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Association between serum estradiol concentrations and carotid atherosclerosis in men with type 2 diabetes mellitus

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Abstract

The aim of this study was to evaluate relationships between serum estradiol concentration and carotid atherosclerosis in addition to major cardiovascular risk factors in men with type 2 diabetes mellitus because previous reports concerning the role of estrogen on atherosclerosis in men are conflicting. Serum estradiol concentrations were measured in 305 consecutive men with type 2 diabetes mellitus. Relationships were evaluated between serum estradiol concentration and carotid atherosclerosis, as determined by ultrasonographically evaluated intima-media thickness (IMT) and plaque score, in a subgroup of 144 diabetic patients, as well as major cardiovascular risk factors, including age, blood pressure, and lipid concentrations. An inverse correlation was found between serum estradiol concentration and IMT ($r = -0.174$, $P = .0369$), but no correlation was found between serum estradiol concentration and plaque score. Patients with serum estradiol concentrations in the lowest tertile displayed significantly higher IMT compared with patients in the highest tertile ($P = .0083$). Serum estradiol concentration was not a determinant of IMT ($\beta = -.121$, $P = .1396$) in the multiple regression analysis. An inverse correlation was found between serum estradiol concentration and triglyceride concentration ($r = -0.136$, $P = .0186$). In conclusion, serum estradiol concentration is inversely associated with carotid atherosclerosis as determined by ultrasonographically evaluated IMT in men with type 2 diabetes mellitus.

1. Introduction

Cardiovascular disease (CVD) is the primary cause of mortality and morbidity in patients with type 2 diabetes mellitus; and several risk factors, including smoking, hypertension, and hyperlipidemia, have been shown to accelerate the progression of CVD [1-3].

Low serum concentrations of endogenous androgens have also been linked with increased CVD risk in men [4]. We recently demonstrated an inverse association between serum testosterone concentrations and carotid atherosclerosis, as determined by ultrasonographically evaluated intima-media thickness (IMT) and plaque score, in men with type 2 diabetes mellitus [5].

Some studies have suggested that the beneficial effects of testosterone would be mediated by estradiol because of conversion of testosterone by aromatase [6]. However, few previous reports have examined the role of estrogen on atherosclerosis in men; and those data are conflicting. Makinen et al [7] reported that serum testosterone concentration was inversely associated with carotid IMT but serum estradiol concentration was not in nondiabetic men. Muller et al [8] demonstrated that higher serum estradiol concentration was related to progression of IMT of the common carotid artery.

To the best of our knowledge, the relationship between serum endogenous estrogen concentrations and carotid

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atherosclerosis has never been explored in men with type 2 diabetes mellitus. The present study evaluated relationships between serum estradiol concentration and carotid atherosclerosis, as determined by ultrasonographically evaluated IMT and plaque score, in addition to major cardiovascular risk factors in men with type 2 diabetes mellitus.

2. Subjects and methods

2.1. Subjects

Serum estradiol concentrations were measured in 305 consecutive men with type 2 diabetes mellitus recruited from outpatient clinics of Kyoto Prefectural University of Medicine and Osaka General Hospital of West Japan Railway Company. The relationship between serum estradiol concentration and carotid atherosclerosis, as evaluated by carotid ultrasonography, was investigated in a subgroup of 144 randomly selected diabetic patients. In addition, relationships between serum estradiol concentrations and major cardiovascular risk factors, including age, blood pressure, plasma lipid concentration, and glycemic control (levels of hemoglobin A_{1c} [HbA_{1c}]); body mass index (BMI); severity of diabetic retinopathy; severity of diabetic nephropathy; current treatment of diabetes; or presence of CVD were evaluated.

Serum estradiol concentrations (reference range, 15-35 pg/mL) were measured by an electrochemiluminescence immunoassay using a Modular Analytics <EE> system (Roche Diagnostics, Tokyo, Japan). Intraassay coefficients of variation were 3.7% and 2.7% and interassay coefficients of variation were 3.2% and 2.8% for free estradiol concentrations of 32.5 and 474 pg/mL, respectively. Plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride concentrations were assessed using standard enzymatic methods. Hemoglobin A_{1c} was assayed using high-performance liquid chromatography. A mean value for urinary albumin excretion was determined from 3 urine collections.

Type 2 diabetes mellitus was diagnosed according to the "Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus" [9]. Retinopathy was graded as follows: no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), and proliferative diabetic retinopathy (PDR). Nephropathy was graded as follows: normoalbuminuria, urinary albumin excretion <30 mg/g creatinine (Cr); microalbuminuria, 30 to 300 mg/g Cr; or macroalbuminuria, >300 mg/g Cr. Mean values for biochemical parameters obtained during the previous year in patients with type 2 diabetes mellitus were used for statistical analysis. Cardiovascular disease was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history or physical examination.

Patients were excluded if they had been castrated for treatment of testicular or prostate cancer or if they were taking any medications known to affect sex hormone concentrations (eg, antiandrogenic agents for prostate cancer). Approval for the study was obtained from the local research ethics committee, and informed consent was obtained from all participants.

2.2. Ultrasonographic measurement of carotid IMT and plaque score

B-mode ultrasonographic imaging of the carotid artery was performed as described previously [10] using a high-resolution, real-time ultrasonograph with a 7.5-MHz transducer. Examination and image analysis were performed by trained sonographers blinded to other data. The IMT was measured in the far wall of the vessel as the distance from the leading edge of the lumen-intima interface to the leading edge of the intima-adventitia interface. The average measurement was taken as the mean IMT. A plaque was defined as a visually distinct area with an IMT greater than that of neighboring sites. Plaque score was determined as the sum of the maximum thicknesses of all plaques measured in millimeters on the near and far walls of the vessels.

2.3. Statistical analysis

Means and frequencies of potential confounding variables were calculated. Unpaired Student *t* tests or analyses of variance were conducted to assess statistical significance of differences between groups using Stat View software (version 5.0; SAS, Cary, NC). Relationships between serum estradiol concentrations and carotid IMT, plaque score, age, glycemic control, and other variables were examined by linear regression analysis. All continuous variables are presented as the mean ± SD. Multiple regression analysis was performed to assess the combined influence of variables on carotid IMT or plaque score. To

Table 1
Clinical characteristics of patients with diabetes

Characteristic	Whole cohort	IMT cohort
n	305	144
Age (y)	64.4 ± 10.8	65.6 ± 9.4
Age at onset (y)	50.6 ± 12.8	51.4 ± 13.4
Duration of diabetes (y)	13.5 ± 11.1	13.5 ± 10.5
BMI (kg/m^2)	23.2 ± 3.4	23.0 ± 3.4
HbA_{1c} (%)	7.2 ± 1.2	7.3 ± 1.1
Systolic blood pressure (mm Hg)	134 ± 15	132 ± 15
Diastolic blood pressure (mm Hg)	78 ± 10	77 ± 10
Total cholesterol (mg/dL)	194 ± 33	202 ± 30
Triglyceride (mg/dL)	139 ± 94	148 ± 100
HDL cholesterol (mg/dL)	52 ± 15	55 ± 14
Retinopathy (NDR/SDR/PDR)	216/48/41	98/26/20
Nephropathy (normo-/micro-/macroalbuminuria)	193/87/25	92/36/16
CVD (−/+)	248/57	122/22
Current treatment (diet/OHA/insulin)	29/199/77	13/92/39
Smoking (none/past/current)	71/135/99	37/57/52
Estradiol (pg/mL)	26.8 ± 9.3	25.4 ± 9.8

Data are mean ± SD or number of patients. OHA indicates oral hypoglycemic agent.

Table 2
Correlation between serum estradiol concentration and other variables in the whole cohort

	r	P
Age	−0.001	.9898
Age at onset	−0.096	.0987
Duration of diabetes	0.104	.0760
BMI	0.078	.1796
HbA_{1c}	0.021	.7159
Systolic blood pressure	0.061	.2899
Diastolic blood pressure	0.013	.8179
Total cholesterol	−0.077	.1845
Triglyceride	−0.136	.0186
HDL cholesterol	−0.043	.4614
Log (urinary albumin excretion)	−0.036	.5985

examine the effects of various factors on carotid IMT or plaque score, serum estradiol concentration, age, duration of diabetes, BMI, HbA1c, systolic blood pressure, diastolic blood pressure, plasma total cholesterol, HDL cholesterol, triglyceride concentrations, and smoking status were considered as independent variables. A P value less than .05 was considered statistically significant.

3. Results

Clinical characteristics of the 305 men with type 2 diabetes mellitus enrolled in this study are shown in Table 1. Mean serum estradiol concentration was 26.8 ± 9.3 pg/mL. Mean IMT was 1.00 ± 0.27 mm and mean plaque score was 4.6 ± 4.4 in a subgroup of 144 diabetic patients.

Relationships between serum estradiol concentration and other variables are shown in Table 2. An inverse correlation was found between serum estradiol concentration and plasma triglyceride concentration (r = −0.136, P = .0186). No significant correlations were found between serum estradiol concentration and age, BMI, HbA1c, plasma total cholesterol, HDL cholesterol, blood pressure, or log (urinary albumin excretion).

An inverse correlation was found between serum estradiol concentration and mean IMT (r = −0.174, P = .0369; Fig. 1A). However, no significant correlation was found between serum estradiol concentration and plaque score (r = −0.085, P = .3138; Fig. 1B). Mean IMT and plaque score with serum estradiol concentrations in the lowest tertile, second tertile, and highest tertile were 1.07 ± 0.29 mm and 5.3 ± 4.1, 1.00 ± 0.24 mm and 4.2 ± 4.7, and 0.92 ± 0.27 mm and 4.2 ± 4.7, respectively. Patients with serum estradiol concentrations in the lowest tertile displayed a significantly higher mean IMT than patients in the highest tertile (P = .0083; Fig. 2A). Patients with serum estradiol concentrations in the lowest tertile tended to display a higher plaque score than patients in the second and highest tertile, although no significant difference was identified (Fig. 2B).

Multiple regression analysis demonstrated that age (β = .438, P < .0001) and duration of diabetes (β = −.191, P = .0395) were independent determinants of mean IMT. Age (β = .238, P = .0282), systolic blood pressure (β = .359, P = .0322), and diastolic blood pressure (β = −.369, P = .0250) were independent determinants of plaque score. Serum estradiol concentration was not a determinant of either mean IMT (β = −.121, P = .1396) or plaque score (β = −.061, P = .4839).

Serum estradiol concentrations did not differ between patients treated with insulin and those treated without insulin (28.2 ± 9.3 vs 26.3 ± 9.3 pg/mL, P = .1095). Serum estradiol concentrations did not differ between patients with and without CVD (25.7 ± 9.8 vs 26.9 ± 9.1 pg/mL, P = .4017). Serum estradiol concentrations did not differ based on the severity of diabetic retinopathy (26.3 ± 9.6 vs 26.5 ± 8.0 vs 28.1 ± 8.4 pg/mL for patients with NDR, SDR, and PDR,

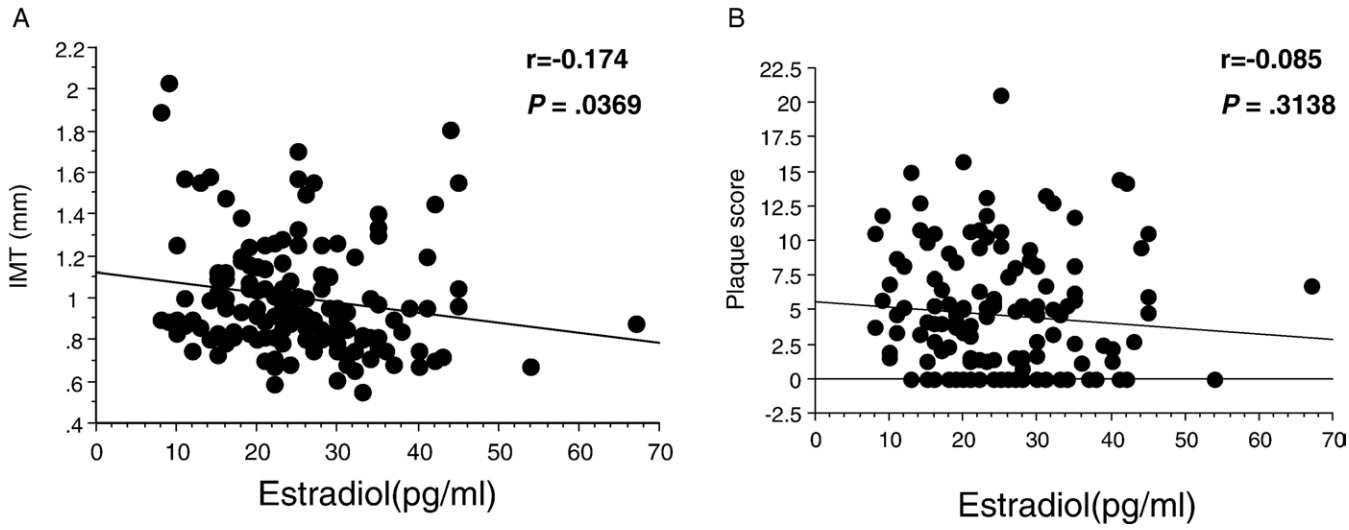

Fig. 1. Correlation between serum estradiol concentrations and carotid mean IMT (A) and between serum estradiol concentrations and plaque score (B) in men with type 2 diabetes mellitus.

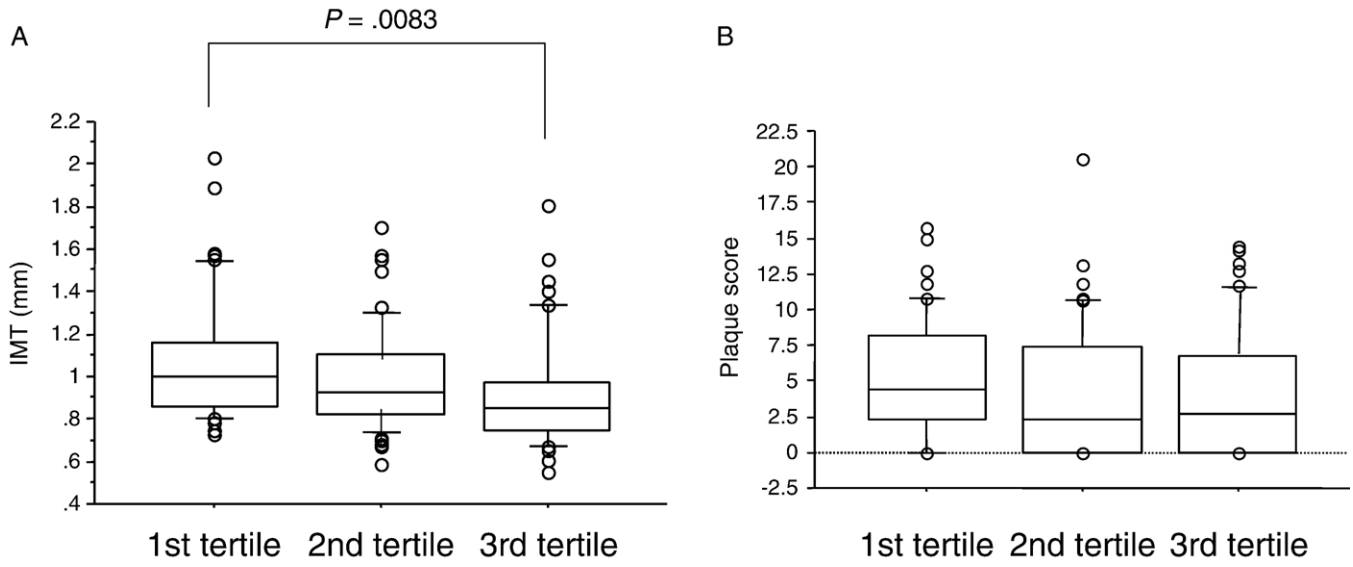

Fig. 2. Carotid mean IMT (A) and plaque score (B) in tertiles of serum estradiol concentrations. Limits in serum estradiol concentrations for different tertiles were as follows: first tertile, 8 to 20 pg/mL; second tertile, 21 to 29 pg/mL; third tertile, 30 to 67 pg/mL. Data are presented as medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers).

respectively) or based on the severity of diabetic nephropathy (26.7 ± 9.4 vs 27.0 ± 8.9 vs 26.2 ± 10.4 pg/mL for patients with normoalbuminuria, microalbuminuria, or macroalbuminuria, respectively).

4. Discussion

We evaluated the relationships between serum estradiol concentration and carotid atherosclerosis, as determined by ultrasonographically evaluated IMT and plaque score, in addition to major cardiovascular risk factors in men with type 2 diabetes mellitus. Serum estradiol concentration was inversely correlated with mean IMT. Patients with serum estradiol concentrations in the lowest tertile exhibited a significantly higher mean IMT compared with patients in the highest tertile. To appreciate these findings, some issues need to be addressed. However, to the best of our knowledge, this study is the first to examine the effect of endogenous estrogen on carotid atherosclerosis in men with type 2 diabetes mellitus.

Studies examining associations between serum estradiol concentrations and CVD risk in men have been inconclusive. Despite several reports suggesting that low concentrations of estradiol are associated with an increased risk of CVD in men [11], some investigators have found no significant association between estradiol concentration and the prevalence of CVD [7]. The present study demonstrated that serum estradiol concentrations did not differ significantly between patients with or without CVD. However, serum estradiol concentration was significantly correlated with ultrasonographically evaluated mean IMT, an early preclinical marker of atherosclerosis. Serum estradiol concentration thus correlated with the severity of atherosclerosis, regardless of the presence of clinical manifestations. Possible mechanisms underlying the inconclusive data concerning the associations between serum estradiol concentrations and CVD in men include the fact that testosterone is aromatized into estradiol at the cellular level. Plasma serum estradiol concentrations do not necessarily reflect tissue-level activity, as peripherally formed estradiol is partially metabolized in situ; thus, not all enters the general circulation. We recently demonstrated an inverse association between serum testosterone concentrations and carotid atherosclerosis in men with type 2 diabetes mellitus [5]. Nathan et al [6] demonstrated that testosterone may attenuate early atherogenesis at least in part by being converted to estradiol by the enzyme aromatase, which is also expressed in endothelial cells.

In the present study, no association was found between serum estradiol concentration and the severity of diabetic microangiopathy (retinopathy and nephropathy). In addition, no significant correlations were found between serum estradiol concentration and log (urinary albumin excretion). As an indication of increased renal endothelial permeability, albuminuria may offer a convenient marker of diffuse endothelial dysfunction [12]. Thus, albuminuria could serve as a readily determined marker of CVD as well as of existing endothelial dysfunction, being likely to reflect both macrovascular and microvascular diseases. We previously reported that serum dehydroepiandrosterone sulfate (DHEA-S) concentration [13], which correlated inversely with degree of urinary albumin excretion, may contribute to the link between elevated urinary albumin excretion and higher CVD mortality in male patients with type 2 diabetes mellitus. Kanauchi et al [14] also demonstrated that serum DHEA-S concentrations were inversely related to the severity of diabetic nephropathy, whereas serum estradiol concentrations did not differ significantly with the severity of diabetic

nephropathy. We think that DHEA might affect albuminuria directly instead of converting to estradiol.

The mechanisms driving the beneficial effects of estrogen on atherosclerosis in men are largely unknown. Nathan et al [15] and Pervin et al [16] demonstrated decreases in vascular cell adhesion molecule 1 [15] and monocyte chemoattractant protein 1 [16] expression in vivo in hypercholesterolemic animals receiving estradiol. Estrogen enhances vasodilatation of coronary artery via an endothelium-dependent mechanism [17]. Moreover, estrogen reduces neointima formation after balloon injury [18]. Estrogens may also offer some degree of protection against CVD by influencing the lipid profile [19].

The regression coefficient was weaker when outliers (2 subjects) were excluded. Without these outliers, however, serum estradiol concentration tended to be inversely associated with carotid atherosclerosis determined by ultrasonographically evaluated IMT in men with type 2 diabetes mellitus, although it did not reach statistical significance ($r = -0.152$, $P = .0707$). We have performed multiple regression analysis to assess the combined influence of variables on mean IMT or plaque score using the following factors: serum estradiol concentration, age, duration of diabetes, BMI, HbA_{1c}, blood pressure, plasma lipid concentration, and smoking status. Neither mean IMT nor plaque score was proven to be inversely associated with serum estradiol concentration in the multivariate analysis. Serum estradiol concentration may be indirectly related to carotid atherosclerosis through an effect on other cardiovascular risk factors, such as plasma lipid concentrations. In our study, serum estradiol concentration was inversely associated with plasma triglyceride concentration ($r = -0.136$, $P = .0186$). The association between serum estradiol concentration and more atherogenic particles derived from triglyceride such as small dense low-density lipoprotein or chylomicron remnants would be of great interest. Unfortunately, we have not measured small dense low-density lipoprotein or chylomicron remnants in the present study.

A few prospective clinical trials [11], intervention studies [20], and experimental studies [6] suggest that estradiol has beneficial effects on the development of atherosclerosis or associated clinical manifestations in men. Large prospective trials and intervention studies are needed to better assess the metabolic and cardiovascular benefits of estradiol in men. In conclusion, serum estradiol concentration is inversely associated with carotid atherosclerosis determined by ultrasonographically evaluated IMT in men with type 2 diabetes mellitus.

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