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Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners

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Summary

Objective: Marathon runners have an increased risk of developing joint disease. During and after a 42-km run, elevation of multiple cytokines occurs in the blood, reflecting inflammatory processes. We compared this cytokine response with serum levels of cartilage oligomeric matrix protein (COMP) and melanoma inhibitory activity (MIA), two markers for joint metabolism and/or damage.

Methods: Serum from eight endurance-trained runners was collected shortly before the start of a marathon run, after 31 km, 42 km, 2 h after the end, on the first and on the second morning after the run. For comparison, serum was obtained from 35 healthy controls and 80 patients with knee joint injury, rheumatoid arthritis or osteoarthritis. Serum levels of C-reactive protein (CRP), interleukin-1β (IL-1β), interleukin-1 receptor antagonist (IL-1RA), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), soluble interleukin-6 receptor (sIL-6R, gp80), soluble tumor necrosis factor receptor II (sTNFRII, p75), COMP and MIA were measured by ELISA.

Results: Compared with healthy controls, the runner's baseline serum levels of TNF-α, sIL-6R, COMP and MIA were significantly increased. COMP and MIA levels, higher than the upper normal limits of 5 μg/ml and 6 ng/ml respectively, were found in seven and five of eight runners. The elevated levels of COMP were similar to those found in joint injury or osteoarthritis, and the elevated levels of MIA were comparable to those reported in rheumatoid arthritis. During the run, the serum levels of IL-1RA, IL-6, TNF-α and COMP rose significantly, and gradually returned to baseline within 24 h. Only modest changes of CRP, sIL-6R, sTNFRII and MIA occurred during the run. Late elevations of CRP and MIA were observed after 24 and 48 h. The correlation analysis suggests associations between COMP, sIL-6R, TNF-α, IL-1RA on one hand and sTNFRII, and MIA and CRP on the other hand.

Conclusions: Elevated baseline levels of COMP and MIA might reflect increased joint matrix turnover and/or damage due to prior extreme physical training. During the run, COMP was increasing possibly due to the severe physical strain on joint structures, associated with the early inflammation. After the run, MIA and CRP increased within 24 h, suggesting a correlation with later inflammatory processes. Thus, our data suggest that COMP and MIA are markers for distinct aspects of joint metabolism and/or damage in both disease and sport. © 2000 OsteoArthritis Research Society International

Key words: Marathon runners, Cytokines, COMP, MIA.

Introduction

Chondrocytes interact with various components of the articular cartilaginous matrix. One of these macromolecules is the 524 kDa pentameric glycoprotein called cartilage oligomeric matrix protein (COMP).[1,2] In humans, COMP is produced in articular cartilage by chondrocytes, and in tendon, meniscus, and synovial tissue by fibroblasts.[3–6] In synovial fluid, increased amounts of COMP have been reported after knee injury, in early stages of osteoarthritis (OA), and in active reactive arthritis.[7,8] In contrast, in patients with advanced destruction of joints due to rheumatoid arthritis (RA) or reactive arthritis, COMP is decreased.[3,9] The COMP fragmentation pattern in synovial fluid appears to be a potential diagnostic marker for RA.

These fragments appear by enzymatic degradation of cartilage mediated by serine proteases and matrix metalloproteinases.[3,10] In serum, elevated levels of COMP occur in knee trauma, early stages of OA, RA and in reactive arthritis.[3,8,9,11–13] Interestingly, in patients with knee injury, a subgroup showed persistently elevated serum levels of COMP.[13] These patients, particularly those with autoantibodies against COMP, appear to have an increased risk of developing post-traumatic OA.

Recently, an 11 kDa protein called melanoma inhibitory activity (MIA) has been purified from metastatic melanoma cell line supernatants.[14] In malignancies, elevated serum levels of MIA are predominantly found in metastatic melanoma and to a lesser extent in ovarian, pancreatic and breast cancer,[15] whereas in non-malignant tissues, MIA production is predominantly found in cartilage.[16,17] Highly elevated serum levels of MIA were also found in patients with RA, associated with positive rheumatoid factors and joint destruction.[18]

Marathon runners have an increased risk of developing joint disease.[19] Erosive joint lesions can frequently be observed.[20,21] In the ankle, degenerative changes of the calcaneous tendon, tendosynovitis of the flexor hallucis longus, joint effusions and cartilage damage have been detected by magnetic resonance tomography.[22] An intensive cytokine response [i.e., interleukin-1 receptor antagonist (IL-1RA), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α)] occurs during and/or after prolonged running.[23–26] The intensive training and the run itself induce inflammation and muscle fiber necrosis which is reflected in rhabdomyolysis and myoglobinuria.[27] Many changes may simply reflect extreme training and the effects of repetitive strain on soft tissue and cartilagenous structures. It is difficult, however, to differentiate between primary cartilage damage and secondary inflammation. We compared the serum levels of COMP and MIA between marathon runners, non-running healthy subjects and patients with various joint diseases, and we examined whether they can be used as markers for joint metabolism in sport medicine. In addition, the kinetics of COMP and MIA concentrations in serum before, during and after the run were compared with levels of markers for inflammation, i.e., C-reactive protein (CRP), circulating cytokines (interleukin-1β (IL-1β), IL-1RA, TNF-α, and IL-6) and soluble cytokine receptors (interleukin-6 receptor gp80 (sIL-6R), and tumor necrosis factor type II p75 (sTNFRII), the most specific receptor for TNF-α). Some of these systemic cytokines are known to be increased during marathon running (TNF-α, IL-6), while other markers may reflect counter-regulatory mechanisms (IL-1RA, soluble receptors). We investigated whether this systemic response is associated with changes in two markers of joint metabolism (COMP and MIA).

Materials and methods

Serum from eight endurance-trained runners (25 to 34 years old) was obtained from the participants of a non-competitive marathon, in whom the metabolic influence of a protein-supplemented carbohydrate drink was studied by other investigators.[28] Since there was no treatment difference for the parameters discussed in the present study, only the control trial data from each runner are presented here. Daily conditioning exercise was allowed, but the athletes did not run for at least 3 weeks before the test. Blood samples were collected from an antecubital vein, at the same arm at all times points: shortly before the start of the run (T0), after 31 km (T1), after 42 km (T2), 2 h after the end of the run (T3), on the first (T4) and on the second morning (T5) after the run. The running time in this non-competitive marathon ranged from 3 h 02 min to 3 h 48 min. At the end of the run, no signs of dehydration was detectable. No physical activity was allowed for 2 days after the run. Serum were also obtained from non-running healthy volunteers (N=35; 16 controls who had never run in any competitive race were age-and sex-matched and used for statistical purposes). In addition, serum was also drawn from patients with knee joint injury (N=30),[13] RA (N=30) and OA (N=20). RA and OA patients fulfilled the criteria of the American College of Rheumatology.[29,30]

C-REACTIVE PROTEIN AND CYTOKINES

Serum levels of CRP (Eurogenetics CRP, Tenssenderlo, Belgium), TNF-α, IL-6 (Coaliza TNF-α and IL-6, Kabi Diagnostica AB, Mölndal, Sweden), IL-1β, IL-1RA, sIL-6R gp80, and sTNFRII p75 (Quantikine IL-1β HS, IL-1RA, sIL-6R and sTNFRII, R&D Systems, Abingdon, United Kingdom) were evaluated by ELISA using commercially available kits. The cut-off of 'normal' serum level of CRP was established by use of 80 sera from healthy donors.

CARTILAGE OLIGOMERIC MATRIX PROTEIN

Serum levels of COMP were measured by competitive ELISA as previously described.[3] Briefly, microtiter plates were coated with 1 μg/ml purified human COMP diluted in phosphate buffered saline (PBS), kept overnight at 4°C and blocked with 1% bovine serum albumin (BSA, Sigma, St. Louis, MO, U.S.A.) in PBS for 2 h at 22–24°C. In a separate plate, serum was diluted in PBS (1:10 to 1:40). The samples were preincubated with an equal volume of rabbit polyclonal antiserum overnight at 4°C. The solutions were transferred to the coated plates and incubated for 1 h at 22–24°C. The plates were washed with PBS containing 0.05% Tween-20 (Merck, Dietikon, Switzerland). The bound anti-COMP antibodies were incubated with horse-radish peroxidase (HRP)-conjugated swine anti-rabbit IgG (Dako, Zug, Switzerland), diluted 1:200 in PBS with 1% BSA, for 1 h at 22–24°C. The wells were washed and bound antibodies were visualized by means of 0.25 mg/ml 5-amino-2-hydroxy benzoic acid (Sigma), pH 6.0, as substrate, in the presence of 0.00024% H_2O_2. The reaction was stopped with 2 N NaOH and the absorbance was read at 490 nm. Standardization was performed with COMP derived from human articular cartilage.

MELANOMA INHIBITORY ACTIVITY

Serum levels of MIA were determined by sandwich ELISA as previously described.[15] Briefly, two monoclonal antibodies directed against 14-meric NH2-terminal and COOH-terminal peptides (monoclonal antibodies 1A12 and 2F7, Boehringer Mannheim, Germany) were conjugated to HRP and biotin, respectively. Ten μl of serum or MIA standards were incubated with 200 μl reagent containing HRP-1A12 and biotinylated-2F7 antibodies in a streptavidin-coated microtiter plate for 45 minutes with continuous shaking. After washing three times in PBS, 200 μl of 2,2′-azino-di-(3)-ethylbenz-thiazoline sulfonate (Boehringer Mannheim) was added to the wells and absorbance was measured at 405 nm. Standardization was performed with MIA derived from transfected Chinese hamster ovary cells.

STATISTICS

Inter- and intra-assay variation and limits of sensitivity for COMP and MIA have been reported previously.[3,15] In our study, COMP and MIA serum concentrations were compared between matched controls and baseline levels in runners (T0), by means of the Mann-Whitney U-test (SPSS 8.0). Concentration changes over the course of time (T0 to T5) were evaluated with the Wilcoxon matched pair test. Mutual relationships among variables were established using Spearman's rank correlation coefficients. P-values <0.05 were considered as significant.
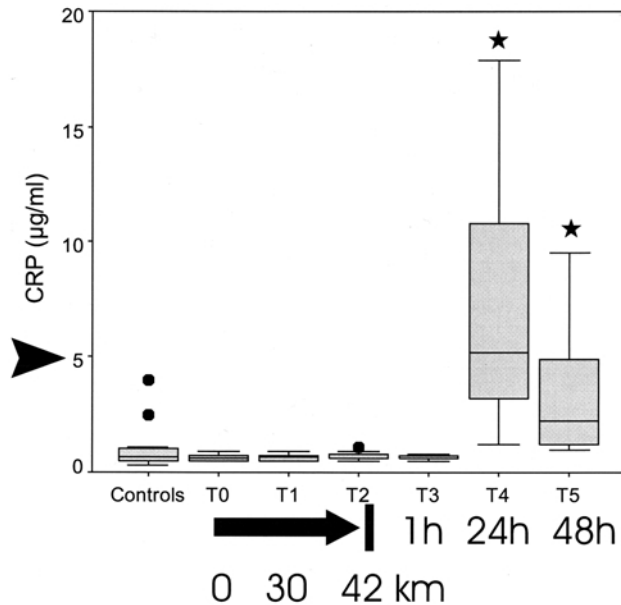
Fig. 1. Box blots of serum C-reactive protein (CRP) in age-matched healthy controls (N=16) and marathon runners (baseline T0, N=8). The upper normal limit (mean plus 2 standard deviations of 35 healthy controls) is shown by the arrow (left). Serum was taken during the run (T1, 31 km), at finish (T2, 42 km), after 2 h of recovery (T3), as well as 24 h (T4) and 48 h (T5) after the run. Serum levels of CRP were significantly elevated at T4 and T5 (P<0.001, compared with T0, closed stars).

Results

C-REACTIVE PROTEIN

Compared with 16 age- and sex-matched controls, serum levels of CRP in marathon runners were not increased before (T0), during (T1, T2) nor immediately after the run (T3) (Fig. 1). On the other hand, CRP increased significantly within 24 h (T4) and remained elevated after 48 h (T5) (P<0.001 for both times, compared with either controls or T0). At T4, four of eight runners showed CRP levels higher than the upper normal limit of 5 μg/ml.

INTERLEUKIN-1β AND INTERLEUKIN-1 RECEPTOR ANTAGONIST

Concentrations of 0.2 to 9.1 pg/ml IL-1β and 85 to 490 pg/ml IL-1RA were measured in the serum of 35 healthy individuals. Regarding the 16 matched controls, medians were 1.9 pg/ml and 110 pg/ml, respectively (Fig. 2). In marathon runners, no significant difference was found for the serum levels of IL-1β, before, during or after the run. In contrast, a significant but transient increase of IL-1RA occurred during the run. At T0, the median level of IL-1RA was similar in runners (95 pg/ml) and controls, whereas the medians at T1, T2 and T3 reached significantly higher values: 260, 485 and 1195 pg/ml (P<0.001, compared with either controls or T0). At T3, six of eight runners showed higher IL-1RA levels than the upper normal limit of 490 pg/ml. This increase returned to baseline within 24 h.

INTERLEUKIN-6 AND SOLUBLE INTERLEUKIN-6 RECEPTOR

Concentrations of 0.3 to 19.0 pg/ml IL-6 and 8 to 57 pg/ml sIL-6R were measured in the serum of healthy individ-
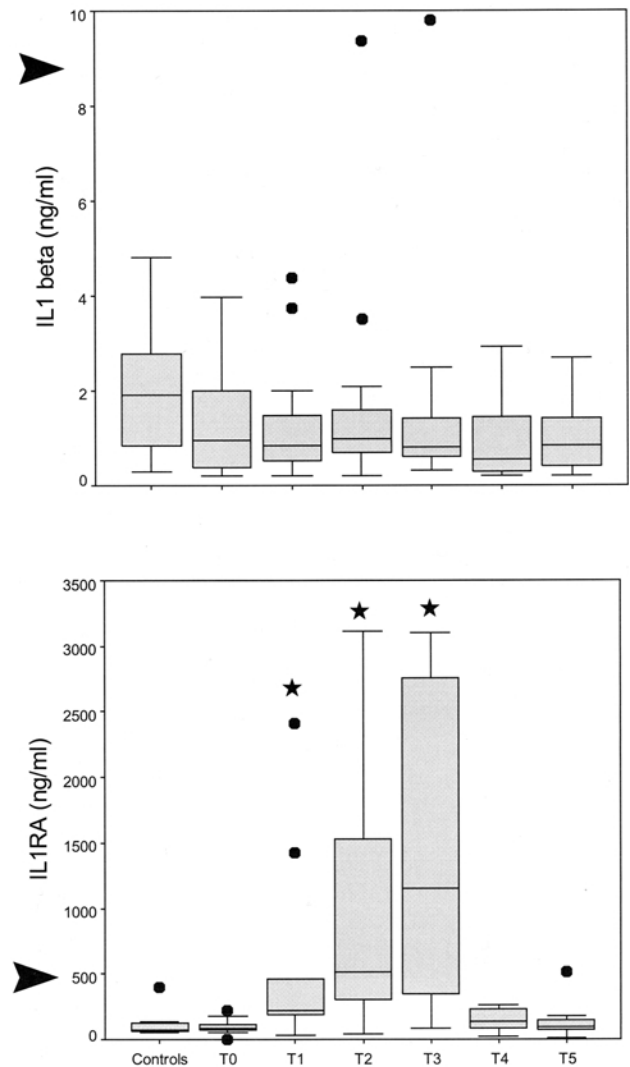

Fig. 2. Serum levels of interleukin-1β (IL-1 beta, upper panel) and interleukin-1 receptor antagonist (IL-1RA, lower panel) in age-matched healthy controls (N=16) and marathon runners (baseline T0, N=8). The upper normal limits (means plus 2 standard deviations of 35 healthy controls) are shown by the arrows (left). The fluctuations of interleukin-1β did not reach a level of significance. In contrast, during (T1, T2) and 2 h after the run (T3), serum levels of IL-1RA were significantly elevated (P<0.001, compared with T0, closed stars).

uals. In matched controls, medians were 5.8 and 21 pg/ml, respectively (Fig. 3). At T0, IL-6 was decreased in runners (median 1.8 pg/ml), while sIL-6R was increased (median 77 pg/ml) (P<0.01 and<0.001, compared with age- and sex-matched controls). Compared with T0, IL-6 increased during the run, at T1 and T2 (medians 8.7 and 9.8 pg/ml, P<0.001 for both), and gradually decreased to baseline within 24 h (T3, 5.1 pg/ml, P<0.001; T4, 2.2 pg/ml and T5, 2.1 pg/ml, not significant). Considering all samples and time points (N=83 values), IL-6 correlated with IL-1RA (r=0.53, P<0.001) and CRP (r=0.29, P<0.01).

Compared with T0, changes in sIL-6R during the run were not statistically significant. However, levels of sIL-6R, in contrast to IL-6, were higher than the normal upper limit of 45 pg/ml, as defined by the mean plus 2 standard
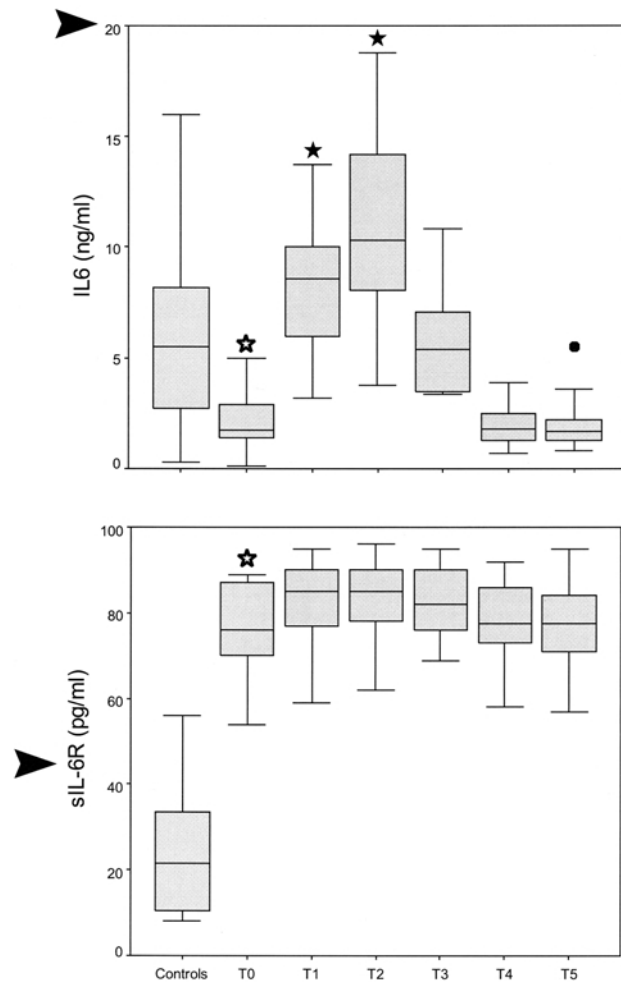
Fig. 3. Serum levels of interleukin-6 (IL-6, upper panel) and soluble interleukin-6 receptor (sIL-6R, lower panel) in matched healthy controls ($N=16$) and marathon runners (baseline T0, $N=8$). The upper normal limits (means plus 2 standard deviations of 35 healthy controls) are shown by the arrows (left). At T0 (start), the serum level of IL-6 was decreased in runners, while the serum level of soluble IL-6R was increased ($P<0.01$ and <0.001, open stars), compared with controls. During the run (T1, T2), the serum level of IL-6 increased ($P<0.001$ for both, closed stars) and gradually decreased to baseline within 24 h (T4). Compared with T0, changes of sIL-6R during the run were not statistically significant.
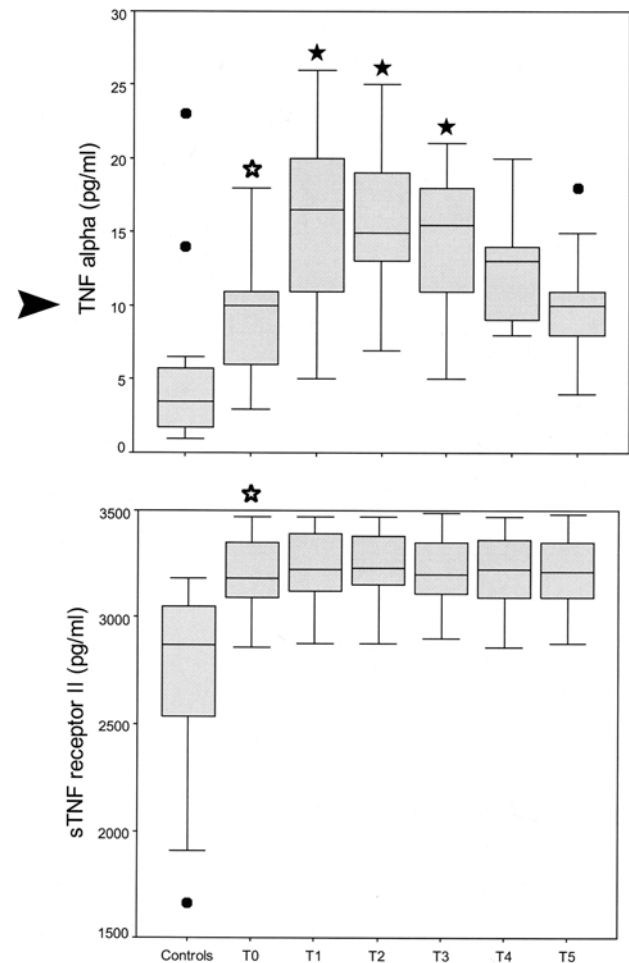

Fig. 4. Serum levels of tumor necrosis factor-α (TNF alpha, upper panel) and soluble tumor necrosis factor receptor II (TNFRII/p75, lower panel) in matched healthy controls ($N=16$) and marathon runners (baseline T0, $N=8$). The upper normal limit of TNF-α (means plus 2 standard deviations of 35 healthy controls) is shown by the arrow (left). The fluctuations of sTNFRII are found within normal limits. At T0 (start), the serum levels of both TNF-α and sTNFRII were increased in runners ($P<0.02$ and 0.01, open stars), compared with controls. During the run (T1 and T2), the serum level of TNF-α increased ($P<0.01$ and <0.005), remained elevated 2 h after the run (T3, $P<0.02$, closed stars) and gradually decreased to baseline within 24 to 48 h (T4 and T5). Before and during the run, fluctuations of sTNFRII are measured within normal limits and were not statistically significant.

deviations of concentrations measured in 35 healthy controls.

TUMOR NECROSIS FACTOR-α AND SOLUBLE TUMOR NECROSIS FACTOR RECEPTOR II

Concentrations of 0 to 7.0 pg/ml TNF-α and 1955 to 3925 pg/ml sTNFRII were measured in the serum of healthy individuals. In matched controls, medians of TNF-α and sTNFRII were 3.2 and 2850 pg/ml, respectively (Fig. 4). In runners, baseline serum levels of TNF-α and sTNFRII were significantly elevated (medians 9.7 and 3180 pg/ml, $P<0.02$ and 0.01 respectively, compared with matched controls). Compared with T0, TNF-α increased during the run, at T1 and T2 (medians 16.6 and 14.3 pg/ml, $P<0.01$ and <0.005), remained elevated 2 h after finish (T3,

15.1 pg/ml, $P<0.02$) and gradually decreased to baseline within 24 to 48 h (T4 and T5, 13.1 and 10.2 pg/ml, non significant). From T1 to T3, seven of eight runners showed higher TNF-α levels than the upper normal limit of 10.1 pg/ml.

In contrast to TNF-α, concentrations of sTNFRII during the run remained within the normal limits (up to 3750 pg/ml) and changes were not statistically significant. Nevertheless, considering all samples and time points ($N=83$ values), the modest alterations of sTNFRII occurred in parallel with TNF-α ($r=0.26$, $P<0.05$).

CARTILAGE OLIGOMERIC MATRIX PROTEIN

Compared with matched controls (median 1.8 µg/ml), serum levels of COMP in runners were increased before
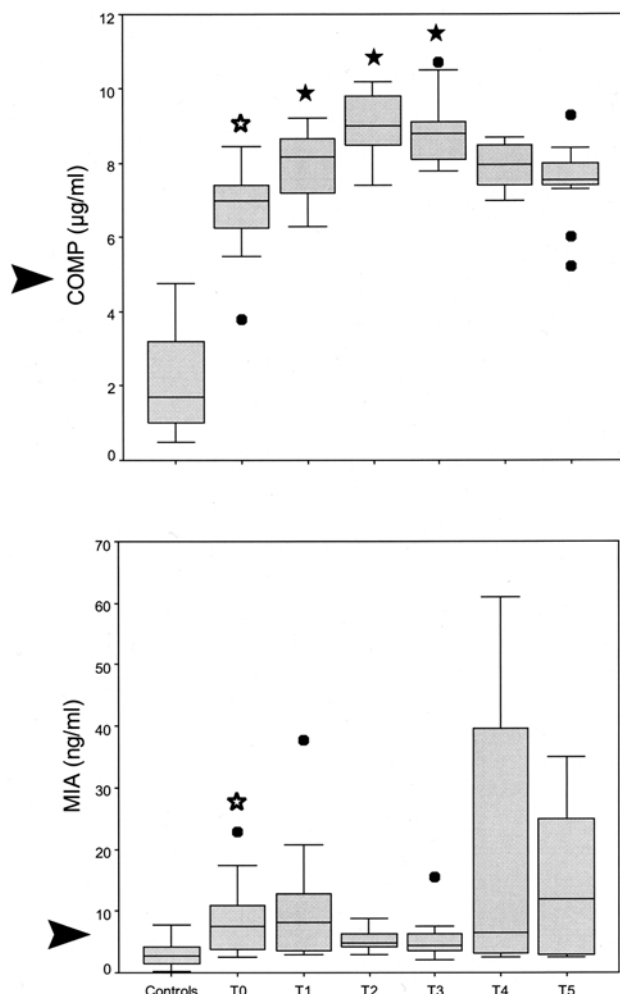
Fig. 5. Serum levels of cartilage oligomeric matrix protein (COMP, upper panel) and melanoma inhibitory activity (MIA, lower panel) in matched healthy controls (*N*=16) and marathon runners (baseline T0, *N*=8). The upper normal limits of COMP and MIA (means plus 2 or 3 standard deviations of 35 or 100 healthy controls, respectively) are shown by the arrows (left). At T0 (start), the serum level of COMP and MIA were increased in runners (*P*<0.001 and <0.01, open stars), compared with controls. During the run, COMP significantly increased (T1 to T3, *P*<0.02 to <0.001 compared with T0, closed stars). After the run, COMP gradually returned to baseline within 24 to 48 h (T4 and T5). During and immediately after the run (T1 and T2), fluctuations of MIA were not statistically significant. In five of eight runners, MIA was found to be elevated 24 to 48 h after the run (T4 and T5).

start (T0, median 7.1 µg/ml, *P*<0.001) (Fig. 5, upper panel). In six healthy individuals, COMP concentrations between 8–10 am and 3–5 pm showed no significant diurnal variation (i.e., between −19 and +8%, *P*=0.89). At T0, baseline levels of COMP in seven of eight runners were higher than the upper normal limit of 5.0 µg/ml. COMP was significantly increased in runners, even when compared with the elevated concentrations in knee joint injury (median 5.7 µg/ml, *P*<0.01) or OA (median 6.0 µg/ml, *P*<0.05). During the run, COMP significantly increased (T1, T2 and T3, medians 8.2, 8.8 and 9.1 µg/ml, *P*<0.02 to <0.001). After the run, COMP returned to baseline within 24 to 48 h (T4, median 7.8 µg/ml, *P*<0.01; and T5, 7.5 µg/ml, not significant).

Table I

Significant Spearman's rank correlation coefficients for serum COMP and MIA in marathon runners (N=8 runners×6 time) and controls (N=35)

Parameters	Significant correlations (r)
All samples and time points pooled (83 values):	
COMP	sIL-6R 0.69***, TNF-α 0.41***, IL-1RA 0.36**, sTNFRII 0.34**
MIA	CRP 0.31**
Runner's baseline (T0, eight values):	
COMP	sIL-6R 0.61*
MIA	No significant correlation
Runners before, during and immediately after the run (T0–T3, 32 values):	
COMP	IL-1RA 0.41**
MIA	sTNRII −0.44**, sIL-6R −0.42**

*P<0.05, **P<0.01, and ***P<0.001.

MELANOMA INHIBITORY ACTIVITY

Compared with matched controls (median 2.5 ng/ml), serum levels of MIA in runners were increased before start (T0, median 7.0 ng/ml, *P*<0.01) (Fig. 5, lower panel). At T0, five of eight runners had baseline levels of MIA higher than the upper normal limit of 6.0 ng/ml. These values were similar to the elevated levels found in patients with RA (median 7.5 ng/ml), and higher than the levels observed in other joint diseases, e.g., OA (median 4.5 ng/ml, *P*<0.01). During the run, MIA transiently increased in 3 runners, but this elevation did not reach a level of significance for the entire group. MIA was elevated above the upper normal limit of 6.0 ng/ml in five of eight runners 24 h after the run (T4) and in six of eight runners at 48 h (T5). Very high levels were detected in two runners, i.e., 62 ng/ml at T4 and 58 ng/ml at T5.

CORRELATIONS

Considering all samples of runners (*N*=48 values) and the controls (*N*=35 values), significant correlation coefficients for COMP could be found with sIL-6R, TNF-α, IL-1RA and sTNFRII (*P*<0.001 to 0.05) (Table I). For runners only, evaluating the samples drawn before run (T0, *N*=8 values), COMP correlated significantly with sIL-6R (*P*<0.05). Considering the samples drawn before, during, and immediately after the run (T0 to T3, *N*=32 values), COMP correlated significantly with IL-1RA (*P*<0.005).

MIA showed a significant correlation with CRP (*P*<0.01), predominantly related to the increase of both CRP and MIA, 24 and 48 h after the run (Table I). Regarding T0 values, MIA did not correlate with any of the parameter tested, including COMP. Regarding T0 to T3 values, MIA negatively correlated with sTNFRII (*P*<0.005) and sIL-6R (*P*<0.01), due to the decrease of MIA from baseline at T2 and T3, and the increases of sTNFRII and sIL-6R during the same time period.

Discussion

In endurance-trained runners, we detected elevated baseline serum levels (T0) of TNF-α, sIL-6R, COMP and MIA. During the run (T1 and T2), the serum levels of IL-1RA, IL-6, TNF-α and COMP rose significantly. Our observations confirmed the intensive cytokine response to

strenuous exercise reported in previous studies.[23–26] After a 5-km run, slight increases in plasma CRP, IL-1RA and soluble TNF receptors were observed.[31] After a 6 h endurance run, increased serum levels of IL-6 and IL-1RA have been reported,[32] associated with decreased mitogen-stimulated production of IL-1β and TNF-α by blood mononuclear cells. Furthermore, 1 h after an exhaustive exercise stress test (cycling, mean duration 68 min), elevated serum levels of IL-6 and TNF-α were detected.[33] At that time, the mitogen-induced cytokine release by blood mononuclear cells was suppressed. Thus, it has been concluded that elevated cytokine levels are present in trained athletes. Nevertheless, the activation of the immune system, caused by strenuous exercise is immediately counter-regulated, e.g., by the release of IL-1RA and TNF receptors.

IL-1RA competes with IL-1 for the same receptor without having intrinsic activity.[34] In our study, serum levels of IL-1RA progressively increased during the run, with a maximum at 2 h after the run. A recent study[26] reported post-run increases of IL-1RA mRNA in a few muscle biopsies and in most blood mononuclear cells. In addition and according to a previous report,[25] serum levels of IL-1β were unchanged, demonstrating that the intensive IL-1RA response was unrelated to IL-1β. On the other hand, as reported in the same study,[25] increased IL-1RA correlated with serum levels of IL-6.

IL-6 is a multifunctional cytokine produced by a wide variety of cell types including T lymphocytes, monocyte/macrophages, fibroblasts, hepatocytes and vascular endothelial cells. Elevated serum levels of IL-6 have been reported to be associated with a variety of diseases, including autoimmune diseases. In patients, serum levels of IL-6 rose in response to injury, surgery and at an early stage of acute phase response.[35] In runners and according to previous reports,[23,25,26] serum concentrations of IL-6 increased during the runs, with a maximum at finish and a rapid decrease within hours. The pre-run serum levels of IL-6 had been low, i.e. within the normal limits. Accordingly, before the run, IL-6 mRNA could not be detected in muscle nor in circulating mononuclear cells, whereas after the run, it was detectable only in muscle biopsies.[26] Thus, it has been suggested that exercise-induced destruction of muscle fibers may trigger the local production of IL-6, which in turn might stimulate the production of IL-1RA by circulating leukocytes.

CRP is an acute-phase protein present in the serum of healthy individuals at concentrations ranging up to 5 μg/ml. CRP increases significantly in case of inflammation, tissue damage and necrosis. As IL-6 is considered to be the mediator of the synthesis of CRP. This process could be detected in our study by the delayed elevation of serum CRP, i.e. 24 to 48 h after the run. In runners of the Swiss Alpine Marathon (Davos, Switzerland, distance 67 km, difference in altitude 2300 m, mean running time 8.5 h), IL-6, CRP and creatine kinase were measured before, during and after the run.[36] IL-6 was found to be elevated at the end of the run. Subsequently, the highest serum levels of CRP and creatine kinase were found 24 h after the run. In combination, these results support the hypothesis of a relation between muscle damage and increased CRP.

A soluble form of the IL-6R (gp80) results from the proteolytic cleavage of membrane-bound receptors. It has been suggested that elevated levels of IL-6 might also be associated with increased production of sIL-6R. Soluble IL-6R has been shown to bind IL-6 and to enhance the activity of IL-6 as a result of the binding of the IL-6/sIL-6R gp80 complex to membrane-bound IL-6R gp130.[37,38] In marathon runners, baseline serum levels of sIL-6R were significantly elevated. The modest change of sIL-6R during the run was correlated with multiple parameters, including COMP.

TNF-α is a pleiotropic cytokine which modulates the inflammatory and immune reactions occuring in response to infection or injury. Concentrations of 0.1 to 10.1 pg/ml TNF-α and 700 to 3250 pg/ml sTNFRII are found in the serum of healthy individuals.

In one study,[31] TNF-α production decreased after a 5-km run, showing a minimum at 24 h. In contrast and confirming previous reports,[24,26] in our marathon runners, serum levels of TNF-α were elevated before and rose further during the run; they remained elevated for 2 h then gradually decreased to baseline within 24 to 48 h. After the run, increased TNF-α mRNA was found neither in muscle biopsies nor in circulating mononuclear cells.[26] Therefore, it must be produced by other cells, e.g., tissue macrophages. Furthermore, sTNFRII (p75) can neutralize the biological activity of TNF-α by competing with cell surface receptors. The levels of sTNFRII show inter-individual variations but are stable for an extended period of time in probates. Similarly to IL-6 and TNF-α, high levels of sTNFRII have been reported in association with infections; however, the mechanism involved in the shedding of the TNFRII is not well understood. Stimuli which cause elevated TNF-α levels can also induce the release of TNFRII. Examining healthy individuals and our marathon runners, the levels of TNFRII correlated with TNF-α. An increase of soluble TNF receptors (types I and II) in serum has been reported after a 5-km run.[32] In our marathon runners, the baseline levels of sTNFRII were slightly elevated, but remained within the normal range. During the run, the concentration of sTNFRII was stable.

In marathon runners, we found elevated baseline serum concentrations of two markers which may reflect joint metabolism and/or damage, namely COMP and MIA. A previous study[39] showed that serum levels of keratan sulfate, a component of aggrecan, are unchanged in runners before and immediately after a marathon, as well as 48 h later. Patients with joint injury or OA showed elevated serum levels of both aggrecan and COMP,[3,7,13] whereas in runners these parameters appeared dissociated. Thus, it has been stated that marathon running causes neither a transient nor a sustained increase in proteoglycan catabolism in articular cartilage, therefore showing no consequence with any pathophysiological process.

COMP is synthesized by chondrocytes or fibroblasts in articular cartilage, meniscus, tendon and synovial tissue,[3–5] and its production may be enhanced by cytokines and growth factors, e.g., transforming growth factor-β1.[6] COMP fragments are present in the serum of healthy individuals at concentrations ranging up to 5.0 μg/ml. Compared with the elevated serum levels of immunoreactive COMP in traumatic joint injury[13] or OA,[3] the median baseline level of COMP in marathon runners was similar or even higher. Thus, before the run, 90% of runners showed COMP levels higher than the upper normal limit. This may reflect increased joint matrix molecule turnover and possibly some joint damage due to extreme physical exercise. It remains uncertain that any of the markers tested reflect changes occurring in joints. Nevertheless, in a subgroup of patients with knee joint injury, persistently elevated serum levels of COMP for over 2 years may be associated with a risk of developing OA.[13] The rise of COMP serum concentrations during the run may be indicative of the severe physical strain on joint structure, but may also be associated with

tendonitis. Considering values of healthy controls and all the values of the marathon runners, significant correlations of COMP with sIL-6R, TNF-α, IL-1RA and sTNFRII could be found, suggesting a correlation of COMP with some aspects of the inflammatory response. Regarding the samples drawn before and immediately after the run (T0 to T3), COMP significantly correlated with IL-1RA only, suggesting that in the joint, damages and/or metabolic changes and the rapid counter-regulation of the inflammatory response occurred simultaneously. The fact that the highest levels of COMP occurs 2 h after the run suggests that other factors beside physical strain influence COMP serum concentrations. One of them could be the kinetics of lymphatic drainage of synovial matrix molecules from the joint space to the blood. In addition, exercise could trigger reactions which persist after cessation, like the creatine kinase efflux from muscle cells.[36]

Studies of protein and mRNA levels indicate that MIA is produced predominantly by malignant melanoma cells and chondrocytes. In healthy organisms, MIA expression is thought to be limited to cartilage.[16,17] In RA, MIA appears to be released by chondrocytes during destructive joint process, and seems only in part due to passive release from necrotic or apoptotic cells.[18] Healthy individuals showed MIA concentrations in serum ranging from 2.0 to 8.5 ng/ml. MIA values above 6.0 ng/ml are considered elevated. Elevated baseline MIA concentrations are not only observed in patients with melanoma[15,17] or RA,[18] but also in runners. In three of eight runners, MIA increased slightly during the run, but the changes were not of statistical significance. However, in six or seven marathon runners 24 to 48 h respectively after the run, MIA increased to levels higher than the upper normal limit. The increase of MIA paralleled the elevation of CRP, suggesting a correlation with late inflammatory processes and—because of the relationship between CRP and creatine kinase[36]—possibly with muscle damage.

For several biomarkers, diurnal variation has been reported, e.g., for hyaluronan after leaving bed in the morning.[40] Hence, it is important to raise the question about diurnal rhythms of COMP and MIA. The time of serum collection from patients and non-running control subjects was standardized (between 8 and 10 am) to the time of baseline collection from the runners. Regarding COMP, no significant difference was observed in six normal subjects between 8–10 am and 3–5 pm. Therefore, change in COMP concentrations during the run was not due to a diurnal variation.

In conclusion, COMP and MIA concentrations increased most likely due to the extreme physical exertion on joint structures. However, there is no direct evidence that they reflect pathological changes occurring in cartilage. COMP and MIA are released by chondrocytes, but also by other cell types which might be activated during or after physical exercise. Thus, considering all the values of the runners and those of healthy controls, correlation of COMP with sIL-6R and TNF-α suggest joint metabolic changes and early inflammatory response occurred simultaneously. COMP also correlated with sTNFRII and the important IL-1RA response, showing that COMP release coincided with early counter-regulatory mechanisms. MIA and CRP increased within 24 h after the run, but the nature of the correlation is yet unclear. All in all, our data suggest a more complex regulatory mechanism than the passive release of matrix proteins from articular cartilage due to joint damage. The kinetics of COMP and MIA suggest different triggering mechanisms and/or sources, i.e. either tendon, meniscus,

synovial fibroblasts or chondrocytes. Since COMP and MIA fluctuations correlated with different phases of the run and the recovery, they are potential markers of different aspects of joint matrix turnover and/or damage in both rheumatic diseases and sport.

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