Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans

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1. The present study investigates to what extent and by which time course prolonged strenuous exercise influences the plasma concentration of pro-inflammatory and inflammation responsive cytokines as well as cytokine inhibitors and anti-inflammatory cytokines.

2. Ten male subjects (median age 27·5 years, range 24–37) completed the Copenhagen Marathon 1997 (median running time 3:26 (h:min), range 2:40–4:20). Blood samples were obtained before, immediately after and then every 30 min in a 4 h post-exercise recovery period.

3. The plasma concentrations of tumour necrosis factor (TNF)α, interleukin (IL)-1β, IL-6, IL-1ra, sTNF-r1, sTNF-r2 and IL-10 were measured by enzyme-linked immunosorbent assay (ELISA). The highest concentration of IL-6 was found immediately after the race, whereas IL-1ra peaked 1 h post exercise (128-fold and 39-fold increase, respectively, as compared with the pre-exercise values). The plasma level of IL-1β, TNFα, sTNF-r1 and sTNF-r2 peaked in the first hour after the exercise (2·1-, 2·3-, 2·7- and 1·6-fold, respectively). The plasma level of IL-10 showed a 27-fold increase immediately post exercise.

4. In conclusion, strenuous exercise induces an increase in the pro-inflammatory cytokines TNFα and IL-1β and a dramatic increase in the inflammation responsive cytokine IL-6. This is balanced by the release of cytokine inhibitors (IL-1ra, sTNF-r1 and sTNF-r2) and the anti-inflammatory cytokine IL-10. The study suggests that cytokine inhibitors and anti-inflammatory cytokines restrict the magnitude and duration of the inflammatory response to exercise.

The integrated cytokine response to infection and injury is complex and tissue responses depend not only on absolute concentrations of tumour necrosis factor (TNF)α and interleukin (IL)-1β, but also on the simultaneous presence of naturally occurring cytokine inhibitors and anti-inflammatory cytokines. The local response to infection or tissue injury involves the production of cytokines which are released at the site of inflammation. These cytokines facilitate an influx of lymphocytes, neutrophils, monocytes and other cells, which participate in the clearing of antigens and healing of tissue. The local inflammatory response is accompanied by a systemic response known as the acute phase response. Injection of TNFα, IL-1β and IL-6 into laboratory animals (Dinarello, 1992) or humans (Richards & Gauldie, 1998) will produce most, if not all, aspects of the acute phase response. These cytokines are therefore usually referred to as 'inflammatory' or 'pro-inflammatory' cytokines, although it may be more reasonable to classify IL-6 as an 'inflammation-responsive' cytokine, since IL-6

alone does not induce inflammation. A pivotal advance in the past years has been the discovery and identification of at least two classes of biological inhibitors of the pro-inflammatory cytokines. These include the IL-1 receptor antagonist (IL-1ra), the soluble type of IL-1 receptors (sIL-1r(I) and sIL-1r(II)) and the two soluble receptors for TNF (sTNF-r1 and sTNF-r2). Furthermore the anti-inflammatory cytokine IL-10 inhibits the release of TNFα and IL-1β (Chernoff *et al.* 1995), and induces the production of IL-1ra (Jenkins *et al.* 1994; Cassatella *et al.* 1994).

Early studies demonstrated that exercise induced an increase in IL-1 (Cannon *et al.* 1986; Evans *et al.* 1986) although it was later pointed out that the biological assay used in the early studies did not distinguish between IL-1 and IL-6 (Bagby *et al.* 1996). Northof & Berg (1991) found increased levels of IL-6, but not IL-1β after a marathon. The finding of markedly elevated levels of IL-6 after exercise is consistent (Sprenger *et al.* 1992; Ullum *et al.* 1994*a*; Drenth *et al.* 1995; Nehlsen-Cannarella *et al.* 1997;

Castell *et al.* 1997; Rohde *et al.* 1997; Hellsten *et al.* 1997; Bruunsgaard *et al.* 1997; Ostrowski *et al.* 1998), and so is the finding of no changes or only slightly elevated levels of IL-1β in plasma (Sprenger *et al.* 1992; Ullum *et al.* 1994*a*; Drenth *et al.* 1995; Bruunsgaard *et al.* 1997; Nehlsen-Cannarella *et al.* 1997; Ostrowski *et al.* 1998). Inconsistent findings have been reported for TNFα. Dufaux & Order (1989) and Espersen *et al.* (1990) reported increased plasma TNFα 2 h after completing a 2·5 h run (2 h 30 min) and 1 h after a 5 km race, respectively, but other studies have failed to detect TNFα after exercise (Rivier *et al.* 1994; Ullum *et al.* 1994*a,b*).

The present study provides measurements of the time course of TNFα, IL-1β and IL-6 in the post-exercise period. Furthermore, it was investigated to what extent exercise induces cytokine inhibitors (IL-1ra, sTNF-r1 and sTNF-r2) and the anti-inflammatory cytokine IL-10.

METHODS

Subjects

Ten male subjects (median age 28 years, range 24–37; median weight 79·5 kg, range 71–91; median $V_{O_2,max}$ 61·2 ml min^{-1} kg^{-1}, range 53·3–70·2) participated in the study. The experimental protocol was approved by the local ethics committee and all subjects were informed of the risks and purposes of the study before their written consent was obtained. The subjects participated in the Copenhagen Marathon, May 1997 (median running time 3 : 27 (h : min), range 2 : 40–4 : 20). Ambient temperature during running was 17 °C .

Determination of maximum oxygen consumption ($V_{O_2,max}$)

For each subject $V_{O_2,max}$ was determined prior to the experiment during an incremental exercise test on treadmill.

Sampling and analysis of blood

Blood samples were drawn from the antecubital vein 1 week prior to, immediately after (the delay from stopping running to the taking of the first blood sample did not exceed 10 min), and every 30 min in the 4 h rest period after the race. Blood was sampled 1 week prior to the race, in order not to influence the performance of the runners. The subjects prepared for the pre-exercise sample, in a similar way as they prepared for the marathon, which involved refraining from exercise for 2 days before the pre-sample was taken. The subjects were allowed to consume fluid and carbohydrate *ad libitum* before, during and after running. Two 10 ml blood samples were drawn into glass tubes containing 35 μmol dipotassium-EDTA and 1500 kallikrein inactivator units Trasylol (Bayer, Leverkusen, Germany). The tubes were kept on ice until centrifuged at 2 150 *g* for 15 min at 4 °C. Plasma was separated from the cells and stored at −80 °C until analysed by commercially available enzyme-linked immunosorbent assay (ELISA; R&D systems, Minneapolis, MN, USA).

ELISA

All measurements were performed in duplicate, and high sensitivity kits were used when available, which was the case for TNFα, IL-1β, IL-6 and IL-10. According to information provided by R&D Systems, the ELISA used for measuring IL-6 and TNFα are insensitive to the addition of the recombinant forms of the soluble receptors (sIL-6r, sTNF-r1 and sTNF-r2, respectively) and

Table 1. Detection limit (DL) and intra-assay coefficient of variation (c.v.) for the cytokine ELISA

Assay	DL (pg ml^{-1})	CV (%)
TNFα (HS)	< 0·1	6·6
IL-1β (HS)	< 0·1	7·8
IL-6 (HS)	< 0·1	6·9
IL-1ra	< 14	4·9
sTNF-r1	< 30	4·1
sTNF-r2	< 10	2·2
IL-10 (HS)	< 0·5	9·7

these measurements, therefore, probably correspond to both soluble and receptor-bound cytokine. Likewise, the ELISA for sTNF-r1 and sTNF-r2 are relatively insensitive to added TNFα or TNFβ, and these measurements, therefore, correspond to the total amount of the soluble receptor present in samples. The detection limit (DL) and the intra-assay coefficient of variation (c.v.) for the ELISA can be found in Table 1.

Correction for plasma volume shifts

Changes in plasma volume were calculated from measurements of haemoglobin and haematocrit according to the method described by Dill & Costill (1974), and cytokine measurements were corrected accordingly.

Statistical analysis

Cytokine measurements were tested for effects of 'time' and 'subject' in a two-way analysis of variance (ANOVA). The model used was:

$$[\text{Cytokine}] = \text{constant} + \text{time} + \text{subject} + \text{error}.$$

If the effect of 'time' tested significant ($P < 0.05$) Tukey's test was used for comparison of the multiple measurements made during and after running, with the pre-exercise value. Before proceding with the statistical analysis, the residuals in the ANOVA were examined for a normal distribution through investigation of a histogram and a normal plot. If residuals were considered not to be normally distributed, data were log transformed and residuals were investigated again. This was the case for IL-1ra, IL-6, IL-1β and IL-10. After log transformation, residuals were considered to be normally distributed and thus for these measurements log-transformed data were used in the subsequent statistical analysis. The cytokine data found not to be normally distributed (IL-1β, IL-6, IL-1ra and IL-10) are plotted as geometric means with a 95 % confidence interval (c.i.). The rest (TNFα and the sTNF receptors) are plotted as means with 95 % c.i.

Statistical calculations were performed using SYSTAT 7.0 for Windows (SPSS Inc., Chicago USA).

RESULTS

The measured cytokines all changed with time (two-way ANOVA: $P < 10^{-9}$ for all except for IL-1β and sTNF-r2: $P < 5 \times 10^{-4}$).

The mean plasma concentration of TNFα peaked immediately after the race (2·3-fold increase compared with pre-race value) and declined slowly during the resting period. It remained significantly different from the pre-race

value until 2·5 h post running (Fig. 1A). The level of IL-1β was slightly elevated (2·1-fold compared with the pre-race value) immediately after the race (Fig. 1B). Three of the subjects had approximately 10-fold higher IL-1β plasma levels than the remaining subjects, which is the reason for the wide confidence interval seen on the figure. The mean plasma concentration of IL-6 peaked immediately after exercise where it had increased 128-fold compared with the pre-race value. The level of IL-6 declined during the 4 h rest period, but remained significantly above the pre-race value (Fig. 1C). The plasma concentration of IL-1ra peaked 1 h after the race (39-fold increase compared with pre-race value; Fig. 1D). The levels of sTNF-r1 and sTNF-r2 increased 2·7- and 1·6-fold, respectively, above the pre-exercise levels within the first hour after exercise (Fig. 1E). The plasma concentration of IL-10 peaked immediately

after the race, where it had increased 27-fold compared with the pre-race value. The level of IL-10 declined steadily in the resting period, but remained significantly above the pre-race value throughout the resting period (Fig. 1F).

DISCUSSION

The present study provides information about changes in the plasma concentration of the cytokines TNFα, IL-1β, IL-6, IL-1ra, sTNF-r1, sTNF-r2 and IL-10 in the 4 h recovery period after the marathon race. The small but significant increase in TNFα found after exercise is in accordance with other studies (Dufaux & Order, 1989; Espersen *et al.* 1990; Ostrowski *et al.* 1998), and so is the finding of high levels of IL-6 (Sprenger *et al.* 1992; Ullum *et al.* 1994a; Drenth *et al.* 1995; Bruunsgaard *et al.* 1997;
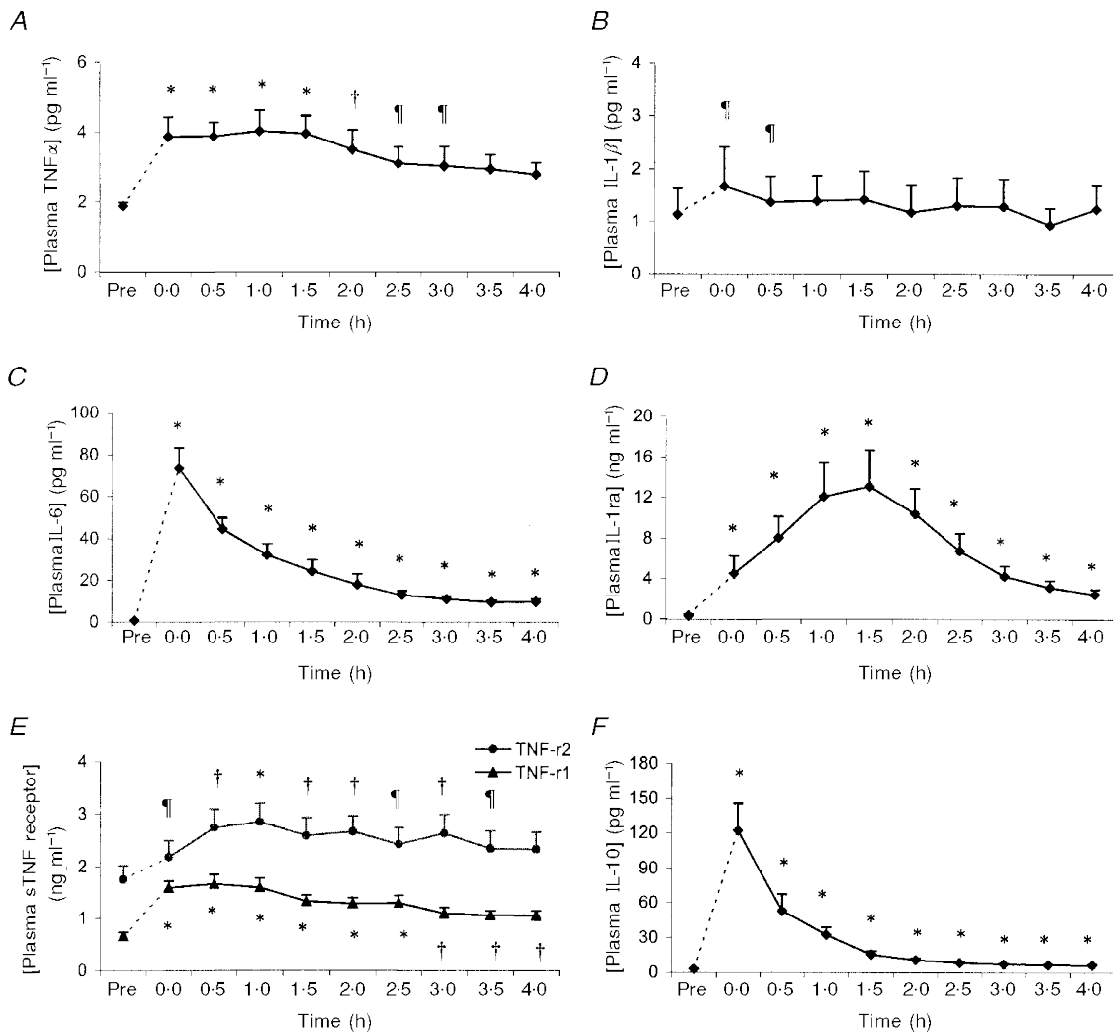

Figure 1. Plasma cytokine concentrations measured before (Pre) and every half hour in the 4 h resting period after a marathon race

Data are plotted as geometric mean with 95% confidence interval (c.i.) (IL-1β, IL-6, IL-1ra and IL-10) or as mean with 95% c.i. (TNFα and sTNF receptors). Legend denotes difference from the pre-exercise value in the Tukey multiple comparison test (*$P < 5 \times 10^{-5}$; †$P < 5 \times 10^{-3}$ and ¶ $P < 0.05$, respectively).

Nehlsen-Cannarella *et al.* 1997; Castell *et al.* 1997; Rohde *et al.* 1997; Hellsten *et al.* 1997; Ostrowski *et al.* 1998). Furthermore, we found a significant increase in plasma IL-1β immediately after running. Previous studies demonstrated increased levels of IL-1 in plasma post exercise (Cannon & Kluger, 1983; Evans *et al.* 1986), but recent studies using more sensitive and specific assays have found only minor, if any, changes in the plasma concentration of IL-1 after exercise (Sprenger *et al.* 1992; Ullum *et al.* 1994*a*; Drenth *et al.* 1995; Bruunsgaard *et al.* 1997; Nehlsen-Cannarella *et al.* 1997; Castell *et al.* 1997; Ostrowski *et al.* 1998). This may be due to less intense exercise regimens studied, or alternatively, to the fact that IL-1β is produced locally and is rapidly cleared from the circulation. The recent finding of IL-1β mRNA in muscle biopsies obtained after strenuous exercise without increase in the IL-1β protein in plasma (Ostrowski *et al.* 1998) and the finding of IL-1β in the urine of runners (Sprenger *et al.* 1992) support this latter idea.

The present study demonstrates that exercise induces a cascade of cytokine inhibitors (IL-1ra, sTNF-r1 and sTNF-r2) and the anti-inflammatory cytokine IL-10. Since this was a field study, blood sampling during running was not possible, and an analysis of changes in plasma cytokines during exercise was therefore not done. It should be noted however, that IL-1ra peaked 1 h after IL-6, which is compatible with the knowledge that IL-6 induces an increase in the IL-1ra plasma concentration (Tilg *et al.* 1994; Jordan *et al.* 1995).

Carbohydrate intake was not controlled in the present study, but subjects can be assumed to be well loaded. It has been shown that carbohydrate loading diminishes the exercise-induced increase in IL-6 and IL-1ra (Nehlsen-Cannarella *et al.* 1997). Thus, with carbohydrate restriction, an even more pronounced increase in plasma cytokine levels might have been found.

Elevated levels of plasma IL-6 have been shown to induce C-reactive protein, but despite the high levels of IL-6 detected after strenuous exercise, only a modest increase in plasma C-reactive protein was detected after a marathon run (Castell *et al.* 1997). Furthermore, other biological effects of the TNF/IL-1/IL-6 system such as myocardium depression, vasodilatation, leukocyte aggregation and dysfunction of kidneys, livers, lungs and brain do not develop in response to exercise. In other words, although the initiation of the acute-phase response develops, exercise is not followed by a fully developed systemic response. The present study suggests that anti-inflammatory cytokines, soluble receptors and receptor antagonists restrict the magnitude and duration of the inflammatory response to exercise.

In conclusion, strenuous exercise induces an increase in the pro-inflammatory cytokines TNFα and IL-1β and a dramatic increase in the inflammation responsive cytokine IL-6. This release is balanced by the release of cytokine inhibitors (IL-1ra, sTNF-r1 and TNF-r2) and the anti-inflammatory cytokine IL-10.

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