Lack of Seasonal Variation in C-Reactive Protein, *Margit Fröhlich,[1] Malte Sund,[2] Barbara Thorand,[3] Winston L. Hutchinson,[4] Mark B. Pepys,[3] and Wolfgang Koenig[1*]* ([1] Department of Internal Medicine II-Cardiology, University of Ulm Medical Center, D-89081 Ulm, Germany; [2] GSF-Institute of Health Economics and Health Care Management, D-85764 Neuherberg, Germany; [3] GSF-National Research Center for Environment and Health, Department of Epidemiology, D-85764 Neuherberg, Germany; [4] Center for Amyloidosis and Acute Phase Proteins, Department of Medicine, Royal Free and University College Medical School, London NW3 2PF, United Kingdom; * address for correspondence: Abteilung Innere Medizin II-Kardiologie, Universitätsklinikum Ulm, Robert-Koch-Strasse 8, D-89081 Ulm, Germany; fax 49-731-500-33872, e-mail wolfgang.koenig@medizin.uni-ulm.de)

Several studies have reported on an increased incidence of cardiovascular events during winter months *(1)*. Some authors *(2)* have suggested that an acute-phase response triggered by upper respiratory tract infections is responsible for the higher incidence of acute coronary syndromes during the winter months.

Serum concentrations of C-reactive protein (CRP), the major acute-phase reactant, have been consistently associated with cardiovascular endpoints *(3–5)*. Because of its short plasma half-life of 19 h, the CRP synthesis rate is the only significant determinant of its plasma concentration and thus precisely reflects inflammatory processes *(6)*. However, this may lead to considerable variability of CRP concentrations, altering its reliability in risk stratification.

We investigated seasonal variation of CRP as measured by means of a high-sensitivity immunoradiometric assay *(7)* in several populations.

We studied seasonal variations of several biochemical markers in 16 healthy volunteers (8 women and 8 men; age range, 20–41 years). After volunteers gave written informed consent, blood samples were taken under identical conditions at each of 12 consecutive months [clinical study; see Ref. *(8)*].

One other group was part of the first cross-sectional survey of the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) center in Augsburg, Germany, which was performed from October 1984 to May 1985. The objectives and design of the MONICA project have been described in detail previously *(9, 10)*. Briefly, 4022 of the 5069 eligible individuals, 25–74 years of age, initially sampled at random from a study population of 282 279 inhabitants of a mixed urban and rural area, participated in the study (response rate, 79.3%). Of these participants, all of the men (45–64 years of age) participated in an 8-year follow-up study. All participants of this cohort who had complete data on all variables studied had been included in the analysis of the association of CRP and coronary heart disease reported earlier *(4)*. This group also served as one study sample for the present analysis (n = 936; MONICA Augsburg Survey 1).

From October 1987 to June 1988, 852 participants in the Survey 1 sample took part in a reexamination (response rate, 91%) and submitted to the same protocol. For 702 of these volunteers, we were able to obtain CRP values. In six participants, at least one of the covariables used was missing. Thus, we had 696 complete cases for the 1987–1988 follow-up (MONICA Augsburg Survey 1 Follow-Up).

Analogous to the Survey 1, another cross-sectional survey was performed at the MONICA center in Augsburg, Germany, from October 1994 to July 1995, in which 2305 men and 2211 women (age range, 25–74 years) were included (MONICA Augsburg Survey 3).

In the clinical study, a standardized interview was performed to assess conditions of life and the medical history. Participants with acute illnesses were excluded from the study. Clinical measurements in the MONICA Augsburg Surveys are described in detail elsewhere *(4)*.

Venous blood samples were drawn into EDTA with the participants in a supine resting position with only short-term venous occlusion and minimal suction. EDTA blood was immediately centrifuged at 3000*g* for 15 min, and the plasma was aliquoted and stored at −70 °C. The CRP concentration was measured in triplicate by an immunoradiometric assay (range, 0.05–10 mg/L) calibrated with WHO reference standard 85/506 *(7)*. The CV for repeated measurements was 12% over all ranges.

The possibility of CRP changing with calendar time was investigated by fitting various parametric and nonparametric regression models to the data. The models included smoothing by local regression (SAS® procedure LOESS), smoothing by generalized additive model techniques with splines (SAS procedure GAM), fitting polynomials with random coefficients (SAS procedure MIXED), and fitting a sinusoidal curve with a period of 1 year to the data (SAS procedure MIXED). The LOESS and GAM procedures were used with their automatic smoothing parameter selection devices. For the polynomial fits, standardized values of the time variable were used. Details for the fitting of the sinusoidal model can be found elsewhere *(8)*. The overall lack-of-fit of the sinusoidal model was assessed by following the concept described by Draper and Smith *(11)*, grouping observations in the same month to estimate the "pure error". CRP was heavily skewed but appeared to be almost perfectly approximated by a lognormal distribution such that the ln-transformation of CRP was used throughout (mg/L CRP). All computations and graphics were performed in Windows NT 4 with SAS software *(12)*.

In the clinical study, participants were seen each month for a period of 1 year, whereas participants in the MONICA studies were measured once, in which the observation period covered 8–10 months with a lack of data in August and September.

Seasonal variations of CRP according to the sinusoidal model are shown in Table 1. In the clinical study, a plot of the individual curves exhibited considerable deviations from the parallelism ideally required for meaningful curve summary analysis. Smoothing of each individual curve separately gave the visual impression that the majority of fits showed practically no change. The signif-

Table 1. Seasonal variation of CRP.

Study	Annual mean, mg/L	Month of maximum CRP value	Seasonal difference		
			Value, mg/L	95% CI[a]	*P*
MONICA S1	1.59	March	1.26	1.02–1.57	0.06
MONICA S1 FU	1.55	May	1.12	0.90–1.40	0.59
MONICA S3					
Men	1.33	May	1.19	1.06–1.34	0.01
Women	1.38	February	1.22	1.01–1.48	0.12
Clinical study	0.36	June	1.58	0.94–2.65	0.22

[a] CI, confidence interval.

icance tests of the individual GAM fits led to only three volunteers showing a significant overall test (no. 3, a concave pattern; no. 11, an increasing inflected line) and one volunteer showing a linear regression term (no. 14, an increasing linear trend) significantly different from the average intercept-only model ($P = 0.0045$). Without this volunteer, the random linear term had to be dropped from the model. Only volunteer 3 exhibited the values expected under the hypothesis of increased CRP concentrations during winter time. The data may, therefore, be summarized by stating that one participant's fitted curve exhibited linearly increasing concentrations of ln (CRP), whereas the remaining 15 volunteers did not indicate significant change, although they varied in their mean concentration of ln (CRP).

Shown in Fig. 1 are data on these 15 volunteers with their original data connected by straight lines, the nonparametric LOESS fit, together with 95% confidence bands (dotted lines), and the best-fitting polynomial, i.e., the no-change (intercept only) model (thick solid line). The plot shows that the intercept-only model is compatible with the nonparametric fit. Seasonal variation was also investigated by fitting a sinusoidal curve with a period of 1 year to the CRP concentrations with the examination day as the time variable. The lack-of-fit test was not significant ($P = 0.23$), indicating a reasonable fit of the model. The test of the two regression slopes to be simultaneously zero was not statistically significant, again leaving a model with only the intercept ($P = 0.22$).

In the MONICA S1 group, the three data-driven models fitted to the data (LOESS, GAM; third-degree polynomial) visually coincided almost completely. Statistical tests were performed with the polynomial model. The overall statistical test of the three regression coefficients to be simultaneously zero was statistically significant ($P = 0.03$) with a significant cubic term ($P = 0.03$). The third-degree polynomial showed a maximum fitted CRP value in March (~0.7 or 2 mg/L CRP) and a minimum in November and December (~0.4 or 1.5 mg/L CRP). The maximum was larger than the minimum by ~35%, which was an irrelevant change. Therefore, a third-degree polynomial, although statistically significant, does not seem to be superior to the null model (intercept only) indicating no change.

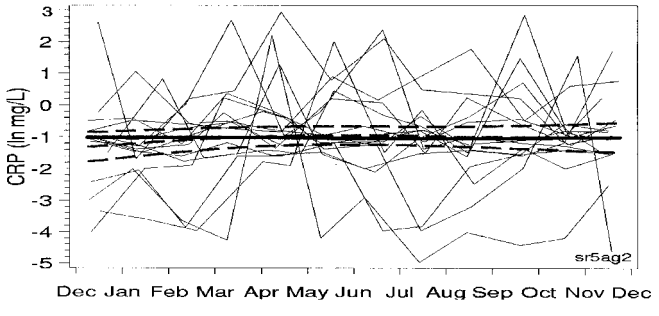

Fig. 1. CRP vs observation time in the clinical study.

Observations (*thin solid lines*), LOESS smooth with 95% confidence bands (*dashed lines*), and the intercept-only fit (*thick solid line*).

In the MONICA S1 follow-up group, the result clearly suggested no change. The LOESS, GAM, and polynomial curves coincided completely (not shown). The overall statistical test for the three regression coefficients to be simultaneously zero in the third-degree polynomial was not significant ($P = 0.62$), suggesting an intercept-only model (no change).

In men participating in MONICA S3, there was an indication of a positive trend. In the cubic polynomial regression model, the overall statistical test for the three regression coefficients to be simultaneously zero was significant ($P = 0.03$). The sequential tests for the cubic, quadratic, and linear terms were $P = 0.52$, $P = 0.73$, and $P = 0.004$, respectively, indicating only the linear trend. The maximum fitted value was larger than the minimum fitted value by ~25%. Therefore, a linear regression model, although statistically significant, does not seem to be superior to the null model indicating no change. In women, the overall test was not significant ($P = 0.17$), suggesting a no-change model.

In summary, we found no strong, consistent evidence for an intraindividual and interindividual seasonal variation of CRP. Except for men in MONICA S3, for whom the seasonal difference was 19% ($P = 0.01$) with the highest CRP values observed in May, our statistical models did not provide significant results in favor of seasonal variation for this acute-phase protein in the remaining populations. In MONICA S3, we found a statistically significant lack-of-fit of the data, which could indicate that our model was not appropriate in this case; thus our findings in men from MONICA S3 have to be interpreted with caution. In the clinical study, however, the seasonal

difference was 58%, which was not statistically significant.

To the best of our knowledge, the present study is the first to systematically investigate interindividual seasonal variations of CRP in several large populations. In two studies, seasonal variation of CRP was investigated on an intraindividual basis. Woodhouse et al. *(2)* reported higher CRP concentrations in winter with a peak in March, and Crawford et al. *(13)* found a significant seasonal variation of CRP with a peak in late February. The lack of seasonal variability of CRP in the present study may be somewhat contradictory to a previous report from our group *(8)* and to others *(2, 13)*, in which seasonal variations of a variety of acute-phase proteins, such as fibrinogen, PAI-1, plasminogen, and α_1-glycoprotein, have been observed. However, in contrast to the major acute-phase reactant CRP, these coagulation proteins are not exclusively related to the acute-phase response.

The predictive value of CRP for cardiovascular events has been consistently established in a variety of prospective studies *(3, 4)*, and highly sensitive assays for CRP are now widely available with low analytical variability. In a recently published study *(14)*, Meier-Ewert et al. demonstrated that baseline CRP concentrations are not subject to time-of-day variation. In the present study, we found no convincing evidence for seasonal variation of CRP; thus there should be no concern about misclassification of participants in population studies and in clinical practice measured during various seasons.

This work was supported by Medical Research Council Program Grant G97900510 (to M.B.). We thank Gerlinde Trischler for excellent technical assistance.

References

1. Kloner RA, Poole WK, Perritt RL. When throughout the year is coronary death most likely to occur? A 12-year population-based analysis of more than 220 000 cases. Circulation 1999;100:1630–4.
2. Woodhouse PR, Khaw KT, Plummer M, Foley A, Meade TW. Seasonal variations of plasma fibrinogen and factor VII activity in the elderly: winter infections and death from cardiovascular disease. Lancet 1994;343:435–9.
3. 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.
4. Koenig W, Sund M, Fröhlich M, Fischer H-G, Löwel H, Döring A, et al. C-Reactive protein, a sensitive marker of inflammation, predicts future risk of coronary heart disease in initially healthy middle-aged men. Results from the MONICA (Monitoring Trends and Determinants in Cardiovascular Disease) Augsburg Cohort Study, 1984 to 1992. Circulation 1999;99:237–42.
5. Liuzzo G, Biasucci LM, Gallimore RL, Grillo RL, Pepys MB, Maseri A. The prognostic value of C-reactive protein in severe unstable angina. N Engl J Med 1994;331:417–24.
6. Vigushin DM, Pepys MB, Hawkins PN. Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. J Clin Invest 1993;91:1351–7.
7. Hutchinson WL, Koenig W, Fröhlich M, Sund M, Lowe GDO, Pepys MB. Immunoradiometric assay of circulating C-reactive protein: age-related values in the adult general population. Clin Chem 2000;46:934–8.
8. Fröhlich M, Sund M, Russ S, Hoffmeister A, Fischer H-G, Hombach V, et al. Seasonal variations of rheological and hemostatic parameters and acute-phase reactants in young, healthy subjects. Arterioscler Thromb Vasc Biol 1997;17:2692–7.
9. WHO-MONICA-Project Principal Investigators. The World Health Organization MONICA Project (monitoring trends and determinants in cardiovascular disease). J Clin Epidemiol 1988;41:105–14.
10. Bothig S. WHO MONICA project: objectives and design. Int J Epidemiol 1989;18(Suppl 1):S29–37.
11. Draper NR, Smith H. Applied regression analysis, 2nd ed. New York: Wiley, 1981.
12. SAS Institute Inc. SAS, Ver. 6.12. Cary, NC: SAS Institute Inc., 1997.
13. Crawford VL, Sweeney O, Coyle PV, Halliday IM, Stout RW. The relationship between increased fibrinogen and markers of infection: a comparison of seasonal cycles. Q J Med 2000;93:745–50.
14. Meier-Ewert HK, Ridker PM, Rifai N, Price N, Dinges DF, Mullington JM. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. Clin Chem 2001;47:426–30.

Serum Vitamin E and Lipid-adjusted Vitamin E Assessment in Friedreich Ataxia Phenotype Patients and Unaffected Family Members, *Moncef Feki,[1] Samir Belal,[2] Habib Feki,[3] Malek Souissi,[1] Mahbouba Frih-Ayed,[4] Naziha Kaabachi,[1] Fayçal Hentati,[2] Mongi Ben Hamida,[2] and Abderraouf Mebazaa[1*]* ([1] Laboratory of Biochemistry, Rabta Hospital, 1007 Tunis, Tunisia; [2] Service of Neurology, National Institute of Neurology, 1007 Tunis, Tunisia; [3] Service of Community Medicine and Epidemiology, Hedi Chaker Hospital, 3029 Sfax, Tunisia; [4] Service of Neurology, Fattouma Bourguiba Hospital, 5000 Monastir, Tunisia; * address correspondence to this author at: Laboratoire de Biochimie Clinique, Hôpital La Rabta, 1007 Eljabbari, Tunis, Tunisia; fax 216-71-570-506, e-mail abderraouf.mebazaa@rns.tn)

Friedreich ataxia (FA) is an autosomal recessive spinocerebellar syndrome with onset before age 25, characterized by progressive cerebellar ataxia, dysarthria, areflexia, sensory loss in lower limbs, pyramidal weakness, and Babinski signs *(1)*. It is caused by an intronic expanded unstable GAA repeat in the frataxin gene *(2)* located on chromosome 9q13-q21 *(3)*. Investigating five Tunisian families with typical FA phenotype, Ben Hamida et al. *(4)* had excluded linkage to the locus of FA in two families and provided evidence for genetic heterogeneity of the disease. Patients belonging to families not linked to the locus of FA showed very low serum vitamin E (VE) with no evidence of lipid malabsorption.

The role of VE in maintaining human nervous system function is established, and the role of VE deficiency in neurologic disorders of a-β-lipoproteinemia and biliary atresia is well accepted *(5, 6)*. Several reports *(4, 7, 8–10)* have described patients with a progressive spinocerebellar syndrome associated with very low serum VE in the absence of fat malabsorption or a-β-lipoproteinemia. This disease, termed ataxia with VE deficiency (AVED), is inherited with an autosomal recessive pattern *(3, 4)*. The abnormal gene was mapped to chromosome 8q *(11)* and identified as the gene encoding for α-tocopherol transfer protein (α-TTP) *(12)*.

Because it is difficult to distinguish on the basis of clinical features between AVED patients in whom VE supplementation may be beneficial *(7, 13, 14)* and those with classic FA, we assessed serum VE, total cholesterol (TC), and triglycerides (TGs) in our patients with FA clinical phenotype and their unaffected family members.