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Some aspects of the acute phase response after a marathon race, and the effects of glutamine supplementation

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Abstract Strenuous exercise may be associated with immune suppression. However, the underlying mechanism is not known. A decrease in the plasma level of glutamine, which is utilised at a high rate by cells of the immune system, and an increase in the plasma level of some cytokines may impair immune functions such as lymphocyte proliferation after prolonged, exhaustive exercise. In two separate studies of the Brussels marathon, using similar protocols, the time course of the changes in the plasma concentrations of some amino acids (glutamine, glutamate, alanine, tryptophan and branched chain amino acids), acute phase proteins and cytokines (interleukins IL-1α, IL-2, IL-6, tumour necrosis factor type a) was measured in male athletes. The numbers of circulating leucocytes and lymphocytes were also measured. Amino acid and cytokine concentrations have not previously been measured concomitantly in marathon runners; the measurement of some of these parameters the morning after the marathon (16 h) is novel. Another novel feature is the provision of glutamine versus placebo to marathon runners participating in the second study. In both studies the plasma concentrations of glutamine, alanine and branched chain amino acids were decreased immediately after and 1 h after the marathon. Plasma concentrations of all amino acids returned to pre-exercise levels by 16 h after exercise. The plasma concentration of the complement anaphylotoxin C5a increased to abnormal levels after the marathon, presumably due to tissue damage activating the complement system. There was also an increase in plasma C-reactive protein 16 h after the marathon. The plasma levels of IL-1α were unaffected by the exercise, while that of IL-2 was increased 16 h after exercise. Plasma IL-6 was increased markedly (≈ 45-fold) immediately after and at 1 h after exercise. Neopterine, a macrophage activation marker, was significantly increased post-exercise. There was a marked leucocytosis immediately after the marathon, which returned to normal 16 h later. At the same time there was a decrease in the number of T-lymphocytes, which was further reduced within 1 h to below pre-exercise levels. Glutamine supplementation, as administered in the second study, did not appear to have an effect upon lymphocyte distribution.

Key words Marathon runners · Acute phase response · Immune cells · Glutamine

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Introduction

Several reports have linked long-term exercise and heavy training with increased susceptibility to infections. Thus, a high incidence of illnesses such as upper respiratory tract infection and gastrointestinal problems has been reported to occur in athletes after prolonged, exhaustive exercise (Linde 1987; Brouns 1991; Fitzgerald 1991; for reviews see Brenner et al. 1994; Hoffman-Goetz and Pedersen 1994; Nieman 1994). Although a considerable leucocytosis occurs in response to this type of exercise (Larrabee 1902), the numbers and functions of circulating immune cells can be adversely affected by prolonged, exhaustive exercise (Nieman 1994; Shephard et al. 1994). The high incidence of infections may thus be associated with impaired function of cells of the immune system; for example, reductions in natural killer cell activity and decreased T-cell responses to mitogenic stimulation have been observed after prolonged, ex-

haustive exercise (Field et al. 1991; Pedersen 1991; Fry et al. 1992; Nieman 1994; Shephard et al. 1994).

Ardawi and Newsholme (1983) and Newsholme et al. (1985) have demonstrated that glutamine is utilised at a high rate by some cells of the immune system and, in particular, have shown that it is essential for proliferation of lymphocytes and for some key functions of macrophages. (Parry-Billings et al. 1990). Hence, a decrease in the plasma level of glutamine may impair immune function.

Skeletal muscle has been shown to be an important site for the synthesis, storage and release of glutamine. There is a decrease in plasma glutamine after prolonged, exhaustive exercise (Parry-Billings et al. 1992). Thus, it has been suggested that restoring glutamine to physiologically normal levels after exercise may help the normal function of the immune system so that the incidence of infections after prolonged exercise may be decreased (Newsholme et al. 1988). Indeed, evidence for this suggested role of glutamine continues to accumulate (Rowbottom et al. 1996).

In addition to a decrease in the plasma levels of glutamine, plasma levels of branched chain amino acids (BCAA) are decreased at the end of a marathon race (Blomstrand et al. 1988; Poortmans et al. 1991) and the plasma level of some cytokines is increased (Shephard et al. 1994). However, to the authors' knowledge, no previous study has measured the plasma concentrations of amino acids, cell numbers and cytokines using the same sample. The present study investigated white blood cell numbers, together with the plasma concentrations of some amino acids, cytokines and some acute phase response markers in athletes after two separate marathon races. In addition, a novel feature in one study was that, after a race, athletes were given drinks containing either placebo or glutamine, and the effects upon the parameters under investigation were monitored.

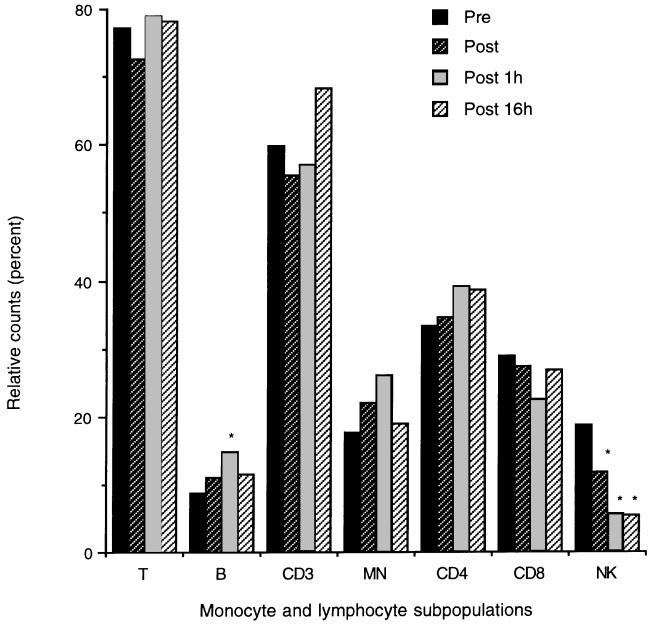

Fig. 1 Relative counts of the lymphocyte subpopulations and monocytes before and after the marathon (Study I) (geometric means). (*T* T-lymphocytes, *B* B-lymphocytes, *CD3* CD3, T-immunocompetent lymphocytes, *MN* monocytes, *CD4* CD4, T-helper lymphocytes, *CD8* CD8, T-suppressor/cytotoxic lymphocytes, *NK* natural killer lymphocytes). Significance of the difference between pre-and post-exercise means is denoted by *($P < 0.05$)

Methods

Two separate studies of male volunteers participating in the Brussels marathon were undertaken (Study I, 1991; Study II, 1993). Ethical permission for both studies was obtained from the committee for Medical Ethics (Faculté du Medecine, Université Libre de Bruxelles). No dietary restrictions were made for either study, since there were considerable logistical difficulties involved in attempting to standardise the nutritional status of athletes in these field studies. The protocol used for both studies was very similar; however, in Study II the athletes were given a glutamine/placebo drink after running the marathon. The details are as follows.

Study I

Twelve male volunteers, aged 20–40 years, participating in the Brussels marathon race 1991 gave their consent to the following experimental protocol.

Blood samples

Four blood samples were taken from the antecubital vein using Vacutainers, without stasis, 30 min prior to the race (Pre), within 15 min after completing the marathon (Post), after the first hour in the recovery period (1 h), and 16 h after finishing the race (16 h), i.e. the next morning. All subjects were supine while the blood samples were taken.

All blood samples were collected using ethylenediaminetetraacetic acid as an anti-coagulant, and the plasma was separated as soon as possible and frozen at −30°C in several vials for each sample. They were thawed only once before analysis.

Assays

The following analyses were made for each sample by appropriate enzyme-linked immunosorbent assays (ELISA), radioimmunoassay or enzymatic method. Total leucocytes were measured with the Hematology System (Technicon), and lymphocyte subsets were determined using two-colour flow cytometry (FACScan) and monoclonal antibodies (Becton Dickinson, UK). Plasma C-reactive protein (CRP) was analysed by laser nephelometry using the immunoglobin G (IgG) fraction of a rabbit anti-(human CRP) antiserum with an automated procedure on an RA 1000 turbidimeter (Technicon). An enzyme immunoassay was used for the determination of complement anaphylotoxin (C5a) (Enzygnost C5a from Boehringer). Tumour necrosis factor type a (TNFα) was determined using a radioimmunoassay (Amersham). Interleukins IL-1α and IL-2 were assayed by ELISA (Medgenix, Brussels, Belgium). Neutralised perchloric acid extracts were analysed using enzymatic assays for levels of glutamine (Windmueller and Spaeth 1974), glutamate (Bernt and Bergmeyer 1974), alanine (Williamson 1974) and BCCA (Livesey and Lund 1980).

Study II

Eighteen male athletes participating in the Brussels Marathon 1993 were studied. Blood samples were taken as outlined in Study I.

Provision of glutamine

Immediately after the second blood sample (Post), subjects were given 330 ml water containing either a placebo (5 g Malto dextrin) or 5 g glutamine and again 1 h later; the drinks were allocated randomly. Subjects were asked not to consume alcohol between the first two post-marathon samples. For medical and ethical reasons we were not able to ask them to abstain from consuming anything else. The following parameters were measured using the same assays as in Study I where appropriate: the total numbers of peripheral blood leucocytes and lymphocytes, and lymphocyte subsets; the plasma concentrations of glutamine, BCAA acute phase markers, CRP and complement C5a; neopterine, a marker of macrophage activation, which was measured using a radioimmunoassay (RIAcid, Henning Berlin, Germany); cytokines IL-6 and interferon-γ (IFγ) which were measured using ELISA methods.

Data analysis

Statistical validations were made by non-parametric analyses (Wilcoxon test between basal value and each post-exercise value – $P < 0.05$). Because most of the data were not distributed normally, logarithmic transformation was applied and geometric means and ranges were reported for the variables. When the Kolmogorov-Smirnov test did not show Gaussian distribution of log values, the statistical differences between resting values and each of the post-exercise values were established by the Wilcoxon matched-pairs signed-ranks test. In order to evaluate the statistical meaning of several related samples (from rest to the next morning, i.e. 16 h), the Friedman test was applied.

Results

Study I 1991

The average time for completion of the marathon race (42.196 km) was 3 h 45 min (range 3 h 06 min - 4 h 24 min). Circulating numbers of B-cells and monocytes were elevated 1 h after exercise, compared with baseline values. By contrast, natural killer (NK) cells were reduced by about 40%; this decrease was maintained until at least 16 h after the marathon Fig. 1.

The following plasma amino acid concentrations were decreased after the marathon: alanine, by 40% at Post and 48% at 1 h; glutamine, by 19% at Post and 26% at 1 h; BCAA, by 25% at Post and at 1 h. All these returned to pre-exercise levels at 16 h (Table 1a). Some plasma cytokine levels in samples taken before and after the marathon are given in Table 1b. There was an increase in the plasma concentrations of IL-2 of 33% at 16 h; those of IL-1α and TNFα remained unaltered. There was approximately a sixfold increase in the plasma concentration of complement C5a at Post, which was decreased ≈ fourfold at 1 h. At 16 h, there was almost a threefold increase in CRP (Table 1c), compared with the earlier samples.

Table 1a Plasma concentrations ($\mu mol \cdot l^{-1}$) of amino acids of Study I. Values are denoted as geometric mean and range (in *parentheses*). (*n* Numbers where complete sets of data were available)

Time	Concentration ($\mu mol \cdot l^{-1}$) of:			
	Glutamine	Alanine	Glutamate	Branched chain amino acids
Pre	571	544	62	487
(n = 12)	(471–658)	(192–707)	(39–76)	(385–601)
Post 5–15 min	462**	319*	62	368*
(n = 12)	(362–542)	(193–419)	(47–77)	(285–460)
Post 1h	421**	281**	55	336*
(n = 12)	(340–502)	(169–337)	(39–66)	(258–625)
Post 16 h	555	441	63	449
(n = 10)	(503–653)	(259–609)	(51–77)	(382–532)

Significance of the difference between pre- and post-exercise means is denoted by *P = 0.01 and **P = 0.001

Table 1b Plasma concentrations ($ng \cdot l^{-1}$) of cytokines interleukin-1 (*IL-1*α), interleukin-2 (*IL-2*) and tumour necrosis factor (TNFα) of Study I. Values are denoted as geometric mean and range (in *parentheses*). (*n* Numbers where complete sets of data were available)

Time	Concentration ($ng \cdot l^{-1}$) of:		
	IL-1α	IL-2	TNFα
Pre	664	1113	659
(n = 12)	(543–771)	(810–1500)	(438–819)
Post 5–15 min	643	1020	705
(n = 12)	(519–775)	(680–1420)	(532–923)
Post 1 h	658	1111	734
(n = 12)	(554–789)	(720–1430)	(585–992)
Post 16 h	685	1479*	760
(n = 10)	(563–828)	(1050–2060)	(596–991)

Significance of the difference between pre- and post-exercise means is denoted by *P = 0.01

Table 1c Plasma concentrations of acute phase C-reactive protein (*CRP*) and complement anaphylotoxin (*C5a*) of Study I. Values are denoted as geometric mean and range (in *parentheses*). (*n* Numbers where complete sets of data were available)

Time	CRP ($mg \cdot l^{-1}$)	C5a ($\mu g \cdot l^{-1}$)
Pre	3.3	1.2
(n = 12)	(1.0–11.0)	(0.4–3.7)
Post 5–15 min	2.0	5.5*
(n = 12)	(1.0–16)	(2.1–10.2)
Post 1 h	2.2	2.4
(n = 12)	(1–14)	(0.6–8.3)
Post 16 h	15.0*	1.5
(n = 10)	(6.0–25.0)	(0.4–4.4)

Significance of the difference between pre- and post-exercise means is denoted by *P = 0.05

Study II 1993

The average time for completion of the marathon race was 3 h 42 min (range 3 h 0 min–4 h 42 min). The total number of leucocytes in the blood tripled immediately and 1 h after the marathon (Post and 1 h); however, there was a 13% decrease in the number of T-cells at Post, and a 30% decrease in the total number of circu-

lating lymphocytes, and a 30% decrease in the number of CD8 cells at 1 h. There was a substantial decrease (85%) in NK cells at 1 h, which remained low (50%) at 16 h after the marathon. The numbers of B-cells and CD4 cells were unchanged (Table 2a).

The following plasma amino acid concentrations were decreased after the marathon: glutamine, by 28% at 1 h in both the placebo and glutamine groups; BCAA, by 16% at Post in the placebo group, and by 38% at 1 h in the glutamine group (Table 2b).

The plasma concentration of complement C5a was increased fourfold in the samples taken immediately after exercise (Post); CRP levels were also increased fourfold, but not until the morning after the marathon, at 16 h. There was an increase in the plasma concentrations of IL-6 (45-fold), and of neopterine (0.2-fold) immediately after the marathon (Post) but no differences were observed between the placebo and glutamine groups (Table 2c).

Discussion

There is considerable information in the literature concerning the influence of exercise on the leucocyte population (for reviews see Mackinnon, 1992; Brenner et al. 1994; Nieman 1994) and the cytokine response (for review see Shephard et al. 1994). By contrast, the literature is much less prolific on the acute phase reactant responses (Liesen et al. 1977, Dufaux et al. 1984, 1989 a and b and 1991; Camus et al. 1994) and the metabolic implications (Blomstrand et al. 1988; Poortmans et al. 1991) involved during prolonged exercise. The present investigation is an attempt to make a link between the lymphocyte changes, some acute phase proteins and cytokine responses and some plasma metabolic implications.

The threefold increase in the total number of peripheral blood leucocytes that occurred immediately after the marathon was sustained for at least an hour, returning to pre-exercise levels the next morning, as usually observed after prolonged, exhaustive exercise. However, we observed a 30% reduction in total lymphocytes in samples taken 1 h post-exercise, and a complete recovery by the next morning. This observation has also been reported recently, by other authors, to occur in marathon runners (Nieman et al. 1995) and in subjects involved in intensive running for 2–4 h (Shek et al. 1995; Bury et al. 1996).

An increase in T-lymphocytes has been observed during and immediately after prolonged exercise on a treadmill, followed by a decrease 30 min later (Shinkai et al. 1992; see Nieman 1994). Thus, it was of interest to note that, in Study II, numbers of total and T-lymphocytes (as a percentage of the total) were decreased in the samples taken immediately (5–15 min) after the marathon (Post), compared with pre-exercise levels.

Conflicting results are reported in the literature as far as the plasma level of IL-1α during exercise is concerned

Table 2a Data on peripheral blood leucocytes and lymphocyte subpopulations of natural killer cells (*NK*), T-helper cells (*CD4*) and T-suppressor cells (*CD8*) in athletes before (*Pre*) and after (*Post*, 1 h, 16 h) the marathon of Study II, with either a glutamine (*Gln*, $n = 10$) or placebo (*Plac*, $n = 8$) drink. Values are denoted as geometric mean and range

Time	Total leucocytes ($\times 10^3 \cdot l^{-1}$)		Total lymphocytes ($\times 10^3 \cdot \mu l^{-1}$)		T-cells (% of total)		NK cells (% of total)		CD4 cells (% of total)		CD8 cells (% of total)		B-cells (% of total)	
	Plac	Gln	Plac	Gln	Plac	Gln	Plac	Gln	Plac	Gln	Plac	Gln	Plac	Gln
Pre	5.17	4.75	1.51	1.54	70	70	17	15	34	34	26	24	11	9
	(3.39–7.09)	(2.98–5.89)	(0.78–2.69)	(0.98–2.07)	(55–80)	(58–77)	(11–27)	(6–26)	(25–43)	(25–48)	(20–33)	(14–43)	(5–23)	(6–13)
Post	16.09***	14.42***	1.42	1.17	61**	60**	6*	9*	37	42	28	24	15	8
5–15 min	(10.59–22.85)	(11.33–18.81)	(0.62–3.41)	(0.59–2.01)	(37–72)	(51–79)	(3–15)	(2–19)	(13–65)	(31–52)	(17–59)	(19–35)	(7–49)	(5–19)
Post	15.83***	15.41***	0.91***	0.77***	59**	64**	2***	2***	40	42	16^{+}	18^{+}	16	15
1 h	(10.39–20.33)	(14.09–17.77)	(0.43–1.99)	(0.34–1.30)	(28–81)	(47–79)	(1–4)	(1–5)	(23–55)	(32–53)	(7–28)	(14–26)	(10–49)	(12–20)
Post	6.58	5.97	1.69	1.78	58	79*	5**	9**	47***	48***	18	22	14	9
16 h	(3.34–10.17)	(4.65–7.50)	(0.84–2.94)	(1.26–2.78)	(13–84)	(76–84)	(1–11)	(5–18)	(44–51)	(44–52)	(3–28)	(19–26)	(6–70)	(7–16)

Statistical significance compared with baseline is denoted by $^{+}P = 0.05$; $*P = 0.01$; $**P = 0.003$; $***P = 0.001$

Table 2b Plasma amino acid concentrations (glutamine and branched chain amino acids) in athletes before (pre) and after (post, 1 h, 16 h) the marathon of Study II with either a glutamine (*Gln*, $n = 10$) or placebo (*Plac*, $n = 8$) drink. Values are denoted as the geometric mean and range (in *parentheses*)

Time	Glutamine (μM)		Branched chain amino acids (μM)	
	Plac	Gln	Plac	Gln
Pre	656 (571–738)	676 (581–800)	444 (322–729)	386 (278–390)
Post 5–15 min	600* (522–685)	594* (519–651)	384 (273–615)	315** (273–392)
Post 1 h	513** (395–643)	541** (433–621)	355*** (308–461)	266*** (198–332)
Post 16 h	646 (533–785)	686 (571–900)	427 (362–496)	408 (317–546)

Statistical significance compared with baseline is denoted by $*P = 0.05$; $**P = 0.01$; $***P = 0.001$

(Shephard et al. 1994). Moreover, blood levels of TNFα and IFγ are not changed by moderate exercise (Haahr et al. 1991; Smith et al. 1992; Pedersen et al. 1994). In the present study, the plasma levels of IL-1α and TNFα were not affected immediately after the marathon race, suggesting that the monocytes and macrophages were not activated by the exercise. However, similar to Dufaux and Order (1989b), we did observe a 10–15% increase of the plasma neopterine level, which is a marker of macrophages. The plasma concentration of IL-6 was greatly elevated immediately after the marathon, suggesting an activation of B- and T- cells; this elevation was maintained for at least 1 h in the recovery period. The increased level of plasma IL-2 observed 16 h after the marathon (Study I) has also been observed by Pedersen (1991).

Complement activation has been observed after prolonged and short-term exercise (Dufaux et al. 1989a, 1991; Camus et al. 1994). Small amounts of complement C5a have been found to induce chemotaxis, and enhance phagocytosis and enzyme release from human polynuclear neutrophils in vitro (Rimmer and Horton 1991). Therefore, we specifically investigated this fragment together with the CRP, which also activates complement and promotes phagocytosis. Complement C5a fragments were elevated (sixfold) in the samples immediately after the race (Study I) and remained above the basal level at 16 h after finishing the event. This observation extends previous results to a more prolonged and severe form of exercise. It is suggested that this increase in the complement cleavage fragments indicates an activation of the complement to enhance the activity of macrophages in clearance of fragments from damaged muscle tissue. The level of acute phase protein CRP in plasma was elevated (fourfold) 16 h after the race in both studies. A similar observation was made by Dufaux et al. (1984) and Liesen et al. (1977), who demonstrated a sixfold increase in the concentration of serum CRP following 2 and 3 h of running, 1 day after the run. These results are consistent with damage to muscle after

Table 2c Plasma concentrations of some acute phase response markers in athletes before (*Pre*) and after (*Post*, 1 h, 16 h) the marathon of Study II with either a glutamine (*Gln*, $n = 10$) or placebo (*Plac*, $n = 8$) drink (*IL-6* Interleukin-6, *CRP* C-reactive protein, *C5a* complement 5a, *IFγ* Interferon-γ). Neopterine is a macrophage marker. Values are denoted as geometric mean and range (in *parentheses*)

Time	Acute phase response marker									
	IL-6 ($ng \cdot l^{-1}$)		C5a ($\mu g \cdot l^{-1}$)		CRP ($mg \cdot l^{-1}$)		IFγ ($ng \cdot l^{-1}$)		Neopterine ($ng \cdot l^{-1}$)	
	Plac	Gln	Plac	Gln	Plac	Gln	Plac	Gln	Plac	Gln
Pre	2.04 (0–8)	0.51 (0–2)	0.26 (0.13–0.48)	0.27 (0.13–0.41)	3.4 (3.0–8.0)	3.3 (3.0–5.0)	0.18 (0.02–0.57)	0.12 (0.04–0.66)	2.00 (1.72–2.45)	2.10 (1.88–2.50)
Post 5–15 min	88*** (34–235)	70*** (31–153)	1.07*** (0.20–4.27)	1.03*** (0.27–4.18)	– –	– –	0.22 (0.04–0.56)	0.28 (0.00–0.55)	2.25** (1.84–2.64)	2.43** (2.17–2.75)
Post 1 h	52*** (20–142)	36.61*** (18–65)	0.33* (0.14–1.65)	0.37** (0.21–0.71)	– –	– –	0.34 (0.08–1.00)	0.11 (0.01–0.36)	2.17 (1.80–2.52)	2.24 (1.93–2.69)
Post 16 h	– –	– –	0.18 (0.08–0.49)	0.24 (0.11–0.39)	11.8* (8.0–18.0)	6.9* (3.0–15.0)	– –	– –	– –	– –

Statistical significance compared with baseline is denoted by $*P = 0.05$, $**P = 0.01$, $***P = 0.001$

such severe and prolonged exercise. This leads to some disruption of muscle cell membranes, which releases cellular components into the blood stream (Appell et al. 1992). Indeed, the marathon races in our studies come in the category of eccentric muscle contractions which induce the production of cytokines. Recently, Pedersen and Bruunsgaard (1995) hypothesised that high-intensity eccentric exercise causes a marked increase in muscle and plasma levels of the cytokines involved in acute inflammatory responses. The increased level of CRP is considered to be regulated by the level of IL-1α and IL-6. The plasma level of IL-1α is either too small, or increases at different times from the sampling time in the present study. By contrast, the increased plasma levels of IL-6 after exercise correspond to the increase in the level of CRP after 16 h.

As in other studies (Blomstrand et al. 1988; Poortmans et al. 1991), the concentrations of plasma BCCA, alanine and glutamine decreased after long-term exercise, at least during the first hour of the recovery phase, and returned to basal values at 16 h. The plasma glutamine concentration in pre-marathon samples was within the normal range (600–700 μM) for fasting samples, which indicated that diet had not affected the glutamine concentration at that stage.

Since glutamine is important for some cells of the immune system (see Introduction) it was considered that administration of glutamine to athletes after completing the marathon might influence some of the changes in the immune system, or factors related to it, after this prolonged exercise. Indeed, the provision of glutamine (vs placebo) as a drink after a race appears to have decreased the number of infections reported by marathon runners (Castell et al. 1996). Thus it was of interest that, statistically, there was a significantly greater number of T-cells in the blood, as a percentage of total lymphocytes, in the glutamine group at 16 h post-marathon compared with the pre-marathon numbers. However, in the sample taken from the control group at the same time there was no statistical significance compared with either pre-marathon numbers or the glutamine group. It was therefore concluded that glutamine supplementation, in the doses and at the times administered in Study II, did not appear to have an effect upon lymphocyte distribution in the blood. Further studies are required to test the effects of glutamine supplementation upon immune function in endurance athletes after exercise.

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