Strikingly Low Circulating CRP Concentrations in Ultramarathon Runners Independent of Markers of Adiposity
How Low Can You Go?

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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:lll-lll.)

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 players 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.

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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 uninformed 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 2 hours weekly of walking or swimming) and were similar in age, BMI, and blood pressure to the ultramarathon group. After the approval of the local bioethical committee, informed consent was obtained from each subject.

Both the study and the control group were divided into 2 categories of lean and nonlean individuals based on the criterion of BMI (< 25 kg/m²). The clinical characteristics of the subjects are presented in Table 1.

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.

Pre-marathon blood samples from 23 marathon runners were collected in resting conditions 2 hours before the warm up preceding the run. Post-marathon 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 immunoassay (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 significantly lower in the ultramarathon group than in the

<table>
<thead>
<tr>
<th>TABLE 1. Clinical and Biochemical Characteristics of the Subjects.</th>
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<tbody>
<tr>
<td><strong>Lean, BMI &lt;25 kg/m²</strong></td>
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<tr>
<td>Marathon Runners</td>
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<tr>
<td>No.</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>BMI, kg/m²</td>
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<td>MAP, mm Hg</td>
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<tr>
<td>sICAM-1, ng/mL</td>
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<tr>
<td>E-selectin, ng/mL</td>
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<td>CRP, mg/L</td>
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<td>Leptin, ng/mL</td>
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<td>IL-6, pg/mL</td>
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MAP indicates mean arterial blood pressure.
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 statement16). 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

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| Table 3. Premarathon and Postmarathon Concentrations of sICAM-1, sE-selectin, CRP, and Leptin |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                             | Premarathon     | Postmarathon    | P               |                 |                 |                 |
| sICAM-1, ng/mL                              | 203.7 (55.7)    | 192.4 (46.6)    | NS              |                 |                 |                 |
| E-selectin, ng/mL                           | 60.2 (28.4)     | 60.1 (28.0)     | NS              |                 |                 |                 |
| CRP, mg/L                                   | 0.3 (0.2–0.7)   | 1.8 (1.0–3.4)   | <0.0001         |                 |                 |                 |
| Leptin, ng/mL                               | 1.7 (1.2–2.2)   | 0.9 (0.5–1.3)   | <0.0001         |                 |                 |                 |
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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 \(\approx 0.45\)-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.\(^8,17\) 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.,\(^8\) 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.\(^8,17\)

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.\(^11\) 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 with hypercholesterolemia.\(^13\) 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,\(^18,19\) we might speculate that insulin’s anti-inflammatory effects, be they on adipocytes, circulating monocytes, or indeed endothelial cells, are heightened in marathon runners. Alternatively, because leptin regulates T cell responses,\(^27\) circulating leptin may polarize T cells toward a proinflammatory Th1 phenotype,\(^22\) and may contribute to arterial thrombosis after vascular injury through the platelet leptin receptor.\(^23\) High leptin levels may promote vascular disease directly.

In addition to a positive effect on CRP and leptin, intense physical exercise exerted a beneficial influence on serum sICAM-1 concentration, at least in the nonlean group of subjects. Serum sE-selectin concentrations were not different among the cases and controls, however. Because there is a selective expression of E-selectin on endothelial cells\(^25\) but several sources of sICAM-1 release (thrombocytes and monocytes), our results suggest that a beneficial effect of regular physical exercise may be not limited to endothelium but rather to a simultaneous effect on several intravascular cellular components.

The lack of difference in circulating concentrations of IL-6 between ultramarathon runners and nonexercising controls is in agreement with the data from a smaller study by Bonsignore et al.\(^26\) and supports the notion that IL-6 and CRP, despite physiological relationships evident in acute inflammatory conditions, may be less strongly associated in the context of low-grade chronic inflammation.\(^26\) IL-6 is a pleiotropic cytokine secreted by many cells, including adipocytes as well as stimulated monocytes, fibroblasts, endothelial cells, and smooth and skeletal muscle cells.\(^27\) Consequently, one possibility of the lack of case-control difference in IL-6 could be a counterbalanced stimulation of IL-6 synthesis in several other tissues, such as skeletal muscle cells in ultramarathon runners. Alternatively, because of diurnal variations in circulating concentrations of IL-6 and considerably longer half-life of plasma CRP, the latter may represent a more stable marker of subclinical inflammatory status and a more appropriate integrated measure of basal IL-6.\(^16\) A final possibility for significantly lower CRP levels in marathon runners despite similar IL-6 levels is that exercise may stimulate synthesis of other, as yet unidentified factors that act either to attenuate the IL-6 stimulation of hepatic CRP synthesis or alter CRP synthesis independently of IL-6. This suggestion clearly requires additional studies.

The lack of changes in sICAM-1 and sE-selectin concentrations after the ultramarathon suggests that even a short-term exposition to a high-intensity aerobic exercise may not affect endothelium negatively in healthy men. Rather, well-known relationships between exercise and endothelial function are probably related to slow-progressing time-dependent mechanisms. These results agree with other data suggesting lack of\(^28\) or minor\(^29\) influence of aerobic performance on adhesion molecules after exercise from small to moderate intensity in healthy subjects. Postexercise CRP levels increased, in agreement with the results obtained after a less strenuous exercise,\(^30\) whereas leptin levels fell. These changes suggest an acute immunological activation after a marathon and likely relate to skeletal muscle injury leading to the same assay and technician performed leptin measurements, we estimate that ultramarathon runners have an approximately 40% lower risk of vascular disease compared with sedentary controls. Mechanistically, circulating leptin may signal vascular risk by virtue of its association with fat mass. Alternatively, because leptin regulates T cell responses, polarizing T cells toward a proinflammatory Th1 phenotype,\(^22\) and may contribute to arterial thrombosis after vascular injury through the platelet leptin receptor,\(^23\) high leptin levels may promote vascular disease directly.

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an inflammatory process followed by repair reaction.\textsuperscript{31} However, it should be noted that the median CRP after exercise was still only 1.8 mg/L. A minor limitation in the postexercise results could have been the potential for confounding by dehydration. However, a lack of changes in markers of plasma volume during marathon (related to compensatory production of metabolic water as well as substantial fluid substitution) shown in several studies\textsuperscript{30,32–34} is unlikely to explain the differences between preexercise and postexercise values of CRP and leptin. Furthermore, the opposite direction of change in CRP and leptin levels after the ultramarathon is in agreement with other studies\textsuperscript{30,35} and suggests genuine changes in the concentrations of these parameters.

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.

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References


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