Potential markers of heavy training in highly trained distance runners

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Markers of a heavy increase in training were examined in ten highly trained distance runners (mean(s.d.) age 29.8(1.7) years, maximal oxygen intake 65.3 ml kg⁻¹ min⁻¹, personal best 10-km time 31 min 4s) who undertook a deliberate 38% increment of training over a 3-week period. Their running performance did not improve, and six of the ten subjects developed sustained fatigue, suggesting that training was excessive, although the full clinical picture of overtraining did not develop. The Profile of Mood States was the best single marker of disturbed function, indicating increased fatigue and decreased vigour. There were no useful changes of resting heart rate or perceived exertion during submaximal running, sleep was undisturbed, and there were no orthopaedic injuries. Two subjects developed rhinoviral infections following the heavy training, and a third complained of symptoms that were diagnosed 2 weeks later as exercise-induced asthma. The increase of serum cortisol normally induced by 30 min of submaximal exercise was no longer seen when the same acute exercise was performed after heavy training. Resting lymphocyte proliferation tended to increase in response to phytohaematoglutinin (PHA) and concanavalin A (Con A), the ratio of helper to suppressor cells (H/S) decreased, and pokeweed mitogen induced smaller increases in IgG and IgM synthesis. Whereas before heavy training, PHA-stimulated lymphocyte proliferation was unchanged by 30 min of acute submaximal exercise, after 3 weeks of heavy training the same bout of exercise caused an 18% suppression of proliferation. Likewise, heavy training brought about a decrease of T-lymphocytes in response to acute submaximal exercise, but an abolition of the acute exercise-induced decrease in the H/S ratio. The previously observed exercise-induced decrease of IgG synthesis did not occur when the same acute bout of exercise was performed after heavy training. We conclude that such minor and transient changes of immune function may possibly be a warning that training is becoming excessive, but they have only a limited significance for overall immune function.

Keywords: Heavy endurance training, staleness, stress reactions, overtraining, immune system, immune function, immunoglobulins, lymphocyte proliferation, helper/suppressor cell ratios, cortisol, creatine kinase

Athletic coaches are all too familiar with the situation of excessive training, characterized by a long-lasting fatigue and worsening of competitive performance with further attempts to improve physical condition¹. The phenomenon has been described, more or less interchangeably, by a multitude of descriptive terms, including staleness, overreaching, overwork, burn-out, chronic fatigue and overstress, as well as overtraining. Diagnosis of the fully developed condition is commonly based on clinical findings, including a decrease of appetite, weight loss, a slow recovery after an exercise bout, increased irritability or emotional lability, and disturbed sleep patterns². Dressendorfer *et al.*³ have reported an increase of heart rate, although their subjects did not show any deterioration of performance. Other authors have not consistently confirmed this observation in athletes with clearer evidence of overtraining². The precise contributions of physiological, biochemical, psychological and pathological changes to the observed clinical syndrome are still unclear, and there is no good agreement on biological markers that can be used to maximize competitive preparation while avoiding overtraining.

Although anaemia, a negative nitrogen balance, a lack of specific amino acids such as glutamine, glycogen depletion, chronic electrolyte depletion and repeated subclinical injuries might all be suspected as potential contributing factors, standard blood chemistry data such as haemoglobin and serum protein concentrations, serum electrolyte levels and muscle enzyme activities have failed to demonstrate any striking changes in affected competitors^{2,4,5}. Some authors have associated excessive training and/or staleness with allergic reactions, head colds and other infections⁶⁻⁸ but it has been less clear whether this is a cause or an effect of the overtraining syndrome. One plausible hypothesis is that heavy training depresses immune function, leaving the body more vulnerable to infection^{6,9-12}. This in turn suggests the possibility of detecting excessive training in terms of immune reactions to exercise.

We have further explored reactions to heavy training in a group of ten competitive distance runners who were well-trained initially. They were normally running 70-100 km per week, and for the purpose of the experiment they volunteered to increase their training volume by an average of 38% for a 3-week period. We studied various measures of

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immune function and other potential markers of excessive training initially, at the end of the 3 weeks of heavy training, and after subjects had repeated 3 weeks of their baseline training. Since lymphocytes are considered the most important link in the relationship between exercise and immune function^{13,14} we elected to make *in vitro* assessments of mitogen-stimulated lymphocyte proliferation rates, percentages of T and B cells, helper: suppressor cell ratios and the capacity of specimens to synthesize immunoglobulins.

Subjects and methods

The subjects for this investigation were all volunteers, ten highly trained male distance runners of mean(s.d.) age 29.8(1.7) years who had been recruited and tested in accordance with a protocol approved by the University of Toronto Committee on Human Experimentation. All had achieved a 10-km performance of <35 min within the preceding 12 months. Their mean(s.d.) personal best speed of 4.76(0.26) m s⁻¹ for the 10-km distance may be compared with national and world records of 5.98 and 6.14 m s⁻¹. We did not feel ethically justified in driving the group into the extreme state of chronic fatigue and exhaustion typical of clinical 'overtraining', but nevertheless the subjects each agreed to undertake a substantial increase over their normal training routine for a 3-week experimental period, boosting their training volume to the maximum that they judged compatible with a consistent 3-week effort while avoiding major injuries. The consensus of the group was that they could sustain a 25% increment for the 3-week period. Compliance was monitored by detailed activity questionnaires, and in fact the average increase of training volume was 38%.

All subjects made five visits to the laboratory at a personally consistent time of day, usually in the late afternoon. Visits 1 and 2 followed 3 weeks of adherence to their normal training plan, visits 3 and 4 were made after 3 weeks of deliberately increased training, and visit 5 followed 3 weeks of reversion to the original training schedule. We wished to examine acute exercise responses as soon as immune function had recovered from the daily training sessions. The usual recovery period is less than 24 h, and accordingly we requested subjects to limit their training to low distance and low intensity work for 36 h immediately preceding each of the laboratory visits. During visits 2, 3 and 5, activity and sleep questionnaires¹⁵ plus the Profile of Mood States questionnaire¹⁶ were completed, anthropometric measurements were made, and the maximal oxygen intake was determined by uphill treadmill running. On day 2 of each visit, subjects ran for 30 min at 80% of their personal maximal oxygen intake, with blood samples being collected before and 5 min and 30 min after the acute exercise bout.

Maximal oxygen intake determinations

Subjects were tested by a progressive treadmill run to exhaustion. After a 3-min warm-up (9.6 km h⁻¹, 0% slope), the treadmill speed was increased to a steady

14.5 km h⁻¹, with a 2% increase of slope every 2 min. Expired gas was collected and analysed using a Beckman Metabolic Cart (Beckman Instruments, Malton, Ontario, Canada). The heart rate (CM₅ electrode placement) was recorded during the final 10 s of each test minute.

Three criteria were used in gauging maximal effort: a plateau of oxygen consumption to within 2 ml kg⁻¹ min⁻¹ with a further increase of work rate; a respiratory gas exchange ratio of 1.15; and a plateauing of heart rate with further increase of work-rate.

Submaximal exercise bout

The treadmill was set at 0% slope, and a speed judged to elicit 80% of the treadmill maximal oxygen intake¹⁷. Expired gas was collected and analysed throughout, and heart rate was recorded every second minute. Perceived exertion was rated in the final minute of exercise, using the ten-point scale of Borg¹⁸.

Blood sampling

Blood samples were collected from the antecubital vein at rest immediately before and 5 min and 30 min after the 30-min acute bout of submaximal exercise. Measurements included cortisol, creatine kinase, lymphocyte proliferation, percentages of T and B cells, helper/suppressor cell ratios, and immunoglobulin synthesis. Analytic techniques are detailed elsewhere¹⁹.

Serum cortisol determinations

Serum cortisol was measured by an automated fluorescent polarizing spectrometer assay (Abbott TDx Cortisol assay (Abbott Laboratories, Mississauga, Ontario, Canada)).

Serum creatine kinase determinations

Serum creatine kinase was determined using Boehringer-Mannheim kits (Boehringer-Mannheim, Montreal, Quebec, Canada). A mixture of creatine phosphate and ADP yields ATP and creatine, and in the presence of glucose, reduces NADP to NADPH. The absorbance at 365 nm is increased in proportion to creatine kinase activity.

Mitogen stimulation of peripheral lymphocytes

Mononuclear cells were separated by centrifugation with Sepracell-MN, (Sepratech, Oklahoma City, USA) a colloidal silica-based medium. They were then resuspended in an appropriate medium and checked to ensure cell viability, using the trypan blue exclusion technique²⁰. Peripheral blood monocytes were incubated in the presence of an appropriate range of concentrations of two plant lectins (phytohaemagglutinin, PHA, 10–20 µg ml⁻¹, and concanavalin A, Con-A, 1–4 µg ml⁻¹) that are specific mitogenic stimulants of T cell proliferation. Responses to PHA are reputedly resistant to cortisone levels, while responses to Con-A are suppressed by cortisone²¹.

At 48 h, the cells were pulsed for 24 h with 1.0 μCi of radioactive (methyl-3H) thymidine, and after 72 h the cells were harvested on to glass fibre filters. The filters were subsequently placed in scintillation fluid and counted by a liquid scintillation counter. Results were expressed as the difference in counts between the stimulated cultures and non-stimulated controls.

Lymphocyte populations

The mononuclear cell suspension was divided and marked by 20 μl volumes of monoclonal antibodies specific for T cells (Anti-Leu-4, CD3⁺), B cells (Anti-Leu-16, CD20⁺), helper cells (Anti-Leu-3a, CD4⁺) and suppressor cells (Anti-Leu-2a, CD8⁺).

The marked cells were resuspended and passed through a flow cytometer, using light at 650 nm to activate the fluorochrome-labelled monoclonal antibodies. The ascites mouse Ig does not bind to any of the specific sites for the human monoclonal antibodies, and the area under the measurable light profile for this antigen was thus used as a control to subtract from the histograms seen with the specific antibodies.

Immunoglobulin synthesis

Cell suspensions were mixed with equal volumes of lyophilized pokeweed mitogen, incubated for 7 days, centrifuged and then frozen for IgG and IgM analysis by enzyme-linked immunosorbent assay. An IgG or IgM peroxidase conjugate was added to each well of the culture plate. After 1 h of incubation and further washing, an *ortho*-phenyldiamine 2HCl substrate catalysed by 0.5% hydrogen peroxide was added to generate a yellow colour that could be measured against an appropriate calibration curve at a wavelength of 492 nm.

Statistical analysis

Repeated measures analyses of variance were used in conjunction with *post hoc* contrast analyses to assess

differences over time. Paired *t* tests were utilized to assess the effects of acute exercise (comparing pre-exercise with post-exercise values at a given testing session). The null hypothesis was rejected at $P < 0.05$.

Results

Subject characteristics

The subjects were typical long-distance runners. Their body mass (mean(s.d.) 66.1(4.7) kg) was low in relation to their height (mean(s.d.) 176.8(4.4) cm), and they had relatively little subcutaneous fat (sum of biceps, triceps, subscapular, suprailiac and medial calf skinfolds (mean(s.d.) 26.1(6.0) mm)). The relative maximal oxygen intake was much higher than in the typical well-trained man of 29 years (initial mean(s.d.) 65.3(4.9) $\text{ml kg}^{-1} \text{min}^{-1}$), and the personal best 10-km times of mean(s.d.) 31 min 43 s (1 min 38 s) confirmed that these were indeed highly trained runners. Despite the narrow range of interindividual performances, the personal best race times were quite closely correlated with the individuals' relative maximal oxygen intakes ($r = -0.86$, $P < 0.0014$).

All subjects were training hard for distance events when first recruited, their initial weekly running distances averaging 98.8(22.0) km.

Modification of training plan

Each runner was consulted individually to establish a schedule of heavy training that could be sustained for the 3-week experimental period without incurring gross orthopaedic injuries. In most cases, subjects elected to increase both the intensity of exercise and the weekly running distance, but subjects 4 and 7 restricted themselves to an increase of training distance (Table 1).

The consensus of the group was that they could sustain a 25% increment of training volume throughout the 3 weeks. In fact, the activity questionnaires showed that the weekly distance had been increased

Table 1. Training plans adopted by individual subjects during the initial 3 weeks (B1), during the 3 weeks of heavy training (HT), and during the 3 weeks of repeated baseline training (B2)

Subject	B1 training distance (km week^{-1})	HT training distance (km week^{-1})	B2 training distance (km week^{-1})	% increase B1 to HT	B1 training volume	HT ¹⁵ training volume	B2 training volume	% increase B1 to HT
1	103.7	121.1	106.2	17	5769	7102	5964	23
2	97.0	108.3	61.1	12	5268	6315	3419	20
3	107.8	131.5	70.7	22	5761	7112	3558	23
4	77.2	126.4	64.6	64	3981	6520	3415	64
5	81.6	135.0	98.8	65	3948	6640	5040	68
6	96.5	130.7	102.4	35	5765	7802	5803	35
7	109.4	135.8	89.2	24	5078	6486	4242	28
8	148.7	193.1	107.7	30	6696	9016	5011	35
9	69.3	121.0	65.0	75	3996	7081	3795	77
10	96.5	100.2	93.1	4	5742	5953	5391	4
Mean(s.d.)	98.8(22.0)	130.3(24.9)	85.9(18.6)	35(25)	5200(943)	7003(877)	4564(996)	38(24)

The training volume was approximated as a simple multiple of the daily training distance and a speed-related assessment of the oxygen cost. For example, if an athlete ran a distance of 16 km at a pace of 4.47 m s^{-1} , the estimated oxygen cost would be 57.1 $\text{ml kg}^{-1} \text{min}^{-1}$ (Reference 17), and the training volume would be 16×57.1 , or 913.6 units. If there were five such sessions in a week, the total volume score for that individual would be 913.6×5 , or 4568 units.

by 35%, and since most subjects also had some increase of speed over the same route, the estimated training volume was increased by an average of 38%. Nine of the ten runners achieved at least a 20% increase of training volume throughout the 3-week period. The largest increase (subject 9) is a little misleading, in that he began the study at a level some 30 km week⁻¹ below his normal schedule. Subject 10 had achieved a 21% increase by the end of the second week, but then complained of excessive tiredness, and managed only five abbreviated workouts in the third experimental week. If his data are excluded, the remaining subjects demonstrated a 41% average increase of training volume. Subjects 2, 3, 4, 7 and 8 also reported sustained tiredness at the end of the experimental period, and thus undertook less than their normal training volume during the recovery period. In terms of both performance and symptomatic responses, we would conclude that at least six of the ten subjects were beginning to show signs of excessive training.

Objective signs of excessive training

Subjects were asked to record waking heart rates on a daily basis throughout the 3 weeks of heavy training and the subsequent 3-week recovery period. There was a small and not statistically significant trend to an increment during the period of heavy training, and a decrease in the first week of recovery, but even in the most fatigued subject (subject 4) the increment from normal training (mean 51.5 beats min⁻¹) to the third week of heavy training (53.4 beats min⁻¹) was less than 2 beats min⁻¹. Plainly, a change of this order, even if statistically significant, would have little value as an early warning of excessive training.

Subjects were also asked to record the quality and the quantity of their daily sleep. In the eight individuals who kept adequate records, there was no evidence of a change of quality or quantity of sleep either during the period of heavy training or during the subsequent recovery period.

The Profile of Mood States (Figure 1) suggested that heavy training led to some changes in the initial

Figure 1. Impact of 3 weeks of increased training upon the Profile of Mood States. Average values for ten endurance athletes initially running 70–100 km week⁻¹. Data obtained by Morgan²⁹ after heavy training of a swimmer is also presented. ●, Before HT; ○, immediately after HT; ■, Morgan's data. (HT = 3-week period of heavy training)

classical 'iceberg' profile. There was an increase of global disturbance (that is, a displacement of the summated mood ratings away from the normal standardized score, $P = 0.03$), with a significant increase of fatigue ($P = 0.03$) and a significant decrease of vigour ($P = 0.02$). Although not statistically significant, there was also a trend for other negative mood states (depression, anger and confusion) to increase in response to heavy training. The subsequent recovery period was associated with a return towards baseline scores for both the global mood rating and scores on the five negative mood scales. However, scores for vigour remained significantly below the initial level.

None of the runners developed any clinical injuries during their 3-week period of heavy training. Subject 10 sensed the onset of a cold, which became manifest immediately after the period of heavy training. Subject 6 felt in good health when tested, yet within 3 days of completing the heavy training, he also developed an acute rhinoviral infection. Subject 9 indicated that he felt as if he were developing a cold at his final laboratory visit. He was diagnosed 2 weeks later as having exercise-induced asthma, although he had not complained of respiratory problems in the years before the study.

Impact on aerobic power

On average, the heavy training did not lead to any gains of maximal aerobic power (Table 2). In all except three subjects, the difference of maximal oxygen intake between the two visits was less than 5%. The largest decrease of 5.9% was seen in a subject who was highly fatigued (subject 4), whereas the largest increase of 8.2% was seen in a subject who ran his personal best time on day 11 of the heavy training schedule. The decrease of peak heart rate, although small, approached statistical significance, being shown by nine of the ten subjects.

There was little difference in the oxygen cost of running during the acute bout of submaximal exercise among visits 2, 3 and 5 (Table 3), although there was a small but statistically significant decrease of the heart rate response to the acute bout of exercise immediately following the period of heavy training.

Table 2. Influence of 3-week period of increased training upon maximal performance – comparison of initial with experimental values

Variable	Mean(s.d.)	Range
Initial $\dot{V}O_{2\max}$ (ml kg ⁻¹ min ⁻¹)	65.3(4.9)	58.4–72.9
Experimental $\dot{V}O_{2\max}$ (ml kg ⁻¹ min ⁻¹)	65.1(4.5)	58.7–70.8
Initial max heart rate (beats min ⁻¹)	186(9.0)	172–204
Experimental max heart rate (beats min ⁻¹)	182(8.0)	174–200
Initial treadmill time (s)	650(83)	530–802
Experimental treadmill time (s)	664(90)	546–823

A paired two-tailed t test suggests a trend to a reduction of maximal heart rate after the period of increased training ($P = 0.058$)

Table 3. Comparison of the oxygen cost of submaximal exercise ($\text{ml kg}^{-1} \text{min}^{-1}$) and heart rates (HR, beats min^{-1}) initially (B1), after 3 weeks of heavy training (HT), and after 3 weeks of repeated baseline training (B2)

Subject	Treadmill speed (m s^{-1})	B1		HT		B2	
		Submax $\dot{V}\text{O}_2$	Submax HR	Submax $\dot{V}\text{O}_2$	Submax HR	Submax $\dot{V}\text{O}_2$	Submax HR
1	4.47	54.9	152	53.6	150	53.0	152
2	4.69	48.6	165	51.7	169	50.6	170
3	4.58	50.8	162	47.6	156	50.8	170
4	4.47	50.7	165	58.9	161	50.9	170
5	4.47	48.3	175	49.6	165	46.1	160
6	4.47	55.6	155	55.2	156	52.8	156
7	4.25	50.7	167	49.9	161	50.3	168
8	4.36	47.6	156	45.2	149	46.6	161
9	4.81	53.6	150	56.1	148	53.3	147
10	4.92	54.8	181	50.7	172	48.4	170
Mean(s.d.)		51.6(3.0)	(A) 162.8(10.0)	51.9(4.1)	(B*) 158.7(8.4)	50.3(2.5)	(A) 161.1(8.2)

* B differs from A ($P < 0.05$ for significant intergroup difference) by application of repeated measures analysis in conjunction with Duncan's Multiple Range Test.

Table 4. Response of serum cortisol to 30 min of exercise at 80% of maximal oxygen intake at the end of 3 weeks of normal training, after 3 weeks of heavy training, and after 3 weeks of return to baseline training (all values nmol l^{-1}). Data obtained at consistent times of day, immediately before exercise (Pre) and 5 min after exercise bout (latter shown as Δ value)

Subject	Initial		Heavy training		Recovery	
	Pre	Δ	Pre	Δ	Pre	Δ
1	229	226	300	22	—	—
2	519	-122	508	14	378	-98
3	—	—	397	-95	334	-49
4	217	85	212	141	—	—
5	506	222	619	-64	445	110
6	297	82	—	—	201	75
7	306	-41	305	-47	240	-71
8	470	-44	456	-97	545	-108
9	222	47	268	-37	110	41
10	295	156	327	32	319	38
Mean(s.d.)	340(124)	68*(121)	377(130)	-15(76)	321(139)	-8(84)

* $P < 0.05$; two-tailed paired t test

Serum cortisol levels

The resting cortisol levels (Table 4) were quite high at all three visits (2, 3 and 4). Resting values tended to be higher after heavy training and a little lower after resumption of baseline training, but these differences were not statistically significant.

At the first laboratory visit, the acute bout of submaximal treadmill exercise led to a small increase of serum cortisol ($P < 0.05$). However, submaximal exercise no longer caused a significant increase after heavy training. Complete datasets were only available on seven of the ten subjects. In these individuals, the cortisol response to the acute bout of exercise at visit 3 was similar to that seen at visit 1, although because of the small sample size the visit 3 response was not statistically significant.

Serum creatine kinase levels

The resting creatine kinase (CK) levels were high in all three training conditions (Table 5). The submaximal treadmill challenge led to a further increase of CK at all three laboratory visits. Datasets were complete

for only nine subjects, the increments immediately after exercise being 25, 27 and 20 units l^{-1} respectively for the three experimental conditions. Two of three subjects who did not show an acute exercise CK response began the experiment with particularly high resting CK values (277 and 293 units l^{-1} respectively, relative to anticipated normal ceilings of 100 units l^{-1}).

Table 5. Response of serum creatine kinase to acute 30-min bout of exercise at 80% of maximal oxygen intake after 3 weeks of normal training, after 3 weeks of heavy training, and after 3 weeks of return to baseline training. Mean(s.d.) of data (units l^{-1})

Sampling time	Initial	Heavy training	Recovery
Resting	155(94)	176(97)	126(94)
5 min after exercise	167(93)*	203(110)*	146(111)*
30 min after exercise	187(96)	193(104)†	141(105)

* Differ significantly from the corresponding resting value ($P < 0.05$), using repeated measures analysis in conjunction with *post hoc* contrast comparisons

† Significantly lower than the corresponding value measured 5 min after exercise ($P < 0.05$), using repeated measures analysis in conjunction with *post hoc* contrast comparisons

Table 6. Effects of acute 30-min bout of exercise at 80% of maximal oxygen intake upon mitogen-induced peripheral blood mononuclear cell proliferation. Data for phytohaemagglutinin (PHA) and concanavalin A (Con-A), expressed as a percentage change of scintillation count rate, after 3 weeks of normal training (B1), 3 weeks of heavy training (HT), and 3 weeks of return to baseline training (B2)

Subject	Percentage change in scintillation count					
	PHA			Con-A		
	B1	HT	B2	B1	HT	B2
1	-10.4	-32.2	-12.6	-12.9	-24.6	-17.6
2	-3.2	-53.5	30.3	-26.5	-43.0	55.5
3	—	-22.8	-14.0	—	-21.8	-6.7
4	3.6	-30.7	-20.0	14.6	-13.7	-10.0
5	-44.5	-18.7	-15.3	-42.0	-30.7	-16.1
6	-18.4	-16.3	-24.7	-26.2	-19.2	-12.2
7	-7.9	9.4	13.6	-13.5	16.6	21.1
8	10.7	15.4	-35.8	17.4	28.3	-18.6
9	55.4	-4.9	26.3	111.2	17.4	10.2
10	-21.5	-25.1	-25.5	-12.2	-26.4	-5.5
Mean(s.d.)	-4.0(27)	-17.9*(20)	-7.8(23)	1.1(45)	-11.7(24)	0.0(23)

* $P < 0.05$; two-tailed paired t test

Rating of perceived exertion

The initial rating of the acute bout of submaximal exertion was of mean(s.d.) 3.55(1.42) (range 2.0–6.5) units. The nearest semantic descriptions on the modified Borg scale are moderate (3 units) and somewhat strong (4 units) of exertion. Scores were closely comparable after the period of heavy training (mean(s.d.) 3.65(0.94) units) and after the 3-week return to baseline training (3.50(0.58) units).

Immune responses

Many previous studies have been content to count lymphocyte numbers. However, this approach ignores the known impact of stressors upon the relative proportions of the various cell types, their ability to proliferate and ingest bacteria, and their ability to secrete antibodies.

Mitogen-induced cell proliferation

Mitogen-induced cell proliferation is a useful overall *in vitro* measure of cell-mediated immune function^{20–23}. The acute bout of submaximal exercise had no consistent impact upon cell proliferation while subjects were following a normal training plan (Table 6). The three individuals who initially were covering the shortest training distances relative to their usual routine (and who were thus the most rested) showed a positive PHA (average +81.4%) and Con-A (average +53.1%) response to the acute bout of submaximal exercise, whereas the remaining six subjects with complete data showed some acute suppression of the response to both PHA (–46.7%) and Con-A (–24.9%). Any effect was quite transient, and at 30 min after exercise the average response for all nine subjects was greater than under resting conditions (+6.2% for PHA, and +14.2% for Con-A).

Immediately after the 3 weeks of heavy training, the resting blood samples showed increased cell proliferation (+24.6% for PHA, +32.2% for Con-A), the latter change being statistically significant ($P < 0.05$). However, relative to the new resting values,

the submaximal test exercise induced a small but statistically significant reduction in response to PHA, with a similar but statistically insignificant trend for Con-A (Table 6). Resting responses remained higher than initial values after 3 weeks of return to baseline training (for PHA, $P = 0.034$, for Con-A, $P = 0.024$), but as initially, the average response to the mitogens was unchanged by the test bout of acute submaximal exercise.

Lymphocyte populations

Three primary categories of lymphocyte are recognized, T cells, B cells and null cells. A combination of monoclonal antibodies and flow cytometry allows differentiation of these various cell types, and of subpopulations of T cells.

Both T and B lymphocyte percentages tended to decrease 5 min after exercise, with recovery 30 min after exercise, these changes being most marked immediately after heavy training (see Table 7 for statistically significant changes).

Table 7. Effects of an acute 30-min bout of exercise at 80% of maximal oxygen intake upon lymphocyte populations after 3 weeks of normal training, 3 weeks of heavy training, and 3 weeks of repeated baseline training. Data collected before exercise bout (Pre), and at 5 min (+5) and 30 min (+30) after exercise

Training regimen	Percentage of total lymphocyte count					
	T lymphocytes			B lymphocytes		
	Pre	+5	+30	Pre	+5	+30
Initial	64.7	57.5	61.0	4.65	4.02	5.01
Heavy training	a62.6	b54.3	61.1	4.64	3.77	5.28
Recovery	a55.2*	49.9	b62.2	a3.51	2.89	b4.68

All values are shown as percentages of the total lymphocyte count as seen in the peripheral blood mononuclear cell suspension. Horizontal items for a given lymphocyte type with differing letter prefixes differ significantly from each other ($P < 0.05$) by Duncan's Multiple Range Test.

* Vertically, significantly different ($P < 0.05$) using repeated measures ANOVA and applying Duncan's Multiple Range Test

Table 8. Response of helper and suppressor cells to an acute bout of 30 min of exercise at 80% of maximal oxygen intake after 3 weeks of normal training, 3 weeks of heavy training, and 3 weeks of return to baseline training. Data collected before the exercise bout (Pre), and at 5 min (+5) and 30 min (+30) after exercise

Variable	Initial	Heavy training	Recovery
Helper cells (%)			
Pre	a38.2	33.2	b30.5
+5	31.6	30.2	26.7
+30	34.5	32.0	29.6
Suppressor cells (%)			
Pre	16.6	a18.0	b12.5
+5	19.2	17.6	14.5
+30	14.0	14.2	11.6
Helper:suppressor ratio			
Pre	2.91	a2.05	a3.49*
+5	1.84	1.89	b2.53
+30	2.74	b3.17	3.98

Horizontally, a differs from b ($P < 0.05$) using repeated measures ANOVA and Duncan's Multiple Range test

Vertically, a differs from b ($P < 0.05$) using repeated measures ANOVA and applying Duncan's Multiple Range test

Horizontally, * is significantly different from both initial and heavy training values ($P < 0.05$).

The resting percentage of helper cells tended to diminish after heavy training, and their number was further decreased after return to baseline training (see Table 8 for statistically significant changes). In contrast, the resting percentage of suppressor cells was increased after heavy training, with corresponding changes in the helper:suppressor cell ratio. Under all three conditions, the helper:suppressor cell ratio tended to diminish 5 min after exercise, with a full recovery or even an overshoot 30 min after exercise (Table 8).

Mitogen-induced immunoglobulin synthesis

Pokeweed mitogen is a known activator of both T and B cells, and is thus commonly used to assess the synthesis of immunoglobulins by the B cells²⁴.

Table 9. Effect of an acute 30-min bout of exercise at 80% of maximal oxygen intake upon pokeweed mitogen-induced IgG synthesis immediately after 3 weeks of normal training, after 3 weeks of heavy training, and after 3 weeks of return to baseline training. Concentrations in ng ml^{-1} . Data collected before exercise bout (Pre), and at 5 min (+5) and 30 min (+30) after exercise

Training regimen	Timing of sample		
	Pre	+5	+30
Initial	ab644 ($n = 10$)	913 ($n = 8$)	701 ($n = 9$)
Heavy training	a537 ($n = 9$)	488 ($n = 10$)	*904 ($n = 9$)
Recovery	b884 ($n = 10$)	847 ($n = 10$)	828 ($n = 10$)

Means with the same letter prefix do not differ significantly from each other ($P > 0.05$) using repeated measures ANOVA and applying Duncan's Multiple Range test

* Data differ significantly from the other two blood sampling times immediately after heavy training ($P < 0.05$)

Table 10. Influence of an acute bout of exercise at 80% of maximal oxygen intake on pokeweed mitogen-induced synthesis of IgM after 3 weeks of normal training, after 3 weeks of heavy training, and after 3 weeks of return to baseline training. All values expressed as ng ml^{-1} IgM. Data collected before exercise bout (Pre) and at 5 min (+5) and 30 min (+30) after exercise

Training regimen	Timing of sample		
	Pre	+5	+30
Initial	a730 ($n = 10$)	765 ($n = 8$)	836 ($n = 9$)
Heavy training	a585 ($n = 10$)	589 ($n = 10$)	*908 ($n = 10$)
Recovery	b1178 ($n = 10$)	1052 ($n = 10$)	1098 ($n = 10$)

Means with the same letter prefix do not differ significantly from each other ($P > 0.05$) using repeated measures ANOVA and applying Duncan's Multiple Range Test

* Value tends to significant difference from the other two blood samples taken following heavy training session ($P < 0.07$)

Resting data showed a trend to decreased IgG production at the end of heavy training, with an increment over the 3-week return to baseline training (see Table 9 for statistically significant changes). The submaximal test exercise tended to increase IgG production at the initial visit. After the period of heavy training, the increment of IgG synthesis was delayed until 30 min after the test exercise, whereas data after the 3-week recovery period showed little effect of the acute submaximal test exercise.

Resting IgM synthesis was greater after the 3-week recovery period than either initially ($P < 0.03$) or after the period of heavy training ($P < 0.01$). Exercise-induced changes in IgM synthesis (see Table 10 for statistically significant changes) followed a somewhat similar pattern to that seen with IgG. After the period of heavy training, production was increased 30 min after the test exercise, but there was little response to the test exercise after the 3-week recovery period.

Discussion

Evidence of excessive training

Ethical limitations constrained the increase of training that our investigation was able to encourage – 3 weeks of exercise to self-selected perceived tolerance levels is not a long period of heavy training. Moreover, subjects did not face the added stress of major competition, and they had the option of tapering or ceasing the activity if fatigued. None of the subjects was clinically ill, and in most the peak aerobic power was unchanged. They cannot thus be considered as pathologically 'over-trained'. Nevertheless, the sudden and sustained 38% increase of training volume was substantial for a group of athletes who were already running 70–100 km week⁻¹, and the symptoms reported by six of the ten participants suggest that the majority of our sample had indeed reached or was close to the threshold of excessive training which most coaches would like to detect. The development of respiratory symptoms in three of the ten subjects is a further indication that training was at the upper limit of the desirable from the viewpoint of immune function. On the other hand, stable readings for maximal oxygen intake and

ratings of perceived exertion, the absence of clinical injuries and the minor nature of changes in serum creatine kinase all confirm that the crest of performance had not been greatly surpassed.

Traditional indicators¹ were not particularly helpful in assessing whether the training was excessive, perhaps in part because of limitations in these markers, and in part because the subjects realized that the heavy training was at their own volition and was not threatening their competitive performance. The resting heart rate³ showed a suspicion of some increase, but even if tachycardia is accepted as a valid index, it would be hard to base recommendations to moderate training upon a 2 beats min⁻¹ increment in an individual's waking heart rate. Likewise, sleep patterns showed no obvious disturbance with heavy training. Some inference of increased stress might be drawn from the increase of resting cortisol levels after the 3 weeks of heavy training, and the decreased cortisol response to an acute bout of submaximal exercise might suggest that such training had surpassed the optimal or eu-stress level²⁵. Barron *et al.*²⁶ earlier suggested that hypothalamic failure, as evidenced by a reduced cortisol response to hypoglycaemia, was a useful indicator of 'overtraining', but their subjects were ill, unlike the present group. Moreover, cortisol estimations are technically difficult to complete, and are notoriously susceptible to the immediate emotional responses of the subject²⁷. Thus, such measures are unlikely to provide a useful marker of excessive training in an athlete who is becoming concerned about deteriorating performance, but has not yet developed gross clinical 'overtraining'.

In keeping with the earlier observations of Morgan *et al.*²⁸, the one simple measure which gave a consistent response to the heavy training was the Profile of Mood States (POMS). This is a rapidly completed procedure, and is well accepted in sports psychology²⁹. The one disadvantage of POMS is that the intent of the questions is fairly obvious to the respondent.

Changes of immune function

Do the various measures of immune function change with heavy training, and if so do they offer a useful alternative measure of training that is becoming excessive^{11, 14}? The group as a whole showed some small changes of both cellular and humoral immunity in response to the period of heavy training, in terms of both their resting status and their response to a standard submaximal exercise challenge. However, despite careful attempts to standardize techniques¹⁵, there was considerable interindividual and intraindividual variation in scores for the various tests, to the point that it would be difficult to place great reliance upon routine data obtained on any single individual. Moreover, the measures of cellular and humoral immune function required highly sophisticated test equipment, and are extremely time-consuming. Although the findings have considerable theoretical interest, it seems unlikely that they will be applied to the evaluation of training status in other than

occasional international competitors within the foreseeable future.

Implications for exercise prescription

Do the group changes in immune function have wider implications for moderation in the choice of an exercise prescription? There have been earlier suggestions that both regular athletic training and single bouts of prolonged exercise, such as a 2-h ski race, can depress immune function^{10, 12, 14}, with potentially adverse consequences for resistance to infection, neoplasms and autoimmune diseases. On the other hand, cross-sectional comparisons have shown that a reasonable training programme is quite compatible with maintenance of normal immune function^{14, 30-33}, and longitudinal studies have demonstrated an enhancement of resting immune function with the moderate training of sedentary subjects^{14, 34, 35}. In keeping with these latter reports, our data suggest that adverse responses are unlikely when subjects continue the pattern of activity to which they are habituated. Furthermore, well-trained individuals such as our sample of distance runners can apparently tolerate a sudden, sustained and substantial increase over their normal pattern of training with only minor, transient and largely subclinical disturbances of immune function. In our experiments, resting immune function was apparently normalized within 3 weeks of a return to the pattern of habitual training, and even at the peak of the heavy training episode, most of the adverse reactions to an acute submaximal exercise challenge disappeared within 30 min of ceasing exercise.

The development of respiratory symptoms in three of the ten subjects may reflect a transient increase of susceptibility to viral infections, of the type previously postulated in athletes^{6-9, 12}, but, in apparent contrast to some earlier studies of less well-trained populations³⁶⁻⁴⁰, the average decrease in helper:suppressor cell ratio at no stage dropped to the critical level of 1.5 where the response to polyclonal T cell mitogens is impaired.

Conclusions

We conclude that well-trained distance runners can increase their habitual training plan by a substantial margin of around 40% without developing symptoms and signs of 'overtraining'. In agreement with earlier reports, resting pulse rate, sleep patterns and hormonal changes do not provide a useful early warning that the peak of performance has been passed, but the POMS does show a consistent pattern of loss of vigour and fatigue during heavy training. Sophisticated tests of immune function show that heavy training induces some small, transient disturbances both at rest and during acute bouts of vigorous submaximal exercise, but the required measurements are time-consuming and costly. Moreover, although the observed changes of immune response seem to indicate that training is becoming excessive, they are likely to have only a minor influence on overall immune function.

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