Contribution of Abdominal Adiposity to Age-Related Differences in Insulin Sensitivity and Plasma Lipids in Healthy Nonobese Women

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OBJECTIVE — We examined the hypothesis that an age-related increase in the compartments of visceral fat would account, in part, for the deleterious changes in insulin sensitivity and blood lipid profile in nonobese women.

RESEARCH DESIGN AND METHODS — We directly assessed visceral and subcutaneous abdominal adipose tissue areas (computed tomography), glucose disposal (hyperinsulinemic-euglycemic clamp), body composition (dual energy X-ray absorptiometry), blood-lipid profile, and aerobic fitness ($V\text{O}_{2max}$) in 178 nonobese women categorized into four age groups: group 1, 28 ± 4 years, $n = 88$; group 2, 46 ± 2 years, $n = 38$; group 3, 53 ± 2 years, $n = 31$; and group 4, 67 ± 6 years, $n = 21$.

RESULTS — Visceral abdominal adipose tissue area increased with age (2.36 cm^2 per year, $P < 0.0001$). We noted an age-related increase in total cholesterol ($P < 0.0003$), triglycerides ($P < 0.0009$), LDL cholesterol ($P < 0.027$), and the ratio of total cholesterol to HDL cholesterol ($P < 0.042$). However, age-related changes in insulin sensitivity exhibited a different age-related pattern. That is, insulin sensitivity, expressed on an absolute basis or indexed per kilogram of fat-free mass, was lowest in group 4 but was not significantly different among groups 1, 2, and 3. After statistical control for visceral fat, lower insulin sensitivity persisted in group 4, although differences were diminished relative to other groups. However, the effect of visceral fat on age-related changes in the blood-lipid profile was stronger. That is, differences in visceral and deep subcutaneous adipose tissue area abolished age-related differences in total cholesterol, triglycerides, and LDL cholesterol. No independent effects of $V\text{O}_{2max}$ or leisure-time physical activity on age-related changes in insulin sensitivity or on the blood–lipid profile were noted.

CONCLUSIONS — We conclude that *1*) visceral fat shows an increase with advancing age, whereas a decrease in insulin sensitivity was noted only in older women; *2*) age-related differences in visceral fat explain only a modest part of the decline in insulin sensitivity in nonobese women; and *3*) unfavorable changes in plasma lipids were strongly associated with the age-related increase in visceral abdominal adipose tissue.

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In the late 1940s, Vague (1) suggested that the relative proportion of body fat in the upper body versus lower body was an important factor to consider when investigating obesity-related health risks. This early hypothesis remained untested for 35 years. It was only until the 1980s that researchers focused on abdominal obesity as an important correlate of dyslipidemia, cardiovascular disease, and diabetes (2–4). These observations stimulated much research that supported the notion that upper body or abdominal obesity was more closely related to the metabolic disturbances associated with cardiovascular disease than was obesity per se (5–9).

Another major advance in our understanding of the health risks associated with body fat accumulation in the late 1980s was the use of powerful imaging techniques, such as computed tomography (CT) and magnetic resonance imaging. With these techniques, the ability to differentiate the mass of adipose tissue located at the abdominal level into visceral and subcutaneous fat became available (10–12). It has been suggested that visceral fat is an independent predictor of alterations in plasma lipid, lipoprotein, and plasma glucose–insulin concentrations and a strong predictor of diabetes, heart disease, and hypertension (3–9,13–15), although this notion is still controversial (16,17).

A number of studies (12,18–21) have shown an increase in CT-measured central fat with advancing age. However, relatively few studies have addressed the potential impact of age-related changes in visceral fat on physiological variables that predict disease outcome, such as plasma lipids and insulin sensitivity (22,23). These studies have generally favored a significant association between visceral fat with alterations in the blood-lipid profile and measures of insulin sensitivity, although this hypothesis remains controversial (17). Discrepancies among studies are in part caused by small sample sizes. In addition, men and women were frequently considered in the same cohort, and a broad age-range was not examined. Perhaps more importantly, previous investigators have relied on surrogate or proxy measures instead of direct determi-

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Abbreviations: CT, computed tomography; FSH, follicle-stimulating hormone; GCRC, General Clinical Research Center; HRT, hormone replacement therapy; LTA, leisure-time activity; OGTT, oral glucose tolerance test.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Physical characteristics and body composition measures according to age-group in the sample of 178 women*

	Group 1	Group 2	Group 3	Group 4	Difference by group
	20–35 years	40–50 years	51–60 years	61–78 years	$P < 0.05$
n	88	38	31	21	—
Age (years)	28.2 ± 3.9	45.8 ± 2.0	53.1 ± 2.5	66.7 ± 5.7	—
Height (cm)	164.8 ± 6.7	165.6 ± 5.1	162.1 ± 4.1	161.7 ± 4.8	NS
Body mass (kg)	59.5 ± 7.5	62.2 ± 8.7	64.1 ± 7.8	68.2 ± 8.7	1 vs. 3 and 4 / 2 vs. 4
BMI (kg/m^2)	21.9 ± 2.1	22.7 ± 3.3	24.4 ± 3.0	25.9 ± 2.7	1 and 2 vs. 3 and 4
Fat mass (kg)	16.6 ± 5.4	17.8 ± 7.7	21.1 ± 6.4	26.1 ± 5.4	1 vs. 3 and 4 / 2 and 3 vs. 4
Fat-free mass (kg)	40.0 ± 4.1	40.7 ± 3.5	39.6 ± 3.5	39.0 ± 3.8	NS
$V\mathrm{O}_{2max}$ (ml · kg^{-1} · min^{-1})*	34.9 ± 6.7	33.6 ± 8.5	29.8 ± 9.5	27.4 ± 3.0	1 and 2 vs. 4

Data are means ± SD. Body composition was determined by dual energy X-ray absorptiometry, and $V\mathrm{O}_{2max}$ was measured by a treadmill test to volitional fatigue. *Group 1, $n = 88$; group 2, $n = 34$; group 3, $n = 8$; and group 4, $n = 21$.

nations of insulin sensitivity and visceral fat.

To address these issues and to extend previous investigations with CT, we quantified compartmental differences in abdominal adiposity in a large cohort of nonobese women spanning a broad age range. Thereafter, insulin sensitivity was assessed using the hyperinsulinemic-euglycemic clamp method and plasma lipids were determined. Our objectives were twofold: to determine age-related differences in abdominal adiposity, insulin sensitivity, and plasma lipids in nonobese women and to examine whether differences in abdominal adiposity explain age-related differences in insulin sensitivity and plasma lipids. We hypothesized that an age-related increase in visceral fat would account, in part, for the deleterious changes in insulin sensitivity and blood-lipid profile in nonobese women.

RESEARCH DESIGN AND METHODS

— A total of 178 healthy normal-weight women (175 Caucasian and 2 of Asian and 1 of American-Indian origin) were considered for this study. These women were recruited from the University of Vermont and the surrounding community of Burlington, Vermont, by advertisements in local newspapers and by radio announcements. Women were excluded from the study if they were a current smoker, had a BMI ≥31 kg/m^2, had acute illness, were currently receiving any medication affecting energy expenditure (e.g., β-blockers), consumed >15 g of alcohol/day, had weight fluctuations >2 kg 6 months before testing, were pregnant or intended to become pregnant during time of study, or had abnormal electrocardiogram at rest or during an exercise test. The presence or absence of a family history of diabetes was obtained during the physical examination. All premenopausal women (groups 1 and 2) were defined as having two menses in the 3 months before testing and a follicle-stimulating hormone (FSH) level <30 IU/l. All postmenopausal women (groups 3 and 4) were defined as having an absence of menses for 6 months to 3 years since the last menstrual period and an FSH level >35 IU/l. All women included in the study also displayed a normal response to an oral glucose tolerance test (OGTT) (blood glucose levels <7.77 mmol/l 2 hr post). All studies were approved by the Committee for Human Research at the University of Vermont, and each participant gave written informed consent before the beginning of the study. The physical characteristics of the four groups of women are presented in Table 1.

Overview of protocol

Each prospective volunteer was evaluated for eligibility via telephone interview. Subjects who met eligibility criteria were invited to the General Clinical Research Center (GCRC) at the Fletcher Allen Health Center for an outpatient screening visit, during which a medical history, a physical examination, biochemical laboratory tests, a 75-g OGTT, and an exercise stress test were performed. The volunteers meeting all screening criteria gave consent and were invited for an overnight visit at the GCRC within 2 months of the screening visit date. For 3 days before admission, all subjects consumed a standardized diet prepared by the metabolic kitchen (1,900 ± 100 kcal/day; 55% carbohydrate, 30% fat, and 15% protein).

Measurements

Plasma lipids and lipoprotein levels. Enzymatic processes were used to determine fasting plasma triglyceride levels (24) and total, LDL, and HDL cholesterol concentrations (25). Cholesterol concentrations in the HDL fraction were determined after precipitation of apolipoprotein B–containing lipoproteins with dextran sulfate (26). LDL cholesterol concentrations were calculated using the Friedewald equation (27).

$V\mathrm{O}_{2max}$. $V\mathrm{O}_{2max}$ was determined from an incremental exercise test on a treadmill to volitional exhaustion, as previously described (28). After an initial 3-min warm-up, the speed was set so that the heart rate would not exceed 70% of the age-predicted maximum heart rate (220-age [years]). Thereafter, the speed was held constant, and the grade was increased by 2.5% every 2 min. The criteria for achieving a $V\mathrm{O}_{2max}$ (ml · kg^{-1} · min^{-1}) were a respiratory exchange ratio >1.0 and a heart rate at or above the age-predicted maximum (220-age [year]). At least one of these criteria was reached by 93% of volunteers. Test-retest conditions for nine individuals (on two occasions 1 week apart) yielded an intra-class correlation of 0.94 and a coefficient of variation of 3.8% in our laboratory. Leisure-time physical activity was measured using the Minnesota leisure-time physical activity survey (29).

Body composition and fat distribution. Body composition was determined by dual energy X-ray absorptiometry using a Lunar DPX-L densitometer (Lunar Radiation, Madison, WI), as previously described (30,31). Measurements included the assessment of fat mass and fat-free

Table 2—*Abdominal adipose tissue compartment areas according to age-group in the sample of 178 women*

	Group 1 20–35 years	Group 2 40–50 years	Group 3 51–60 years	Group 4 61–78 years	Difference by group $P < 0.05$
n	84	38	31	21	—
L4 Visceral adipose tissue area (cm^2)	42 ± 21	54 ± 30	82 ± 41	133 ± 45	All different
L4 Subcutaneous adipose tissue area (cm^2)	181 ± 87	221 ± 107	256 ± 88	300 ± 69	1 and 2 vs. 4 / 1 vs. 3
L4 Superficial SQ adipose tissue area (cm^2)*	96 ± 46	120 ± 54	131 ± 36	143 ± 36	1 vs. 3 and 4
L4 Deep SQ adipose tissue area (cm^2)*	90 ± 45	104 ± 62	134 ± 55	157 ± 38	1 vs. 3 and 4 / 2 vs. 4

Data are means ± SD. *Group 1, $n = 78$; group 2, $n = 35$; group 3, $n = 29$; and group 4, $n = 21$. SQ, subcutaneous.

mass. Percent body fat was calculated by dividing fat mass by body weight.

Abdominal adipose tissue areas were measured by CT with a High Speed Advantage CT scanner (General Electric Medical Systems, Milwaukee, WI), as previously described (30). Subjects were examined in the supine position with both arms stretched above the head. The scan was performed at the L4–L5 vertebrae level using a scout image of the body to establish the precise scanning position. Intra-abdominal adipose tissue area was quantified by delineating the intra-abdominal cavity at the internal most aspect of the abdominal and oblique muscle walls surrounding the cavity and the posterior aspect of the vertebral body with the computer interface of the scanner. Deep subcutaneous adipose tissue area was defined as the area between the subcutaneous fascia and the muscle wall. Superficial subcutaneous adipose tissue was obtained by subtracting deep subcutaneous area from total subcutaneous area. Adipose tissue areas were highlighted and computed using an attenuation range from –190 to –30 Hounsfield units.

Hyperinsulinemic/euglycemic clamp. Basal and insulin-stimulated glucose kinetics were measured by the hyperinsulinemic-euglycemic clamp technique, as described by DeFronzo et al. (32) and as previously implemented in our laboratory (15,30). All subjects were tested after a 12-h overnight fast at the GCRC and 3 days of standardized meals. An intravenous catheter was placed in an antecubital vein at 0600 h for infusion of insulin. A second catheter was placed retrograde in the contralateral hand for blood sampling. The hand was warmed in a box by a gentle stream of heated air (50–55°C) to produce arterialized-venous blood. At 0900 h, the insulin infusion began and continued for an additional 2 h. Insulin was infused at a rate of 40 mU · m^{-2} · min^{-1} to attain postprandial peripheral insulin levels and to suppress endogenous glucose production. Blood glucose was monitored every 5 min during the insulin infusion, and euglycemia was maintained throughout the clamp by infusing 20% dextrose at a variable rate. The glucose levels attained during the last 30 min of the clamp were 76.7 ± 4.2, 85.8 ± 4.3, 83.4 ± 5.7, and 81.8 ± 5.3 mg/dl for groups 1, 2, 3, and 4, respectively. The duration of the insulin infusion was such that the rate of infused glucose reached a constant value by the 2nd hour of the clamp. Aliquots of blood were placed in heparinized tubes and stored on ice until the plasma was prepared by centrifugation at 4°C, frozen, and stored at –60°C for later analysis.

Glucose and insulin levels. Glucose levels were measured by the glucose oxidase method using an automated analyzer (YSI Instruments, Yellow Springs, OH). Serum insulin was determined with a double antibody radioimmunoassay (Diagnostic Products, Los Angeles, CA). Intra-assay and interassay coefficients of variation were 4 and 10%, respectively.

Statistical analysis. A one-way analysis of variance was used to test the main effects of aging (age-group) on the physical characteristics, abdominal adipose tissue compartments, and glucose disposal in our sample of women. Homogeneity of variances among groups was assured using the Levene test (acceptance of unequal variances at $P < 0.10$). Variances were unequal for the following variables: triglycerides, visceral adipose tissue area, $V_{O_{2max}}$, BMI, height, and percent body fat. For these variables, the Welch analysis of variance was used to test the main age-group effect. Post hoc comparisons were performed with the Tukey-Kramer test. Correlational analysis was also used to examine the relationship between variables. Stepwise regression analysis was used to examine the independent predictors of glucose disposal. To examine the effects of abdominal adipose tissue compartments, fat-free mass, and $V_{O_{2max}}$ on glucose disposal and lipid profile, least square means were derived from an analysis of covariance, with adiposity measures as covariates. Statistical significance was accepted at an alpha level ≤0.05. Values are expressed as means ± SD, unless otherwise specified.

RESULTS — Table 1 shows physical characteristics for the four age-groups. The source of the differences among age-groups are presented in the last column. There was no difference in height among groups. Body mass and adiposity measures increased with age. Body mass was less in group 1 than in groups 3 and 4, whereas group 2 weighed less than group 4 ($P < 0.05$). Women in groups 1 and 2 had a lower BMI than individuals in groups 3 and 4 ($P < 0.05$), although there was no difference in the BMI between groups 1 and 2. Fat mass was higher in groups 3 and 4 than in group 1. Group 4 also had a higher fat mass than groups 2 and 3. Fat-free mass was not different among groups. $V_{O_{2max}}$ was lower in group 4 than in groups 1 and 2 ($P < 0.05$).

Table 2 shows CT-measured abdominal adipose tissue compartment areas among age-groups. We noted a progressive increase in visceral and subcutaneous adipose tissue areas ($P < 0.05$) with age, so that all groups were different from each other. A regression equation yielded an age-related increase of 2.36 cm^2 per year for visceral adipose tissue area. Subcutaneous adipose tissue area was further divided into superficial subcutaneous tissue area and deep subcutaneous adipose area. Groups 3 and 4 had significantly higher superficial and deep subcutaneous adipose tissue areas than group 1 ($P < 0.05$).

Table 3—*Glucose disposal and blood-lipid profile according to age-group in the sample of 178 women*

	Group 1 20–35 years	Group 2 40–50 years	Group 3 51–60 years	Group 4 61–78 years	Difference by group $P < 0.05$
n	88	38	31	21	—
Glucose disposal (mg/min)	404 ± 128	439 ± 127	444 ± 119	308 ± 90	1, 2, and 3 vs. 4
Glucose disposal (mg · FFM^{-1} · min^{-1})	10.0 ± 2.8	10.8 ± 2.8	11.4 ± 3.5	8.0 ± 2.4	1, 2, and 3 vs. 4
Fasting insulin (pmol/l)	44 ± 22	41 ± 14	46 ± 16	56 ± 16	2 vs. 4
Fasting glucose (mmol/l)	4.2 ± 0.2	4.6 ± 0.4	4.6 ± 0.4	4.6 ± 0.3	1 vs. 2, 3, and 4
Triglyceride (mmol/l)	1.1 ± 0.4	1.0 ± 0.6	1.4 ± 0.9	1.6 ± 0.9	1 and 2 vs. 4
Total cholesterol (mmol/l)	4.7 ± 0.8	4.6 ± 1.0	5.0 ± 1.1	5.6 ± 0.8	1 and 2 vs. 4
LDL cholesterol (mmol/l)	2.8 ± 0.8	2.7 ± 0.8	2.9 ± 1.0	3.3 ± 0.6	2 vs. 4
HDL cholesterol (mmol/l)	1.4 ± 0.4	1.5 ± 0.3	1.4 ± 0.3	1.6 ± 0.5	NS
Total cholesterol–to–HDL cholesterol	3.7 ± 1.2	3.2 ± 1.0	3.6 ± 0.8	3.9 ± 1.2	1 vs. 2

Data are means ± SD. FFM, fat-free mass.

Group 2 had less deep subcutaneous adipose tissue area than group 4.

Table 3 shows insulin sensitivity and blood lipid data among the four groups. Glucose disposal, whether expressed on an absolute basis or indexed per kilogram of fat-free mass, was lower in group 4 than in groups 1, 2, and 3 ($P < 0.05$). In regression-based analysis, the correlation between age and glucose disposal was nonsignificant ($r = -0.144$). No differences in glucose disposal were noted among groups 1, 2, or 3. Fasting glucose was lower in group 1 than in groups 2, 3, and 4 ($P < 0.0001$), although no differences were noted among groups 2, 3, and 4. Fasting insulin was lower in group 2 than in group 4 ($P < 0.03$). Group 4 showed higher fasting triglyceride and total cholesterol levels than groups 1 and 2 ($P < 0.05$). LDL cholesterol was also higher in group 4 than in group 2. No effect of age was found on HDL cholesterol levels. Total cholesterol–to–HDL cholesterol ratios were higher in group 1 than in group 2 ($P < 0.05$).

Table 4 shows least square means data of glucose disposal and blood-lipid profile according to age-group after adjustment for visceral adipose tissue area. From a statistical standpoint, the use or control of visceral adipose tissue area is justified because it was the first variable selected ($r^2 = 12\%$) in a multiple regression model, with glucose disposal as the dependent variable. No independent effect of $V\text{O}_{2\text{max}}$ or leisure-time activity (LTA) was found in the model, although these variables were moderately related to glucose disposal on a univariate basis ($r = 0.310$ for $V\text{O}_{2\text{max}}$ vs. glucose disposal and $r = 0.212$ for LTA vs. glucose disposal). Information in Table 4 provides mean differences among groups after statistical control for differences in visceral adipose tissue area.

The main age-group effect on glucose disposal, whether expressed on an absolute basis or indexed per kilogram of fat-free mass, persisted after adjustment ($P < 0.004$) for visceral adipose tissue area. A significantly lower glucose disposal persisted in older postmenopausal women (group 4) compared with group 3. Adjusted values in group 4 tended to be lower than groups 2 and 3, but these did not reach statistical significance. Unexpectedly, glucose disposal was also found to be lower in group 1 than in groups 2 and 3 after adjustment for visceral fat. A similar pattern was observed when glucose disposal values were expressed per kilogram of fat-free mass.

We also examined the possibility that other abdominal fat components may influence rates of glucose disposal (not shown in table form). That is, we examined whether statistical control for total fat mass, total subcutaneous abdominal fat, as well as deep subcutaneous and superficial subcutaneous adipose tissue area influenced glucose disposal. None of

Table 4—*Least square means of glucose disposal and blood-lipid profile according to age-group in the sample of 178 women after adjustment for visceral adipose tissue area*

	Group 1 20–35 years	Group 2 40–50 years	Group 3 51–60 years	Group 4 61–78 years	Difference by group $P < 0.05$
n	88	38	31	21	—
Glucose disposal (mg/min)	379.7 ± 14.4	431.0 ± 19.5	462.8 ± 22.2	376.6 ± 33.7	1 vs. 2 and 3 / 3 vs. 4
Glucose disposal (mg · FFM^{-1} · min^{-1})	9.4 ± 0.3	10.5 ± 0.45	11.9 ± 0.5	10.1 ± 0.8	1 vs. 2 and 3 / 2 vs. 3 / 3 vs. 4
Triglyceride (mmol/l)*	1.2 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	1.1 ± 0.2	NS
Total cholesterol (mmol/l)*	4.8 ± 0.1	4.6 ± 0.1	4.9 ± 0.2	5.3 ± 0.3	NS
LDL-cholesterol (mmol/l)*	2.9 ± 0.1	2.7 ± 0.1	2.9 ± 0.2	3.1 ± 0.2	NS
HDL-cholesterol (mmol/l)*	1.3 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	1 vs. 2 and 4
Total cholesterol–to–HDL cholesterol	3.9 ± 0.1	3.2 ± 0.2	3.5 ± 0.2	3.4 ± 0.3	1 vs. 2

Data are means ± SEM. *Group 1, $n = 87$; group 2, $n = 38$; group 3, $n = 22$; and group 4, $n = 21$. FFM, fat-free mass.

these compartments abolished the effect of age on glucose disposal when data were expressed on an absolute basis or indexed for fat-free mass.

Table 4 also shows age-related differences in plasma lipids after control for visceral fat. We found no age effect on fasting triglycerides, total cholesterol, and LDL cholesterol after statistical control for visceral adipose tissue area. On the other hand, the age-group differences persisted for HDL cholesterol and total cholesterol–to–HDL cholesterol ratio after statistical control for visceral fat ($P < 0.01$ and $P < 0.02$, respectively).

We also examined the possibility that other abdominal fat components may influence age-related differences in plasma lipids. The only other variable (other than visceral fat) that abolished age-related differences in plasma lipids was statistical control for deep subcutaneous adipose tissue (data not shown). Statistical control for total fat mass, total subcutaneous adipose tissue area, and superficial subcutaneous adipose tissue area had marginal or no effect on age-related differences in plasma lipids.

Both oral contraceptive and hormone replacement therapy (HRT) use were examined as confounding factors by comparing users in groups 1 and 4, respectively, with age-matched nonusers. Users of oral contraceptives or HRT in groups 1 and 4, respectively, showed no significant differences in adipose tissue area or glucose disposal data. However, HRT users did show a trend for a higher HDL cholesterol ($P < 0.08$) and improved total cholesterol–to–HDL cholesterol ratio ($P < 0.06$). Oral contraceptive users in group 1 showed higher triglycerides ($P < 0.01$), higher cholesterol ($P < 0.03$), and lower total cholesterol–to–HDL cholesterol ratio ($P < 0.03$).

CONCLUSIONS — We tested the hypothesis that increasing abdominal adiposity with advancing age would be a strong predictor of the deterioration in the metabolic profile. To address this issue, we determined the role of abdominal adiposity (and its compartments) as a modulator of age-related changes in insulin sensitivity and plasma lipids in a relatively large group of women spanning a broad age range (20 to 78 years of age). Our major findings are as follows: *1*) visceral fat showed a progressive increase with advancing age; *2*) despite the age-related increase in visceral fat, a deterioration in insulin sensitivity was noted only in the oldest group of women (>60 years of age); *3*) differences in visceral fat explained a modest portion of the age-related decline in insulin sensitivity; and *4*) unfavorable changes in plasma lipids with age were strongly associated with the increase in visceral adipose tissue.

To our knowledge, this is one of the largest studies to directly measure insulin sensitivity using euglycemic/hyperinsulinemic clamp methodology and intra-abdominal fat using radiologic imaging across a broad age spectrum (20 to 78 years of age) of women. We have intentionally recruited nonobese women in this protocol to reduce the confounding influence of obesity and high levels of body fat on insulin sensitivity and plasma lipids. Moreover, this study extends previous works by providing new information on the time course of changes in intra-abdominal fat, insulin sensitivity, and blood-lipid profile among aging women. That is, the majority of previous studies have relied on a two-group comparison (i.e., younger versus older), with relatively little consideration of changes observed in middle-aged women.

We noted a progressive increase in abdominal adipose tissue from all abdominal adipose tissue compartments with advancing age. The regression equation between age and visceral fat yielded an increase of 2.36 cm^2 per year of visceral fat. However, we noted considerable divergence among investigators regarding the rate of change in intra-abdominal fat with advancing age. For example, Enzi et al. (18) (62 men and 68 women ages 20–60+ years), reported a lower rate (0.49 cm^2/year) of change in visceral fat with age, as compared with the present investigation. Borkan et al. (20) reported an increase in visceral fat of 1.41 cm^2/year, with data derived from a study involving 21 middle aged (46.3 ± 2.6 years) and 20 older aged (69.4 ± 4.1 years) nonobese men. Lemieux et al. (33) prospectively examined 32 moderately obese young women (aged 35.1 ± 5.4 years) and found an increase of 4.57 cm^2/year after a 7 year follow-up. Divergence in the rate of change among investigators is likely caused by differences in experimental design (i.e., cross-sectional versus longitudinal), low statistical power related to small sample sizes, and differences in age range and obesity level of the volunteers. Nonetheless, in our relatively large sample size, our results suggest an incremental increase in visceral fat with advancing age, with the highest values observed in older women.

In the present study, insulin sensitivity was determined using euglycemic/hyperinsulinemic clamp methodology. We paid strict attention to the experimental conditions before the assessment of insulin sensitivity. That is, all measurements were performed under inpatient conditions; dietary intake was standardized before the clamp measurements to diminish the confounding effects of the fluctuation in the antecedent diet; all individuals refrained from regular exercise 3 days before measurement; and all individuals were weight-stable within the previous 6 months (<2 kg fluctuation in body weight).

We used both a regression-based approach and an age-cutpoint approach to analyze our data. Using a regression-based approach, the correlation between age and glucose disposal was nonsignificant ($r = -0.144$). Using an age-cutpoint approach, we found that measured values for insulin sensitivity displayed a different pattern with advancing age (Table 3), unlike the incremental age-related increase in visceral fat. That is, there was no significant difference in insulin sensitivity among the three younger age-groups (20 to 60 years of age), but a lower insulin sensitivity was noted only in the older cohort (61 to 78 years of age). On a relative basis, older women (group 4) displayed a 28% lower glucose disposal value relative to the other groups, suggesting a breakpoint for diminished insulin action in the women who are ~10–15 years postmenopausal. This finding is consistent with and extends the work of others (22,23,34–36) by attempting to identify at which age range insulin sensitivity may start to deteriorate. Moreover, our results are consistent with other investigators who found a modest effect of age on insulin sensitivity (37).

We originally hypothesized that an age-related increase in the compartments of visceral fat would account, in part, for the deleterious changes in insulin sensitivity. We found a modest effect of differences in intra-abdominal adiposity on age-related deterioration in glucose disposal. Two lines of evidence support this assertion. In multiple regression analysis, the level of visceral fat was selected as the

only significant predictor of insulin sensitivity but only accounted for 12% of the unique variance. This suggests that the contribution of visceral fat to variation in insulin sensitivity among a nonobese population is modest and that 88% of the variance remains unaccounted for. To further investigate this issue, we statistically controlled for group differences in visceral fat in an attempt to abolish the lower insulin sensitivity in the oldest group of women (group 4). We found that statistical control for age-related differences in visceral fat (Table 4) tended to reduce differences in glucose disposal among groups, but a lower insulin sensitivity persisted in oldest group of women (group 4, 61 to 78 years of age).

We also considered the notion that an age-related decline in maximal aerobic capacity ($V\text{O}_{2max}$) may be a modulator of changes in insulin sensitivity. Our data replicate the well-known age-related decline in aerobic fitness (Table 1). Despite this observation, $V\text{O}_{2max}$ was not an independent predictor of age-related changes in insulin sensitivity. That is, regardless of our statistical approach, $V\text{O}_{2max}$ failed to account for any unique variance in insulin sensitivity among age-groups. We were not totally surprised at this finding, as other investigators have reported no independent contribution of $V\text{O}_{2max}$ to age-related changes in insulin sensitivity (23). Furthermore, we have shown that despite an increase in $V\text{O}_{2max}$ and insulin sensitivity with aerobic training, changes in these variables showed no relationship with each other (30). Similarly, we found no independent effect of leisure-time physical activity on glucose disposal, as measured from a questionnaire.

Although a growing body of evidence supports a relationship between visceral fat accumulation and insulin sensitivity (15,23,38), our findings, at least in nonobese women, suggest that this effect may be moderate. It is also possible that the magnitude of differences in visceral fat across age-groups may be too small to negatively impact insulin sensitivity. Indeed, it has been suggested that a threshold of 130 cm^2 (39) is needed to negatively impact glucose disposal. The only group that approached this threshold was the oldest cohort of women (group 4, 61 to 78 years of age), who also displayed the lowest level of insulin sensitivity. Collectively, we would suggest that in a nonobese cohort, the relationship between visceral fat and insulin sensitivity is less pronounced compared with an obese cohort (15).

As expected, we found that the blood-lipid profile worsened with increasing age. Specifically, triglycerides, total cholesterol, and LDL cholesterol increased within each age-group (31, 16, and 15%, respectively). No consistent effect of age was noted on HDL cholesterol and the total cholesterol–to–HDL cholesterol ratio, which is consistent with earlier reports (40–42). Despite the lack of age difference in HDL cholesterol, the trends for an increase noted in triglyceride, cholesterol, and LDL cholesterol suggest a worsening of the blood-lipid profile that is supported by other studies (41,42). It should be kept in mind that age-related changes in the blood-lipid profile were modest and did not exceed levels that would warrant pharmacological intervention (43). These findings may be attributable to the fact that recruitment criteria used in this study led to the selection of an apparently healthy cohort.

Another objective in this study was to examine whether the relationship between age and blood-lipid profile was related to differences in body fat distribution. Statistical control for visceral fat abolished age-related differences in plasma lipids. This suggests that the accumulation of visceral fat is an important component associated with deleterious changes in the blood-lipid profile with advancing age. These findings are consistent with those of other investigators (8,21,38). The mechanism of action for the deleterious effects of visceral adipose tissue on the lipid profile has been suggested to be related to its anatomical location. That is, adipose tissue located within the abdominal cavity releases high levels of fatty acids into portal circulation, which may in turn impair hepatic metabolism (44). Increased delivery of free-fatty acids to the liver is associated with reduced hepatic insulin clearance (45,46), increased production of triglyceride-rich lipoproteins (VLDL), and hepatic glucose production by the liver (47). The only other abdominal adipose tissue compartment that was shown to abolish age-related differences in plasma lipids was the deep subcutaneous adipose tissue. Collectively, our results support a prominent role for visceral fat as a modulator of age-related changes in plasma lipids. Moreover, the deep subcutaneous adipose tissue may be the most important compartment within the abdominal cavity as a correlate of deleterious changes in lipids with advancing age.

Several caveats of the present study need to be mentioned. Due to the cross-sectional design of the present study, it is impossible to infer causal relationships. In addition, the women recruited in the present study were healthy volunteers that were nonobese and nondiabetic. Thus, it remains unclear whether findings of the present study can be extrapolated to obese and insulin resistant/glucose intolerant populations of women and other ethnic groups. Clearly, longitudinal studies are needed to confirm our findings in women at different disease risk levels.

In summary, we conclude the following: *1*) visceral fat shows an increase with advancing age, whereas a decrease in insulin sensitivity was noted only in older women; *2*) age-related differences in visceral fat explain a modest part of the decline in insulin sensitivity in nonobese women; and *3*) unfavorable changes in plasma lipids were strongly associated with the age-related increase in visceral abdominal adipose tissue.

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