Immune and Neuroendocrine Alterations in Marathon Runners

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ABSTRACT

Marathon running is a stressful event that can significantly affect virtually any of the physiologic systems of a runner. The goal of this study was to investigate effects of marathon running on human immune and neuroendocrine parameters and their interaction. Blood samples were collected from 15 male runners (38.3 ± 6.9 years) 18 hours before finish time, then within 20 minutes (0h), 1 hour, 24 hours, 48 hours, 5 days, and 8 days after the marathon. Complete blood count, secretion of cytokines in mitogen-activated cell culture and plasma, and plasma concentration of β-endorphin, Adrenocorticotropic hormone (ACTH), cortisol, and growth hormone (GH) were analyzed. Significant increase in granulocyte and MID-cell count and lymphopenia were seen immediately after the marathon. Secretion of interleukin (IL)-2 and interferon (IFN)-γ significantly declined at 0 and 1 hour after the marathon. Secretion of TNF-α declined at 0 hours and remained suppressed until 5 days. Suppression in the secretion of IL-1β was observed at 48-hour and 5-day intervals. Activated secretion of IL-6 decreased at 24 and 48 hours. Peak concentrations of ACTH, cortisol, β-endorphin, and GH were registered after the race (0 and 1 hour). We concluded that marathon-associated stress factors can alter physiologic balance of cell-mediated versus humoral and anti-inflammatory versus proinflammatory cytokines. Results suggest that hypothalamic–pituitary–adrenocortical axis hormones played a significant role in the regulation of the observed changes. This information may be beneficial for development of new stress countermeasures to preserve wellness in subjects undergoing intensive physiological stress.

INTRODUCTION

A competitive marathon race is a very stressful event and can significantly affect virtually any of the physiologic systems of a runner. Dehydration, weight loss, gastrointestinal problems (bleeding), hypo- or hyperthermia, collapse, muscle damage and microtrauma are often seen after a marathon race.[1-6] Particular interest has been paid to the incidences of upper respiratory infections (URTI) among marathoners.[7-10] A number of epidemiologic studies have shown increased risk of URTI in athletes up to 2 weeks after endurance races.[11,12] Immunologic studies in marathon runners were performed in two main directions: the assessment of pro- and anti-inflammatory response and the evaluation of host protective immunity. In vitro and in vivo response of pro- and anti-inflammato-

Table 1. Demographic and Fitness Data for Runners Who Participated in the Studies

Marathon	Age (yrs)	Marathon time (h:min)	Best time (h:min)	Body wt (kg)		Training (miles/wk)
				Before Marathon	After	
WR, n=8	37.8±3.9	3:53±0:34	3:31±0:26	78.2±12.7	76.7±12.5	38.7±15.9
CT, n=7	40.3±7.7	4:17±0:23	4:05±0:19	79.8±11.1	76.8±10.4	45.0±10

Data are presented as mean ± SD.

ry cytokines to marathon running has been studied intensively.[5,13-18] However, there is limited and often contradictory information pertaining to cytokine-promoted cell-mediated (type-1) versus humoral (type-2) immune response to endurance running or exercise. Weinstock et al.[19] showed a significant decrease in mitogen-activated secretion of interleukin (IL)-2 and interferon (IFN)-γ one hour after exhaustive competitive exercise (sprint triathlon). Similarly, a significant decrease of endotoxin-B induced IFN-γ secretion was observed 30 minutes after exhaustive ergometer exercise.[20] At the same time, no changes in the plasma concentration of IFN-γ, IL-1∫, IL-12, IFN-α, and tumor necrosis factor (TNF)-α were registered after the marathon in the study Suzuki et al. It was accompanied by a significant increase in concentration of IL-10, although concentrations of IL-4 and TGF-β were unaffected by the race.[6] In contrast, a significant elevation of plasma IL-2 level without changes in IL-1α plasma level was observed 16 hours after completing the marathon, as reported in the study of Castell et al.[21] Moreover, prolonged physical exercise resulted in a significant decrease in the relative percentage and absolute number of T-cells ($CD3^+$) and NK-cells ($CD16^+$), which are major producers of type-1 and type-2 cytokines.[22]

Physiologic and psychological stress factors of exhaustive endurance exercise may affect the cell mediated-humoral immune equilibrium through perturbations in the neuroendocrine system. One of the major events that occurs during the neuroendocrine stress reaction to a marathon run is the activation of the hypothalamic–pituitary–adrenocortical (HPA) axis.[23-26] Dramatic elevation of ACTH, endorphins, and cortisol is a classic representation of the HPA axis response to a prolonged bout of exercise.[27-29] Cortisol is well known for its anti-inflammatory and immunosuppressive properties,[30,31] but this stress hormone is also able to unequally modulate cell-mediated and humoral immune reactions.[32-34] Thus, stress (marathon)-induced neuroendocrine mediators and especially corticosteroids may alter cell-mediated or humoral equilibrium and therefore result in increased susceptibility to infections, autoimmune reactions and allergies.

This study was designed to investigate effects of marathon-associated stressors on cell-mediated versus humoral and anti-inflammatory versus proinflammatory equilibrium as well as their correlations to neuroendocrine response. We measured secretion of cytokines, which support cell-mediated reactions or favor humoral immunity and are involved in inflammatory mechanisms. Additionally, we assessed plasma concentrations of hormones to investigate their involvement in the stress-associated immune alterations.

MATERIALS AND METHODS

Subjects and Experimental Design

This study presents data from two marathon races. Eight runners participated in the White Rock (WR) marathon race in December 1996 (Dallas, Tex), and seven runners participated in the Cowtown (CT) marathon in February 1997 (Forth Worth,

Table 2. Morphologic and Hematologic Changes in Whole Blood of Marathon Runners

Study Intervals	*Complete Blood Count (10^3cells/μL)*				
	Granulocytes	**Lymphocytes**	**MID-cells**	**Hemoglobin (g/dl)**	**Hematocrit(%)**
PRE	5.6±0.5	2.3±0.2	0.5±0.1	14.4±0.6	41.1±1.7
0h	18.8±1.3*	2.1±0.1	1.2±0.1*	15.5±0.2	44.0±0.5
1h	16.3±0.8*	1.5±0.1*	0.8±0.1	15.2±0.3	43.1±0.6
24h	7.7±0.5	2.6±0.2	0.6±0.1	14.4±0.2	41.2±0.6
48h	6.2±0.3	2.2±0.17	0.5±0.1	14.1±0.2	40.2±0.6
5d	5.8±0.4	2.1±0.1	0.5±0.1	14.5±0.3	41.5±0.7
8d	6.6±0.4	2.1±0.1	0.5±0.1	14.7±0.3	41.8±0.7

Data are presented as mean ± SEM.
*Significant difference (P ≤.05) against all intervals.

Tex). All athletes were trained for the marathon run, although the running experience varied from 1 to 16 years. Demographic and fitness data on subjects are presented in Table 1. The study protocol was approved by the UT Southwestern Medical School at Dallas Review Board for Human Studies. Informed consent was obtained from all subjects before the study.

Blood samples were collected via venipuncture 24 hours before (PRE) expected finish time (between 11:00 AM and 1:30 PM) to avoid circadian rhythm effects. The postrace blood samples were collected at the finish site within 20 minutes (0h) and 1 hour (1h) after completion of the marathon. The next samples were collected 24 hours, 48 hours, 5 days, and 8 days (24h, 48h, 5d, and 8d, respectively) after the marathon race. During the race, runners drank ad libitum. Runners were asked to refrain from any training during a recovery period.

A complete blood count (CBC) was performed on EDTA-blood samples using an I-1800 Hematology Analyzer (Infolab, Inc., Round Rock, TX).

Heparinized whole blood in 1:10 dilution was incubated with 5 mg/mL of phytohemagglutinin (PHA) from *Phaseolus vulgaris*, and 10 mg/mL of lipopolysaccharide (LPS) from *E coli* O26:B6 in RPMI-1640 supplemented with 2 mM glutamine, 75 U/mL gentamicin sulfate (Sigma, St-Louis, Mo) for 24 hours at 37°C in a 95% humid atmosphere containing 5% of CO_2. Supernatants were collected, and levels of IL-2, IFN-γ, IL-10, and TNF-α were assayed with commercial EASIA kits (Medgenix Diagnostic distributed by INC-STAR, Stillwater, Minn) in all 15 subjects. Additionally, secretion of IL-1β and IL-6 in PHA/LPS stimulated cultures as well as plasma levels were assessed in the seven subjects who participated in the CT marathon. Minimal detectable levels of EASIA kits were (IL-2 - 0.1 IU/mL, IFN-γ - 0.1 IU/mL, IL-10 - 1 pg/mL, TNF-α - 3 pg/ml, IL-1β - 2 pg/mL, and IL-6 - 2 pg/mL).

Serum or EDTA plasma samples were collected on ice and stored at -70°C until assays were performed. Plasma levels of cortisol (CORT), ACTH, β-endorphin (βENDO), growth hormone (GH), and creatine phosphokinase (CPK) were analyzed with commercial RIA and IRMA kits (Diagnostic Products Corporation, Los Angeles; Nichols Institute Diagnostics, San Juan Capistrano, Calif). Plasma volume shift due to marathon-induced dehydration was calculated by the method of Dill and Costill.[35]

Statistical Analysis

Two-way repeated measures (RM) analysis of variance (ANOVA) was used to deter-

Table 3. Cytokine Secretion in PHA/LPS Activated Cell Cultures

	N=15 (WR and CT subjects)		N=7 (CT subjects only)			
	IL-2 (IU/mL)	IFN-γ (IU/mL)	IL-10 (pg/mL)	TNF-α (pg/mL)	IL-1β (pg/mL)	IL-6 (pg/mL)
PRE	6.0±1.5	210.8±26.6	445.3±69.3	16937±1800.8	4377.1±664.5	16571.4±2058.1
0h	2.0±0.4*	17.7±3.5*	310.0±44.3	9594±1421.5*	2937.1±696.8	13585.7±3105.4
1h	1.6±0.2*	14.4±3.3*	463.7±146.9	11394±1522.8*	3162.9±617.1	16200.0±1940.2
24h	6.6±1.0	196.4±15.8	262.3±27.0	12859±1585*	3520.0±743.3	6514.3±985.0*
48h	5.0±0.6	154.8±20.0	288.2±33.2	12899±1720.8*	2342.8±359.6*	8414.3±1470.9*
5d	8.4±1.2*	272.8±23.9*	441.8±73.9	12276±1276.7*	2388.6±481.9*	10642.8±2291.1
8d	5.7±1.0	141.0±18.1	355.0±47.9	14043±1231.2	2817.1±243.6	14271.4±1331.8

Data are presented as mean ± SEM.
* statistical significance (P ≤.05) against PRE interval
WR, White Rock Marathon (Dallas, Tex); CT, Cowtown Marathon (Fort Worth, Tex).

mine differences within two marathon races, and one-way RM ANOVA was used to analyze interval-dependent differences. SigmaStat (SPSS Inc., Chicago, Ill), statistical software package was used to determine normality of variances and where parametric or nonparametric (Neuman-Keuls and Bonferroni tests) methods were appropriate. Pearson product moment correlation test and random-effects regression model were used to analyze possible relations between studied variables. A value of $P \leq .05$ was considered statistically significant. Power of performed tests with alpha .05 for both marathons (n = 15) was equal to 1.000 and 0.714 for CT marathon (n = 7). All data are presented as mean ± standard error of the mean (SEM) of its corresponding units (except Table 1, in which demographic and performance data are presented as mean ± standard deviation [SD]).

RESULTS

No differences in the studied variables were observed between two groups of subjects at any intervals and thus the results from two groups were analyzed as data from one cohort.

Hematological Data

Significant increases in granulocyte and monocyte (MID-cell) counts along with a significant decline in lymphocyte count were seen at 0h and 1h compared with all other intervals. A significant increase in hemoglobin level was seen at the 0h interval, with recovery by the next hour. Hematocrit increase, seen at 0h, was nonsignificant ($P = .075$) compared with PRE level, but it significantly differed from the 48h interval (Table 2).

Immune Responses (in vitro and in vivo Cytokine Secretion)

Results on cytokine secretion in PHA/LPS activated whole blood cell cultures are presented in Table 3. A significant threefold decrease in IL-2 and more than 10-fold decrease in IFN-γ secretion were registered immediately after (0h) and 1 hour after completion of the marathon race. Secretion of IL-2 and IFN-γ fully recovered the day after the marathon (24h). In addition, a significant elevation of IL-2 and IFN-γ secretion compared to PRE data was observed 5 days after the marathon (5d). A near-significant ($P = .052$) decline in IL-10 secretion (13% decrease) was seen immediately (0h) after completion of the race. Mitogen-activated secretion of TNF-α significantly declined immediately after the marathon and remained suppressed until the 5d interval.

A significant decline in the mitogen-activated secretion of IL-1β was observed at the 48h interval and it remained suppressed until the 5d interval. Mitogen-activated

secretion of IL-6 decreased significantly at 24h and 48h after the marathon (Table 3).

A nearly 20-fold increase of plasma IL-6 level was found immediately after the marathon (190.6 ± 29.9 pg/mL), and it remained significantly elevated at the 1h interval (94.3 ± 15.8 pg/mL) compared to PRE level (10.0 ± 1.6 pg/mL). Plasma level of TNF-α was significantly elevated (28.6 ± 4.71 pg/mL) immediately (0h) after the marathon compared with PRE interval (15.4 ± 2.54 pg/mL). No significant changes were seen in IL-1β plasma levels (data not shown).

Immune Responses (in vitro Cytokine Equilibrium)

For further analysis we calculated ratios of cytokine secretion in cell cultures. Ratios between cytokine pairs which support cell-mediated (type 1) or humoral (type 2) immune reactions are presented in Figure 1A, and they reflect results from all 15 subjects. A significantly higher IL-2/IFN-γ ratio was observed at 0h and 1h after the marathon compared with the PRE interval. This parameter returned to the baseline level at 24h and remained at the same level until the end of the study. On the contrary, significant decline in IL-2/IL-10 and IFN-γ/IL-10 ratios were seen immediately (0h) and 1h after marathon. These parameters significantly increased 24h post marathon. While IFN-γ/IL-10 ratio returned to baseline level by 48h, IL-2/IL-10 ratio remained significantly higher the PRE level until the 5d post marathon measurement (Figure 1A). Ratios between cytokine pairs that support proinflammatory versus anti-inflammatory reactions are presented in Figure 1B. Significant elevations in TNF-α/IL-10 and IL-1β/IL-10 ratios were observed 24h after completion of the marathon, while an elevation in IL-6/IL-10 ratio was registered immediately after the marathon (Figure 1B).

Neuroendocrine and Humoral Responses

Plasma levels of ACTH, cortisol (CORT), and β-endorphin (βENDO) increased dramatically immediately (0h) and 1h after the race and returned to the base-level 24h post marathon (Figure 2A). Similarly, plasma growth hormone (GH) level significantly elevated at 0h, then started to decline. However, it was still higher than the baseline at 1h recovery. Further, it continued to decline and became significantly lower at the 24h interval compared with PRE level. Recovery of plasma GH level was observed by the 48h interval (Figure 2B). Significant near threefold elevation of serum CPK level was observed immediately (0h) and 1 hour after the marathon, but the highest concentration for this parameter was registered 24 hours after the marathon. Increased CPK level was seen until the fifth day of recovery (5d), and it returned to the base level at 8 days after the marathon (Figure 2B).

Immuno-Endocrine Interrelations

As a next step in data analysis, we performed correlation tests to investigate interval-dependent neuroendocrine and immune responses as well as the interactions. Highly significant correlations ($P < .001$) were observed for ACTH versus β-endorphin (r = .816), ACTH versus cortisol (r = .604), and cortisol versus β-endorphin (r = .81) plasma levels, which reflect systemic HPA axis response to stress. These results represent a "classic" stress response, and they are supported by data from the literature.[25,27-29,36,37] No correlative interactions for other studied hormones were found.

Consequently, correlative analysis of cytokine secretion in mitogen-activated culture was performed. On the one hand, highly significant correlative interactions were found between IL-2 and IFNγ (r = .74). Conversely, IL-10 secretion significantly correlated to TNF-α and IL-6 (r = 0.67 and r = 0.56, correspondingly), and TNF-α secretion correlated with IL-1β (r = .556). Significant interactions were found for IL-1β and IL-6 secretion as well (r = .553).

To investigate the effect of HPA axis hormones (in vivo activity) on immune responsiveness in vitro we performed correlation tests between plasma levels of ACTH, β-

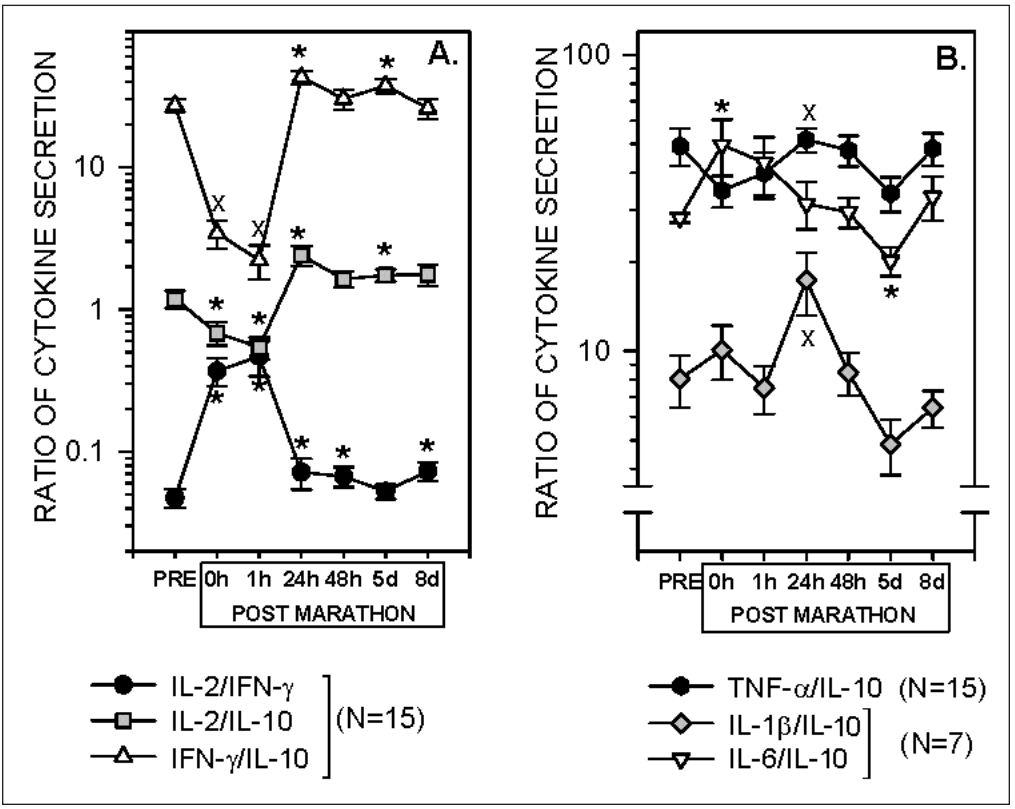

Figure 1. Effects of marathon-associated stress factors on cytokine balance in cell cultures. Data are calculated as ratios of cytokines secretion in mitogen-activated cultures and presented as mean ± SEM. Secretion units for IL-2 and IFN-γ were transferred to pg/mL before calculation of ratios according to NIBSC 86/504 and NIH Gg 23-901-530 standards. (*statistical significance ($P \leq .05$) against PRE interval; X-statistical significance against all other intervals.) Panel A represents data from all 15 subjects, and Panel B reflects results from both WR (TNF-α/IL-10, N = 15) and CT (IL-1β/IL-10 and IL-6/IL-10, N = 7) marathoners.

endorphin, cortisol, and PHA/LPS-induced cytokine secretion in cell culture. Data on correlation between cortisol and cytokines was presented in Figure 3. A similar pattern was seen for β-endorphin and ACTH. These results suggest that the HPA response (plasma cortisol, βENDO, and ACTH): (1) predominantly affects the functional properties of cell-mediated immune responsiveness (IL-2, IFN-γ) but not humoral (IL-6, IL-10) reactions and (2) affects mostly secretion of lymphocyte-derived but not monocyte-derived cytokines.

DISCUSSION

A marathon race is a stressful event that comprises of a variety of physical as well as physiologic cognitive and noncognitive factors, and it is often associated with immunosuppression.[6,12,38-42] Several earlier epidemiologic studies showed increased risk of URTI in athletes up to 2 weeks after participation in marathon running.[11,12] Despite a large number of publications on the issue, the variety of employed methods, lack of homogeneity in the population sample (gender, running experience), and the effect of environmental factors (climate, humidity, altitude) make the comparative analysis difficult. Our data indicate that marathon running produces significant alterations in the distribution of leucocytes (Table 2), affects immune and neuroendocrine homeostasis (Figures 2 and 3), and leads to skeletal mus-

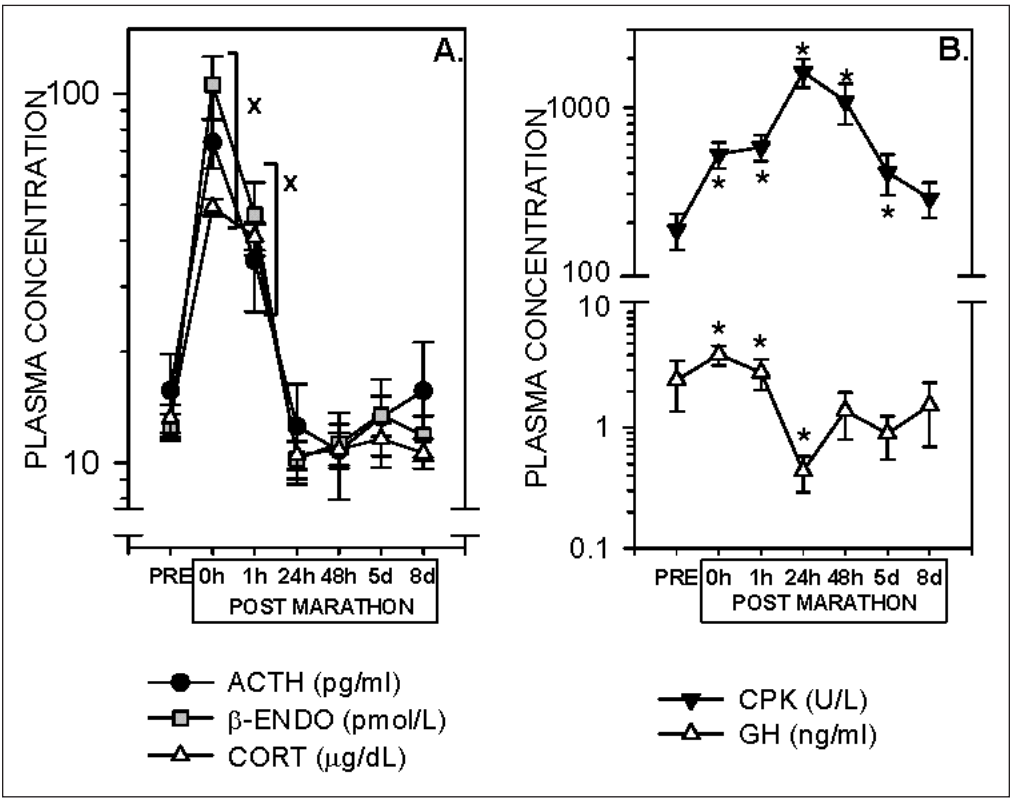

Figure 2. Plasma concentration of ACTH, cortisol, and (β-endorphin before and after marathon race. (*statistical significance (P ≤.05) against PRE interval.) Data are presented as mean ± SEM in the absolute corresponding units for each hormone (N = 15).

cle damage (Figure 3) in subjects with wide experience. The majority of changes were seen immediately after marathon completion. In most of the studies pertaining to prolonged exhaustive exercise, data collection and analysis of recovery were limited to a few days. In the present study we attempted to evaluate the immune and neuroendocrine response to marathon run throughout a more extended recovery period. Additionally, we analyzed correlative relations between studied variables to reveal potential immune and neuroendocrine interactions.

The present study uses both in vitro (cell cultures) and in vivo (plasma) immunologic models and evaluates the neuroendocrine response to stress to understand the underlying physiologic mechanisms of observed changes. We used a mixture of PHA and LPS as a nonspecific activator for cytokine secretion in cell culture.[43-45] LPS is a very potent, physiologically relevant activator of monocytes and can be used to optimally stimulate cytokine production in cell cultures. It has been shown that combination of PHA and LPS gives the most reliable production of IL-1β, IL-2, IL-6, IFN-γ, TNF-α, and GM-CSF compared with production of these cytokines after activation with either PHA or LPS alone.[46] However, the use of this stimulus makes the identification of source cells for a number of cytokines difficult. For example, although IL-10 is produced by lymphocytes after PHA activation, it can also be secreted by monocytes in response to LPS challenge.

The observed diverse results in the

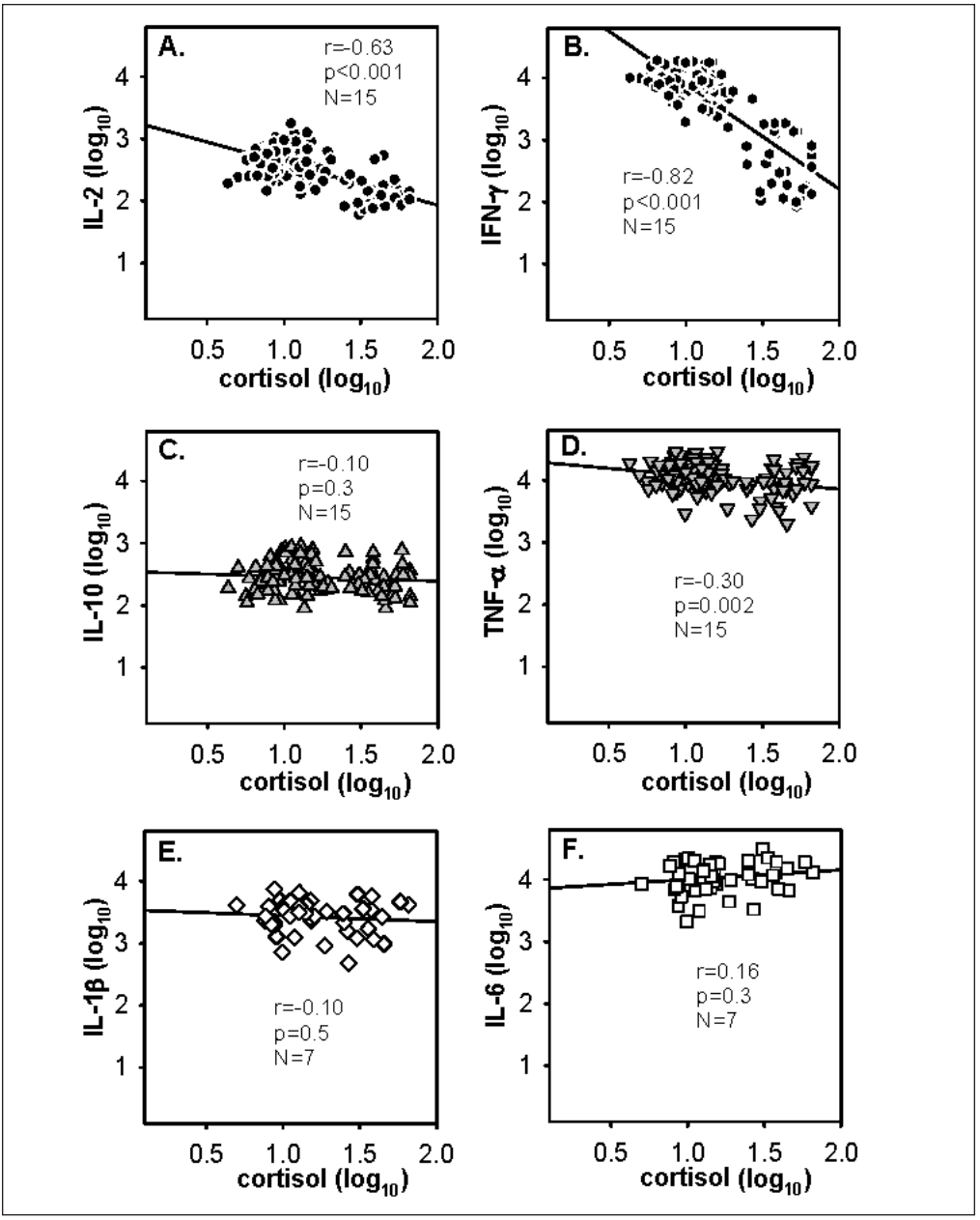

Figure 3. Time-effects of marathon-associated stress factors on immuno-endocrine interactions. Data is analyzed using random-effects regression model to test interactions between plasma cortisol level and the secretion of cytokines in PHA/LPS activated whole blood cell culture, and plotted as means of cortisol (X axis) vs. means of cytokine (Y axis) at all studied intervals. Figures 4A-D represent data from all 15 subjects, while Figures 4E and 4F reflect results from CT marathoners (N=7).

PHA/LPS activated secretion of IL-2 and IFN-γ on the one hand, and IL-10 and IL-6 on the other hand, suggest a shift in the cell-mediated or humoral immune balance, as assessed in the in vitro model. These findings correspond very well to the current model of stress induced immune changes, in which corticosteroids play a significant

role.[47-51]

Additionally, downregulated secretion of IL-2 and IFN-γ can be explained by three nonexclusive mechanisms. First, it can be related to the lymphopenia seen in our and other studies[52] and especially by the reported decrease in the number of the major producers of these cytokines (T- and NK-cells) after a marathon race.[21,22,53] Second, elevation in the number of target cells (granulocytes, monocytes) may play a significant role in the observed decrease of cytokine secretion. The third possible mechanism is a decrease in the percentages of IL-2 and IFN-γ secreting CD4+ and CD8+ T-cells registered after a 2.5-hour run in the study of Steensberg et al.[54] One of the explanations of this phenomenon is that dramatic elevation of HPA axis stress hormones may significantly affect functional properties of immune cells in vivo. Also, because we used whole blood culture, the presence of high concentrations of stress mediators in the culture could be responsible for in vitro changes.

One of the study objectives was to correlate in vitro and in vivo immune responses; therefore we used a whole blood cell culture (WBC). It appears that WBC represents the closest and may be the most appropriate in vitro model to study immune reactions because it preserves cellular and humoral in vivo milieu. However, in this study, in vitro immune response registered in the cell culture did not reflect changes seen in plasma cytokine (in vivo) concentrations. On one hand, we observed elevation in MID-cell count and an increase in plasma concentration of IL-6 and TNF-α, and this observation is supported by the literature.[5,21,55] Conversely, a significant decrease in the secretion of these cytokines was registered in mitogen-stimulated cell culture at the same intervals. The revealed opposed pattern of IL-6, and TNF-α secretion in cell culture and their plasma levels suggests that not only immune cells are involved in the postmarathon immune changes (high level of plasma IL-6 can be tissue originated). This observation is supported by the literature data.[15,56] These findings suggest that these changes are not leukocyte dependent and that marathon-associated stress factors significantly alter functional properties of immunocytes and other cytokine-secreting cells.

Analysis of activated cytokine ratios suggests a shift in cytokine equilibrium. Assuming that IL-2/IL-10 ratio reflects type-0/type-2, IL-2/IFNγ ratio to type-0/type-1, and IFN-γ/IL-10 ratio to type-1/type-2 balances, it appears that marathon race favors the type-2 immune response. The fact that these alterations were registered immediately after marathon suggests the involvement of neuroendocrine stress mediators (especially HPA axis), which are predominantly released during the marathon. This data corresponds to the current concept of neuroendocrine regulation of immune stress-response and is supported by the literature.

The emotional component of marathon race and psychological profile of runners should not be omitted while discussing the results. A premarathon raise in saliva cortisol and testosterone levels has been documented.[29,57]

Correlative analysis revealed a number of high and significant interrelations between immune and endocrine parameters, which were primary driven by time-effect. Plasma ACTH level significantly and negatively correlated with in vitro secretion of IL-2 and IFN-γ ($r = -0.53$ and $r = -0.536$, respectively). The plasma cortisol level correlated with IL-2 and IFN-γ secretion ($r = -0.63$ and $r = -0.82$, respectively) in a similar manner. Analogous interactions were seen for β-endorphin. No other correlative interactions were found for these hormones and cytokines secretion. A significant but low correlation was observed between IL-2 secretion and plasma level of growth hormone ($r = -0.4$).

Observed changes in the plasma ACTH, β-

endorphin, and cortisol levels reflect the "classic" stress-induced neuroendocrine response and correspond to the results from other studies.[27-29] Similarly, our results on increase in plasma growth hormone level are consistent with data of Scavo et al.[29] and Suzuki et al.[6]

The current model of stress response is becoming further complicated. It involves both inter- and intrasystemic interactions in practically all physiologic bodily systems. Close interactions between neuroendocrine and immune systems are well documented, and their homeostatic balance is critically important for development of the appropriate immune response. Our data supports the current model of stress-induced neuroendocrine immunomodulation and shows that stress factors of marathon running alter the immune response by shifting the cytokine balance. It appears that marathon-associated stress factors predominantly affect type-1 immunity, and, to a lesser degree, type-0 and type-2 immune responses. Downregulation of type-0 and type-1 cytokine secretion presignifies a decrease in the secretion of type-2 cytokine. Stress-induced modulation of cytokines is primary mediated by the HPA axis, and the secretion of type-0 and type-1 cytokines are mainly affected. These changes have an acute pattern and return to base level after 24 hours. Alterations in the secretion of proinflammatory and anti-inflammatory cytokines can be registered immediately at finish line and can be detectable for as long as 5 days after a marathon. This information may be beneficial for the development of new countermeasures to preserve the wellness of athletes and subjects undergoing extensive physical stress.

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