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Diabetes and menopause aggravate age-dependent deterioration in arterial stiffness

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Abstract

Objective: The present study was designed to evaluate the effects of menopause status and diabetes on arterial stiffness, metabolic parameters, and inflammatory parameters in premenopausal and postmenopausal women with and without type 2 diabetes mellitus.

Methods: In the present study, 186 women were divided into three groups: group 1 includes 42 premenopausal women without type 2 diabetes mellitus, group 2 includes 85 postmenopausal women without diabetes, and group 3 includes 59 postmenopausal women with diabetes. Blood glucose, hemoglobin A1c, insulin, lipids, C-reactive protein, homeostasis model assessment–insulin resistance, aldosterone, and renin were measured. Pulse wave velocity (PWV) and augmentation index (AI) were determined using SphygmoCor (version 7.1; AtCor Medical, Sydney, Australia).

Results: PWV and AI values increased from group 1 to group 3 in a continuous fashion. Postmenopausal women with and without diabetes exhibited significantly increased AI compared with premenopausal women without diabetes ($P < 0.0001$ and $P < 0.0001$, respectively). PWV was significantly higher in postmenopausal women with diabetes mellitus than in premenopausal and postmenopausal women without diabetes mellitus ($P = 0.007$ and $P = 0.002$, respectively).

Conclusions: Postmenopausal women without diabetes have significantly higher AI compared with premenopausal women without type 2 diabetes mellitus. The combination of diabetes and postmenopause status is associated with further deterioration of AI and PWV independently of age, body mass index, and other cardiovascular risk factors.

Key Words: Arterial stiffness – Menopause status – Type 2 diabetes mellitus.

In general, women have a lower risk of cardiovascular disease (CVD) compared with men.[1] However, this sex protection goes missing after menopause.[2-4] Advancing age mediates arterial stiffening, leading to the development of atherosclerotic vascular disease, especially in women with diabetes. Several preexisting risk factors, including hypertension, dyslipidemia, estrogen withdrawal, and increased adiposity, may further raise the cardiovascular risk associated with hyperglycemia, leading to excess CVD risk in women with diabetes at an earlier age.[5-8] However, the exact relationship between type 2 diabetes mellitus and menopause status has not been clearly elucidated. Even less information is available regarding the effects of menopause status in women with and without diabetes on surrogate markers of CVD, one of which is arterial stiffness. Pulse wave velocity (PWV) and augmentation index (AI), which are considered to be reliable and valid measures of vascular stiffness, are significantly and independently associated with target organ damage, cardiovascular morbidity, and cardiovascular mortality.[9-12]

The present study was designed to evaluate the effects of aging, menopause status, and presence of type 2 diabetes mellitus on arterial stiffness, metabolic parameters, and inflammatory parameters.

METHODS

Participants

One hundred eighty-six women were recruited from the outpatient clinic at Wolfson Medical Center (Holon, Israel) and evaluated for the present study. The participants were divided into three groups: group 1 includes premenopausal women without type 2 diabetes mellitus, group 2 includes postmenopausal women without diabetes, and group 3 includes postmenopausal women with diabetes. Study participants were classified as diabetic if the fasting plasma glucose level was 126 mg/dL or higher on at least two blood samples or if they were taking antidiabetic medications. Cardiovascular risk factors were defined using the National Cholesterol Education Program risk factors: hypertension (systolic blood pressure ≥140 mm Hg and/or diastolic blood pressure ≥90 mm Hg and/or pharmacological treatment), dyslipidemia (triglyceride level ≥150 mg/dL

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and/or high-density lipoprotein [HDL] cholesterol level <50 mg/dL and/or pharmacological treatment), and current cigarette smoking.

Women with a history of major disease or surgical operation within the 6 months preceding study entry were excluded. Women reporting a history of clinical CVD were excluded, as were women with plasma creatinine levels higher than 2 mg/dL, elevation of liver enzymes to more than twice the upper normal limit, and electrolyte abnormalities. Women included in the study were stabilized on their previous medical treatment in the outpatient clinic for up to 3 months before study entry to stabilize their conditions, such as diabetes and hypertension, in an effort to minimize treatment changes during the study. Participants who were unbalanced during the 3-month run-in period were not included in the study. This study was approved by the local scientific committee, and all participants gave an informed consent form before study entry.

Blood pressure and PWV measurement

Investigations were performed between 8 AM and 10 AM in a quiet temperature-controlled laboratory. Blood pressure was measured using an automated digital oscillometric device (Omron model HEM 705-CP; Omron Corp, Tokyo, Japan), and the mean of three readings was taken. Radial pressure waveform was recorded and subsequently transformed using a validated generalized transfer function incorporated into SphygmoCor (version 7.1; AtCor Medical, Sydney, Australia) to give an estimate of the corresponding central ascending aortic pulse wave. With the integral software, the central augmented pressure was calculated as the difference between the early systolic peak and the late systolic peak of the estimated central pressure waveform. Central aortic AI was calculated as the augmented pressure expressed as a percentage of pulse pressure. PWV was measured via a simultaneous recording of the right carotid and right radial artery pulse waveforms by two pressure transducers using the SphygmoCor Vx PWV System. This technique, which has been validated for its reproducibility and used extensively, estimates PWV between the two artery sites and has been accepted to be substantially equivalent to aortic pressure measured by invasive catheterization.[13,14]

Biochemical parameters

Blood sampling for full chemistry and metabolic parameters, including total cholesterol, HDL cholesterol, low-density lipoprotein cholesterol, triglycerides, fasting glucose, hemoglobin A1c, fasting insulin, and C-reactive protein, was performed. Blood tests were performed after a 12-hour overnight fast and before the women took their medications. Blood samples for fasting blood glucose were centrifuged at 1,500*g* for 10 minutes at room temperature and analyzed on the same day. Concentrations of plasma glucose were measured with an Olympus AU2700 analyzer using the manufacturer's kit. Serum aldosterone and plasma renin activity were measured using commercially available radioimmunoassays. The lower limits of serum aldosterone and plasma renin activity were 0.016 nmol/L and 2.37 pmol/L/hour, respectively. The samples were measured in duplicate. Homeostasis model assessment–insulin resistance was calculated using the following formula: fasting plasma insulin (mU/mL) × fasting plasma glucose (mg/dL) / 405.

Statistical analysis

Data analysis was carried out using SPSS 11.0 statistical analysis software (SPSS Inc, Chicago, IL). For continuous variables, such as hemodynamic, arterial compliance, and chemistry parameters, descriptive statistics were calculated and reported as mean (SD). Normalcy of the distribution of continuous variables was assessed using Kolmogorov-Smirnov test (cutoff at $P = 0.01$). Continuous variables were compared across groups using one-way analysis of variance. Variables for which across-group differences were detected underwent post hoc pairwise testing using Bonferroni test. Categorical variables, such as comorbidities and prescribed medications, were described using frequency distributions and presented as frequency (%). Categorical variables were compared across groups using χ^2 test (exact as needed). Pearson correlation analysis was used to calculate correlation coefficients to describe associations between continuous variables. PWV and AI were modeled using multiple linear regression analysis with a backward stepwise approach. The probability F was set to 0.05 for inclusion and to 0.15 for exclusion. Variables for inclusion were identified in univariate associations. A backward approach was used to develop the most parsimonious model. In this model, group was included as a fixed factor. All tests are two-sided and considered significant at $P < 0.05$.

RESULTS

The demographic and clinical characteristics of all the three groups are presented in Table 1. As can be seen, group 1 participants were younger than group 2 and group 3 participants, whereas groups 2 and 3 did not differ from each other. Women without diabetes (groups 1 and 2) were similar in body mass index (BMI) and presence of concomitant cardiovascular risk factors, whereas postmenopausal women with type 2 diabetes mellitus (group 3) had higher BMI and greater number of cardiovascular risk factors. As expected, parameters of glucose homeostasis, including fasting blood glucose and hemoglobin A1c, were significantly lower in women without diabetes than in women with diabetes (group 3). HDL cholesterol was significantly lower and C-reactive protein levels were significantly higher in group 3 than in the other groups. Plasma aldosterone levels and aldosterone-to-renin ratio were significantly higher in women with diabetes compared with the two other groups. Systolic blood pressure, diastolic blood pressure, and prevalence of hypertension increased from group 1 to group 3 in a continuous fashion. Overall, group 1 had more favorable cardiovascular profiles and metabolic parameters compared with postmenopausal women with type 2 diabetes mellitus, whereas group 2 participants exhibited an intermediate outline.

As shown in Figure 1 and Table 1, PWV values increased from group 1 to group 3 in a continuous fashion. PWV was

TABLE 1. *Demographic and clinical characteristics of study participants*

Variable	Group 1 M^-DM^- (n = 42)	Group 2 M^+DM^- (n = 85)	Group 3 M^+DM^+ (n = 59)
Age, mean (SD), y	42.7 (6.2)	64.7 (6.6)[a]	64.8 (6.7)[a]
Body mass index, mean (SD), kg/m^2	25.2 (4.7)	26.5 (4.7)	31.1 (5.7)[a]
Type 2 diabetes mellitus	0	0	59[a]
Hypertension, n (%)	2 (4.7)	37 (43.5)[a]	49 (83.1)[a]
Dyslipidemia, n (%)	2 (4.7)	48 (56.5)[a]	45 (76.3)[a]
Current smoker, n (%)	6 (14.3)	8 (9.4)	7 (11.9)
Family history of CVD, n (%)	16 (38.1)	28 (32.9)	24 (40.7)
Menopause duration			
Diabetes medications, n (%)			
Oral			54 (91.5)[a]
Insulin			15 (25.4)[a]
Aspirin use, n (%)	2 (4.7)	13 (15.3)[a]	27 (45.8)[a]
ACEI/ARB use, n (%)	0	17 (20)[a]	27 (45.8)[a]
β-Blocker use, n (%)	1 (2.4)	36 (42.4)[a]	20 (33.9)[a]
Statin use, n (%)	1 (2.4)	24 (28.2)[a]	34 (57.6)[a]
Fasting plasma glucose, mean (SD), mg/dL	90.2 (7.8)	93.1 (10.8)	152.2 (47.4)[a]
HbA1c, mean (SD), %	5.6 (0.3)	5.9 (0.4)	7.9 (1.5)[a]
Low-density lipoprotein cholesterol, mean (SD), mg/dL	119.4 (33.3)	125.8 (35.4)	96.5 (31.8)[a]
High-density lipoprotein cholesterol, mean (SD), mg/dL	54.9 (13.3)	55.5 (11.1)	51.3 (12.7)
Triglycerides, mean (SD), mg/dL	117.4 (66.9)	118.7 (47.7)	136.5 (58.3)
C-reactive protein, mean (SD), mg/dL	0.3 (0.3)	0.4 (0.4)	0.7 (1.4)[a]
Fasting insulin, mean (SD), IU	9.4 (9.0)	9.6 (9.2)	18.4 (23.4)[a]
Creatinine, mean (SD), mg/dL	0.8 (0.1)	0.9 (0.2)	0.9 (0.2)
Aldosterone-to-renin ratio, mean (SD)	14.4 (9.9)	19.2 (29.1)	25.1 (5.8)[a]
PWV, mean (SD), m/s	6.1 (1.3)	6.2 (1.7)	7.1 (2.2)[a]
AI, mean (SD), %	22.9 (8.3)	31.2 (8.7)[a]	39.9 (8.6)[a]
Aortic SP, mean (SD), mm Hg	108.2 (13.9)	120.7 (16.1)[a]	141.3 (19.7)[a]
Aortic DP, mean (SD), mm Hg	68.8 (9.4)	70.7 (9.4)	75.5 (10.1)[a]

By-group comparisons simultaneously comparing all three groups using one-way analysis of variance.
M^-DM^-, premenopausal women without diabetes mellitus; M^+DM^-, postmenopausal women without diabetes mellitus; M^+DM^+; postmenopausal women with diabetes mellitus; CVD, cardiovascular disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; HbA1c, hemoglobin A1c; PWV, pulse wave velocity; AI, augmentation index; SP, systolic pressure; DP, diastolic pressure.
[a]*P* value versus group 1 (significant at 0.05).

significantly higher in postmenopausal women with diabetes mellitus than in premenopausal and postmenopausal women without diabetes mellitus ($P < 0.007$ and $P < 0.002$, respectively).

AI differed significantly between groups such that AI increased from group 1 to group 3 in a continuous fashion. Compared with premenopausal women without diabetes, postmenopausal women with and without diabetes exhibited significantly increased AI ($P < 0.0001$ and $P < 0.0001$, respectively). Postmenopausal women without diabetes had significantly higher AI compared with premenopausal women without diabetes ($P < 0.0001$). Central aortic pressure (systolic and diastolic) significantly increased from group 1 to group 3 (Table 1).

Multiple linear regression analysis (Table 2) was arrived at using a backward stepwise approach with probabilities (F) of 0.05 for entry and 0.15 for removal from the model. A

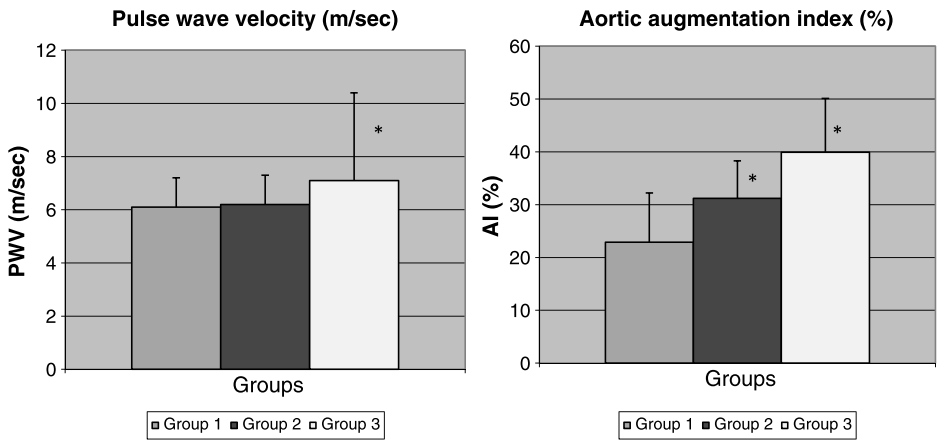

FIG. 1. Pulse wave velocity (PWV) and augmentation index (AI) parameters by study group. Group 1 includes premenopausal women without diabetes, group 2 includes postmenopausal women without diabetes, and group 3 includes postmenopausal women with diabetes. **P* value versus group 1 (significant at <0.05).

TABLE 2. *Multiple linear regression analysis for AI and PWV*

	PWV		AI	
Variables	*P*	Mean square	*P*	Mean square
Group	0.003	8.825	0.0001	541.925
Age	0.586	0.380	0.003	548.379
Body mass index	0.684	0.169	0.137	137.007
Glucose	0.061	4.759	<0.0001	541.925
Error		37.694		5,724.100

Multiple linear regression analysis was arrived at using a backward stepwise approach with probabilities (*F*) of 0.05 for entry and 0.15 for removal from the model.
AI, augmentation index; PWV, pulse wave velocity.

backward approach was used to develop the most parsimonious model. In this model, group was included as a fixed factor. Backward elimination was performed to identify variables independently associated with PWV and AI. In this model, group was significantly associated with PWV ($P < 0.003$) even after adjustment for other cardiovascular confounders. The model was significant and explains 53.8% of the variability in PWV. In a separate model, backward elimination was performed to identify variables independently associated with AI. In this model, group was significantly associated with AI ($P < 0.0001$) even after adjustment for other cardiovascular confounders. The model was significant and explains 50.65% of the variability in AI. In multiple linear regression analysis, menopause status and presence of diabetes were significantly associated with PWV and AI even after adjustment for age.

DISCUSSION

In the present study, AI and PWV were significantly higher in postmenopausal women with diabetes compared with premenopausal and postmenopausal women without type 2 diabetes mellitus. Menopause status was associated with an adverse effect on blood vessels, independently of age. The combination of diabetes and postmenopause status was associated with significant deterioration in arterial stiffness, independently of age, BMI, and other cardiovascular risk factors.

Many studies have investigated the vascular effects of sex hormones. Animal model data and experimental studies in vascular cells have suggested that estrogen may protect against CVD. Moreover, a randomized clinical trial showed favorable effects of hormone therapy on lipid levels,[15] whereas observational studies suggested a 40% to 50% risk reduction in coronary heart disease.[16,17] However, large randomized clinical trials such as the Heart and Estrogen/progestin Replacement Study and the Women's Health Initiative, which examined the effects of conjugated equine estrogens on women with and without established CVD, failed to demonstrate protective vascular effects of estrogen treatment on older women with established CVD. Despite these disappointing results, considerable evidence indicates the beneficial vascular effects of estrogens on the early stages of atherogenesis.[18,19]

Estrogen might affect arterial stiffening, in the short term and in the long term, through a number of mechanisms. Estrogen promotes endothelium-dependent relaxation by increasing the production, release, and bioactivity of endothelium-derived relaxing factors (such as nitric oxide and prostacyclin) and by improving nitric oxide–mediated vasodilation.[20,21] Estrogen also inhibits the elements involved in the mechanisms of vascular smooth muscle contraction, including $[Ca^{2+}]$, protein kinase C, and ρ-kinase. Estrogen may have protective effects on the cardiovascular system, including modification of the composition of circulating lipoproteins (eg, decreased low-density lipoprotein cholesterol and Lp(a)), increased HDL cholesterol, decreased lipid peroxidation, decreased insulin resistance, changes in blood coagulation, inhibition of intravascular accumulation of collagen, decreased vascular smooth muscle cell growth and proliferation, and direct vasodilation of blood vessels.[22-24]

In the present study, systolic blood pressure and diastolic blood pressure differed between groups; whether measurement of arterial stiffness reflects merely blood pressure change has been questioned, and there is passive reduction in arterial stiffness with decreased blood pressure. Although we observed no correlations between systolic/diastolic blood pressure and PWV/AI parameters in each group, the possibility that inequality in blood pressure levels in the study groups can contribute to between-group differences in arterial stiffness parameters must be considered. Moreover, additional markers of subclinical atherosclerosis, such as coronary artery calcification,[8] are required to definitively establish the vascular effects of menopause status on cardiovascular outcomes in women with and without diabetes.

Another important observation in our study is the increase in aldosterone levels and aldosterone-to-renin ratio in postmenopausal women with diabetes compared with the two other groups. Studies in women with established CVD have shown the detrimental effects of aldosterone on endothelial function.[25,26] Aldosterone can mediate vascular fibrosis of cardiac arterioles and large arteries.[27] An increase in intracellular calcium in vascular smooth muscle and endothelial cells after application of aldosterone suggests a direct vasoconstrictor effect, together with endothelium-mediated vasoconstriction.[28] Because aldosterone is involved in the development of vascular hypertrophy, fibrosis, and endothelial dysfunction, high aldosterone levels—as a potential mechanism of deteriorated arterial stiffness in postmenopausal women with type 2 diabetes mellitus—may also be considered.

The present study has some limitations. Our study includes a relatively small number of participants, and larger, long-term, prospective studies are required to definitively establish the vascular effects of menopause status and its clinical impact on cardiovascular outcomes in women with diabetes.

In addition, because premenopausal women with diabetes mellitus have a higher cardiovascular risk even before menopause, the inclusion of an additional group (ie, premenopausal women with diabetes) would have added an interesting dimension to the present study and would have elucidated the pathophysiological mechanism of menopause in the vasculature among women with and without diabetes.

CONCLUSIONS

This study has shown that menopause status is associated with adverse effects on blood vessels in women with and without diabetes. The combination of diabetes and postmenopause status is associated with significant deterioration in arterial stiffness independently of age, BMI, and presence of other cardiovascular risk factors. The findings of the present study justify future investigations of the vascular effects of sex hormones on cardiovascular outcomes in populations with diabetes.

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