence intervals.

P-values (two-tailed) < 0.05 were considered statistically significant.

In *a priori* power simulations that assumed almost equal number of subjects across 4 quartiles of estrogens distribution (n≈233) and the nominal level of statistical significance (α=0.05) our study had 100%, 99.8%, 87.0% and 61.0% power to detect a clinically meaningful difference (defined as an incremental 10% increase or decrease in means from the lowest to the highest quartile) in quantitative cardiovascular variables with standard deviations of respective 10%, 20%, 30% and 40% of the population means.

The statistical analysis was conducted using SPSS (14.0 for Windows) and Minitab (12.21 for Windows) packages.

Results

Clinical characteristics of the subjects

The general demographic and clinical characteristics of the subjects presented in Table 1 are representative of a young (median age – 19 years), apparently healthy male population.

Cardiovascular risk profile and circulating concentrations of estrogens – unadjusted analysis

Circulating concentrations of estradiol showed statistically significant associations with all measured lipid fractions in the crude analysis (Table 2). The most significant associations were revealed between estradiol and HDL-C. Indeed, HDL-C levels decreased linearly in subjects from the bottom to the top quartile of estradiol distribution. (Table 2).

Estrone was associated with 3 metabolic parameters (TC, LDL-C, HDL-C) in the unadjusted analysis (Table 3). Both TC and LDL-C showed apparent incremental linear trends across increasing quartiles of estrone distributions (Table 3).

There was a statistically significant, positive linear correlation between estrone and androstenedione ($r=0.28$, $p<0.001$) and no correlation between total estradiol and testosterone ($r=0.03$, $p=0.441$) in the YMCA subjects.

Association between lipids and estrogens – adjusted analysis

Estradiol remained a statistically significant, independent associate of both total cholesterol and HDL-C levels after adjustment for other confounding factors (including estrone) in multiple regression analysis (Table 4). In the multivariable model in which other independent variables were held constant one standard deviation increase in estradiol level was associated with a 6%-standard deviation increase in TC (standardized beta 0.06) and a 6%-standard deviation decrease in HDL-C (standardized beta -0.06) (Table 4). When included as an additional independent covariant in these models, total testosterone showed a

positive association with HDL-C (standardized beta 0.07, p=0.029) but not TC. However, neither significance nor magnitude of the association between estradiol and HDL-C or TC was affected by inclusion of total testosterone as and extra independent variable in the multiple regression equations (Table 5).

Estrone retained its linear association with both TC and LDL-C after controlling for demographic, metabolic and hormonal confounders in the multiple regression equations (Table 4). In the multivariable model in which other independent variables were held as constant, an increase in estrone levels by one standard deviation was associated with a 12%- and 13%-standard deviation increase in TC and LDL-C levels (standardized beta coefficient of 0.12 and 0.13), respectively (Table 4). Added as an extra variable to these models, androstenedione showed positive independent association with TC (standardized beta 0.08, p=0.007) and LDL-C (standardized beta 0.09, p=0.002) but had minimal impact on the significance and the magnitude of the associations between estrone and both TC and LDL-C (Table 5).

Associations between LDL-C, triglycerides (TG) and estradiol as well as between HDL-C and estrone were no longer significant after adjustment for other covariates in multiple linear regression analysis.

Discussion

Our data show that both estradiol and estrone are associated with lipid profile in young men well before overt clinical manifestations of cardiovascular disease. These associations are mediated by different lipid fractions and are independent of other demographic, metabolic and hormonal confounders.

Indeed, our results indicate that the relationships between lipids and estrogens are independent of their direct biochemical precursors – testosterone and androstenedione. In fact, estradiol and testosterone showed independent opposite associations with HDL-cholesterol in our analysis. These data suggest differential effects of estradiol and testosterone on lipid profile in men and are in agreement with the reported reverse trend in associations between each of the sex steroids and terminal manifestations of cardiovascular disease (11). Although testosterone is the major precursor of estradiol in men (21), the ultimate concentrations of the latter are largely dependent on interindividual variation in several endogenous [enzymatic activity of aromatase (18) and estrogen degrading catechol-O-methyltransferase (17)] as well as exogenous factors. Consistently, estimates of phenotypic correlation between estradiol and testosterone differ across studies (22). In addition, the biological actions exerted by testosterone and estradiol on cardiovascular system are mediated by different types of receptors (23). This molecular variation may underlie differences in associations of cardiovascular phenotypes with each of these sex hormones.

The moderate magnitude of the independent associations between estrogens and cholesterol fractions reflects the expected effect arising from an interaction between complex heterogeneous quantitative traits such as lipids and sex steroids. Indeed, a number of genetic and environmental determinants contribute to the overall phenotypic variation in cholesterol levels (24) and sex hormones (22) and the majority of the effects exerted by each individual factor is modest. Nevertheless, the demonstrated associations are likely to have a

clinical significance. Circulating concentrations of LDL-cholesterol and HDL-cholesterol in adolescence are good predictors of lipid profile (25) and vascular phenotype (including arterial stiffness and subclinical atherosclerosis) in the adulthood (26-27). Given that estradiol level shows little variation with age in men (28), its associations with lipids are likely to track into adulthood and may explain observed correlations between estrogens and the terminal manifestations of atherosclerosis in middle-age and elderly men (11,13-15). Future long-term prospective studies on estrogens and cardiovascular risk factors are needed to provide a direct verification of our cross-sectional observations.

Accumulated data from experimental models and clinical studies suggest that there are substantial differences in molecular mechanisms underlying estrogens actions between male and female cardiovascular system (23). This sexual dimorphism is apparent both at the biochemical (circulating levels of hormones) and cellular (expression profile of sex steroid receptors) level and may be related to sex-specific genetic mechanisms and differences in response to modulating effect of environmental factors in males and females. Indeed, common polymorphic variation within estrogen receptor α gene was associated with pro-atherogenic lipid profile in women but not men (29) and negative interaction of smoking with estradiol metabolism on cardiovascular system is a widely recognised risk factor in women. Therefore it is not surprising that estrogens, which are generally perceived as cardioprotective in pre-menopausal women, may show differential effects on cardiovascular phenotypes in men.

Our study has a number of advantages. Firstly, it is one of the first reports of independent association between estrogens and lipids in a well-sized sample representative of the general population of young, apparently healthy men. Secondly, the reported associations are unlikely to be biased by factors such as concomitant illnesses or medications – well recognised confounders of lipids and sex hormones. Finally, taking into account of estrogens substrates (total testosterone and androstenedione) in the adjusted analysis has eliminated a potential confounding effect driven by correlations among sex steroids and excluded the role of estrogens precursors as the drivers of the demonstrated associations. Nevertheless, we are aware of several limitations of this investigation. Firstly, unavailability of sex-hormone binding globulin (SHBG) measures does not permit to evaluate free and bioavailable hormonal fractions responsible for the ultimate biological effects on cells. Although biologically available fractions of estradiol concentrations are less dependent than androgens on SHBG levels and the key correlate of SHBG (BMI) was taken into account in the adjustment analysis, we cannot exclude that using free rather than total hormonal fraction of estradiol would provide a better estimate of its association with lipids. Secondly, lack of measurements of fat mass does not allow us to fully exclude its contribution to the detected associations. However, it should be noted that BMI shows an excellent correlation with direct markers of obesity (such as total and percent body fat) in adolescents (30). Therefore, although estimates of fat mass may better account for contribution of adipose tissue in the current study they are unlikely to abolish the detected associations between lipids and estrogens. Finally, cross-sectional character of this study

does not allow for ascertaining the causative nature to the observed associations. Future prospective investigations are warranted to confirm the hypothetical negative effect of estrogens on lipid profile in men.

Our study shows that an association between estrogens and lipids in men has its roots early in life, before any overt clinical manifestations of cardiovascular disease. In addition, within the framework of interpretational limitations discussed above, our data suggest that increased circulating concentrations of estrogens may have negative impact on the cardiovascular risk profile in men. Future prospective, long-term studies are needed to confirm that endogenous estrogens are likely to contribute to hyperlipidaemia in men and aid in the dissection of sex-specific associations between sex steroids and cardiovascular morbidity and mortality.

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Table 1. Demographic and clinical characteristics of subjects

phenotype	values
n	933
age (years)	19.0 (18.0 – 19.0)
weight (kg)	70.0 (65.0 – 79.0)
height (m)	1.78 (1.73 – 1.83)
body mass index (kg/m^2)	22.5 (20.6 – 24.2)
systolic blood pressure (mmHg)	118.3 (108.3 – 126.7)
diastolic blood pressure (mmHg)	73.3 (70.0 – 80.0)
mean arterial pressure (mmHg)	88.9±8.6
total cholesterol (mmol/L)	4.3±0.9
LDL-cholesterol (mmol/L)	2.5 (2.0 – 3.1)
HDL-cholesterol (mmol/L)	1.10 (0.90 – 1.30)
triglycerides (mmol/L)	1.00 (0.80 – 1.38)
total estradiol (pmol/L)	71.4 (51.6 – 93.6)
estrone (pmol/L)	125.4 (86.5 – 182.0)
total testosterone (nmol/L)	19.0 (13.4 – 24.2)
androstenedione (ng/mL)	2.6 (1.8 – 3.6)
number of alcoholic drinks per week (range)	1 (0 – 20)
smokers (%)	301 (30.0%)

data medians and 25-75% percentiles or means and standard deviations

Table 2. Characteristics of subjects across 4 quartiles of total estradiol – unadjusted analysis

Phenotype	1st quartile	2nd quartile	3rd quartile	4th quartile	P-value
n	233	234	234	232	-
total estradiol (pmol/L)	35.5 (33.6 – 37.5)	61.5 (60.8 – 62.2)	81.63 (80.8 – 82.4)	120.3 (117.0 – 123.7)	<0.001
age (years)	18.5 (18.3 – 19.0)	18.5 (18.3 – 18.6)	18.3 (18.2 – 18.5)	18.5 (18.4 – 18.6)	0.286
BMI (kg/m^2)	22.3 (22.0 – 22.7)	22.4 (22.1 – 22.8)	22.7 (22.3 – 23.1)	22.8 (22.4 – 23.2)	0.225
SBP (mmHg)	116.6 (115.0 – 118.2)	117.9 (116.3 – 119.5)	118.1 (116.4 – 119.8)	117.2 (115.6 – 118.8)	0.578
DBP (mmHg)	73.4 (72.5 – 74.4)	74.3 (73.3 – 75.3)	74.3 (73.3 – 75.4)	73.6 (72.6 – 74.6)	0.901
MAP (mmHg)	88.3±8.2	89.3±8.6	89.5±9.2	88.6±8.5	0.627
TC (mmol/L)	4.1±0.9	4.3±0.9	4.3±0.9	4.3±0.9	0.006
LDL-C (mmol/L)	2.3 (2.2 – 2.4)	2.4 (2.3 – 2.5)	2.5 (2.4 – 2.6)	2.6 (2.4 – 2.7)	0.001
HDL-C (mmol/L)	1.14 (1.10 – 1.19)	1.10 (1.06 – 1.16)	1.07 (1.03 – 1.12)	1.01 (0.95 – 1.05)	<0.001
TG (mmol/L)	1.02 (0.97 – 1.07)	1.03 (0.98 – 1.09)	1.06 (1.01 – 1.12)	1.09 (1.03 – 1.16)	0.041
total testosterone (nmol/L)	16.4 (15.2 – 17.6)	17.3 (16.2 – 18.6)	17.0 (15.9 – 18.1)	17.5 (16.1 – 19.0)	0.294

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure. MAP – mean arterial pressure, TC – total cholesterol, HDL-C – HDL-cholesterol, LDL-C – LDL-cholesterol, TG – triglycerides; data are means and standard deviations or geometric means ± 95% confidence intervals

Table 3. Characteristics of subjects across 4 quartiles of estrone – unadjusted analysis

phenotype	1st quartile	2nd quartile	3rd quartile	4th quartile	P-value
n	233	235	232	233	
estrone (pmol/L)	61.5 (59.0 – 64.1)	104.1 (102.7 – 105.5)	150.6 (148.5 -152.7)	261.7 (252.2 – 271.5)	<0.001
age (years)	18.1 (18.0 – 18.30)	18.6 (18.5 – 18.8)	18.5 (18.4 – 18.6)	18.5 (18.4 – 18.7)	<0.001
BMI (kg/m^2)	22.3 (22.0 – 22.7)	22.5 (22.2 – 22.9)	22.5 (22.1 – 22.9)	22.9 (22.5 – 23.3)	0.053
SBP (mmHg)	117.3 (115.8 – 118.8)	117.4 (115.7 – 119.1)	116.6 (115.0 – 118.2)	118.4 (116.8 – 120.0)	0.524
DBP (mmHg)	73.8 (72.8 – 74.7)	73.4 (72.4 – 74.5)	73.9 (72.9 – 74.9)	74.6 (73.5 – 75.6)	0.243
MAP (mmHg)	88.7±8.1	88.6±9.1	88.6±8.5	89.7±8.8	0.239
TC (mmol/L)	4.1±0.8	4.2±0.9	4.3±0.9	4.4±0.9	<0.001
LDL-C (mmol/L)	2.2 (2.1 – 2.4)	2.4 (2.3 – 2.5)	2.6 (2.4 – 2.7)	2.6 (2.5 – 2.7)	<0.001
HDL-C (mmol/L)	1.11 (1.07 – 1.15)	1.13 (1.09 – 1.18)	1.01 (0.96 – 1.06)	1.08 (1.03 – 1.13)	0.032
TG (mmol/L)	1.05 (1.00 – 1.12)	1.01 (0.96 – 1.07)	1.06 (1.00 – 1.12)	1.08 (1.02 – 1.14)	0.455
androstenedione (ng/ml)	2.1 (1.9 – 2.2)	2.4 (2.2 – 2.5)	2.7 (2.5 – 2.9)	2.9 (2.8 – 3.1)	<0.001

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure. MAP – mean arterial pressure, TC – total cholesterol, HDL-C – HDL-cholesterol, LDL-C – LDL-cholesterol, TG – triglycerides; data are means and standard deviations or geometric means ± 95% confidence intervals

Table 4. Significant associations between lipids and circulating concentrations of estrogens – adjusted multiple regression analysis

Model No	Dependent variable	Independent variable	Other significant independent variables in the model*	β-coefficient	95% confidence interval	Standardized B-coefficient	p-value
1	TC	estradiol	age, BMI, HDL-C, TG, smoking, alcohol, estrone	0.26	0.02 – 0.50	0.06	0.038
2	TC	estrone	age, BMI, HDL-C, TG, smoking, alcohol, estradiol	0.42	0.21 – 0.64	0.12	<0.001
3	LDL-C	estrone	age, HDL-C, TG, smoking	0.08	0.04 – 0.12	0.13	<0.001
4	HDL-C	estradiol	BMI, MAP, LDL-C, TG, smoking, alcohol	-0.04	-0.08 – -0.003	-0.06	0.036

*models based on forward entry of variables into the multiple regression equation (criterion: probability of F-statistics ≤ 0.05), TC – total cholesterol, BMI – body mass index, HDL-C – HDL-cholesterol, TG – triglycerides, alcohol – number of alcohol units consumed weekly, LDL-C – LDL-cholesterol, MAP – mean arterial pressure

Table 5. Significant associations between lipids and circulating concentrations of estrogens – adjusted multiple regression analysis after inclusion of androstenedione and total testosterone in the models

Model No	Dependent variable	Independent variable	Other significant independent variables in the model*	β-coefficient	95% confidence interval	Standardized B-coefficient	p-value
1	TC	estradiol	age, BMI, HDL-C, TG, smoking, alcohol, estrone	0.26	0.02 – 0.50	0.06	0.038
2	TC	estrone	age, HDL-C, TG, smoking, alcohol, estradiol, androstenedione	0.36	0.14 – 0.58	0.10	0.001
3	LDL-C	estrone	age, HDL-C, TG, smoking, androstenedione	0.07	0.03 – 0.11	0.10	0.002
4	HDL-C	estradiol	BMI, MAP, LDL-C, TG, smoking, alcohol, testosterone	-0.04	-0.08 – -0.01	-0.07	0.029

*models based on forward entry of variables into the multiple regression equation (criterion: probability of F-statistics ≤ 0.05), TC – total cholesterol, BMI – body mass index, HDL-C – HDL-cholesterol, TG – triglycerides, alcohol – number of alcohol units consumed weekly, LDL-C – LDL-cholesterol, MAP – mean arterial pressure