Sex Steroids, Bone Turnover and Bone Mineral Density in Pre-, Peri-, and Postmenopausal Women

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Abstract

To examine 1) the relationships between endogenous androgens and bone mineral density (BMD), 2) the relationships between sex-hormone binding globulin (SHBG) and BMD, and 3) the associations of endogenous androgens and SHBG with biochemical markers of bone turnover, a cross-sectional study was carried out in 88 healthy pre-, peri-, and postmenopausal women aged 35 to 74. Measurements of BMDs at the ultradistal radius and ulna, and the distal radius (using DEXA), estrogens, androgens, deoxypyridinoline (D-Pyr) and intact bone gla protein (I-BGP) were performed. In the multivariate regression models testosterone (T) was positively correlated with BMD at the ultradistal radius and ulna in perimenopausal women, and was positively correlated with BMD at the ultradistal radius and ulna, and the distal radius in postmenopausal women. T was positively associated with I-BGP in premenopausal women ($r = 0.65$, $p < 0.01$), and negatively associated with D-Pyr in pre- ($r = -0.53$, $p < 0.05$) and postmenopausal women ($r = -0.49$, $p < 0.001$). On the other hand, SHBG was negatively correlated with BMD at the ultradistal radius and ulna, and the distal radius in pre- and postmenopausal women in the models. SHBG was positively related to D-Pyr in pre- ($r = 0.57$, $p < 0.05$) and postmenopausal women ($r = 0.41$, $p < 0.01$), and negatively related to I-BGP in postmenopausal women ($r = -0.38$, $p < 0.01$). These findings suggest that endogenous androgens may exert positive influences on BMD, and that SHBG may have negative effects on BMD.

Key words: estrogens, androgens, sex-hormone binding globulin, biochemical marker of bone turnover, bone mineral density

Introduction

Osteoporotic fracture is a major cause of illness in older women¹⁾. Although the causes of these fractures are complex, bone mineral density (BMD) is one of the critical determinants of risk for fracture^{2,3)}. BMD in postmenopausal women is mainly dependent on both the amount of peak bone mass achieved during childhood and adolescence, and the rate of bone loss that occurs with menopause and aging following menopause. Estrogen is an important determinant of the bone loss around and after menopause, since such loss can be prevented by estrogen therapy⁴⁾. Late menarche and menstrual disturbances are related to lower BMD in young women^{6,7)}. Estrogen exerts an important

influence on the amount of peak bone mass in young women. However, not all women develop lower BMD in the face of estrogen deficiency. Androgen excess can prevent the detrimental effects of estrogen deficiency in young women^{8,9)}. Significant differences in the serum levels of androgens were found between postmenopausal women with and without osteoporosis¹⁰⁾. BMD has been positively correlated with androgens in pre- and postmenopausal women^{11,12)}. However, some researchers failed to prove these associations^{13,14)}.

On the other hand, sex-hormone binding globulin (SHBG) is related to the transport of estrogens and androgens. Higher SHBG levels have been found in patients with osteoporotic fractures^{15,16)}. BMD has been negatively associated with SHBG in pre- and postmenopausal women^{17,18)}.

Therefore, this study was designed to examine: 1) the relationships between endogenous androgens and BMD, 2) the relationships between SHBG and BMD, and 3) the associations of endogenous androgens and SHBG with biochemical markers of bone turnover, in pre-, peri- and postmenopausal women.

Received Jun. 8, 1998/Accepted Aug. 26, 1998

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Subjects and Methods

Subjects

364 women aged 35 to 74 received medical checkups. They lived in a single community (population 6,000). Women were excluded if they had a current or past history known to affect bone turnover including oophorectomy, diabetes mellitus, gastrectomy, and disorders of the thyroid and the parathyroid ($n = 89$), if they took medications known to affect bone turnover including exogenous estrogens and corticosteroids ($n = 75$), or if they had a history of smoking or alcohol abuse ($n = 18$).

Premenopausal women were eligible for study if their ages were from 35 to 44, if they were menstruating regularly and had FSH concentrations < 20 mIU, and if their menstrual cycles were during the early follicular phase at the time of study. Perimenopausal women were eligible if their ages were from 45 to 54, if they were menstruating irregularly and had FSH ≥ 20 mIU, and if their menstrual cycles were during the early follicular phase at the time of study. Women who had stopped the cycle for one or more years were considered to be postmenopausal. Women were excluded if they did not meet the definitions of pre-, peri-, or postmenopausal women ($n = 94$).

Pre- ($n = 18$), peri- ($n = 18$) and postmenopausal ($n = 52$) women were recruited to participate in this study.

BMD measurements

Bone mineral density (BMD) measurements were made using dual energy X-ray absorptiometry (model pDEXA; Norland Co., Fort Atkinson, WI). The measurements were made on the dominant forearms at the ultradistal of the radius and ulna, and the distal one-third of the radius. The mean coefficients of variation (CV) of repeated measurements on 30 subjects were 1.2 % with a range of 0.1 to 3.7% (ultradistal radius and ulna), and 1.0% with a range of 0.1 to 3.4% (distal radius).

Serum and urinary assays

Blood and urinary samples were collected in the morning between the hours of 0900-1200 h after at least 8 hours of fasting, and stored frozen until assayed. All measurements of sera were done using commercially available kits. Estrone (E1) was analyzed by RIA (Diagnostic System Laboratories, Webster, TX). Estradiol (E2), testosterone (T), and androstenedione (A), and dehydroepiandrosterone (DHEA) were determined by RIA (Diagnostic Products Corporation, Los Angeles, CA). Sex-hormone binding globulin (SHBG) and FSH were measured by time-resolved fluoroimmunoassay (Wallac Oy, Turku, Finland). Intact bone gla protein (I-BGP) was measured using a sandwich enzyme immunoassay kit (Teijin Bio Lab, Tokyo, Japan)¹⁹. The sensitivities for E1, E2, T, A, DHEA, SHBG, FSH, and I-BGP were 10.0 pg/mL, 10.0 pg/mL, 0.05 ng/mL, 0.1 ng/mL, 0.2 ng/mL, 6.3 nmol/L, 0.5 mIU, and 1.0 ng/mL, respectively. The intra- and interassay CVs for these factors were 4% and 7%, 6% and 8%, 7% and 7%, 6% and 8%, 4% and 6%, 6% and 9%, 6%

and 7%, and 7% and 7%, respectively. The urinary excretion of deoxypyridinoline (D-Pyr) was determined using ion-pair reversed-phase HPLC²⁰, and corrected for creatinine. The sensitivity for D-Pyr was 3 pmol/mL. The intra- and interassay CVs were 8% and 10%, respectively.

Statistical analysis

All statistical analyses were performed using a statistical analysis package (SAS Inst. Inc., Cary, NC). Multiple group comparisons were done by ANOVA with Dunnett's test. Associations among variables were examined with Pearson correlations. To test the independences of the variables and to predict BMD, multivariate regression models for BMD were constructed. Statistical significance was defined as having p-value less than 0.05.

Results

The descriptive data are shown separately for pre-, peri-, and postmenopausal women (Table 1). Both estrogens (E1 and E2) and androgens (T, A and DHEA) were significantly lower in postmenopausal women than in premenopausal women. The three groups did not differ significantly from one another in SHBG. Postmenopausal women had lower BMD than premenopausal women. With respect to the biochemical markers of bone turnover, both D-Pyr and I-BGP were significantly higher in postmenopausal women than in premenopausal women.

Table 2 demonstrates the correlations among sex steroids, and age and body size. The expected correlations were found between E1 and E2 and between T and A in all three groups. SHBG was significantly higher in premenopausal women with higher E2 concentrations, but lower in pre- and postmenopausal women with higher A concentrations. In premenopausal group, age was negatively correlated with T, A, and DHEA. In postmenopausal group, age was negatively correlated with E1, T, and DHEA.

Table 3 demonstrates the correlations of sex steroids and biochemical markers of bone turnover with BMD at the ultradistal radius and ulna, and the distal radius. The expected correlations were found between BMD at the ultradistal radius and ulna, and BMD at the distal radius in all three groups. BMD at both sites showed negative associations with age in postmenopausal women. In peri- and postmenopausal women, BMD was positively correlated with either E1 or E2, depending on skeletal site. In perimenopausal women, BMD at the ultradistal radius and ulna ($r = 0.60$, $p < 0.01$), and the distal radius ($r = 0.51$, $p < 0.05$) were related to T. In postmenopausal women, BMD at the ultradistal radius and ulna ($r = 0.71$, $p < 0.001$), and the distal radius ($r = 0.63$, $p < 0.001$) were associated with T. BMD at the ultradistal radius and ulna ($r = -0.58$, $p < 0.05$), and the distal radius ($r = -0.49$, $p < 0.05$) negatively correlated with SHBG in premenopausal women. BMD was lower in peri- and postmenopausal women with higher D-Pyr, and in perimenopausal women with higher I-BGP.

Table 1 Comparisons of levels of sex steroids, bone mineral density and biochemical markers of bone turnover in women classified.

	Menopausal Status		
	Premenopause (n = 18)	Perimenopause (n = 18)	Postmenopause (n = 52)
Physical characteristics			
Age (yr)	40.6 (2.8)	49.7 (2.7) ^c	61.7 (8.3) ^c
Height (cm)	153.8 (7.0)	153.7 (3.9)	152.2 (3.7)
Weight (kg)	54.9 (8.0)	54.0 (5.1)	53.1 (2.6)
Sex steroids			
FSH (mIU)	10.2 (6.2)	47.9 (24.5) ^c	79.9 (19.1) ^c
E1 (pg/mL)	63.4 (22.0)	36.1 (12.3) ^c	19.1 (6.2) ^c
E2 (pg/mL)	77.7 (18.6)	36.4 (10.4) ^c	14.5 (3.0) ^c
T (ng/mL)	0.28 (0.04)	0.27 (0.04)	0.22 (0.05) ^c
A (ng/mL)	1.05 (0.46)	0.74 (0.36)	0.68 (0.36) ^b
DHEA (ng/mL)	2.23 (1.13)	1.81 (0.86)	1.22 (0.71) ^c
SHBG (nmol/L)	81.2(22.8)	80.1 (22.1)	70.9 (20.0)
BMD			
Ultradistal radius and ulna (g/cm ²)	0.41 (0.03)	0.38 (0.04)	0.29 (0.06) ^c
Distal radius (g/cm ²)	0.74 (0.04)	0.71 (0.06)	0.60 (0.10) ^c
Biochemical markers of bone turnover			
D-Pyr (pmol/ μ mol creatinine)	2.7 (0.3)	5.2 (1.4) ^c	5.1 (1.1) ^c
I-BGP (ng/ml)	3.8 (0.7)	4.8 (1.5)	6.5 (1.5) ^c

Values are shown as means (SDs).

E1, estrone; E2, estradiol; T, testosterone; A, androstenedione; DHEA, dehydroepiandrosterone; SHBG, sex-hormone binding globulin; BMD, bone mineral density; D-Pyr, deoxypyridinoline; I-BGP, intact bone gla protein. ^b: p < 0.01, ^c: p < 0.001 (compared with premenopausal women) .

Tbale 2 Correlations among sex steroids, and age and body size.

	Ht	Wt	FSH	E1	E2	T	A	DHEA	SHBG
Premenopausal (n = 18)									
Age	-0.12	-0.01	0.24	-0.39	-0.28	-0.51 ^a	-0.55 ^a	-0.55 ^a	-0.05
Height		0.47 ^a	0.27	-0.16	-0.16	0.23	0.16	-0.23	-0.23
Weight			0.43	-0.27	0.02	0.10	0.29	-0.23	-0.43
FSH				-0.15	-0.18	0.09	0.12	-0.08	-0.13
E1					0.57 ^a	0.10	-0.07	0.29	0.41
E2						-0.24	-0.16	0.06	0.47 ^a
T							0.74 ^c	0.61 ^b	-0.44
A								0.44	-0.51 ^a
DHEA									-0.15
Perimenopausal (n = 18)									
Age	-0.24	-0.19	0.38	0.05	-0.15	-0.29	-0.13	-0.20	0.17
Height		0.21	-0.09	0.22	0.06	0.13	-0.02	0.17	0.01
Weight			0.03	0.03	0.26	0.21	0.01	0.22	-0.41
FSH				-0.32	-0.43	-0.19	-0.19	-0.09	-0.14
E1					0.71 ^b	0.52 ^a	0.41	0.36	0.41
E2						0.34	0.30	0.21	0.34
T							0.58 ^a	0.76 ^c	-0.41
A								0.70 ^b	-0.26
DHEA									-0.35
Postmenopausal (n = 52)									
Age	-0.15	0.08	0.18	-0.32 ^a	-0.16	-0.66 ^c	-0.17	-0.47 ^c	-0.22
Height		0.21	-0.10	-0.03	0.09	0.14	0.04	0.12	0.06
Weight			-0.18	-0.10	-0.07	-0.01	-0.12	-0.13	-0.37
FSH				-0.19	-0.32 ^a	-0.26	-0.14	-0.25	0.13
E1					0.44 ^b	0.23	0.24	0.30	0.37
E2						0.20	0.11	0.33 ^a	0.21
T							0.52 ^c	0.54 ^c	-0.24
A								0.49 ^c	-0.31 ^a
DHEA									0.07

See footnote of Table 1 for explanation of abbreviations.

^a: p < 0.05, ^b: p < 0.01, ^c: p < 0.001.

Table 3 Correlations with bone mineral density at the ultradistal radius and ulna (RU), and the distal radius (R).

	Premenopause (n = 18)		Perimenopause (n = 18)		Postmenopause (n = 52)	
	RU	R	RU	R	RU	R
Age	-0.10	-0.18	-0.17	-0.40	-0.62 ^c	-0.61 ^c
Height	-0.08	0.23	0.23	0.13	0.10	0.21
Weight	0.18	0.29	0.24	0.39	0.19	0.16
FSH	-0.21	-0.02	-0.04	-0.23	-0.30	-0.27
E1	-0.02	0.04	0.54 ^a	0.42	0.37 ^b	0.43 ^b
E2	0.06	0.11	0.50 ^a	0.52 ^a	0.30 ^a	0.26
T	0.35	0.35	0.60 ^b	0.51 ^a	0.71 ^c	0.63 ^c
A	0.32	0.33	0.28	0.71 ^b	0.35 ^a	0.37 ^b
DHEA	0.22	-0.03	0.38	0.61 ^b	0.49 ^a	0.40 ^b
SHBG	-0.58 ^a	-0.49 ^a	-0.20	-0.28	-0.23	-0.22
D-Pyr	-0.44	-0.39	-0.71 ^c	-0.59 ^b	-0.51 ^c	-0.48 ^c
I-BGP	0.29	0.29	-0.58 ^a	-0.56 ^a	-0.17	-0.21
R	0.74 ^c		0.51 ^a		0.83 ^c	

See footnote of Table 1 for explanation of abbreviations.

^a: p < 0.05, ^b: p < 0.01, ^c: p < 0.001.

To further examine the relationships between androgens and BMD, multivariate regression models were constructed from estrogen and androgen (Table 4). Dependent variables were BMD at the ultradistal radius and ulna, and the distal radius. Independent variables entered were E2, T, age, height, and weight. D-Pyr and I-BGP were not included in these models because they were considered to be reflections of the effects of other independent variables. In perimenopausal women T accounted for 31 % of the variability in BMD at the ultradistal radius and ulna. In postmenopausal women age, weight, and T together accounted for 58 % of the variability in BMD at the ultradistal radius and ulna, and age and T together accounted for 48 % of the variability in BMD at the distal radius. In these models, T was positively correlated with BMD. Although all models used E2 as an independent variable, E1 (a weaker estrogen than E2) was also examined, and yielded essentially similar results. Likewise, the inclusion of A (a weaker androgen than T) yielded essentially similar results to that of T.

Table 4 Multivariate regression models constructed from estrogen and androgen for the ultradistal radius and ulna bone mineral density (RU BMD), and the distal radius bone mineral density (R BMD).

Independent variables	Coefficient ± SE	Significance
Premenopausal (n = 18)		
RU BMD =		
None		
R BMD =		
None		
Perimenopausal (n = 18)		
RU BMD =		
T (ng/ml)	0.63 ± 0.21	0.009
		R ² = 31 % ^b
R BMD =		
E2 (pg/ml)	0.0023 ± 0.0013	0.089
T (ng/ml)	0.61 ± 0.34	0.096
		R ² = 31 % ^a
Postmenopausal (n = 52)		
RU BMD =		
Age (yr)	-0.0023 ± 0.0009	0.020
Weight (kg)	0.0031 ± 0.0013	0.026
T (ng/ml)	0.70 ± 0.17	0.000
		R ² = 58 % ^c
R BMD =		
Age (yr)	-0.0040 ± 0.0016	0.016
T (ng/ml)	0.84 ± 0.29	0.006
		R ² = 48 % ^c

See footnote of Table 1 for explanation of abbreviations.

^a: p < 0.05, ^b: p < 0.01, ^c: p < 0.001.

Independent variables entered were age, height, weight, E2 and T.

Similarly, to further investigate the relationships between SHBG and BMD, multivariate regression models were constructed (Table 5). Independent variables entered were SHBG, age, height, and weight. In premenopausal women SHBG accounted for 19 % of the variability in BMD. In postmenopausal women SHBG and age together accounted for 48 to 51 % of the variability in BMD. In these models, SHBG was negatively correlated with BMD.

Table 5 Multivariate regression models constructed from SHBG for the ultradistal radius and ulna bone mineral density (RU BMD), and the distal radius bone mineral density (R BMD).

Independent variables	Coefficient $\pm$ SE	Significance
Premenopausal (n = 18)		
RU BMD =		
SHBG (nmol/L)	-0.0008 $\pm$ 0.0003	0.012 R ² = 29 % *
R BMD =		
SHBG (nmol/L)	-0.0008 $\pm$ 0.0004	0.037 R ² = 19 % *
Perimenopausal (n = 18)		
RU BMD =		
None		
R BMD =		
None		
Postmenopausal (n = 52)		
RU BMD =		
Age (yr)	-0.0054 $\pm$ 0.0008	0.000
SHBG (nmol/L)	-0.0012 $\pm$ 0.0003	0.000 R ² = 51 % *
R BMD =		
Age (yr)	-0.0081 $\pm$ 0.0012	0.000
SHBG (nmol/L)	-0.0018 $\pm$ 0.0005	0.000 R ² = 48 % *

See footnote of Table 1 for explanation of abbreviations.

*: p < 0.05, *: p < 0.001.

Independent variables entered were age, height, weight and SHBG.

The correlations of sex steroids and SHBG with D-Pyr and I-BGP are shown in Table 6. In perimenopausal women E2 was negatively associated with D-Pyr and I-BGP. In perimenopausal women D-Pyr showed a positive correlation with I-BGP. T was higher in premenopausal women with higher I-BGP ($r = 0.65$, $p < 0.01$), and higher in pre- ($r = -0.53$, $p < 0.05$) and postmenopausal women ($r = -0.49$, $p < 0.001$) with lower D-Pyr. SHBG was lower in pre- ($r = 0.57$, $p < 0.05$) and postmenopausal women ($r = 0.41$, $p < 0.01$) with lower D-Pyr, and in postmenopausal women with higher I-BGP ($r = -0.38$, $p < 0.01$).

Table 6 Correlations with deoxypyridinoline (D-Pyr) and intact bone gla protein (I-BGP).

	Premenopause (n = 18)		Perimenopause (n = 18)		Postmenopause (n = 52)	
	D-Pyr	I-BGP	D-Pyr	I-BGP	D-Pyr	I-BGP
Age	0.44	-0.13	0.19	0.23	0.13	0.04
Height	-0.20	0.16	-0.36	-0.35	-0.07	0.01
Weight	-0.22	-0.28	-0.39	-0.05	-0.23	0.04
FSH	0.04	0.06	0.32	0.24	0.28	0.27
E1	0.18	0.28	-0.42	-0.44	-0.19	-0.30*
E2	0.19	-0.18	-0.45*	-0.73*	-0.26	0.13
T	-0.53*	0.65*	-0.38	-0.32	-0.49*	-0.04
A	-0.44	0.46	-0.21	-0.24	-0.40*	0.09
DHEA	-0.45	0.49*	-0.31	-0.33	-0.25	-0.05
SHBG	0.57*	-0.44	0.40	0.13	0.41*	-0.38*
D-Pyr		-0.30		0.70*		-0.13

See footnote of Table 1 for explanation of abbreviations.

*: p < 0.05, *: p < 0.01, *: p < 0.001.

Discussion

In this study, 1) the relationships between endogenous androgens (T, A, and DHEA) and BMD at the ultradistal radius and ulna (a trabecular bone-rich site²¹), which is more sensitive to the declining estrogens that accompany menopause than a cortical bone-rich site²²), and the distal radius (a cortical bone-rich site²¹), 2) the relationships between SHBG and BMD at both sites, and 3) the associations of endogenous androgens (T, A, and DHEA) and SHBG with biochemical markers of bone turnover (D-Pyr as a marker of bone resorption and I-BGP as a marker of bone formation), were examined in 88 healthy pre-, peri-, and postmenopausal women.

Menopausal estrogen deficiency causes an increase in bone resorption. As a result of coupling phenomenon, increased bone resorption results in an increase in bone formation²³. Therefore, in perimenopausal women lower E2 was associated with higher biochemical markers of bone turnover (D-Pyr and I-BGP). D-Pyr and I-BGP were significantly higher in postmenopausal women than in premenopausal women.

In the multivariate regression models constructed from estrogen and androgen, T was positively correlated with BMD at the

ultradistal radius and ulna in perimenopausal women, and was positively correlated with BMD at the ultradistal radius and ulna, and the distal radius in postmenopausal women. Androgens may have positive roles in the maintenance of BMD. Additionally, T was positively associated with bone formation in premenopausal women, and negatively associated with bone resorption in pre- and postmenopausal women. Although the roles of androgens in bone turnover are uncertain, these findings may support the hypothesis that androgens may exert positive influences on BMD¹⁰⁻¹².

SHBG was negatively correlated with BMD in pre- and postmenopausal women in the multivariate regression models. SHBG is positively related to bone resorption in pre- and postmenopausal women, and negatively related to bone formation in postmenopausal women. Therefore, SHBG may have negative roles in the determination of BMD. SHBG is related to the transport of estrogens and androgens, and is known to regulate their free (biologically available) fraction²⁴. With respect to androgens, SHBG was negatively correlated with A in pre- and postmenopausal women. Therefore, lower SHBG may be associated with higher free androgens. If androgens have positive roles in BMD, it is possible that lower SHBG is related to higher BMD through the increase in free androgens.

Greater body weight correlates with increased skeletal mass at all ages^{25,26}. Body weight is the load by gravity and weight-bearing exercise, and the skeleton responds to the greater mechanical loading by increasing skeletal BMD^{27,28}. This mechanism, however, plays a more important role at weight-bearing sites than at non-weight-bearing sites²⁹. This may be one of the reasons that the correlations between body weight and BMD at the ultradistal radius and ulna, and the distal radius are relatively weak in the present study.

Since cigarette smoking^{30,31} and alcohol abuse³² are known to influence skeletal mass, women were excluded from this study if they had a history of smoking or alcohol abuse. Other potential confounders affecting skeletal mass, including physical activity^{33,34} and calcium intake^{35,36} were also examined in the univariate and

multivariate analyses, but they did not achieve statistical significance possibly due to the design of this study which, while specifically testing the relationships of endogenous androgens and SHBG of BMD, did not have adequate statistical power to detect the effects of these variables.

This study also had several limitations. In pre- and perimenopausal women, measurements of sex steroids and biochemical markers of bone turnover were made during the early follicular phase of the menstrual cycle, but it is possible that the use of only single measurements of each of these factors failed to reflect the true status of the subjects. Thus, multiple measurements throughout the menstrual cycle may be necessary to adequately determine the true status of subjects, particularly in pre- and perimenopausal women. Moreover, menopausal bone loss is a generalized phenomenon, affecting all sites of the skeleton, and estrogen therapy for such loss is effective in all sites³⁷. Therefore, measurements at the radius and ulna should also be useful in terms of the evaluation of skeletal mass, and were performed here. However, to conclusively determine the associations between sex steroids and SHBG with BMD at the spine and femur, further investigations specific to each of these sites will be needed. Finally, the design of this study was cross-sectional, and some information was obtained by recall. Therefore, longitudinal evaluations not relying on recalled information would be necessary to confirm these results.

Acknowledgements

The author would like to thank Professor Takefumi Kondo and Guest Professor Haruo Kondo, Keio University Department of Preventive Medicine and Public Health for their continuing guidance and encouragement. The author is deeply indebted to Professor Kazuyuki Omae, Dr. Naoki Shimada, Dr. Michiko Miyakawa, and Koji Shirasaya, Keio University Department of Preventive Medicine and Public Health for their helpful suggestions and discussions. This study was supported in part by a grant from Keio University.

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