Subcutaneous Fat Accumulation Shows a Beneficial Correlation with Serum Cholesterol in Postmenopausal Japanese Women

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This study aimed to investigate whether accumulation of subcutaneous abdominal fat has a beneficial correlation with lipid metabolism in premenopausal and/or postmenopausal Japanese women. The study enrolled 146 premenopausal women, ranging in age from 19 to 54 years, and 82 postmenopausal women, ranging in age from 47 to 66 years. Fat distribution, including abdominal visceral fat area (VFA) and abdominal subcutaneous fat area (SFA), were measured in an outpatient clinic by magnetic resonance imaging. Homogeneity of the regression slopes for SFA to total cholesterol ($P = 0.030$), low-density lipoprotein cholesterol ($P = 0.020$), apolipoprotein B (apoB) ($P = 0.001$), and the ratio of apoB to apolipoprotein A-I (apoA-I) ($P = 0.003$) were not found between premenopausal and postmenopausal women, even after adjustment for both VFA and age. However, the regression slopes for VFA to all measured lipid parameters, as well as apolipoproteins, were homogeneous between the premenopausal and postmenopausal groups. Abdominal SFA in postmenopausal women correlated negatively with total cholesterol ($P = 0.007$), low-density lipoprotein cholesterol ($P = 0.002$), apoB ($P < 0.001$), and the ratio of apoB to apoA-I ($P = 0.001$), after adjustment for age and VFA, but this was not the case in premenopausal women. The mechanisms involved in the beneficial effects of subcutaneous fat accumulation in postmenopausal women remain obscure, but upregulated aromatase expression, derived from adipose tissue, may possibly improve lipid and apolipoprotein metabolism. Exp Biol Med 232:1064–1070, 2007

Key words: subcutaneous fat accumulation; postmenopausal women; LDL-cholesterol; aromatase activity in adipocytes

Introduction

A cluster of multiple risk factors contributes to the development of coronary artery disease and includes insulin resistance (1); impaired glucose metabolism (1); elevated blood pressure (2); increased serum total cholesterol (TC) (1, 3), triglyceride (TG) (1, 4), and low-density lipoprotein cholesterol (LDL-C) (1, 3); and a decrease in high-density lipoprotein cholesterol (HDL-C) (3, 4). In addition, it is well known that visceral fat accumulation plays an important role in the occurrence of coronary artery disease. However, precisely how subcutaneous fat contributes to atherosclerosis is as yet unclear. This view is reinforced by the observation that there is no detectable, definite correlation between subcutaneous fat accumulation and abnormal lipid metabolism (4, 5). We previously demonstrated that a powerful negative correlation was evident, particularly in females, between subcutaneous leg fat deposition and parameters of lipid metabolism (6). Higher subcutaneous thigh fat was associated with a reduction of TC (7), LDL-C (7), and TG (7–9) but an elevation of HDL-C (8, 9), indicating that subcutaneous adiposity is carried to lessen health risks (6–9). Subcutaneous adiposity has thus far been reported to be rather difficult to compare with visceral fat in terms of pathophysiological implications.

There is a growing body of evidence that postmenopausal women treated with hormone replacement therapy (HRT) show evidence of lower levels of TC (10–13), LDL-C (10, 12, 13), and apolipoprotein B (apoB) (12, 13) and an elevated level of HDL-C (13). In the case of postmeno-

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pausal women with accumulated subcutaneous fat, androstenedione from the adrenal gland is converted actively into estrone by aromatase in the adipose tissue stroma, which, in turn, provides estrogen (14, 15). On the basis of these findings, we focused on the pathophysiological roles of subcutaneous adiposity in postmenopausal women, who lack female hormone secretion from the ovaries. The aims of the present study were to clinically investigate the advantageous correlation between subcutaneous fat accumulation and serum lipids and apolipoproteins in postmenopausal women compared with that in premenopausal women and to discuss the possible pathophysiological implications of subcutaneous adiposity.

Materials and Methods

Subjects. Through public advertisements, we recruited obese women with a body mass index $\geq$25 kg/m^2 every year for 11 years for admission to a weight-reduction program carried out by the Nakamura-Gakuen University Health Promotion Center. The total number of obese participants in the program was 318. Subjects were excluded from the study for the following reasons: 5 subjects were incapable of undergoing magnetic resonance imaging (MRI) because of claustrophobia or a metal implant in their body, 17 subjects had undergone ovary extirpation and/or uterus amputation, 13 subjects were taking antidyslipidemic medications, and 55 subjects had an unclear menopause history. After exclusion of the above mentioned subjects, 146 premenopausal and 82 postmenopausal women were enrolled in the present study.

Blood Sampling and Assay. All of the enrolled subjects fasted overnight and rested for at least 10 minutes before blood sampling. Blood samples were taken in the morning, after the 10- to 14-hour fasting period. Blood samples taken at each sampling period were kept at room temperature (between 15°C and 25°C), which was adjustable depending on seasons, until the samples were assayed at SRL Inc. (Tokyo, Japan). All the blood samples were centrifuged less than 3 hours after collection, and measurements were made within 12 hours after blood was taken from the subjects. TC and TG were assayed by enzymatic and glycerol blanking methods, respectively, using commercially available kits (L-Type Cho H and L-Type Triglyceride H; Wako Pure Chemical Industries, Ltd., Osaka, Japan). HDL-C was assessed by a direct method using a commercially available kit (Cholestest N HDL; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Concentrations of serum apolipoprotein A-I (apoA-I) and apoB were determined with a modified commercially available turbidimetric assay (APO A-I AUTO N 'DAIICHI' and APO B AUTO N 'DAIICHI'; Daiichi Pure Chemicals). LDL-C was calculated by using Friedewald's formula (16), and the cholesterol balance was determined by the ratio of apoB to apoA-I (17).

Fat Distribution. The cross-sectional of visceral fat area (VFA) and subcutaneous fat area (SFA) were measured by use of MRI. The MR images of subjects in the supine position were scanned at an umbilical level for both VFA and SFA (18). By applying image software devised by the National Institutes of Health (NIH Image 1.62; Scion Inc. Available at: http://rsb.info.nih.gov/nih-image/), we defined a scanned area between the skin and muscle, on the MRIs, as the subcutaneous fat layer. An area inside the peritoneum, which was identified by fat density, was also scanned as a visceral fat layer. In the present study, a single-slice scan method was utilized at the umbilical level in the assessment of abdominal fat masses, on the basis of evidence that a tight correlation (r = 0.993) existed in the fat masses between the single-slice and multiple-slice scan methods (18).

Classification of Menopausal Status. Premenopausal women were defined as those who had shown natural menstrual cycles for more than 2 years prior to the initiation of the program, while postmenopausal subjects were defined as those who had been menopausal for more than 2 years prior to the start of the program. However, some of the subjects were not confident as to when their menstrual cycles became irregular prior to the start of the program; therefore, we reconfirmed menstrual status to exclude subjects whose menstrual status at the 2-year follow-up period differed from that at the start of the program.

Confounding Factors. Disturbance of glucose metabolism, including impaired glucose tolerance and diabetes mellitus, was diagnosed on the basis of the criteria issued by the Japan Diabetes Society (19). A family history of coronary artery disease was defined as a previous diagnosis of the disease in a first-degree or second-degree relative.

General Procedures. The following were performed at the start of the program: measurement of body weight, height, and fat distribution and sampling of blood. A questionnaire about family history as defined in the previous section was given to all subjects enrolled at the start of the study. Menopausal history of and possible receipt of antidyslipidemic medications by all enrolled subjects were investigated at the start of the program and at the 1- and 2-year follow-up periods. In addition, ovary extirpation and uterus amputation were ascertained at the start of the study.

Statistical Analysis. The unpaired Student's t test was performed to compare mean values between the premenopausal and postmenopausal groups. To examine the relationships between fat distribution and serum lipids, apolipoproteins, and the ratio of apoB to apoA-I, multiple regression analyses were performed. Serum lipids, apolipoproteins, and the ratio of apoB to apoA-I were evaluated as dependent variables, and SFA, VFA, and age were evaluated as independent variables. At the first step, the homogeneity of regression slopes between the premenopausal and postmenopausal groups, in relation to fat distribution (SFA or VFA) and serum parameters, was tested by fitting a model that consisted of the main effects of menopause and fat distribution (SFA or VFA) as well as the interaction between menopause and fat distribution, which

Table 1. Comparison of the Mean Values Between Premenopausal and Postmenopausal Women[a]

Parameter	Pre-menopausal	Post-menopausal
No. of subjects	146	82
Age	44.2 ± 7.9	56.4 ± 3.4***
Body mass index	29.7 ± 3.2	28.6 ± 2.2**
SFA	287 ± 80	269 ± 54*
VFA	81 (64, 108)[b]	101 (82, 122)[b,c,***]
TC	206 ± 37	233 ± 34***
TG	96 (68, 139)[b]	110 (73, 154)[b,c]
LDL-C	127 ± 33	151 ± 31***
HDL-C	57 ± 13	58 ± 13
ApoA-I	140 ± 19	149 ± 24**
ApoB	99 ± 22	114 ± 22***
Ratio of apoB to apoA-I	3.3 ± 0.8	3.7 ± 0.9**

[a] Data are the mean ± SD. The unpaired *t* test was used to determine the *P* values.
[b] The median value (25th percentile, 75th percentile) is shown.
[c] The *P* value was assessed after logarithmic transformation.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

was adjusted by the nonmain factors of age and fat distribution (SFA or VFA) together with family history and disturbance of glucose metabolism. The interaction effect provided the test of the null hypothesis of homogeneous slopes (20). At the second step, multiple linear regression analyses of the premenopausal and postmenopausal groups were carried out when the regression slopes were not homogenous between the two groups. However, multiple linear regression analysis, including the main effects but not including the interaction effect, was applied to data from all subjects, when homogeneity of regression slopes between the two groups was detectable. VFA and TG were logarithmically transformed, as these data did not fit normal distributions. The results were considered statistically significant if the result of a two-tailed α was <0.05. All data were analyzed by the SPSS 13.0 software package (SPSS Inc., Chicago, IL).

Results

Comparison of Parameters Between Premenopausal and PostMenopausal Groups. Age, VFA, TC, LDL-C, apoA-I, apoB, and the ratio of apoB to apoA-I were significantly higher in the postmenopausal group than in the premenopausal group (Table 1). In contrast, the postmenopausal group had significantly lowered body mass index and SFA when compared with those of the premenopausal group.

Homogeneity of Regression Slopes Between the Premenopausal and Postmenopausal Groups. The homogeneity of the regression slopes for SFA to TC, LDL-C, apoB, and the ratio of apoB to apoA-I between premenopausal and postmenopausal groups was rejected (Table 2), thus indicating that regression lines were not parallel between the premenopausal and postmenopausal groups. The results obtained with regression lines were not altered after adjustment of the data by factors that included impaired glucose metabolism and family history. Scatter plots and regression lines between SFA and TC, TG, LDL-C, HDL-C, apoB, or the ratio of apoB to apoA-I were adjusted for both VFA and age and are provided in Figure 1.

The homogeneity of the regression slopes for VFA to TC, TG, LDL-C, HDL-C, apoA-I, apoB and the ratio of apoB to apoA-I between the premenopausal and postmenopausal groups was accepted (Table 2). The results shown in Table 2 were not altered after adjustment of the data by factors that included impaired glucose metabolism and family history. Scatter plots and regression lines between VFA and TC, TG, LDL-C, HDL-C, apoB, or the ratio of apoB to apoA-I were adjusted for SFA and age and are shown in Figure 2.

Multiple Regression Analysis Between Fat

Table 2. Homogeneity of Regression Slopes Between Premenopausal and Postmenopausal Women and Results of Multiple Regression Analysis

	SFA							VFA[a]		
	Homogeneity of regression slopes	Multiple regression analysis						Homogeneity of regression slopes	Multiple regression analysis	
		All		Premenopausal		Postmenopausal			All	
Parameter	*P* value	B[b]	*P* value	B[b]	*P* value	B[b]	*P* value	*P* value	B[b]	*P* value
TC	0.030	—	—	−0.011	0.787	−0.195	0.007	0.576	13.606	0.034
TG[a]	0.185	<0.001	0.727	—	—	—	—	0.469	0.474	<0.001
LDL-C	0.020	—	—	−0.021	0.588	−0.197	0.002	0.212	11.013	0.058
HDL-C	0.172	0.015	0.277	—	—	—	—	0.496	−8.310	0.001
ApoA-I	0.717	−0.014	0.523	—	—	—	—	0.839	−6.057	0.120
ApoB	0.001	—	—	<0.001	0.991	−0.167	<0.001	0.603	16.282	<0.001
Ratio of apoB to apoA-I	0.003	—	—	<0.001	0.750	−0.006	0.001	0.727	0.482	0.002

[a] Assessed by logarithmic transformation.
[b] Regression coefficient. The dependent variables were serum lipids. The independent variables were VFA, SFA, and age.

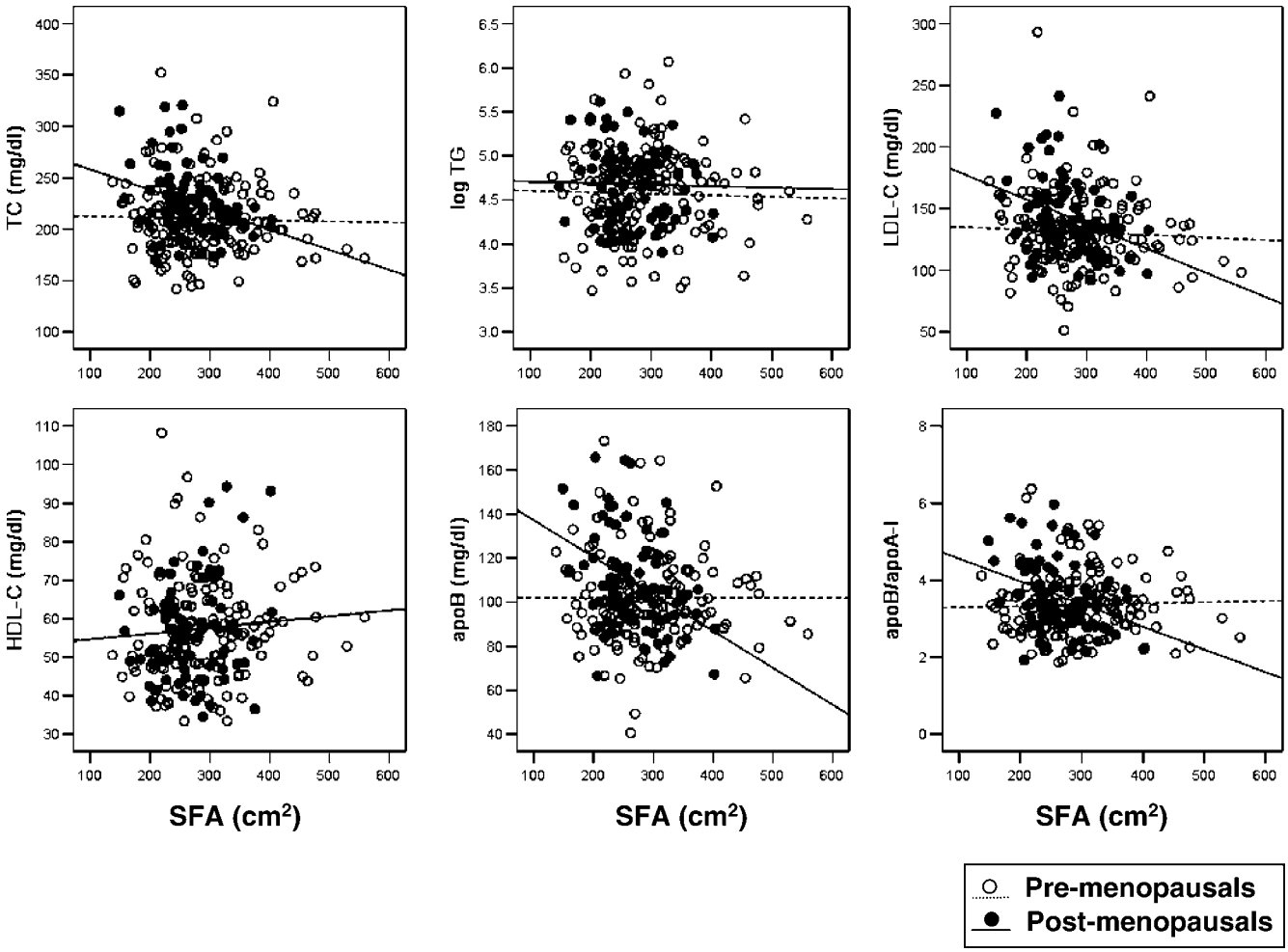

Figure 1. Scatter plots and regression lines between the SFA and TC, TG, LDL-C, HDL-C, apoB, and the ratio of apoB to apoA-I. Each serum parameter was adjusted by age and VFA. Regression lines were drawn by using the regression coefficients shown in Table 2. The regression slopes in TC, LDL-C, apoB, and the ratio of apoB to apoA-I were not homogeneous between premenopausal and postmenopausal women, while those of TG and HDL-C were homogeneous between the two groups. The results indicate that subcutaneous fat accumulation in postmenopausal women causes a reduction in TC, LDL-C, apoB, and the ratio of apoB to apoA-I.

Distribution and Serum Parameters. According to multiple regression analyses, TC ($P = 0.007$), LDL-C ($P = 0.002$), apoB ($P < 0.001$), and the ratio of apoB to apoA-I ($P = 0.001$) were negatively associated with SFA when adjusted for age and VFA in the postmenopausal group but not in the premenopausal group (Table 2). VFA had a positive association with TC, TG, apoB, and the ratio of apoB to apoA-I, but an inverse association was evident with HDL-C in all of the subjects enrolled, when adjustments were made for both age and SFA (Table 2).

Discussion

The major findings from this research effort were that the regression slopes between SFA and TC, LDL-C, apoB, or the ratio of apoB to apoA-I in the premenopausal group were different from those seen in the postmenopausal group. SFA was inversely correlated with TC, LDL-C, apoB, and the ratio of apoB to apoA-I in postmenopausal women but not in premenopausal women. In contrast, the regression slopes between VFA and serum lipids, apolipoproteins and the ratio of apoB to apoA-I were homogenous between the premenopausal and postmenopausal groups.

These findings raise the question about the antiatherosclerotic mechanisms of subcutaneous fat accumulation, independent of visceral fat accumulation, in postmenopausal women. One possible explanation is that large amounts of adipose tissue, such as subcutaneous adiposity, become an active locus after menopause, at which time androgens are converted into estrogens through aromatase catalysis. Androgens, in particular androstenedione, are produced primarily in the adrenal glands and are converted into estrogens by the catalytic action of aromatase at certain extraglandular loci such as mesenchymal cells (both in adipose tissue and skin), osteoblasts, osteogenic chondrocytes, vascular endothelial cells, aortic smooth muscles, and numerous sites in the brain (15). However, after menopause, adipose tissue is altered into a main locus of systemic estrogen production (14, 15), while in premenopausal and

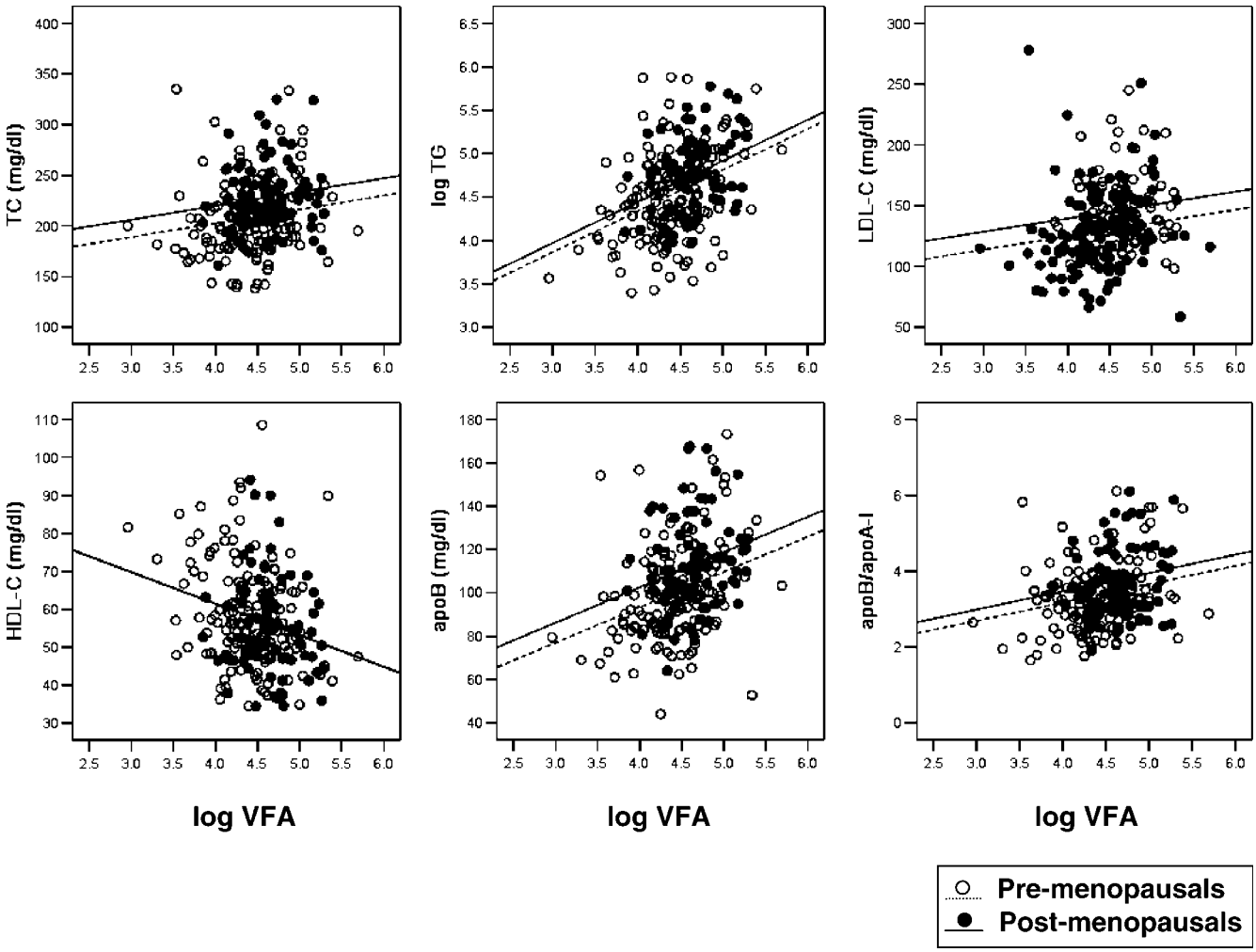

Figure 2. Scatter plots and regression lines between VFA and TC, TG, LDL-C, HDL-C, apoB, and the ratio of apoB to apoA-I. Each serum parameter was adjusted by age and SFA. Regression lines were drawn by using the regression coefficients shown in Table 2. The regression slopes in all serum parameters were homogeneous between the premenopausal and postmenopausal groups. The results indicate that the relation between VFA and serum lipids was not different between the premenopausal and postmenopausal groups.

nonpregnant women, the ovaries are the principal source of systemic estrogen. Oophorectomy increases aromatase protein and mRNA expression in the subcutaneous adipose tissue of rats (21, 22) and induces the gradual elevation of E_2 in the circulation following oophorectomy (21). In human studies a strong positive correlation exists between age and aromatase mRNA level in abdominal subcutaneous adipose tissue (23). Thus, extragonadal aromatization increases to compensate for deficient circulatory estrogen concentration following menopause or oophorectomy (21). Estrogen indeed accelerates LDL receptor activity in the liver (11), elevates the fractional catabolic rate for LDL apoB (24), and suppresses the absorption of dietary cholesterol (24). In addition, there is a growing body of evidence suggesting that estrogen replacement therapy has beneficial effects on lipids and lipoproteins in postmenopausal women (10–13, 24). In view of these findings, fat accumulation may play a crucial role in postmenopausal women to improve atherosclerotic parameters.

The present results suggest that the beneficial effects on lipid metabolism in postmenopausal women may be due to subcutaneous fat accumulation but not visceral adiposity. One possible explanation for the beneficial effects of subcutaneous fat is that basal aromatase activity is significantly higher in subcutaneous preadipocytes than in visceral preadipocytes (25). Another possibility is that the abdominal SFA is enormously larger than the abdominal VFA. In addition, abdominal visceral adipocytes are more sensitive to catecholamine-induced lipolysis and less sensitive to insulin-induced antilipolytic action than abdominal subcutaneous adipocytes (26).

In contrast to the beneficial actions of subcutaneous fat, our present results revealed that postmenopausal women have a greater ratio of visceral to subcutaneous fat accumulation than premenopausal women. The acceleration of visceral fat accumulation in postmenopausal women was consistent with the results seen in previous studies (27, 28). Estrogen-deficient women demonstrated an elevated ratio of

omental to subcutaneous lipoprotein lipase activity when compared with that in regularly cycling women (29). Lipoprotein lipase catalyzes hydrolysis of circulating triglycerides to produce free fatty acids, which are, in turn, re-esterified and stored in adipocytes (30). Thus, lipoprotein lipase activity is one of the important determinants for storage of triglycerides in adipocytes (31). Additionally, visceral adiposity accelerated basal and adrenergic receptor–stimulated lipolysis of subcutaneous fat (32). These findings in postmenopausal women are well matched with the notion of fat redistribution toward the visceral fat compartment following menopause and enhancement of impaired lipid metabolism. Taken together, menopause-induced visceral adiposity makes subcutaneous fat lipolysis more potent and thereby reduces the mass of subcutaneous adipose tissue. This acceleration of visceral fat accumulation alters circulating triglyceride concentration and allows a flux of free fatty acids into the portal circulation (26). The elevated concentration of portal free fatty acids is associated with an increase in the synthesis of very-low-density lipoprotein TG (26). However, visceral adipocytes are the dominant contributors of adiponectin (26), cholesteryl-ester transfer protein (26), and visfatin (33), all of which play crucial roles in lipid metabolism. A decrease in adiponectin and an increase in cholesteryl-ester transfer protein and visfatin (26, 33), which are induced by visceral fat accumulation, cause unfavorable effects on lipid metabolism beyond the favorable effects of aromatase that are activated in the subcutaneous adipose tissue. Indeed, our present results revealed that VFA correlates positively with serum TC, TG, apoB, and the ratio of apoB to apoA-I but negatively with HDL-C in all subjects.

It has been a matter of debate as to whether subcutaneous fat is simply a passive storehouse of excess body fat or actively involved in the regulation of lipid metabolism and/or weight control. Previous studies demonstrated that oophorectomized female rats have elevated aromatase activity in subcutaneous fat tissues (21, 22), suggesting that aromatase activity augmented by subcutaneous fat accumulation may produce beneficial effects on the metabolism of lipids and apolipoproteins, so that postmenopausal women can be protected from the development of atherosclerosis. Although the present study of the relationship between subcutaneous adiposity and serum lipid concentration does not clarify a definite mechanism by which subcutaneous adiposity confers beneficial effects on lipid metabolism after menopause, it does provide a new insight into the roles of fat distribution after menopause not only in lipid metabolism but also in the development of atherosclerosis.

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