ARTICLE COVER SHEET

LWW

Journal of The North American Menopause Society

FLA, EDI

Article : GME20475
Creator : cq16
Date : Wednesday December 12th 2007
Time : 17:29:23
Article Title : Centrally located body fat is related to inflammatory markers in healthy postmenopausal women
Number of Pages (including this page) : 11
Template Version : 2.4
08/25/07
Scripts:
1. sc_Extract_Xml
2. AssymFrame
3. sc_Multifig_Marker
4. Autopagination compliant
5. sc_TwoColAff–for 2-column affiliation

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

Menopause: The Journal of The North American Menopause Society
Vol. 15, No. 4, pp. 000-000
DOI: 10.1097/gme.0b013e318159f1a2
© 2007 by The North American Menopause Society

Centrally located body fat is related to inflammatory markers in healthy postmenopausal women

Courtney D. Perry, MS, RD,[1] *D. Lee Alekel, PhD,*[1] *Laura M. Ritland,*[1] *Shilpa N. Bhupathiraju, MS,*[1] *Jeanne W. Stewart, MS,*[1] *Laura N. Hanson, MS,*[1] *Oksana A. Matvienko, PhD,*[2] *Marian L. Kohut, PhD,*[3] *Manju B. Reddy, PhD,*[1] *Marta D. Van Loan, PhD,*[4] *and Ulrike Genschel, PhD*[5]

Abstract

Objective: C-reactive protein and fibrinogen are established atherosclerotic cardiovascular disease risk factors. These acute-phase proteins and the proinflammatory cytokines tumor necrosis factor α, interleukin-6, and interleukin-1β may be elevated in obesity and with menopause. The purpose of this multicenter study was to identify whether centrally located fat and/or overall adiposity were related to these inflammatory markers in healthy postmenopausal women.

Design: We used dual-energy x-ray absorptiometry to assess overall and regional body composition (fat mass in particular) in 242 postmenopausal women in relation to plasma fibrinogen, serum C-reactive protein, and these proinflammatory cytokines.

Results: Multiple regression analyses revealed that 36% of the variability in C-reactive protein (F = 32.4, $P \leq$ 0.0001) was accounted for by androidal fat mass (16.1%, $P \leq 0.0001$), white blood cells (5.6%, $P \leq 0.0001$), and age (2.3%, P = 0.0045). Regression analyses revealed that 30% of the variability in fibrinogen (F = 24.5, $P \leq$ 0.0001) was accounted for by white blood cells (3.1%, P = 0.0015), hip fat mass (2.2%, P = 0.0081), years since menopause (0.9%, P = 0.082), and geographic site ($P \leq 0.0001$). Our results indicated that androidal fat mass and hip fat mass contributed to C-reactive protein and fibrinogen, respectively, whereas we found no association between whole-body or regional fat measures and cytokines.

Conclusion: Further study is warranted to determine the responsiveness of these acute-phase proteins and cytokines to loss of body fat through exercise and dietary intervention in postmenopausal women.

Key Words: C-reactive protein – Fibrinogen – Tumor necrosis factor α – Interleukin-6 – Interleukin-1β – Cardiovascular disease.

Menopause is a universal transition, marking an important stage in every woman's life, associated with decreased estrogen and increased follicle-stimulating and luteinizing hormones.[1] Estrogen deficiency and increasing age are associated with body composition changes and hence an increased risk of metabolic syndrome that may lead to diseases such as atherosclerotic cardiovascular disease (CVD) and diabetes. Postmenopausal women typically experience untoward changes in body fat distribution that have garnered attention recently because they contribute to an increased risk of CVD.

Fat is typically distributed in two areas: the central or androidal region and the gluteal-femoral or gynoidal region. Estrogen promotes fat accumulation in the gluteal-femoral

Received July 27, 2007; revised and accepted August 29, 2007.

From the [1]Department of Food Science and Human Nutrition, Human Metabolic Unit, Center for Designing Foods to Improve Nutrition, Iowa State University, Ames, IA; [2]School of Health, Physical Education, and Leisure Services, University of Northern Iowa, Cedar Falls, IA; [3]Department of Kinesiology, Iowa State University, Ames, IA; [4]U.S. Department of Agriculture ARS, Western Human Nutrition Research Center, University of California, Davis, CA; and [5]Department of Statistics, Iowa State University, Ames, IA.

AQ1

Funding/support: The overall project described was supported by a grant (RO1 AR046922 A2) from the National Institute of Arthritis and Musculoskeletal and Skin Diseases. It was also supported by a grant (P01 ES012020) from the National Institute of Environmental Health Sciences and the Office of Dietary Supplements (ODS) and by a grant (95P50AT004155) from the National Center of Complementary and Alternative Medicine and ODS of the National Institutes of Health. Support was also provided by a special grant (2006 3411517184) from the U.S. Department of Agriculture (USDA) to the Center for Designing Foods to Improve Nutrition at Iowa State University, by the USDA/ARS, Western Human Nutrition Research Center, and the CTSC Clinical Research Center at the University of California, Davis (1M01RR19975-01), and the National Center for Medical Research (UL1 RR024146). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of these granting agencies.

AQ1
AQ2

Financial disclosure: None reported.

Reprints of this article are not available.

Address correspondence to: D. Lee Alekel, PhD, Human Metabolic Unit, Center for Designing Foods to Improve Nutrition, Department of Food Science and Human Nutrition, 1127 Human Nutritional Sciences Building, Iowa State University, Ames, IA 50011. E-mail: alekel@iastate.edu

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

region[2]; correspondingly, estrogen deficiency in postmenopausal women plays a role in increasing centrally deposited fat.[3] This increase in centrally located fat is associated with greater disease risk than overall body fat, including metabolic syndrome, non–insulin-dependent diabetes,[4] heart disease,[5,6] and stroke.[7] The location or distribution of fat also plays a major role in determining morbidity risk associated with increased body fat. Evidence indicates that indices of abdominal obesity, such as waist-to-hip ratio[8] and waist circumference,[9] are better predictors of disease risk than body mass index (BMI) alone.

Adipose tissue is a complex and active secretory organ that both transmits and receives signals to modulate energy expenditure, appetite, endocrine and reproductive functions, insulin sensitivity, bone metabolism, inflammation, and immunity.[10] Due to a surfeit of energy intake or insufficient energy expenditure, excess lipid in adipose tissue and the liver accumulates with obesity, which in turn can trigger a chronic, subacute state of inflammation, causing changes in inflammatory cells and biochemical markers of inflammation. Obesity-induced inflammation may play a role in the development and progression of atherosclerotic CVD and type 2 diabetes.

Adipose tissue has been described as a metabolically active endocrine organ, releasing proteins such as leptin and adiponectin[11]; cytokines such as tumor necrosis factor α (TNF-α)[12] interleukin (IL)-6,[13] and interleukin-1β (IL-1β)[14]; as well as acute-phase proteins, such as C-reactive protein (CRP) and fibrinogen.[15] Collectively, these hormones, cytokines, and acute-phase proteins (adipocytokines or adipokines) released from adipocytes, activated macrophages, and other immune cells produce TNF-α, IL-6, and IL-1β, although the relative amount produced by each type of cell is unclear.[10] The inflammatory response that emerges with obesity seems to be triggered and to reside predominantly in adipose tissue, although other metabolically critical sites may also be involved.[16] A decline in ovarian function with menopause may also be associated with increases in proinflammatory cytokines.[17] The exact mechanisms by which estrogen interferes with cytokine activity are still unknown but may include interactions of the estrogen receptor with transcription factors,[18] modulation of nitric oxide activity,[19] antioxidant effects,[20] plasma membrane actions,[21] and changes in immune cell function.[22] Clinical studies have shown a strong link between increased proinflammatory cytokine activity and postmenopausal bone loss[23,24] and atherosclerotic CVD.[25]

The main purpose of this cross-sectional study was to identify whether centrally located fat and overall adiposity were related to inflammatory markers, specifically TNF-α, IL-6, IL-1β, CRP, and fibrinogen, and to determine whether centrally located fat or overall adiposity was more closely related to each of these inflammatory markers in healthy postmenopausal women. Taking into account dietary intake and energy expenditure, we hypothesized that these cytokines and acute-phase proteins would be higher with greater fat mass.

METHODS

Research design

We enrolled healthy postmenopausal women (45.8-65.0 y of age) as part of a randomized, double-blind, placebo-controlled multicenter (Iowa State University [ISU], Ames, IA, and University of California at Davis [UC-Davis], Davis, CA) National Institutes of Health–funded clinical trial. This ongoing parent study (Soy Isoflavones for Reducing Bone Loss [SIRBL]) was designed to examine the effect of two doses of isoflavones extracted from soybeans on bone loss during the course of 3 years in at-risk postmenopausal (eg, less than 10 y since their last menses) women. Eligible participants (nonosteoporotic, without diseases or conditions, not taking hormones or medications) were enrolled in the ongoing 5-year parent clinical trial starting in 2003. This ancillary project focused on overall and regional body composition using dual-energy x-ray absorptiometry (DXA) in which we report only baseline data for 242 women. We excluded 13 women at UC-Davis from this analysis because they did not meet the entry criteria (11 due to a thickened endometrium, 1 with breast cancer, and 1 without a blood sample at baseline).

Participant screening, selection, and characteristics

We recruited women throughout the state of Iowa and the Sacramento region in California primarily through direct mailing lists, stories in local newspapers, and local/regional radio advertisements, as well as other recruiting avenues. As part of the SIRBL trial, we screened women who responded (N = 5,255) to outreach materials initially via a telephone questionnaire to identify healthy women who went through a natural menopause (cessation of menses 9 mo to 10 y), were not experiencing excessive vasomotor symptoms, were 65 years of age or younger, were nonsmokers, and had a BMI (kg/m^2) ranging from 18.5 through 29.9 (inclusive) to exclude women at the extremes of adiposity. We excluded vegans and high alcohol consumers (more than seven servings per week) because alcohol interferes with isoflavone metabolism. The inclusion/exclusion criteria were established by the parent SIRBL project; thus, we also excluded women with current or previous diagnosis of diseases known to affect bone metabolism and/or those women who had a first-degree relative with breast cancer based on a medical history questionnaire and blood chemistry profiles. We also excluded women who used medications long term, such as cholesterol-lowering and antihypertensive medications. Use of oral hormone or estrogen therapy, selective estrogen-receptor modulators, or other hormones within the past 12 months; use of estrogen or progestogen creams or calcitonin within the past 6 months; use of antibiotics within the past 3 months; and/or any previous use of bisphosphonates were grounds for exclusion.

Women who met the initial criteria via telephone (N = 677) attended a prebaseline appointment to determine eligibility for additional entry criteria. To determine eligibility, we measured height and weight to confirm BMI status and

© 2007 The North American Menopause Society

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

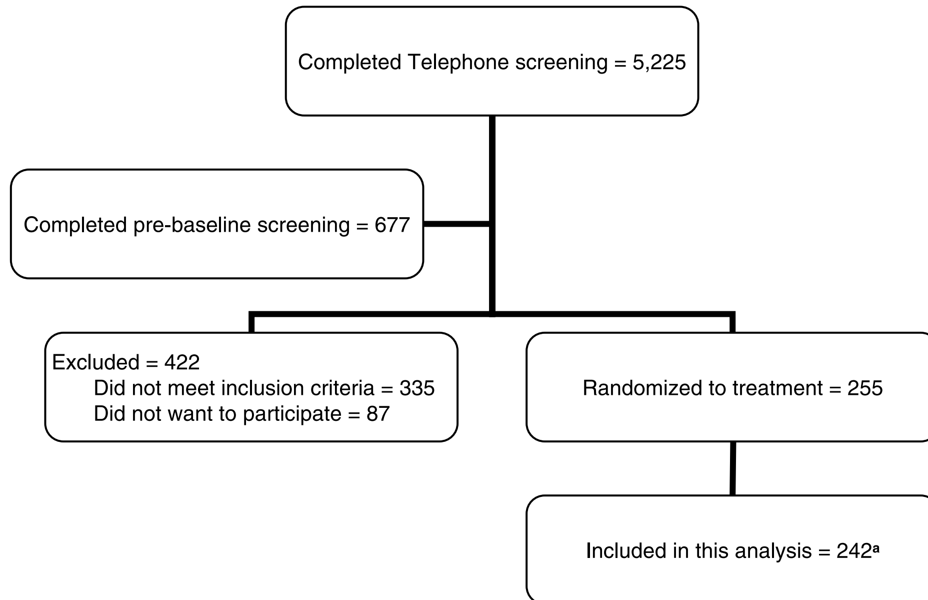

FIG. 1. Participant screening and enrollment flow chart. [a]We excluded 13 women at UC-Davis from this analysis because they did not meet entry criteria (11 due to a thickened endometrium, 1 with breast cancer, 1 without a baseline blood sample).

used DXA to assess bone mineral density (BMD) of the lumbar spine and left total proximal femur. Because the SIRBL project focused on prevention rather than treatment of disease, women with evidence of osteopenia or osteoporosis based on lumbar spine and/or proximal femur BMD (using >1.5 SDs below the young adult mean as cutoff) and women with evidence of previous or existing spinal fractures were excluded. We also excluded women with spine and/or femur BMD more than 1.0 SD above the mean. If a woman qualified based on her BMD, our phlebotomist drew blood for a chemistry profile. We excluded women if their fasted blood values indicated diabetes mellitus (fasting blood glucose >126 mg/dL); abnormal renal (elevated creatinine), liver (elevated aspartate aminotransferase and alanine aminotransferase), and/or thyroid function (both abnormal thyroid-stimulating hormone and free thyroxine); or abnormal lipid profile (low-density lipoprotein cholesterol >160 mg/dL, triacylglycerol >200 mg/dL). For this ancillary project using DXA, we included 242 women who met our entry criteria (Fig. 1). F1

The study protocol, consent form, and participant-related materials were approved by the respective institutional review boards at ISU (ID# 02-199) and at UC-Davis (ID# 200210884-2). We obtained approval for the DXA procedures from each institution's institutional review board and appropriate safety boards. We obtained informed consent from all women at the start of prebaseline screening.

Data collection

Questionnaires

At the prebaseline visit to ensure the health status of participants, trained interviewers administered three questionnaires: health and medical history,[26] reproductive history,[27] and nutrition history.[26] Women were asked to cease taking herbal therapies and/or nutritional/dietary supplements before baseline testing. At baseline testing, we assessed dietary intake using a semiquantitative food frequency questionnaire from Block Dietary Data Systems (Berkeley, CA). We assessed physical activity using the Paffenbarger physical activity recall[28] to obtain information about the previous year's activity, including walking, climbing stairs, sport/recreational activity, and time spent engaged in activities ranging from light to heavy activity. Each reported recreational or work-related activity was summed using metabolic equivalents of 4, 6, or 8 for activities classified as light, moderate, or heavy,[29] respectively, to provide an estimate of weekly energy expenditure.

Body size and composition measurements

A trained anthropometrist measured standing and sitting heights (Model S100; Ayrton Corp., Prior Lake, MN), weight (Abco Health-o-meter; Bridgeview, IL), waist circumference, and sagittal abdominal diameter (Holtain-Kahn abdominal caliper; Crosswell, Crymych Dyfed, UK). Sagittal abdominal diameter, an index of centralized adiposity, was measured with women in the supine position (knees bent) at the narrowest section between the small of the back and the navel. AQ3

Body composition measurements using DXA (Delphi W; Hologic Inc., Waltham, MA) were approved by the State Department of Public Health in both Iowa and California. Matching instruments at each site and daily calibration ensured that the DXA instruments provided comparable results. One certified DXA operator at ISU and one at UC-Davis performed all DXA scans, with cross-training for DXA scanning between sites to ensure comparable quality control. Placement of the women for the scans was standardized and adhered to manufacturer's guidelines, with body composition assessed on each woman's whole-body scan by one DXA

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

operator. We also assessed centralized adiposity from the whole-body DXA scans performed on each woman. One evaluator sectioned each whole-body DXA scan into waist, hip, and thigh regions based on bone landmarks,[30,31] and these subregions were analyzed using special software (Discovery Version 12.3:7). The waist region included the first through the fourth lumbar vertebrae. The hip region began below the fourth lumbar vertebra and extended just above the greater trochanter of the femur. Last, the thigh region extended superiorly from the greater trochanter to the approximate midpoint between the top of the thigh region and the lateral condyle of the femur. The lateral edge of each region was extended distally to encompass all tissue. We estimated fat and lean mass within each of these three regions using the DXA data.

Laboratory measurements

Phlebotomists collected fasted (9 h) blood samples between 7:00 and 8:00 AM. We separated serum and plasma from whole blood by centrifuging for 15 minutes (4°C) at 1,000*g* and stored aliquots at −80°C until analyses. Blood samples were analyzed by certified clinical laboratories (LabCorp, Kansas City, KS, at the ISU site and the UC-Davis Medical Center Laboratory, Sacramento, CA at the UC-Davis site) for a complete blood count (CBC) with differential, general chemistry panel, and thyroid screen (thyroid-stimulating hormone with reflex to free thyroxine if thyroid-stimulating hormone was abnormal). TNF-α, IL-6, and IL-1β concentrations were determined in serum with a high-sensitivity human cytokine (LINCOplex kit; LINCO Research, St. Charles, MO) using a BioPlex (Luminex 100; Bio-Rad Laboratories, Inc., Hercules, CA). Serum CRP concentration was determined in duplicate with a high-sensitivity sandwich enzyme-linked immunosorbent assay kit (ALPCO Diagnostics, Salem, NH) using a microtiter plate reader (ELx808; Bio-Tek Instruments, Inc., Winooski, VT). Plasma (heparinized) fibrinogen concentration was determined in duplicate with a sandwich enzyme-linked immunosorbent assay kit (AssayPro; St. Charles, MO) using a microtiter plate reader (ELx808, Bio-Tek Instruments, Inc.). We used manufacturer-provided quality controls and in-house quality control sera/plasma for calculating intra- and interassay coefficients of variation (CVs). The intra-assay CVs for TNF-α, IL-6, IL-1β, CRP, and fibrinogen were 5.8%, 6.0%, 14.5%, 3.7%, and 2.7%, respectively. The interassay CVs for TNF-α, IL-6, IL-1β, CRP, and fibrinogen were 6.0%, 3.6%, 5.4%, 6.0%, and 2.3%, respectively.

Statistical analyses

Statistical analyses were performed using SAS (version 9.1, Cary, NC)[32] with results considered statistically significant at $P \leq 0.05$. Descriptive statistics included means for normally distributed data (age, body size, overall body composition measures, serum fibrinogen) and medians for data that were not normally distributed (years since menopause, regional body composition measures, dietary intake, energy expenditure; serum CRP, TNF-α, IL-6, IL-1β; white blood cell count [WBC], lymphocyte count, and neutrophil count). To examine relationships between the outcomes of interest (CRP, fibrinogen, TNF-α, IL-6, IL-1β) versus the independent variables, we used Spearman's ρ correlation analyses because the majority of outcome variables were not normally distributed. These variables (CRP, TNF-α, IL-6, IL-1β) were log-transformed before the regression analysis because they did not follow a normal distribution, causing a violation of assumptions. Classes of variables in modeling the outcomes included all independent variables that were biologically plausible and/or significantly related using the Spearman's ρ correlation coefficient analysis. We used stepwise regression analyses to assess the combined contribution of independent variables to CRP, fibrinogen, TNF-α, IL-6, and IL-1β. Classes of variables in modeling each of these five outcomes included age or years since menopause, overall body composition (fat mass and lean mass), indices of centralized fat mass (waist circumference, sagittal abdominal diameter, waist fat mass, hip fat mass, or androidal fat mass), likelihood of concomitant infection (WBC, lymphocyte count, or neutrophil count), energy expenditure, energy intake–related factors (total energy, total fat, saturated fatty acids, or trans fatty acids), and dietary fiber. In modeling each outcome, we removed variables that exhibited multicollinearity as indicated by the variance inflation factor. The variance inflation factor measures the impact of collinearity among the independent variables in a regression model and the degree to which multicollinearity degrades the precision estimate. A value exceeding 10 is typically of concern, but in weaker regression models, a value exceeding 2.5 may be cause for concern.[32] All models included site as an obligatory variable to account for potential study site differences.

RESULTS

Participant characteristics

The baseline characteristics of the studied women are presented in Table 1. At baseline, women ranged from 45.9 to 65.5 years of age and from 0.8 to 10.0 years since menopause. The majority of women were white, although the ISU site enrolled one African American and one woman of more than one race, whereas the UC-Davis site enrolled two African Americans, one Native Hawaiian, one Native American, three Asians, six women of more than one race, two of unknown race, and two who chose not to report race. Women had a wide range (17.8-32.7 kg/m^2) of BMI values, with half having a BMI less than 25.0 kg/m^2; the UC-Davis site enrolled nine women beyond our BMI inclusion criteria. Overall and regional body composition as assessed by DXA (Table 1) indicated wide variability among these women, particularly in the regional and overall body fat measures. The median values for dietary intake of nutrients are listed in Table 2, with the minimal and maximal values also illustrating wide variability. Values for circulating analytes are presented in Table 3, demonstrating that median (or mean) values were within the range reported in the literature. However, 65 women had low (<4.5 but $>2.3 \times 10^9$/L) and

T1 T2 T3

© 2007 The North American Menopause Society

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 1. *Characteristics of women[a] at baseline*

	Mean ± SD	Median	Min-Max
Age, y	54.6 ± 3.4		
Years since menopause[b]		2.8	0.8-10.0
Energy expenditure, kJ/wk[b]		10370	0-51077
Body size			
Height, cm	164.7 ± 6.3		
Weight, kg	67.7 ± 9.3		
BMI, kg/m^2	24.9 ± 3.1		
Waist circumference, cm	77.9 ± 8.0		
Hip circumference, cm	100.3 ± 6.8		
Sagittal abdominal diameter, cm	18.4 ± 2.7		
Overall body composition[c]			
Lean mass, kg	43.18 ± 4.60		
Fat mass, kg	23.26 ± 6.35		
Regional body composition[b,c]			
Waist fat mass, kg		2.24	0.39-5.59
Hip fat mass, kg		3.27	0.72-6.41
Thigh fat mass, kg		5.21	2.12-10.85
Androidal (waist + hip) fat mass, kg		5.42	1.12-11.78

[a]The number of women was 242 for all variables, except sagittal abdominal diameter (n = 237) because five sagittal abdominal diameter values were missing due to instrument malfunction.
[b]Distributions for these variables were not normal; thus, median (min-max) values are reported.
[c]Assessed by dual-energy x-ray absorptiometry.

TABLE 3. *Circulating analytes of women[a] at baseline*

Analyte	Mean ± SD	Median (min-max)	Range reported from literature
Serum TNF-α, pg/mL[b]		3.9 (0.5-121.0)	<15.0[c]
Serum IL-6, pg/mL[b]		8.3 (0-255.4)	<12.5[c]
Serum IL-1β, pg/mL[b]		0.6 (0-106.8)	0.0-5.0[d]
Serum CRP, mg/L[b]		1.0 (0.01-29.9)	≤1.5[e]
Plasma fibrinogen, mg/mL[f]	3.4 ± 1.1		1.8-4.3[g]
White blood cell count, $\times 10^9$/L[b]		5.0 (2.3-8.4)	4.5-11.0[h]
Lymphocyte count, $\times 10^9$/L[b]		1.6 (0.9-2.9)	1.0-4.8[h]
Neutrophil count, $\times 10^9$/L[b]		2.7 (0.7-6.60)	1.8-7.7[h]

TNF-α, tumor necrosis factor α; IL, interleukin; CRP, C-reactive protein.
[a]The number of women was 242 for the acute-phase proteins and proinflammatory cytokines; the number of women was 237 for white blood cell count and 232 for the lymphocyte and neutrophil variables.
[b]Distributions for these analytes were not normal; thus, median (min-max) values are reported.
[c]Cutoff values reported for TNF-α and IL-6 based on Yom et al[37] for adults.
[d]Range reported for IL-1β based on Narbutt et al[38] for adults.
[e]Cutoff value reported for CRP based on Bassuk et al[35] for postmenopausal women not on hormone therapy.
[f]Distribution for fibrinogen was normal; thus, mean ± SD is reported.
[g]Range reported for fibrinogen based on Nicoll[36] for adults.
[h]Range reported for white blood cell, lymphocyte, and neutrophil counts based on DeMott[42] for adults.

none of the women had an elevated WBC; by including the WBC of all participants (N = 237) in the regression analyses, we accounted for this variability in inflammatory markers. Four (1.7%) women had nondetectable values for IL-6 and 50 (20.6%) women had nondetectable values for IL-1β. We replaced these nondetectable values with 0.01 pg/mL (lowest detectable value was 0.03 for IL-6 and 0.01 for IL-1β) to retain all data (nonmissing) in the regression models subsequent to log transformation for regression analysis.

Correlation analyses

Spearman's ρ correlation analysis (Table 4) indicated a positive correlation between CRP and fibrinogen (r = 0.29, P < 0.0001), but not between CRP or fibrinogen and TNF-α, IL-6, or IL-1β. However, positive correlations were noted between TNF-α and IL-6 (r = 0.20, P = 0.002), TNF-α and IL-1β (r = 0.26, P < 0.0001), and IL-6 and IL-1β (r = 0.26, P < 0.0001). These data indicated that the acute-phase proteins were related to each other, but not to the cytokines, whereas the cytokines were also related to each other.

Regression analyses

We performed regression analyses to examine independent factors contributing to the variability in CRP, fibrinogen, TNF-α, IL-6, and IL-1β as our primary outcomes of interest, log-transforming all but fibrinogen. No notable multicollinearities emerged among the independent variables, as indicated by the low variance inflation factors in all regression models (Table 5). Residual analyses indicated that the model assumptions of normality of error terms and homogeneity of residual variance were satisfied for the final regression models. Although geographic site did not reach significance in the CRP, TNF-α, IL-6, and IL-1β models, we retained site to account for potential site differences. After

TABLE 2. *Dietary intake of women[a] at baseline*

Nutrient intake from food, based on food frequency questionnaire[b,c]		
	Median	Min-Max
Total energy, kJ	6,455	1,772-19,096
Carbohydrate, g	174	27-476
Protein, g	61	15-168
Total fat, g	65	17-247
Saturated fatty acid, g	19	5-65
Trans fatty acid, g	5	1-25
Dietary fiber, g	16	4-49

[a]The number of women was 242 for all variables.
[b]Reported from a semiquantitative food frequency questionnaire.
[c]Distributions for these variables were not normal; thus, median (min-max) values are reported.

TABLE 4. *Relationship[a] among inflammatory markers*

Inflammatory marker	Fibrinogen	TNF-α	IL-6	IL-1β
CRP	r = 0.29, P < 0.0001	r = 0.025, P = 0.70	r = −0.073, P = 0.26	r = 0.0062, P = 0.92
Fibrinogen		r = −0.12, P = 0.057	r = 0.036, P = 0.57	r = −0.084, P = 0.19
TNF-α			r = 0.20, P = 0.002	r = 0.26, P < 0.0001
IL-6				r = 0.26, P < 0.0001

TNF-α, tumor necrosis factor α; IL, interleukin; CRP, C-reactive protein.
[a]Based on Spearman's ρ correlation analysis (N = 242); correlation coefficients and P values presented.

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

TABLE 5. *Regression analyses[a]: contributors to inflammatory markers*

Parameter	Parameter estimate	Percentage variance[c]	P^d	Variance inflation factor[e]
C-reactive protein[b]				
Overall model R^2 = 35.8% (Adj R^2 = 34.7%) (F = 32.4; df = 4, 232) $P \leq 0.0001$				
Intercept	0.7458		0.51	
Study site	−0.06282	0.06	0.65	1.06
Androidal fat mass	0.0002631	16.1	≤0.0001	1.11
White blood cell count	0.3117	5.6	≤0.0001	1.15
Age	0.05776	2.3	0.0045	1.04
Fibrinogen[b]				
Overall model R^2 = 29.7 % (Adj R^2 = 28.5%) (F = 24.54; df = 4, 232) $P \leq 0.0001$				
Intercept	3.4109		≤0.0001	
Study site	−0.9477	16.6	≤0.0001	1.05
White blood cell count	0.2051	3.1	0.0015	1.11
Hip fat mass	0.0001690	2.2	0.0081	1.09
Years since menopause	−0.05562	0.9	0.082	1.03
Tumor necrosis factor α[f]				
Overall model R^2 = 4.5% (Adj R^2 = 3.3%) (F = 3.6; df = 3, 228) P = 0.014				
Intercept	0.03239		0.96	
Study site	−0.1520	1.2	0.087	1.07
Age	0.02337	1.4	0.068	1.01
Lymphocyte count	0.1807	1.4	0.074	1.06

[a]Because C-reactive protein and tumor necrosis factor α did not follow a normal distribution causing a violation of the assumptions for regression analysis, these variables were log-transformed before the regression analysis.
[b]n = 237 for the C-reactive protein and fibrinogen models because five women were missing complete blood count values.
[c]Squared semipartial type II correlation coefficient, accounts for shared variance among variables.
[d]Variables (except site) left in the model were significant at the $P \leq 0.10$ level.
[e]Measures the impact of collinearity among the independent variables in a regression equation and the degree to which multicollinearity degrades the precision estimate.
[f]n = 232 for the tumor necrosis factor α model because eight women from University of California, Davis were missing the differential analysis (lymphocyte cell count) that accompanies complete blood count and two women from Iowa State University were missing complete blood count at baseline.

variable elimination was completed, multiple regression analyses revealed that 36% of the variability in CRP (F = 32.40, P < 0.0001) was accounted for by androidal fat mass (16.1%), WBC (5.6%), and age (2.3%). Regression analyses revealed that 30% of the variability in fibrinogen (F = 24.54, P < 0.0001) was accounted for by WBC (3.1%) and hip fat mass (2.2%). Years since menopause contributed negatively to fibrinogen (P = 0.082); however, site (16.6%) was a significant contributor. Regression analyses revealed that 4.5% of the variability in TNF-α (F = 3.60, P = 0.014) was accounted for by age and lymphocyte count, but these variables were not statistically significant (P < 0.10). The IL-6 and IL-1β multiple regression models were poor (not shown), as indicated by their F statistic and P values, explaining approximately 3% of the variability in each of these cytokines (data not shown). Regression analyses revealed that 2.7% of the variability in IL-6 (F = 3.25, P = 0.040) and 3.2% of the variability in IL-1β (F = 3.89, P = 0.022) was accounted for by dietary fiber (1.9%, P = 0.032 and 3.0%, P = 0.0069, respectively).

DISCUSSION

Postmenopausal women experience a decrease in estrogen and change in body composition that may contribute to the risk of developing atherosclerotic CVD, diabetes, and/or stroke.[1] Age, increased adiposity[17] associated with menopause, and the increase in circulating analytes, such as CRP, fibrinogen, TNF-α, IL-6, and IL-1β, may also play a role in increasing disease risk. A few published studies[17,33,34] have been designed to examine the relationship between CRP, fibrinogen, TNF-α, IL-6, and/or IL-1β with respect to regional body composition in healthy postmenopausal women. However, the DXA-derived centralized fat mass is advantageous because it is specific for fat and encompasses both internal and external fat. This report focuses solely on baseline data, with one measurement per variable per woman, and is thus a cross-sectional snapshot of the inflammatory markers in relation to centralized body fat.

Although these women were deemed healthy (not known to be in an inflammatory state), as evidenced by average cytokine and acute-phase protein values (Table 3), some women exhibited high concentrations of these markers. For example, 39% of women had CRP above 1.5 mg/L[35] and 24% had fibrinogen above the 4.3 mg/mL[36] cutoff values. Less than 1% had TNF-α above 15.0 pg/mL,[37] whereas 41% had IL-6 above 12.5 pg/mL[37] and 10% had IL-1β above the 5.0 pg/mL[38] cutoff values. Our findings are corroborated by the literature indicating that menopause is associated with increased proinflammatory markers,[17] particularly IL-6.[39] However, the ranges reported in the literature, except for CRP listed in Table 3, are for adults in general and not specifically for postmenopausal women, perhaps explaining why some of our participants did not fall within these ranges. Similar to previous findings,[40] we confirmed a relationship between CRP and fibrinogen, as well as between the cytokines. However, contrary to previous findings,[15,33] we did not demonstrate a relationship between the acute-phase proteins and cytokines.

Androidal or hip (predominant component of androidal) fat mass was the key component related to CRP or fibrinogen, respectively, although correlation analysis revealed associations between whole-body fat mass and CRP (r = 0.50, P < 0.0001) and fibrinogen (r = 0.19, P = 0.0024). Once we accounted for fat mass, lean mass (total or regional) did not contribute to either CRP or fibrinogen, suggesting that fat mass is more closely related than lean mass to these proinflammatory markers. Our results are consistent with those of Manns et al,[34] who demonstrated in healthy postmenopausal women (N = 133) that higher CRP was associated with higher BMI, larger waist circumference (>88 cm), and greater centralized fat mass, the latter explaining 35% of the variance in serum CRP. Similarly,

© 2007 The North American Menopause Society

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

circulating fibrinogen has been reported[33,41] to be positively associated with body fat percentage, BMI, waist circumference, and subcutaneous fat in postmenopausal women.

Because CRP and fibrinogen are inflammatory markers, it made sense to account for potential concomitant infection by including WBC, lymphocyte count, or neutrophil count as a contributing factor. Infection has been shown to play a role in elevating CRP and fibrinogen, as indicated by Kritchevsky et al,[40] who found a positive correlation ($P < 0.001$) between WBC and CRP and fibrinogen. Yet, few of our women reported having an infection at baseline, as corroborated by the WBC and lymphocyte and neutrophil counts that were within the normal range[42] for the majority of women (low values were noted in 65, 8, and 11 women, respectively). We did not anticipate that WBC would contribute so strongly to CRP and fibrinogen, yet WBC explained slightly more of the variability in CRP and fibrinogen than either lymphocytes or neutrophils. Being overweight or obese has been shown to increase WBC,[43] which may explain why WBC was a significant contributor to CRP and fibrinogen in these women, who ranged from lean to modestly overweight. Only 11% of women had a waist circumference greater than 88 cm, but androidal fat mass was positively related ($r = 0.29$, $P < 0.001$) to WBC, suggesting that centralized adiposity was coincident with an overall inflammatory state.

Geographic site was significant only in the fibrinogen model, corroborated using a *t* test to indicate that women at the UC-Davis site had a significantly lower mean fibrinogen concentration than those at the ISU site. Although we do not have a definitive explanation for this disparity, this may have been due to climate differences between the two sites. Northern California is warmer during a far greater proportion of the year than central Iowa. A large (N = 9,377) cross-sectional study[44] examining the effect of seasonal fluctuation in fibrinogen reported a 12% greater value in January compared with August, with consistently lower fibrinogen values during the warmer months. This is consistent with the fact that more than half of the women at ISU underwent baseline testing during the colder months (fall through winter 2003). In contrast, a greater proportion of women at UC-Davis underwent baseline testing during the spring than at ISU. Rudnicka et al[44] did not find any effect of delay in sample processing on fibrinogen values, but they reported significant diurnal differences. However, our samples were collected in the morning in the fasted state; thus, this could not account for the site difference.

Although the contributors to TNF-α (age, lymphocyte count, site) were not statistically significant (remained in the model at $P < 0.10$), these factors were similar (with the exception of centralized body fat) to those related to CRP and fibrinogen. The vast majority of these women were not considered obese based on BMI, but some nevertheless had excess centrally located fat based on waist circumference, suggesting that TNF-α might have been related to androidal fat mass. However, this was not the case, perhaps because only six women had elevated TNF-α. Roubenoff et al[45] also demonstrated that age was not related to TNF-α, but that IL-6 increased with age. Manns et al[34] noted that serum CRP was positively ($P = 0.047$) associated with age. Likewise, age or years since menopause contributed to CRP and fibrinogen, respectively, although only age was statistically significant. Also, Pearson et al[46] demonstrated associations between fibrinogen and age, menopause, obesity, and inflammation. Further, we verified a link between centralized obesity and age with these two acute-phase proteins, both of which are considered good predictors of atherosclerotic CVD risk.[15,46] Interestingly, age was related to waist circumference ($r = 0.15$, $P = 0.019$), but age was not related (using Spearman ρ correlation analysis) to other indices of central adiposity in these women. However, time since menopause, a reflection of estrogen deficiency after menopause, was related to androidal fat mass ($r = 0.15$, $P = 0.017$), hip fat mass ($r = 0.15$, $P = 0.021$), waist circumference ($r = 0.19$, $P = 0.0037$), and sagittal diameter ($r = 0.19$, $P = 0.0028$). This *suggests* that in these women lack of estrogen *may* have been more important than age per se in relation to androidal fat mass. Nevertheless, because this is a cross-sectional study, we cannot determine cause and effect.

The regression models for both IL-6 and IL-1β were extremely weak. Obesity has been associated with elevated IL-6,[47] but we did not demonstrate this in our women. Adipocytes produce as much as 30% of total circulating IL-6,[15] with higher amounts produced in abdominal visceral versus subcutaneous fat.[13,48] Many of our women had excess centrally located fat, but our results indicated no link between centralized adiposity and TNF-α, IL-6, or IL-1β. Yet, DXA does not distinguish between visceral and subcutaneous fat. Intriguingly, we noted that all 100 women with elevated IL-6 also had androidal fat mass above the median. Circulating IL-1β is relatively low compared with TNF-α or IL-6. This may be because a large amount of immature or proIL-1β remains intracellular, never maturing into the typically measured form of IL-1β, binding to large proteins and the IL-1 receptor,[49] making it difficult to detect in circulation.

It is important to note that the postmenopausal women included in our study were selectively chosen because they were "disease free" and the majority was nonobese. This may have contributed to the lack of association between overall fat/centrally located fat and TNF-α, IL-6, or IL-1β. Elevated concentrations of these analytes in a large group of unhealthy and/or obese women may have shown stronger associations with fat mass indices. However, the median value for each analyte fell within the reference range for adults, although some women had values higher than the reference range, as discussed previously. Nonetheless, despite the inclusion of healthy women to comprise a homogeneous sample, we evidently had sufficient variability in adiposity to demonstrate a strong relationship between androidal fat mass versus CRP and fibrinogen. These women were likely representative of healthy postmenopausal women, but not necessarily of all postmenopausal women; thus, we cannot generalize our results to all women.

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

CONCLUSION

Regression analyses indicated that androidal fat mass and hip fat mass, respectively, contributed to CRP and fibrinogen, but these analyses did not indicate an association between the whole-body or regional fat measures and the cytokines. Although this study describes relationships among cross-sectional rather than longitudinal data and thus does not indicate causality, centralized adiposity was strongly related to CRP and fibrinogen but showed no relationship to cytokines. This may suggest that, in healthy postmenopausal women, acute-phase proteins may be used as earlier indicators of CVD risk than inflammatory cytokines. Decreasing centralized body fat may in turn decrease a variety of inflammatory markers, thereby potentially reducing CVD risk. Further study is warranted to determine the responsiveness of these acute-phase proteins and cytokines to loss of body fat (particularly centralized fat) through exercise and dietary intervention in postmenopausal women.

Acknowledgments: The authors thank Dr. Marian Kohut for the use of her equipment (BioPlex Luminex 100; Bio-Rad Laboratories, Inc., Hercules, CA). They thank the phlebotomists Cindy Kruckenberg, Marilyn Chrusciel, and Shirley Nelson. They also thank all the participants who volunteered for this study.

REFERENCES

1. Grady D. Management of menopausal symptoms. *N Engl J Med* 355; 2006:2338-2347.
2. Krotkiewski M, Björntorp P, Sjöström L, Smith U. Impact of obesity on metabolism in men and women: importance of regional adipose tissue distribution. *J Clin Invest* 1983;72:1150-1162.
3. Carr M. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 2003;88:2404-2411.
4. Carr DB, Utzschneider KM, Hull RL, et al. Intra-abdominal fat is a major determinant of the national cholesterol education program adult treatment panel III criteria for the metabolic syndrome. *Diabetes* 2004;53:2087-2094.
5. De Michele M, Panico S, Iannuzzi A, et al. Association of obesity and central fat distribution with carotid artery wall thickening in middle-aged women. *Stroke* 2002;33:2923-2928.
6. Kannel WB, Cupples LA, Ramaswami R, Stokes J III, Kreger BE, Higgins M. Regional obesity and risk of cardiovascular disease, the Framingham study. *J Clin Epidemiol* 1991;44:183-190.
7. Rexrode KM, Hennekens CH, Willett WC, et al. A prospective study of body mass index, weight change, and risk of stroke in women. *JAMA* 1997;277:1539-1545.
8. Azizi F, Esmailzadeh A, Mirmiran P, Ainy E. Is there an independent association between waist-to-hip ratio and cardiovascular risk factors in overweight and obese women? *Int J Cardiol* 2005;101:39-46.
9. Klein S, Allison DB, Heymsfield SB, et al. Waist circumference and cardiometabolic risk: a consensus statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Am J Clin Nutr* 2007;85:1197-1202.
10. Shoelson SE, Herrero L, Naaz A. Obesity, inflammation, and insulin resistance. *Gastroenterology* 2007;132:2169-2180.
11. Matsubara M, Maruoka S, Katayose S. Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. *Eur J Endocrinol* 2002;147:173-180.
12. Katsuki A, Sumida Y, Murashima S, et al. Serum levels of tumor necrosis factor-α are increased in obese patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 1998;83:859-862.
13. Fried SK, Bunkin DA, Greenberg AS. Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. *J Clin Endocrinol Metab* 1998;83: 847-850.
14. Di Renzo L, Bigioni M, Del Gobbo V, et al. Interleukin-1 (IL-1) receptor antagonist gene polymorphism in normal weight obese syndrome: relationship to body composition and IL-1 alpha and beta plasma levels. *Pharmacol Res* 2007;5:131-138.
15. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunctions: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999;19:972-978.
16. Hirosumi J, Tuncman G, Chang L, et al. A central role for JNK in obesity and insulin resistance. *Nature* 2002;420:333-336.
17. Pacifici R, Brown C, Puscheck E, et al. Effect of surgical menopause and estrogen replacement on cytokine release from human blood mononuclear cells. *Proc Natl Acad Sci U S A* 1991;88:5134-5138.
18. Pottratz ST, Bellido T, Mocharla H, Crabb D, Manolagas SC. 17 beta-Estradiol inhibits expression of human interleukin-6 promoter-reporter constructs by a receptor-dependent mechanism. *J Clin Invest* 1994;93:944-950.
19. Armour KE, Ralston SH. Estrogen upregulates endothelial constitutive nitric oxide synthase expression in human osteoblast-like cells. *Endocrinology* 1998;139:799-802.
20. Massafra C, Gioia D, De Felice C, et al. Effects of estrogens and androgens on erythrocyte antioxidant superoxide dismutase, catalase and glutathione peroxidase activities during the menstrual cycle. *J Endocrinol* 2000;167:447-452.
21. Kelly MJ, Levin ER. Rapid actions of plasma membrane estrogen receptors. *Trends Endocrinol Metab* 2001;12:152-156.
22. Olsen NJ, Kovacs WJ. Gonadal steroids and immunity. *Endocr Rev* 1996;17:369-384.
23. Jilka RL, Hangoc G, Girasole G, et al. Increased osteoclast development after estrogen loss: mediation by interleukin-6. *Science* 1992;257: 88-91.
24. Ammann P, Rizzoli R, Bonjour JP, et al. Transgenic mice expressing soluble tumor necrosis factor-receptor are protected against bone loss caused by estrogen deficiency. *J Clin Invest* 1997;99:1699-1703.
25. Maxwell SR. Women and heart disease. *Basic Res Cardiol* 1998;93: 79-84.
26. Alekel DL, St. Germain A, Peterson CT, Hanson KB, Stewart JW, Toda T. Isoflavone-rich soy protein isolate attenuates bone loss in the lumbar spine of perimenopausal women. *Am J Clin Nutr* 2000;72:844-852.
27. Morabia A, Costanza MC. International variability in ages at menarche, first live birth, and menopause: World Health Organization collaborative study of neoplasia and steroid contraceptives. *Am J Epidemiol* 1998;148: 1195-1205.
28. Paffenbarger RS, Wing AL, Hyde RT. Physical activity as an index of heart attack risk in college alumni. *J Epidemiol* 1978;108:161-175.
29. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000;32:S471-S480.
30. Moeller LE, Peterson CT, Hanson KB, Dent SB, Lewis DS, Alekel DL. Isoflavone-rich soy protein prevents loss of hip lean mass but does not prevent the shift in regional fat distribution in perimenopausal women. *Menopause* 2003;10:322-331.
31. Glickman SG, Marn CS, Supiano MA, Dengel DR. Validity and reliability of dual-energy X-ray absorptiometry for the assessment of abdominal adiposity. *J Appl Physiol* 2004;97:509-514.
32. Allison PD. *Logistic Regression Using the SAS System: Theory and Application*. Cary, NC: SAS Institute Inc, 1999.
33. Piché ME, Lemieux S, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J. Relation of high-sensitivity C-reactive protein, interleukin-6, tumor necrosis factor-alpha, and fibrinogen to abdominal adipose tissue, blood pressure, and cholesterol and 34. triglyceride levels in healthy postmenopausal women. *Am J Cardiol* 2005;96:92-97.
34. Manns PJ, Williams DP, Snow CM, Wander RC. Physical activity, body fat, and serum C-reactive protein in postmenopausal women with and without hormone replacement. *Am J Hum Biol* 2003;15:91-100.
35. Bassuk SS, Rifai N, Ridker PM. High-sensitivity C-reactive protein: clinical importance. *Curr Probl Cardiol* 2004;29:439-493.
36. Nicoll CD. In: Tierney LM, McPhee SJ, Papdikis MA, eds. *Current Medical Diagnosis and Treatment*, 45th ed. Columbus, OH: McGraw-Hill, 2006:1736, 1738.
37. Yom SS, Busch TM, Friedberg JS, et al. Elevated serum cytokine levels in mesothelioma patients who have undergone pleurectomy or extrapleural pneumonectomy and adjuvant intraoperative photodynamic therapy. *Photochem Photobiol* 2003;78:75-81.

© 2007 The North American Menopause Society

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

38. Narbutt J, Lesiak A, Skibinska M, et al. Repeated doses of UVR cause minor alteration in cytokine serum levels in humans. *Mediators Inflamm* 2005;2005:298-303.
39. Ushiroyama T, Ikeda A, Ueki M. Elevated plasma interleukin-6 (IL-6) and soluble IL-6 receptor concentrations in menopausal women with and without depression. *Int J Gynaecol Obstet* 2002;79:51-52.
40. Kritchevsky SB, Bush AJ, Pahor M, Gross MD. Serum carotenoids and markers of inflammation in nonsmokers. *Am J Epidemiol* 2000;152: 1065-1071.
41. DeSouza CA, Stevenson ET, Davy KP, Jones PP, Seals DR. Plasma fibrinogen levels in healthy postmenopausal women: physical activity and hormone replacement status. *J Gerontol A Biol Sci Med Sci* 1997; 52:M294-M298.
42. Hematology. In: Jacobs DeMott RW, Demott DS, Grady WR, Horvat HJ, Huestis RT, Kasten DW Jr. *Laboratory Test Handbook*, 4th ed. Hudson, OH: Lexi-Comp, 1996:338.
43. Womack J, Tien PC, Feldman J, et al. Obesity and immune cell counts in women. *Metabolism* 2007;56:998-1004.
44. Rudnicka AR, Rumly A, Lowe GDO, Strachan DP. Diurnal, seasonal, and blood-processing patterns in levels of circulating fibrinogen, fibrin D-dimer, C-reactive protein, tissue plasminogen activator, and von Willebrand factor in a 45-year-old population. *Circulation* 2007;115: 996-1003.
45. Roubenoff R, Harris TB, Abad LW, Wilson PW, Dallal GE, Dinarello CA. Monocyte cytokine production in an elderly population: effect of age and inflammation. *J Gerontol A Biol Sci Med Sci* 1998;53:M20-M26.
46. Pearson TA, LaCava J, Weil HF. Epidemiology of thrombotic-hemostatic factors and their associations with cardiovascular disease. *Am J Clin Nutr* 1997;65(Suppl):1674S-1682S.
47. Pradhan AD, Manson JE, Rifai N, Buring JE, Ridker PM. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *JAMA* 2001;286:327-334.
48. Krauss S, Zhang CY, Lowell BB. The mitochondrial uncoupling-protein homologues. *Nat Rev Mol Cell Biol* 2005;6:248-261.
49. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood* 1996;87:2095-2147.

AQ4

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES

AQ1 = Please expand ARS.

AQ2 = Please expand CTSC.

AQ3 = Name of the manufacturer of Health-o-meter?

AQ4 = Is this the chapter title of a book edited by the authors listed? Please provide author of chapter if so.

END OF AUTHOR QUERIES

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.

Author Reprints

For *Rapid Ordering* go to: **www.lww.com/periodicals/author-reprints**

Menopause

Order

Author(s) Name ______________________

Title of Article ______________________

*Article # ______________ *Publication Mo/Yr ________

**Fields may be left blank if order is placed before article number and publication month are assigned.*

		Reprint Pricing	Shipping
Quantity of Reprints ____	$______	100 copies = $375.00	$5.00 per 100 for orders shipping within the U.S.
Covers (Optional) ______	$______	200 copies = $441.00	$20.00 per 100 for orders shipping outside the U.S.
Shipping Cost	$______	300 copies = $510.00	**Tax**
Reprint Color Cost	$______	400 copies = $585.00	U.S. and Canadian residents add the appropriate tax or submit a tax exempt form.
Tax	$______	500 copies = $654.00	
Total	$______	**Covers**	
		$108.00 for first 100 copies	
		$18.00 each add'l 100 copies	
		Reprint Color	
		($70.00/100 reprints)	

You may have included color figures in your article. The costs to publish those will be invoiced separately. If your article contains color figures, use Rapid Ordering **www.lww.com/periodicals/author-reprints**.

Payment

MC VISA Discover American Express

Account # ______ / ______ / ______ Exp. Date ______

Name ______________________

Address ______________________ Dept/Rm ______

City ______ State ______ Zip ______ Country ______

Telephone ______________________

Signature ______________________

Ship to

Name ______________________

Address ______________________ Dept/Rm ______

City ______ State ______ Zip ______ Country ______

Telephone ______________________

Use this form to order reprints. Publication fees, including color separation charges and page charges will be billed separately, if applicable.

Payment must be received before reprints can be shipped. Payment is accepted in the form of a check or credit card; purchase orders are accepted for orders billed to a U.S. address.

Prices are subject to change without notice.

Quantities over 500 copies: contact our Pharma Solutions Department at **410.528.4077**

Outside the U.S. call **4420.7981.0700**

MAIL your order to:
Lippincott Williams & Wilkins
Author Reprints Dept.
351 W. Camden St.
Baltimore, MD 21201

FAX:
410.528.4434

For questions regarding reprints or publication fees,
E-MAIL:
reprints@lww.com

OR **PHONE:**
1.800.341.2258

For *Rapid Ordering* go to: **www.lww.com/periodicals/author-reprints**

Copyright © 2007 The North American Menopause Society. Unauthorized reproduction of this article is prohibited.