Strikingly Low Circulating CRP Concentrations in Ultramarathon Runners Independent of Markers of Adiposity

How Low Can You Go?

Maciej Tomaszewski, Fadi J. Charchar, Malgorzata Przybycin, Lynne Crawford, A. Michael Wallace, Katarzyna Gosek, Gordon D. Lowe, Ewa Zukowska-Szczechowska, Wladyslaw Grzeszczak, Naveed Sattar, Anna F. Dominiczak

Objective—This study was undertaken to evaluate to what extent C-reactive protein (CRP) can be reduced by exercise by examining its circulating concentrations in male ultramarathon runners and to determine if low leptin as a robust circulating marker of fat mass could account for low CRP in such men.

Methods and Results—Sixty-seven male ultramarathon runners and 63 sedentary male controls of similar age and body mass index were recruited. CRP and leptin were measured by ELISA and radioimmunoassay, respectively. Median CRP concentration in lean (body mass index <25 kg/m²) marathon runners was less than half control median (0.4 [0.2 to 0.9] mg/L versus 0.9 [0.5 to 2.7] mg/L, P=0.0013) and, more strikingly, in nonlean runners was approximately 26% of control median (0.4 [0.3 to 0.8] mg/L versus 1.5 [0.9 to 2.5] mg/L, P=0.0002). Circulating leptin levels were also substantially lower in lean (45% less) and nonlean (63% less, both P<0.0001) ultramarathon runners. However, interleukin-6 levels were not different. Furthermore, leptin adjustment only minimally attenuated the case-control difference in CRP, suggesting that mechanisms other than fat mass reduction contribute to low concentrations of CRP in marathon runners.

Conclusions—This study suggests that circulating CRP concentrations can be markedly suppressed, independently of total adiposity or indeed fat mass, by intense regular physical exercise. (Arterioscler Thromb Vasc Biol. 2003;23:1640-1644.)

Key Words: exercise ■ inflammation ■ leptin ■ C-reactive protein ■ cardiovascular risk

Mounting evidence indicates that increasing levels of physical activity can reduce the number of long-term diseases, including diabetes and coronary heart disease (CHD).1 Favorable effects of regular physical exercise on classical risk factor pathways are well documented. More recently, an improvement of endothelial function2 has been reported, and Wannamethee et al3 noted beneficial effects of ongoing but not a past history of moderate physical activity on hemostasis and inflammation pathways. The latter observation is particularly relevant because inflammatory biomarkers are increasingly recognized as potential predictors or players4,5 in the pathogenesis of both CHD and type 2 diabetes in men and women.6-9

The mechanisms for the beneficial effects of exercise on inflammation-related pathways are not clear, however. A reduction in total adiposity could contribute, because CRP increases with body mass index (BMI) in many studies, and recent data show a reduction in CRP with weight loss.10 However, one could speculate that a specific reduction in fat mass independent of a decrease in total BMI might contribute to CRP reduction, because fat cells synthesize and elaborate several inflammatory markers, including interleukin-6 (IL-6),11 the major driver of hepatic CRP synthesis. In support of this, we noted that leptin, which is an accepted robust circulating surrogate of percent fat mass,12 correlated with CRP independently of BMI in middle-aged men.13 Interestingly, in the latter study we also noted that leptin independently predicted vascular events, and data from other laboratories have also linked elevated leptin to the development of type 2 diabetes.14,15 However, presently there is no joint analysis of CRP and leptin in the context of regular physical exercise. Therefore, it is not known whether a potential inflammation-modulating effect of chronic exercise is entirely dependent on expected parallel reduction in body fat mass. In addition, the extent to which CRP and leptin can be reduced by exercise, important in terms of cardiovascular risk estimates, has not been established.
To address these issues, we compared CRP and leptin serum concentrations in ultramarathon runners (an excellent supraphysiological model of intense physical activity) against sedentary controls of the same sex with similar age, blood pressures, and BMI. We also examined circulating concentrations of IL-6 and adhesion molecules, inflammatory biomarkers with a potential to influence cardiovascular risk.16

Methods

Subjects

Sixty-seven regular long-distance runners along with 63 untrained healthy subjects were included in the study. All participants represented a white, normotensive, nonobese, nonsmoking male population. Chronic disorders, infections, and use of any medication were excluded in each participant. The long-distance runners were recruited before an ultramarathon, Calissia 2000. Each subject was an experienced long-distance runner with a history of participation in at least 2 marathons and a regular weekly training distance of 40 to 100 km per week for at least 2 years. The control subjects reported average low physical activity for a Polish male population (less than 25 kg/m2 for at least 2 years and a regular weekly training distance of 40 to 100 km per week for at least 2 years). Sixty-seven regular long-distance runners along with 63 untrained healthy subjects were included in the study. All participants represented a white, normotensive, nonobese, nonsmoking male population. Chronic disorders, infections, and use of any medication were excluded in each participant. The long-distance runners were recruited before an ultramarathon, Calissia 2000. Each subject was an experienced long-distance runner with a history of participation in at least 2 marathons and a regular weekly training distance of 40 to 100 km per week for at least 2 years. The control subjects reported average low physical activity for a Polish male population (less than 25 kg/m2 for at least 2 years and a regular weekly training distance of 40 to 100 km per week for at least 2 years) and were similar in age, blood pressures, and BMI. We also examined circulating concentrations of IL-6 and adhesion molecules, inflammatory biomarkers with a potential to influence cardiovascular risk.16

Study Design

The Calissia 2000 ultramarathon was an annual amateur sports competition organized in October in Kalisz, Poland. The route of 100 km was delineated in a relatively flat area (138 meters above sea level on average). Phenotyping included taking clinical history by means of standardized questionnaires, physical examination, weight, height, blood pressure measurements, and collection of a blood sample for biochemical analysis.

Each ultramarathon runner was assessed in a resting state at baseline, which was 1 day before the ultramarathon, and a fasting blood sample for biochemical analysis was taken by a standard antecubital venipuncture in a sitting position.

Premarathon blood samples from 23 marathon runners were collected in resting conditions 2 hours before the warm up preceding the run. Postmarathon blood samples were collected approximately 10 minutes after the ultramarathon.

The controls’ phenotyping protocol was arranged in the same baseline conditions as in case of the ultramarathon runners. To avoid any potential bias that may arise from circadian variation of biochemical parameters, the baseline procedures were completed in the morning for both groups. The same medical staff performed phenotyping and collecting of blood samples.

Blood samples were left for clotting at room temperature. Serum was separated from blood samples by centrifugation (for 10 minutes) within 2 hours and stored at −70°C until biochemical analysis. All analyses were measured on samples that had not been previously thawed.

Biochemical Parameters

Soluble intercellular cell adhesion molecule (sICAM-1) and E-selectin serum concentrations were assessed by quantitative sandwich enzyme immunoassay technique (R&D Systems). The interassay and intraassay coefficients of variance were below 5% and 10%, respectively. CRP was measured using a sensitive double antibody sandwich ELISA with rabbit antihuman CRP and peroxidase-conjugated rabbit anti-human CRP, as described in detail before.8,17 The interassay and intraassay coefficients of variation were less than 10% across the range of measured results. Circulating concentrations of leptin were measured according to the protocol used previously in the West of Scotland Coronary Prevention Study (WOSCOPS)13 by an in-house radioimmunoassay validated thoroughly against the commercially available Linco assay. The interassay and intraassay coefficients of variation were below 7% and 10%, respectively, over the sample concentration range. Circulating concentrations of IL-6 were evaluated using a high-sensitivity human IL-6 immunosay (R&D Systems). The interassay and intraassay coefficients of variance were both less than 10%.

Statistical Analysis

The data were expressed as mean (±SD) or median (25th to 75th percentile). Unpaired t test (with Welch’s correction where appropriate) and Mann–Whitney test were used to compare quantitative traits between 2 groups, as appropriate. The comparisons between preexercise and postexercise values were analyzed by a parametric one-sample t test or a nonparametric Wilcoxon’s test. Pearson’s linear correlation was used to test for associations between biochemical and clinical variables within the groups using log-transformed CRP, leptin, and IL-6 values. Multiple linear regression was used to examine whether adjusting for BMI, leptin, and IL-6 concentrations attenuated differences in CRP between groups.

Results

Baseline Characteristics

There were no statistically significant differences in age or BMI and mean arterial pressure between ultramarathon runners (cases) and the control subjects (controls) either in the lean or nonlean category (Table 1). sICAM-1 serum level was

<table>
<thead>
<tr>
<th>TABLE 1. Clinical and Biochemical Characteristics of the Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean, BMI &lt; 25 kg/m2</td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
</tr>
<tr>
<td>sICAM-1, ng/mL</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
</tr>
<tr>
<td>CRP, mg/L</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
</tr>
<tr>
<td>IL-6, pg/mL</td>
</tr>
</tbody>
</table>

Data are mean (SD) or median (interquartile range). MAP indicates mean arterial blood pressure.
The percent of individuals with CRP <1 mg/L in lean ultramarathon runners, lean controls, nonlean ultramarathon runners, and nonlean controls. Note that whereas significantly fewer nonlean controls had CRP <1 mg/L compared with lean controls, the percent of marathon runners with CRP <1 mg/L was similar in the lean and nonlean groups (P=0.85). MR indicates marathon runners.

significantly lower in the ultramarathon group than in the controls only among nonlean individuals. There were no significant differences in E-selectin and IL-6 levels between the groups (Table 1).

The median CRP concentration in lean ultramarathon runners was less than half that of controls and in nonlean cases less than a third of controls (Table 1). Indeed, the 75th percentile of CRP in lean ultramarathon runners was less than the median in controls, and in nonlean cases it was similar to the 25th percentile of controls despite similar BMI.

The proportion of lean and nonlean ultramarathon runners with CRP <1 mg/L was calculated (this cut off was proposed as the reference CRP value in evaluation of cardiovascular risk by the recent American Heart Association consensus statement). A total of 76% and 83% of lean and nonlean marathon runners, respectively, were below this cut off compared with 57% of lean and 30% of nonlean controls (Figure). The median leptin concentration in lean marathon runners was approximately half that of controls, and in nonlean cases it was approximately one third that of controls (Table 1).

**Relationships Among CRP, BMI, Leptin, and IL-6 in Cases and Controls**

Serum CRP concentration did not correlate with BMI in the ultramarathon (n=67) or the control (n=63) groups. However, BMI correlated positively with serum leptin levels in both the ultramarathon (r=0.31, P=0.01) and the control (r=0.28, P=0.02) groups. Serum CRP levels correlated positively with leptin (r=0.33, P=0.009) and sICAM-1 (r=0.451, P=0.0002) concentrations in controls but not cases. A positive correlation between circulating concentrations of CRP and IL-6 was evident in sedentary men (r=0.62, P<0.0001) and ultramarathon runners (r=0.32, P=0.01). Leptin levels correlated positively with IL-6 levels in controls (r=0.34, P=0.007) but not in cases.

The influence of markers of adiposity (BMI and leptin) on the difference in CRP between cases and controls was examined in Table 2. BMI and leptin adjustments only modestly attenuated the difference in CRP concentration between cases and controls in both lean and nonlean subjects. Additional adjustment for IL-6 also had minimal effect.

**Acute Ultramarathon Effects on Adhesion Molecules, CRP, and Leptin Concentrations**

There were no differences in postmarathon sICAM-1 and sE-selectin serum concentrations compared with the baseline values (Table 3). There was a 6-fold rise (P<0.0001) in median CRP concentration in the 23 ultramarathon runners who gave samples both before and 10 minutes after running the ultramarathon (Table 3). The postrun median CRP was only 1.8 mg/L, however. Median leptin concentration fell by half after the ultramarathon (P<0.0001) (Table 3).

**Discussion**

The results of this study show that CRP concentrations are markedly lower in the resting state in ultramarathon runners compared with sedentary controls of similar BMI. Indeed, the median CRP 0.4-mg/L (or geometric mean ~0.45-mg/L) concentrations for lean and nonlean marathon runners were identical, and more than 75% had a CRP concentration <1 mg/L, a value below which diabetes and CHD risk are very low. Moreover, whereas 8 of the 12 nonlean marathon runners (67%) had a CRP <0.66 mg/L, the cut off for the lowest quintile of CRP in a recent study by Freeman et al, only 2 of 33 (6.1%) nonlean controls did so, an 11-fold difference. These observations help place the CRP data from ultramarathon runners into a clinical context, because the lowest quintile of CRP in WOSCOPS was associated with one third of the risk of diabetes and a 50% lower CHD risk compared with the highest CRP quintile after adjustment for classical predictors.

**TABLE 3. Premarathon and Postmarathon Concentrations of sICAM-1, E-selectin, CRP, and Leptin**

<table>
<thead>
<tr>
<th></th>
<th>Premarathon</th>
<th>Postmarathon</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>sICAM-1, ng/mL</td>
<td>203.7 (55.7)</td>
<td>192.4 (46.6)</td>
<td>NS</td>
</tr>
<tr>
<td>E-selectin, ng/mL</td>
<td>60.2 (28.4)</td>
<td>60.1 (28.0)</td>
<td>NS</td>
</tr>
<tr>
<td>CRP, mg/L</td>
<td>0.3 (0.2–0.7)</td>
<td>1.8 (1.0–3.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Leptin, ng/mL</td>
<td>1.7 (1.2–2.2)</td>
<td>0.9 (0.5–1.3)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are mean (SD) or median (interquartile range).
We sought to examine if lower leptin as a surrogate of fat mass partly explained the lower CRP concentrations in marathon runners, because fat cells elaborate a range of inflammatory markers, such as IL-6. In support of our hypothesis, we noted that magnitudes of difference in CRP and leptin concentrations between cases and controls were remarkably similar in lean and nonlean groups. We also noted that leptin correlated with CRP in controls but that BMI did not. This finding agrees with previous evidence of an independent correlation of leptin with CRP in middle-aged men. However, there was no linear correlation between CRP and leptin levels in ultramarathon runners, in contrast with nonexercising controls. Furthermore, both lean and nonlean ultramarathon runners were almost identical in terms of their CRP levels despite a significant difference in BMI. Finally, adjustment for markers of adiposity (BMI and leptin) only modestly attenuated the difference in CRP between cases and controls. These data indicate that factors other than simple reductions in percent body fat might account for the lower CRP concentration in marathon runners. Given that exercise increases insulin sensitivity and recent evidence that insulin exhibits anti-inflammatory effects, we might speculate that insulin’s anti-inflammatory effects, be they on adipocytes, circulating monocytes, or indeed endothelial cells, are heightened in marathon runners. This suggestion requires direct examination in future studies, but it is notable that several studies report a strong correlation between CRP and insulin sensitivity and that CRP predicts diabetes development.

The lower leptin levels in ultramarathon runners can also be placed into cardiovascular context, because we have reported that high leptin levels predict risk for vascular events independently of classical risk factors and CRP in middle-aged men. The case-control leptin difference in this study was particularly marked in the nonlean subjects; ultramarathon runners had a median leptin of 2.8 mg/L, whereas control leptin was nearly 3-fold higher at 7.5 mg/L (Table 1). Extrapolating from data in the WOSCOPS, we might speculate that insulin’s anti-inflammatory effects, be they on adipocytes, circulating monocytes, or indeed endothelial cells, are heightened in marathon runners. This suggestion requires direct examination in future studies, but it is notable that several studies report a strong correlation between CRP and insulin sensitivity and that CRP predicts diabetes development.

In summary, a supraphysiological model of aerobic performance used in the present study helped to assess the extent to which inflammatory status (and thus cardiovascular risk) may be influenced by regular intense physical exercise. It also indicated that metabolic pathways other than reduction in percent body fat mass or indeed IL-6 levels might be involved in regulation and maintenance of this effect. Finally, this study showed that engagement in ultrastrenuous aerobic
performance, although leading to a transitory minor increase in CRP, does not affect negatively endothelial markers of cardiovascular risk.

Acknowledgments

This study was supported by International Society of Hypertension Clinical Research Fellowship, the Wellcome Trust International Research Development Award (PG2000023), and the European Commission EURUNETGEN QLG1-2001-01137 Program within EU Framework 5 (to A.F.D.). F.J.C. is supported by a Wellcome Trust Traveling Fellowship.

References


Strikingly Low Circulating CRP Concentrations in Ultramarathon Runners Independent of Markers of Adiposity: How Low Can You Go?
Maciej Tomaszewski, Fadi J. Charchar, Małgorzata Przybycin, Lynne Crawford, A. Michael Wallace, Katarzyna Gosek, Gordon D. Lowe, Ewa Zukowska-Szczechowska, Władysław Grzeszczak, Naveed Sattar and Anna F. Dominiczak

Arterioscler Thromb Vasc Biol. 2003;23:1640-1644; originally published online July 17, 2003; doi: 10.1161/01.ATV.0000087036.75849.0B
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/9/1640