

Seasonal and Sex Variation of High-Sensitivity C-Reactive Protein in Healthy Adults: A Longitudinal Study

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BACKGROUND: Cross-sectional studies have reported seasonal variation in high-sensitivity C-reactive protein (hsCRP). However, longitudinal data are lacking.

METHODS: We collected data on diet, physical activity, psychosocial factors, physiology, and anthropometric measurements from 534 healthy adults (mean age 48 years, 48.5% women, 87% white) at quarterly intervals over a 1-year period between 1994 and 1998. Using sinusoidal regression models, we estimated peak-to-trough amplitude and phase of the peaks.

RESULTS: At baseline, average hsCRP was 1.72 mg/L (men, 1.75 mg/L; women, 1.68 mg/L). Overall seasonal variation amplitude was 0.16 mg/L (95% CI 0.02 to 0.30) and was lower in men (0.10 mg/L, 95% CI –0.11 to 0.31) than in women (0.23 mg/L, 95% CI 0.04 to 0.42). In both sexes, hsCRP peaked in November, with a corresponding trough in May. Relative plasma volume, waist and hip circumference, diastolic blood pressure, and depression scores were major factors associated with changes in amplitude of seasonal variation of hsCRP, and taken together explain most of the observed seasonal change. There was a 20% increase in the percentage of participants classified in the high-risk category for hsCRP (≥ 3 mg/L) during late fall and early winter compared with late spring and early summer.

CONCLUSIONS: Concentrations of hsCRP were modestly increased in fall and winter compared to summer, with greater seasonal amplitude of variation observed in women. Conventional classification methods fail to consider seasonality in hsCRP and may result in substantial misclassifications in the spring and fall. Future

clinical practice and research should take these variations into account.

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Epidemiologic, experimental, and clinical evidence supports a relationship between inflammatory processes and atherogenesis (1–3), diabetes (4), and cancer (5). The most widely studied inflammatory biomarker is C-reactive protein (CRP),⁶ an acute-phase protein produced in the liver under the influence of cytokines such as interleukin-6 and tumor necrosis factor- α . Data from epidemiologic studies have shown a significant association between increased concentrations of CRP and increased risk of first and recurrent cardiovascular events (6). Some studies report a greater predictive value of high-sensitivity CRP (hsCRP) than that of traditional laboratory markers such as total cholesterol, HDL cholesterol, and LDL cholesterol and novel markers such as lipoprotein, homocysteine, and apolipoproteins AI and B (7). Furthermore, some have found that the combination of hsCRP and the total-to-HDL cholesterol ratio has the greatest power to predict a future first coronary event (7). There also is evidence that a number of pharmacologic agents used in the treatment of coronary heart disease (CHD)—statins (8), aspirin (9), and β -blockers (10)—reduce serum hsCRP, suggesting that reduced inflammation contributes to the beneficial effects of these medications.

A statement from the CDC and the American Heart Association (6) suggests that hsCRP values of <1 , 1–3, and >3 mg/L should be used to define low, average, and high cardiovascular risk categories, respectively. These values correspond to approximate

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⁶ Nonstandard abbreviations: CRP, C-reactive protein; hsCRP, high-sensitivity CRP; CHD, coronary heart disease; SEASONS, Seasonal Variation of Cholesterol Levels Study; 24HR, telephone-administered 24-h recall interview; BMI, body mass index.

tertiles in the general population, although controversy exists regarding these clinical cutoff points (11). Systemic inflammatory status can fluctuate substantially over time (12), and therefore it is recommended to use the average of 2 assays, optimally obtained 2 weeks apart, to provide a more reliable estimate (13). A greater degree of variability between measurements of hsCRP has been noted among patients with established CHD, raising the possibility that patients with CHD have a dynamic systemic inflammatory status that may affect acute coronary risk (6). This variability between measurements becomes a potential limitation of using serial measurements to monitor risk status or the response to medical therapies in these patients. Sources of variability of hsCRP values include age (14), body weight (15), diet (16), level of physical activity (17), depression (18), medication effects (8–10), and infectious and inflammatory processes (19). HsCRP values >10 mg/L should trigger a search for a source of infection or inflammation (6).

Several cross-sectional studies have found that hsCRP values are higher in the fall and winter than in the spring and summer (20, 21); however, no longitudinal data have been reported to determine if season of the year has an independent effect on hsCRP. We proposed to address this gap using data from the Seasonal Variation of Cholesterol Levels Study (SEASONS) (22), an observational longitudinal study that collected detailed anthropometric, physiologic, dietary, physical activity, and psychosocial data, at baseline and every 3 months for a year, with corresponding blood samples from each participating individual. The SEASONS study provides a unique opportunity to explore seasonal variation of hsCRP among healthy adult men and women, while controlling for several factors known to be associated with hsCRP.

Materials and Methods

Participants in the SEASONS study were recruited primarily from the Fallon Healthcare System, a health management organization serving central Massachusetts. Eligibility criteria included being a resident of Worcester County and age 20–70 years and having telephone service. Study participants were not taking cholesterol-lowering medications and were not actively on lipid-lowering or weight-control diets, did not have possible causes of secondary hyperlipidemia, had not been diagnosed as having CHD, and were free of life-threatening illness. Subjects were recruited between December 1994 and February 1997 and enrollment occurred throughout the calendar year. The Institutional Review Boards of the Fallon Healthcare System and the University of Massachusetts Medical School approved all subject recruitment and data col-

lection procedures. Each subject signed an approved informed consent form before entering the study.

Demographic data and health information were collected by self-administered questionnaires. Anthropometric data, including body mass, height, and waist and hip circumference, as well as fasting blood samples were obtained at baseline and then every 3 months, within a 3-week window on either side of the individual's quarterly appointment date, to the 1-year anniversary point (total of 5 assessments). The hsCRP analyses were performed in 2004, using stored serum (–80 °C) from the original study conducted between 1994 and 1998. Previous studies have demonstrated that appropriately frozen hsCRP samples are stable for long periods of time (up to 20 years) (23). Analyses for hsCRP were performed in the laboratory of Nader Rifai at Children's Hospital, Boston, MA. We described the methodology in our previous publication (16). Inter-assay and intraassay CVs for hsCRP were in compliance with CDC-accepted ranges. We excluded hsCRP concentrations >10 mg/L (n = 66, 3%) from this analysis because such increased values are likely to be caused by acute infection or underlying inflammatory problems (6).

Telephone-administered 24-h recall interviews (24HRs) were used for dietary intake and physical activity assessment, with interviews conducted at baseline and then every 3 months. At each quarterly data collection point, three 24HRs were conducted on randomly selected days (including 2 weekdays and 1 weekend day). All dietary data for the 24HRs were entered into and analyzed using the Nutrition Data System (NDS DOS version 2.6, NDS 2.9) software (24). Estimates of physical activity energy expenditure (MET hours/day) were calculated from the 24HRs in 3 domains: household, occupational, and leisure-time activities, using methods described by Ainsworth et al. (25). We have reported the validation of this method elsewhere (26).

STATISTICAL ANALYSIS

We compared selected baseline demographic characteristics by sex using 2-sample *t*-tests (for continuous variables) and χ^2 tests (for categorical variables). The primary outcome variable was hsCRP. We analyzed seasonal variation of hsCRP using the SAS mixed (random and fixed) effect model procedure (Proc Mixed) with restricted maximum likelihood methods (27). Two methods were used to estimate seasonal variation using mixed models. First, we used the date of blood draw to classify each hsCRP measure into a season of the year, using the "light season" definition, centered at the equinoxes, so as to maximize variation in light exposure (winter, November 6 to February 4; spring, February 5 to May 6; summer, May 7 to August 5; fall,

August 6 to November 5). Season was used as a fixed effect (with 4 levels) in the mixed model analyses, and estimates of change in hsCRP between seasons were constructed. Similar to analysis for hsCRP, we obtained estimates in seasonal variation of covariates using mixed models, with season fitted as a fixed effect and subject as a random effect. These covariates include anthropometric measures, physical activity, diet, physiology, and other variables. Second, we used the date of blood draw to define sine and cosine coefficients for a sine-shaped seasonal model that assumed a period of 365 days. Estimates of fixed-effect regression coefficients for the sine and cosine terms in the mixed model were transformed to estimate the amplitude (peak–trough hsCRP difference) and phase (date of peak hsCRP) of the seasonal effects. We used a first-order Taylor series expansion to construct estimates of the variance of the amplitude and phase from the variance estimates of the sine and cosine coefficients. Detailed documents explaining calculations of the amplitude and phase and their standard errors can be found in the appendix from the article by Matthews et al. (28).

Sex-specific mixed models were fitted solely with seasonal effects, using subjects as random effects. Subsequent models were fitted that controlled for various time-dependent covariates separately, including body mass index (BMI), percent calories from saturated fat, physical activity (total and leisure time), relative plasma volume (29) (calculated from a subject-specific mean hemoglobin value, assuming that hemoglobin mass remains constant over the year in healthy subjects), and age. We evaluated the impact of including the covariates by assessing the extent to which the estimated amplitude of the seasonal effect changed when the covariates were considered. Percent change in seasonal amplitude was used for this purpose and was obtained by comparing amplitude from the model with covariates to that of the model without covariates. We also conducted similar analyses for subjects in the upper or lower quartile distribution of hsCRP.

Finally, we obtained percentages of the study population with hsCRP ≥ 3 mg/L, by sex, in both winter and summer using the subject average at each season.

Results

Study subjects were required to have at least 2 quarterly hsCRP measures for inclusion, resulting in an analysis subset of 534 (83%) of the 641 SEASONS subjects. Baseline demographic data (Table 1) demonstrate that the study group was predominantly composed of white, married, well-educated, and overweight individuals. Average age was 48 years. Men had more years of formal education than women. Women were less

likely to be overweight than men. More than 50% of the study participants worked in service industries or in white-collar occupations. Smokers comprised about 25% of the study population, with no significant sex differences in smoking rates.

Anthropometric data did not demonstrate significant seasonal changes, whereas diet and physical activity showed marked seasonal variation (Table 2). Women had significantly higher self-reported caloric intake during the winter compared with other seasons, whereas men reported a significantly higher intake of total fat and saturated fat, as percentages of total caloric intake, during the winter compared with the rest of the year. Both men and women reported higher levels of physical activity during the summer, particularly related to changes in the leisure-time and household physical activity domains. As a whole, though, the study group had a relatively low level of physical activity. As described in a previous publication (30), relative plasma volume showed significant seasonal variation, suggesting a hemodilution effect during the summer and the converse during the winter (30).

Average hsCRP was 1.72 mg/L (men, 1.75 mg/L; women, 1.68 mg/L). Overall, the estimate of the seasonal amplitude of variation of hsCRP was 0.16 mg/L (95% CI 0.02 to 0.30), a 9% increase in hsCRP from late spring to late fall. Among men, the amplitude of variation was 0.10 mg/L (95% CI -0.11 to 0.31) (6%), whereas among women, it was 0.23 mg/L (95% CI 0.04 to 0.42) (14%). Both peaked in November, with a corresponding trough in May (Fig. 1). The seasonal amplitude was of similar relative magnitude (10%–15% of the average value) independent of baseline hsCRP quartile (data not shown).

Several factors were associated with the amplitude of seasonal change of hsCRP, primarily changes in relative plasma volume, waist circumference, and diastolic blood pressure among women and changes in relative plasma volume, depression score, and diastolic blood pressure among men (Table 3). After multivariable analyses, amplitude of seasonal variation was no longer statistically significant in either sex (Table 4), indicating that these factors explained most of the seasonal variation in hsCRP.

Using the raw values, there was a 20% increase in the percentage of participants classified in the high-risk category for hsCRP (values ≥ 3 mg/L) during the peak period, corresponding to late fall and early winter (88 of 428 participants) compared with the trough period, corresponding to late spring and early summer. By sex, the corresponding increases were 23.1% among men and 17.9% among women, a sex difference that was not statistically significant (Table 5). The peak and trough periods were calculated using the corresponding peak

Table 1. Baseline demographic characteristics of the seasonal study cohort by sex, SEASONS.^a			
	Men	Women	P^b
n	275	259	
Mean age, years (SD)	48.9 (12.5)	47.5 (12.1)	0.20
White ethnicity	240 (87.3)	211 (81.5)	0.17
Education			0.003
High school diploma or less	13 (4.7)	16 (6.2)	
Some college	54 (19.7)	77 (30.0)	
College graduate	92 (33.6)	73 (28.4)	
Postgraduate degree	115 (42.0)	91 (35.4)	
Occupation			<0.0001
Unemployed/retired	45 (16.4)	68 (26.3)	
Blue-collar	80 (29.1)	36 (13.9)	
Service worker	21 (7.6)	25 (9.7)	
White-collar	129 (47.0)	130 (50.2)	
Marital status			0.002
Single	24 (8.8)	26 (10.1)	
Married	219 (80.0)	172 (66.7)	
Living with partner	9 (3.3)	11 (4.3)	
Separated	2 (0.7)	2 (0.8)	
Divorced	16 (5.8)	28 (10.9)	
Widowed	4 (1.5)	19 (7.4)	
BMI (kg/m ²)			0.0002
Normal weight (17.1–24.9)	81 (29.5)	121 (46.7)	
Overweight (25–29.9)	125 (45.5)	85 (32.8)	
Obese (≥30)	69 (25.1)	53 (20.5)	
Current smoker	48 (24.4)	41 (26.5)	0.65
Reported minor infection/inflammation	80 (40.6)	79 (46.5)	0.26
Antiinflammatory medication use	38 (13.8)	29 (11.2)	0.36

^a Data are frequency (%) unless noted otherwise.
^b H₀: equal mean based on test of difference between sexes.

and trough dates from the sinusoidal models, ± 45 days, with a similar pattern apparent in both sexes.

Discussion

To our knowledge, this is the first study to report seasonal variation of hsCRP in the general population using longitudinal data. The estimated seasonal amplitude of variation was greater among women, but the difference in amplitudes was not significantly different from that observed in men. The results also suggest that the magnitude of seasonal variation (10%–15%) is independent of the baseline hsCRP value. We also observed a 20% relative increase in the number of participants classified in the high-risk category for hsCRP

(hsCRP values ≥ 3 mg/L) during the late fall and early winter, compared with late spring and early summer.

Our results are consistent with suggestions from previous cross-sectional studies reporting higher concentrations of hsCRP in the winter compared with the summer. A study in a large, healthy adult population in Korea (12 064 men and 6381 women, average age 47.2 years), reported that, on average, hsCRP concentrations were 0.25 mg/L higher in the winter than in the summer (1.76 vs 1.51 mg/L, respectively), suggesting that increased plasma hsCRP during the winter months could be related to the observed increased risk of cardiovascular events, and other conditions that are known to have seasonal peaks, ranging from the common cold to cancer (20, 31).

Table 2. Selected general characteristics by light seasons and sex, SEASONS.^a

	Men (n = 275)				Women (n = 259)			
	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall
BMI, kg/m ²	27.8 (0.28)	27.8 (0.28)	27.8 (0.28)	27.7 (0.28)	26.6 (0.38)	26.6 (0.38)	26.6 (0.38)	26.5 (0.38)
Waist circumference, inches	38.2 (0.28)	38.2 (0.28)	38.2 (0.28)	38.3 (0.28)	31.9 (0.32)	32.0 (0.32)	32.1 (0.32)	32.0 (0.32)
Hip circumference, inches	41.0 (0.20)	41.0 (0.20)	41.0 (0.20)	41.1 (0.20)	40.8 (0.30)	40.8 (0.30)	40.7 (0.30)	40.8 (0.30)
Diet ^b								
Caloric intake, kcal/day	2231.4 (69.8)	2168.8 (69.4)	2162.0 (69.8)	2249.5 (74.7)	1827.0 (44.6) ^c	1739.6 (47.2)	1763.9 (46.9)	1717.7 (48.7)
Total fat intake, percent of daily caloric intake	38.3 (0.54) ^c	37.6 (0.54)	37.4 (0.54)	36.8 (0.57)	37.1 (0.53)	36.1 (0.55)	35.9 (0.55)	36.7 (0.57)
Saturated fat intake, percent of daily caloric intake	13.1 (0.21) ^c	13.0 (0.21)	12.9 (0.21)	12.6 (0.22)	12.5 (0.21)	12.2 (0.22)	12.1 (0.22)	12.4 (0.23)
Fiber intake, g/day	17.9 (0.46)	18.4 (0.46)	17.1 (0.46) ^c	18.1 (0.48)	14.4 (0.36)	14.6 (0.38)	14.1 (0.38)	14.0 (0.39)
Physical activity ^b								
Total physical activity, MET ^d	31.6 (0.43)	31.0 (0.43)	32.2 (0.43)	31.6 (0.45)	28.3 (0.24)	28.7 (0.26)	29.3 (0.26) ^c	28.5 (0.27)
Leisure time physical activity, MET ^d	1.6 (0.19) ^c	2.0 (0.19)	2.6 (0.19) ^c	2.1 (0.20)	1.2 (0.16) ^c	1.6 (0.17)	2.2 (0.17) ^c	1.8 (0.18)
Occupational physical activity, MET ^d	6.2 (0.48)	5.6 (0.48)	5.4 (0.49)	6.1 (0.51)	3.0 (0.27)	3.2 (0.28)	2.5 (0.28)	2.9 (0.29)
Household physical activity, MET ^d	4.5 (0.34)	4.1 (0.34)	5.2 (0.35) ^c	4.3 (0.37)	4.9 (0.25)	4.9 (0.27)	5.5 (0.27) ^c	4.7 (0.28)
Physiologic and other variables								
hsCRP, mg/L	1.82 (0.11)	1.70 (0.11)	1.85 (0.11)	1.77 (0.12)	1.86 (0.12)	1.68 (0.13)	1.74 (0.13)	1.82 (0.13)
Diastolic blood pressure, mmHg	77.2 (0.61)	77.8 (0.61)	76.8 (0.61)	77.6 (0.65)	72.5 (0.64)	72.9 (0.67)	72.2 (0.66)	73.2 (0.68)
Systolic blood pressure, mmHg	125.5 (1.02)	125.5 (1.02)	122.8 (1.02)	124.0 (1.07)	113.7 (1.08)	114.0 (1.12) ^c	112.4 (1.11)	112.0 (1.13)
Heart rate, beats per min	68.5 (0.72)	67.2 (0.72)	65.6 (0.72)	67.5 (0.76)	73.7 (0.72)	73.1 (0.76)	70.7 (0.75)	71.7 (0.78)
Relative plasma volume, percentage of average yearly value	98.7 (1.01)	100.6 (0.71)	99.8 (0.36)	100.0 (0.38)	102.4 (2.76)	101.7 (1.05) ^c	100.4 (0.45)	99.4 (0.50)
Minor infection or inflammation, %	48.03 ^c	39.57	28.94	36.80	50.00 ^c	43.81	31.30	45.87
Psychological variables								
Anxiety score, Beck Inventory	3.5 (0.28)	3.6 (0.28)	3.2 (0.28)	3.5 (0.29)	5.3 (0.35)	5.4 (0.37)	4.7 (0.37)	5.4 (0.38)
Depression score, Beck Inventory	5.2 (0.34)	5.4 (0.34)	5.2 (0.34)	4.9 (0.36)	7.0 (0.41)	6.5 (0.43)	6.2 (0.43)	6.3 (0.44)

^a Light seasons: centered in the equinoxes. Data are mean (SE) unless noted otherwise.
^b Data obtained from 24-h recall interviews.
^c P < 0.05 for comparison among seasons, within each sex.
^d MET, metabolic equivalent; 1 MET h/day is roughly equivalent to 1 kcal/kg/day.

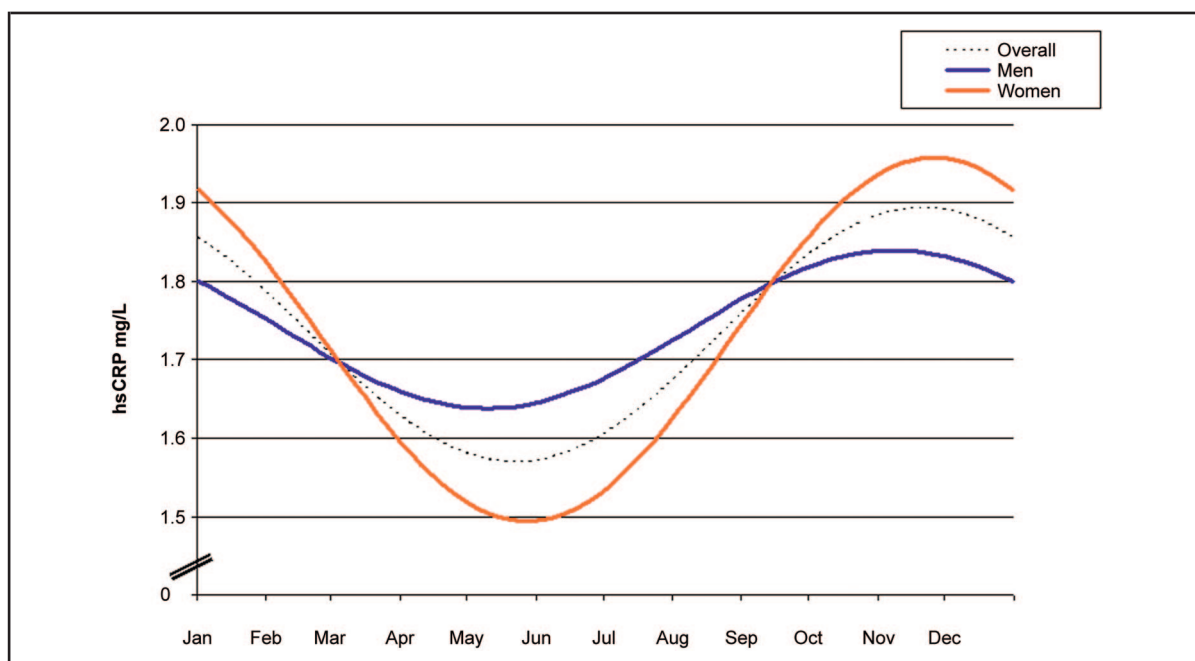


Fig. 1. Seasonal variation in hsCRP, SEASONS.

Table 3. Summary of regression analysis by sex, sinusoidal model, SEASONS.

	Women			Men		
	Amplitude of seasonal variation, mg/L	95% CI	Change in seasonal amplitude, %	Amplitude of seasonal variation, mg/L	95% CI	Change in seasonal amplitude, %
Unadjusted hsCRP	0.23	0.04 to 0.42		0.10	-0.11 to 0.31	-
Adjusted for ^a						
BMI	0.24	0.05 to 0.43	4.35	0.11	-0.11 to 0.32	10.00
Hip circumference	0.24	0.05 to 0.43	4.35	0.12	-0.10 to 0.33	20.00
Waist circumference	0.26	0.07 to 0.45	13.04	0.11	-0.11 to 0.33	10.00
Systolic blood pressure	0.21	0.01 to 0.41	-8.70	0.08	-0.15 to 0.30	-20.00
Diastolic blood pressure	0.20	0.0003 to 0.40	-13.04	0.06	-0.17 to 0.28	-40.00
Resting heart rate	0.22	0.01 to 0.43	-4.35	0.09	-0.13 to 0.31	-10.00
Relative plasma volume	0.27	0.06 to 0.48	17.39	0.24	0.02 to 0.46	140.00
Age	0.23	0.04 to 0.42	0	0.11	-0.11 to 0.32	10.00
Total cholesterol	0.23	0.04 to 0.42	0	0.11	-0.10 to 0.32	10.00
Triglycerides	0.23	0.04 to 0.42	0	0.12	-0.10 to 0.33	20.00
Total caloric intake	0.22	0.02 to 0.42	-4.35	0.08	-0.13 to 0.30	-20.00
Dietary fiber intake	0.23	0.04 to 0.42	0	0.08	-0.14 to 0.29	-20.00
Beck depression score	0.25	0.04 to 0.46	8.70	0.21	-0.02 to 0.43	110.00
Beck anxiety score	0.24	0.04 to 0.44	4.35	0.12	-0.10 to 0.34	20.00
Minor infection or inflammation	0.21	0.02 to 0.40	-8.70	0.11	-0.10 to 0.32	10.00

^a Change in amplitude of seasonal variation in hsCRP when controlled for each of the listed variables, one at a time, to assess the relative change in magnitude of seasonal variation, with respect to the unadjusted model.

Table 4. Multivariate regression analysis, sinusoidal mixed model by sex, SEASONS.

Effect	Women ^a			Men ^b		
	Estimate	SE	P	Estimate	SE	P
Intercept	-12.535	1.955	<0.0001	-7.526	2.563	0.004
Sine	-0.086	0.061	0.158	-0.108	0.063	0.084
Cosine	0.065	0.062	0.291	-0.030	0.064	0.640
BMI	0.083	0.035	0.019	0.035	0.040	0.382
Hip circumference	0.048	0.015	0.002	0.009	0.023	0.699
Waist circumference	0.010	0.017	0.558	0.032	0.019	0.094
Systolic blood pressure	0.005	0.005	0.392	0.007	0.005	0.211
Diastolic blood pressure	-0.007	0.008	0.337	-0.004	0.008	0.642
Heart rate	0.022	0.005	<0.0001	0.014	0.006	0.017
Age	0.009	0.009	0.329	0.014	0.008	0.088
Total cholesterol	-0.001	0.002	0.626	0.000	0.002	0.881
Triglycerides	0.002	0.001	0.020	-0.0006	0.0005	0.254
Total caloric intake	-0.0001	0.0001	0.751	-0.00003	0.0001	0.786
Beck depression score	-0.005	0.012	0.683	0.012	0.015	0.418
Beck anxiety score	0.022	0.012	0.080	0.005	0.016	0.768
Minor inflammation or infection	0.158	0.101	0.117	0.327	0.106	0.002
Dietary fiber intake	-0.031	0.012	0.013	-0.004	0.010	0.711
Relative plasma volume	0.047	0.015	0.002	0.021	0.019	0.259

^a Amplitude of seasonal variation in women: 0.22 mg/L, 95% CI -0.02 to 0.47.
^b Amplitude of seasonal variation in men: 0.22 mg/L, 95% CI -0.02 to 0.45.

Another cross-sectional study performed in the UK, in 9377 middle-aged adults, showed a seasonal variation in hsCRP of about 9%, between a low value in the summer and a high value in the winter (32). Our finding of a 20% relative increase in the number of patients labeled as being in the high hsCRP risk category between summer and winter, although not statistically significant, needs to be taken into account as another source of variability in hsCRP risk categorization. Clearly, if this were indicative of a more general-

izable pattern, it would have important implications for a host of conditions with known seasonal periodicity and for treatments that are known to be affected by timing (31, 33). We found that there is much more variation among women than among men, yet the percentages increasing into the high-risk category are similar in both men and women. The discrepancy may be related to differences in the time distribution of CRP by sex.

Average concentrations of hsCRP appeared to be similar in men and women, as reported for the general US population (23); however, the amplitude of seasonal variation in hsCRP was statistically significant only among women, and >2 times the magnitude of the estimated amplitude in men.

Significant advances have been made concerning the differential effects of cardiovascular risk factors as they relate to sex (34). Diabetes and concentrations of HDL cholesterol and triglycerides have been found to have a greater impact on CHD risk in women than in men (35). Greater variability in autonomic responses, particularly heart rate variability, is seen in women, and this variability has been associated with improved cardiovascular health (36). We have reported that women

Table 5. Comparison of percentages of study participants with high-risk hsCRP values during the peak and trough periods, SEASONS.

hsCRP concentrations ≥ 3 mg/L	Trough, n (%)	Peak, n (%)	Relative difference, %	P ^a
All	76/445 (17.1)	88/428 (20.6)	20.5%	0.19
Men	40/231 (17.3)	46/216 (21.3)	23.1%	0.29
Women	36/214 (16.8)	42/212 (19.8)	17.9%	0.43

^a χ^2 Test for difference between peak and trough periods calculated as hsCRP seasonal amplitude peak and trough \pm 45 days.

appear to have greater seasonal variation of lipid concentrations than men (30) and have observed a similar sex pattern of greater seasonal variation in other physiologic variables, including systolic blood pressure and relative plasma volume (Table 2). This raises the possibility that the greater variability in physiologic variables seen in women suggests an overall physiology that may be more adaptable to changing circumstances, a factor which we speculate might contribute in some way to the overall lower morbidity and incidence of chronic diseases in women and, concomitantly, a longer lifespan.

Factors significantly associated with seasonal variation of hsCRP included relative plasma volume and anthropometric factors. We have described (30) the effect of a relative winter hemoconcentration, which could be related to the clustering of peak values of hemostatic coronary risk factors during the winter months. Seasonal variation in hsCRP may be another factor related to the excess CHD mortality in the winter compared to the summer (37). The positive correlation between anthropometric measurements and hs-CRP has been described extensively (38, 39). In our study, waist and hip circumference, but also BMI, were positively correlated with changes in amplitude of seasonal variation of hsCRP. Similarly, depression scores (18) and blood pressure levels (40) have been correlated with hsCRP, and appear to also have differential correlation with seasonal variation of hsCRP, with higher depression scores and lower systolic and diastolic blood pressures correlating with higher amplitude of seasonal variation of hsCRP. Minor infectious or inflammatory processes also were correlated with change in amplitude of seasonal variation of hsCRP. Given that CRP is an acute-phase protein, it would be expected that hsCRP follows the seasonal pattern of a higher incidence of minor viral infections during the colder months (41). We attempted to reduce this potential by excluding hsCRP measures >10 mg/L; however, the presence of minor infectious or inflammatory processes remained a significant predictor of seasonal variation of hsCRP.

Our study has several strengths, including the longitudinal design with multiple and detailed measurements of demographic, anthropometric, dietary, physical activity, psychosocial, physiologic, and blood parameters related to hsCRP concentrations. We were therefore able to determine the relative contribution of these factors to the seasonal variation in hsCRP concentrations. We also kept track of minor inflammatory and infectious processes and controlled, to the extent possible, for these factors in the statistical analyses. Limitations

include the derivation of our study participants from a volunteer sample, composed of primarily white, well-educated individuals living in central Massachusetts; therefore caution should be taken in extrapolating our results to other populations. An additional limitation is the relatively small number of hsCRP measures (5) per subject, limiting analysis to the simplest seasonal models.

In conclusion, clinically significant seasonal variation in hsCRP was observed in this cohort of healthy adults. Women had greater seasonal variation than men, 14% vs 6% fluctuation between late spring and late fall, respectively. Relative plasma volume, anthropometric measurements, diastolic blood pressure, and depression scores are major factors correlated with seasonal variation of hsCRP, with differential magnitude of effect by sex. Together, the analyzed factors explained all the observed seasonal variation of hsCRP. Further research is needed to understand the biological mechanisms and clinical implications of higher concentrations of hsCRP during late fall/early winter and to determine the presence and potential connotations of seasonal variation of hsCRP among patients with established CHD and other chronic diseases including diabetes and cancer.

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References

- Bassuk SS, Rifai N, Ridker PM. High-sensitivity C-reactive protein: clinical importance. *Curr Probl Cardiol* 2004;29:439–93.
- Bisoendial RJ, Kastelein JJ, Levels JH, Zwaginga JJ, van den Bogaard B, Reitsma PH, et al. Activation of inflammation and coagulation after infusion of C-reactive protein in humans. *Circ Res* 2005;96:714–6.
- Schwedler SB, Amann K, Wernicke K, Krebs A, Nauck M, Wanner C, et al. Native C-reactive protein increases whereas modified C-reactive protein reduces atherosclerosis in apolipoprotein E-knockout mice. *Circulation* 2005;112:1016–23.
- Dehghan A, van Hoek M, Sijbrands EJ, Stijnen T, Hofman A, Witteman JC. Risk of type 2 diabetes attributable to C-reactive protein and other risk factors. *Diabetes Care* 2007;30:2695–9.
- Siemes C, Visser LE, Coebergh JW, Splinter TA, Witteman JC, Uitterlinden AG, et al. C-reactive protein levels, variation in the C-reactive protein gene, and cancer risk: the Rotterdam Study. *J Clin Oncol* 2006;24:5216–22.
- Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;107:499–511.
- Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. *Clin Chem* 2001;47:403–11.
- Kleemann R, Verschuren L, de Rooij BJ, Lindeman J, de Maat MM, Szalai AJ, et al. Evidence for anti-inflammatory activity of statins and PPAR α activators in human C-reactive protein transgenic mice in vivo and in cultured human hepatocytes in vitro. *Blood* 2004;103:4188–94.
- Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med* 1997;336:973–9.
- Jenkins NP, Keevil BG, Hutchinson IV, Brooks NH. Beta-blockers are associated with lower C-reactive protein concentrations in patients with coronary artery disease. *Am J Med* 2002;112:269–74.
- Ridker PM. High-sensitivity C-reactive protein and cardiovascular risk: rationale for screening and primary prevention. *Am J Cardiol* 2003;92:17K–22K.
- Ockene IS, Matthews CE, Rifai N, Ridker PM, Reed G, Stanek E. Variability and classification accuracy of serial high-sensitivity C-reactive protein measurements in healthy adults. *Clin Chem* 2001;47:444–50.
- Kushner I, Sehgal AR. Is high-sensitivity C-reactive protein an effective screening test for cardiovascular risk? *Arch Intern Med* 2002;162:867–9.
- Kushner I. C-reactive protein elevation can be caused by conditions other than inflammation and may reflect biologic aging. *Cleve Clin J Med* 2001;68:535–7.
- Tchernof A, Nolan A, Sites CK, Ades PA, Poehlman ET. Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation* 2002;105:564–9.
- Ma Y, Griffith J, Chasan-Taber L, Olenzki B, Jackson E, Stanek E III, et al. Association between dietary fiber and serum C-reactive protein. *Am J Clin Nutr* 2006;83:760–6.
- Geffken DF, Cushman M, Burke GL, Polak JF, Sakkinen PA, Tracy RP. Association between physical activity and markers of inflammation in a healthy elderly population. *Am J Epidemiol* 2001;153:242–50.
- Kop WJ, Gottdiener JS, Tangen CM, Fried LP, McBurnie MA, Walston J, et al. Inflammation and coagulation factors in persons > 65 years of age with symptoms of depression but without evidence of myocardial ischemia. *Am J Cardiol* 2002;89:419–24.
- Whicher JT, Chambers RE, Higginson J, Nashef L, Higgins PG. Acute phase response of serum amyloid A protein and C reactive protein to the common cold and influenza. *J Clin Pathol* 1985;38:312–6.
- Sung KC. Seasonal variation of C-reactive protein in apparently healthy Koreans. *Int J Cardiol* 2006;107:338–42.
- Kelly GS. Seasonal variations of selected cardiovascular risk factors. *Altern Med Rev* 2005;10:307–20.
- Merriam PA, Ockene IS, Hebert JR, Rosal MC, Matthews CE. Seasonal variation of blood cholesterol levels: study methodology. *J Biol Rhythms* 1999;14:330–9.
- Rifai N, Ridker PM. Population distributions of C-reactive protein in apparently healthy men and women in the United States: implication for clinical interpretation. *Clin Chem* 2003;49:666–9.
- Buzzard IM, Price KS, Warren RA. Considerations for selecting nutrient-calculation software: evaluation of the nutrient database. *Am J Clin Nutr* 1991;54:7–9.
- Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, et al. Compendium of physical activities: an update of activity codes and MET intensities. *Med Sci Sports Exerc* 2000;32:S498–504.
- Matthews CE, Freedson P, Hebert J, Stanek E, Ockene I, Merriam P. Comparison of physical activity assessment methods in the Seasonal Variation of Blood Cholesterol Levels Study. *Med Sci Sports Exerc* 2000;32:976–84.
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD. SAS system for mixed models. Cary (NC): SAS Institute Inc.; 1996.
- Matthews CE, Freedson PS, Stanek EJ, Hebert JR, Merriam PA, Rosal MC, et al. Seasonal variation of household, occupational, and leisure-time physical activity: longitudinal analyses from the Seasonal Variation of Cholesterol Study. *Am J Epidemiol* 2001;153:172–83.
- Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974;37:247–8.
- Ockene I, Chiriboga DE, Stanek EJJ, Harmatz MG, Nicolosi R, Saperia G, et al. Seasonal variation in serum cholesterol: treatment implications and possible mechanisms. *Arch Intern Med* 2004;164:863–70.
- Hrushesky WJ. Circadian timing of cancer chemotherapy. *Science (Wash DC)* 1985;228:73–5.
- Rudnicka AR, Rumley A, Lowe GD, 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.
- Mormont MC, Levi F. Cancer chronotherapy: principles, applications, and perspectives. *Cancer* 2003;97:155–69.
- Roeters van Lennep JE, Westerveld HT, Erkelens DW, van der Wall EE. Risk factors for coronary heart disease: implications of gender. *Cardiovasc Res* 2002;53:538–49.
- Bello N, Mosca L. Epidemiology of coronary heart disease in women. *Prog Cardiovasc Dis* 2004;46:287–95.
- Virtanen R, Jula A, Kuusela T, Helenius H, Voipio-Pulkki LM. Reduced heart rate variability in hypertension: associations with lifestyle factors and plasma renin activity. *J Hum Hypertens* 2003;17:171–9.
- Seretakis D, Lagiou P, Lipworth L, Signorello LB, Rothman KJ, Trichopoulos D. Changing seasonality of mortality from coronary heart disease. *JAMA* 1997;278:1012–4.
- Bermudez EA, Rifai N, Buring J, Manson JE, Ridker PM. Interrelationships among circulating interleukin-6, C-reactive protein, and traditional cardiovascular risk factors in women. *Arterioscler Thromb Vasc Biol* 2002;22:1668–73.
- Rawson ES, Freedson PS, Osganian SK, Matthews CE, Reed G, Ockene IS. Body mass index, but not physical activity, is associated with C-reactive protein. *Med Sci Sports Exerc* 2003;35:1160–6.
- Blake GJ, Rifai N, Buring JE, Ridker PM. Blood pressure, C-reactive protein, and risk of future cardiovascular events. *Circulation* 2003;108:2993–9.
- O'Kelly EA, Hillary IB. Epidemiology of respiratory syncytial virus infection among infants over three winter seasons. *Ir J Med Sci* 1991;160:12–6.