Increasing running volume elicits hematological changes in trained endurance runners: a case study

El aumento del volumen de entrenamiento de carrera induce alteraciones hematológicas en corredores entrenados: un estudio de caso

José Augusto Rodrigues dos Santos
University of Porto (Portugal)

Abstract. Background: Endurance running training induces several hematological changes that increase the capture, transport and delivery of the oxygen to the exercising muscles. Objective: This study aimed to verify how a dramatic increase in running volume induced new alterations in several hematological indicators in previously trained endurance runners. Methods: Three subjects (PL: 26 years, 169.5 cm; HP, 27 years, 167.9 cm; MC, 27 years, 180.7 cm) running 10-12 km/day, increased their running volume to prepare the participation in a 100-km ultra-marathon. New training program included 10-12 training sessions per week, totaling 200-260 km. Average daily running volume was 35.8±6.2 km. The parameters analyzed were: hemoglobin, erythrocytes, hematocrit, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), leukocytes, neutrophils, eosinophils, lymphocytes, and monocytes. Results: Erythrocyte count, hemoglobin, and hematocrit decreased 6.5%, 5.1% and 6.7%, respectively for the average of the three runners. Leukocytes, neutrophils, eosinophils, lymphocytes, and monocytes showed different alterations among participants with all the values remaining within normal reference values. Conclusion: Well-trained runners show further hematological alterations when training volume is dramatically increased, which can be seen as the specific adaptation to the new training level. It seems that hemogram is more sensitive than leucogram to the increase in running volume.

Keywords: endurance; training; running; hemogram; leucogram.

Introduction

It is well known that long-distance running training induces several hematological adaptations according to the intensity, volume and frequency of training loads. Training loads are dependent on the subjects’ training level and their nutritional status (Ohtani et al., 2001). It seems that chronic endurance training significantly reduced hemoglobin, mean corpuscular hemoglobin (MCHC) and increased lymphocyte count (Bredbent, 2011). Available cross-sectional and longitudinal studies indicate that blood of endurance athletes is more dilute and this has been attributed to blood volume expansion, particularly plasma volume as a result of chronic training (El-Sayed et al., 2005).

It seems that exercise-induced hemolysis in counterbalanced by elevated serum erythropoietin concentration and reticuloocyte count with no impact on total erythrocyte volume and hemoglobin mass (Robach et al., 2012). So, hemodilution is the main hematological feature that characterizes endurance runners. This adaptation facilitates muscle microcirculation and oxygen delivery to exercising muscles. Plasma expansion is also related to thermoregulation because metabolic heat production consequent to muscle contraction creates an internal heat load proportional to exercise intensity (Cheuvront & Haymes, 2001) which must be dissipated mainly through sweating. Even in intermittent sport athletes, e.g. soccer players, hemoglobin and hematocrit mean values decrease throughout the season (Malcovati et al., 2003). While hematocrit rises immediately after marathon (Kratz et al., 2006) due to hemococoncentration significant declines in red blood cell, hemoglobin and hematocrit were detected two days and nine days after a 24 h ultramarathon race (Wu et al., 2004). It seems that the continuity of training and/or exertion induces a continuous accumulation of total body water (Knechtle et al., 2008) which can decrease hemoconcentration and affecting some hematological parameters.

In well-trained athletes increasing training intensity during the course of the season decreases hemoglobin and hematocrit (Bardi et al., 2011). The results obtained from the manipulation of training volume are not so clear and depend on the subjects’ training status. While in sedentary individuals, hematological changes are easily achieved, in elite athletes with several years of hard training, long-term endurance training does not largely alter hematological status (Rietjens et al., 2002).

Therefore, this study sought to verify if a dramatic increasing in running volume during a training period of 17 weeks induced hematological alterations in well-trained endurance runners.

Methods

Participants

Three subjects well trained for endurance events voluntarily participated in this study. Participant’s age and height were as follows: PL (26 years; 169.5 cm), HP (27 years; 167.9 cm), and MC (27 years; 180.7 cm). All subjects were non-smokers and regularly drank alcoholic beverages (beer and wine) during the meals. This study was conducted in accordance with the policy statement of the Declaration of Helsinki, adopted by the World Medical Association, regarding the ethical principles for medical research involving human subjects and approved by the Scientific Council of the Faculty of Sport of the University of Porto, Portugal. The participants were informed of the risks associated with their participation before giving voluntary written consent. All subjects participated regularly in road running, orientation, and cross-country races. Medical screening showed no health constraints in the beginning of the study. During the last three years prior to the study they usually run 10-12 km daily with one day of complete rest. Subject HP was prone to gastrointestinal distress during exertion and reported...
episodic gastrointestinal complaints during normal running workouts. He was medically supervised. Abal et al. (2013) stated a significant correlation between running training volume and the tendency for lower limb injury. However, training program was accomplished by all the participants without major and impetuous injuries.

**Training protocol**

With the objective to compete in an ultramarathon (100 km), subjects realized 10-12 training sessions per week, totaling 200-260 km during 17 weeks. Average daily running volume was 35.8±6.2 km. Running training intensity was controlled by thoracic frequency counter. Low to moderate running pace was selected (130–160 beats per minute corresponding to 70-85% maximum heart rate) for continuous uniform running with 2 fartlek sessions per week (10 accelerations of 300m) inducing heart rates close to the maximum. Every four weeks a performance test (30 km) was conducted.

**Nutrition**

Nutrition status was not controlled but participants were requested to maintain their usual dietary diversity increasing carbohydrate intake. Although this general nutritional counseling mean body mass was markedly reduced in all subjects during the period of the study. Subjects did not take any mineral or vitamin supplementation during the study. During workouts, isotonic beverages (Isostar®) were ingested for hydration and carbohydrate supplementation.

**Body mass**

Body mass measurement was made by the same technician in the two moments of evaluation and with the same device (Seca Alpha Model 770 Digital Weighing Scales, UK).

**Hematology**

Hemogram and Leucogram were assessed with the Automated Hematology Analyzer S890 Coulter Counter. All blood collections were made at 9 A.M. after an overnight fasting (12 hours after the last meal), and after a 24 hours of compulsory rest period following the last workout to attenuate the effects of hemodynamic variations and acute hemodilution induced by prior workout (Sawka et al., 2000). Venous blood samples (5 ml) were drawn from antecubital vein using standard venotomy techniques with the subjects in the sitting position. Blood samples were collected into vacutainers containing ethylenediaminetetraacetic acid (EDTA) and processed within 6 h.

**Statistical analysis**

Alterations are expressed as the percentage of variation (Å).

**Results**

The results presented in Table 1 express the reduction in body mass and in all hemogram parameters. Leukocyte and leukocyte subsets changed differently among subjects with subject HP showing the largest alterations.

**Discussion**

Training volume is one of the best predictors for 100-km race performance (Knechtle et al., 2010). We hypothesize that even well-trained endurance runners a marked increasing of running volume can induce significant hematological changes.

**Table 1.** Weights and hematological alterations induced by training

| Variables | Pilot | Mean | Training | Mean | ?  (%) | Mean | ?  (%) | Mean | ?  (%) |
|-----------|-------|------|----------|------|--------|------|--------|------|--------|
| Body mass (kg) | 68.5 | 68.5 | 68.5 | 68.5 | -0.5 | 68.5 | -0.5 | 68.5 | -0.5 |
| Hemoglobin (g/dL) | 14.3 | 14.3 | 14.3 | 14.3 | -0.3 | 14.3 | -0.3 | 14.3 | -0.3 |
| Erythrocytes (x1012/L) | 4.72 | 4.72 | 4.72 | 4.72 | -0.6 | 4.72 | -0.6 | 4.72 | -0.6 |
| Hematocrit (%) | 42.4 | 42.4 | 42.4 | 42.4 | -0.2 | 42.4 | -0.2 | 42.4 | -0.2 |
| MCV (fl) | 86 | 86 | 86 | 86 | -0.3 | 86 | -0.3 | 86 | -0.3 |
| MCHC (gg/L) | 35 | 35 | 35 | 35 | 0.5 | 35 | 0.5 | 35 | 0.5 |
| MCHC (%) | 30 | 30 | 30 | 30 | 0.3 | 30 | 0.3 | 30 | 0.3 |
| Lymphocytes (x109/L) | 4.5 | 4.5 | 4.5 | 4.5 | 0.1 | 4.5 | 0.1 | 4.5 | 0.1 |
| Neutrophils (%) | 30 | 30 | 30 | 30 | -0.2 | 30 | -0.2 | 30 | -0.2 |
| Eosinophils (%) | 3 | 3 | 3 | 3 | 0.0 | 3 | 0.0 | 3 | 0.0 |
| Monocytes (%) | 4 | 4 | 4 | 4 | 0.2 | 4 | 0.2 | 4 | 0.2 |

**Discussion**

The results presented in Table 1 express the reduction in body mass and in all hemogram parameters. Leukocyte and leukocyte subsets changed differently among subjects with subject HP showing the largest alterations.
compensatory reticulosis (Schumacher et al., 2010) provoked by the traumatic nature of running (Telford et al., 2003) that induces intravascular hemolysis, intramuscular destruction and osmotic stress. In our study it seems that red blood cell destruction was not completely compensated by reticulosis.

It is generally accepted that at rest highly trained individuals present lower leukocyte counts than non-trained (Pedersen, 1991), however the results are conflicting (Broadbent, 2011). Watson et al. (1988) found no differences between trained and non-trained subjects in relation to the leukocyte profile, while Nieman et al. (1995) verified decreased leukocyte and neutrophil counts in marathon runners when compared to sedentary.

In this study, although the values remained within the range of clinical normality, leukocyte concentration changed differently among subjects. It seems that the dramatic increase in the volume of training tends to reduce resting leukocyte count (Lehanm et al., 1997) what was seen in two subjects. The significant increasing (10.9%) verified in subject PL highlights the variability between subjects for leukocyte response to long lasting running training.

Exhaustive training training attenuates neutrophils activity (Gleeson, 2007) while chronic moderate exercise improves neutrophil functions (Syu et al., 2012). It seems that intensive training is deletorous for some immune functions mainly neutrophil respiratory burst (Pyne et al., 1995). In this study all subjects decrease neutrophil percentage with subject HP showing the greater decreasing (almost 30%) eventually related to their gastric constraints and subsequent inflammatory processes (Lamprecht & Frauwallner, 2012). When exercise is exhaustive neutrophils can be mobilized into the circulation and migrate to the muscle tissue several hours after exertion (Kanda et al., 2013). Recurrent long lasting endurance training can elicit a chronic framework characterized by low basal neutrophil values. Our results are corroborated by Morgado et al. (2012) who found reduced number of neutrophils in swimmers undergoing long-term intensive training.

Lymphocytosis occurs during and immediately after exercise under a variety of conditions but return to basal levels within 24 hours (Gleeson & Walsh, 2012). Resting lymphocyte number is usually normal in athletes, although low lymphocyte counts have been reported in marathon runners (Green et al., 1981; Kratz et al., 2002). All the subjects in this study showed normal lymphocyte values (higher than 1500/mm3) in the two moments of evaluation. After the training period subjects PL and HP increased sharply their lymphocyte count and percentage while subject MC reduced both indicators. This variability is corroborated by other studies (Broadbent, 2011; Rodrigues dos Santos et al., 2006) and can be related to the individual response to the accumulation of the physical loads. Markers of immune function in athletes at least 24 hours after the last exercise bout are generally not different from those of their sedentary counterparts, except when athletes are engaged in periods of intensified training (Gleeson & Walsh, 2012). Our results are similar to those found by Gleeson et al. (2011) in athletes of different endurance sports but slightly higher than the values found by Kratz et al. (2002) in marathonees.

In subject HP, the combination of high leukocyte counts, marked neutrophil reduction and marked lymphocyte increasing are an index of hyperresponsiveness of his immune system eventually related to their gastrointestinal complaints.

Eosinophils counts and percentages changed different in the three subjects but remained within normal reference values. The individual variations could be related to exercise-induced bronchoconstriction associated to airway inflammation and subsequent recovery. Endurance athletes have high prevalence of bronchial abnormalities caused, among other reasons, by hyperventilation in cold environments that can induce inflammation of upper respiratory tract (Hellemuis et al., 2005). The inflammatory response may induce increases in the percentage of eosinophils, a situation that occurred in 2 subjects of this study which is corroborated by Vergès et al. (2005).

Circulating monocytes increase significantly after exercise (Lombardi et al., 2011) but return to basal values 24 hours later (Starkie et al., 2001) eventually due to remarginalization or tissue recruitment. The changes verified in this study, all within normal reference values, are partially confirmed by the study of Fallon et al. (1999) but conflict with the results of Shimizu et al. (2011) who found no alterations in basal monocyte counts of elderly subjects submitted to resistance training. The discrepancies can be related to the training hardness in this study. Relative high monocyte values seen in subjects HP and MC can be an index of increased muscle inflammation characteristic of ultra-endurance runners (Mastaloudis et al., 2006).

Conclusion

This study concluded that endurance trained subjects adapt to a dramatic increase in running volume reducing red blood cell mass, hemoglobin and hematocrit without significant changes in MCV, MCH and MCHC. Leucocytes and leukocyte subsets changed differently among subjects but always within laboratory range. High running volume can induce gastrointestinal constraints which are reflected in the behavior of some leukocyte subsets.

References

Abal, F.R., Soidán, J.L.G. & Arufe-Giráldez, V. (2013). Factores de riesgo de lesión en atletas. Retos, 23, 70-74.
Banfi, G., Lundby, C., Robach, P. & Lippi, G (2011). Seasonal variations of haematological parameters in athletes. Eur J Appl Physiol, 111, 9-16.
Banfi, G., Ori, G.S., Dolci, A. & Susta, D. (2004). Behaviour of haematological parameters in athletes performing marathons and ultramarathons in altitude (okýrunners). Clin Lab Haematol, 26, 373-377.
Berger, N.J., Campbell, I.T., Wilkerson, D.P. & Jones, A.M. (2006). Influence of acute plasma volume expansion on VO2 kinetics, VO2peak, and performance during high-intensity cycle exercise. J Appl Physiol, 101, 707-714.
Broadbent, S. (2011). Seasonal changes in haematology, lymphocyte transferring receptors and intracellular iron from ironman triathletes and untrained men. Eur J Appl Physiol, 111, 93-100.
Brun, J.F., Varlet-Marie, E., Connes, P. & Aloulou, I. (2010). Hemorheological alterations related to training and overtraining. Biorheology, 47, 95-115.
Burgomaster, K.A., Howarth, K.R., Phillips, S.M., Rakobowchuk, M., MacDonald, M.J., McGee, S.L. & Gibala, M.J. (2008). Similar metabolic adaptations during exercise after low volume sprint interval and traditional endurance training in humans. J Physiol, 586, 151-160.
Cheuvront, S.N. & Haymes, E.M. (2001). Thermoregulation and marathon running: biological and environmental influences. Sports Med, 31(10), 743-762.
Dubois, R., Paillard, T., McGrath, D., Chamari, K., Maurelli, O., Polly, S. & Pioux, J. (2017) Changes in training load, running performance, lower body power and biochemical characteristics of back players throughout a professional Rugby Union season. JHSE, 121(1), 1-16.
El-Sayed, M.S., Ali, N. & Al-Bayatti, M. (2009). Aerobic power and the main determinants of blood theology: is there a relationship? Blood Coagul Fibrinolysis, 20, 679-685.
El-Sayed, M.S., Ali, N. & El-Sayed, A. Z. (2005). Haemorheology in exercise and training. Sports Med, 35, 649-670.
Fallon, K.E., Sivyver, G., Sivyver, K. & Dare, A. (1999). Changes in haematological parameters and iron metabolism associated with a 1600 kilometre ultramarathon. Br J Sports Med., 33, 27-31.
Gjovaga, T.F. & Dahl, H.A. (2008). Effects of training with different intensities and volumes on muscle fibre enzyme activity and cross sectional area in the m. triceps brachii. Eur J Appl Physiol, 103, 399-409.
Gleeson, M. & Walsh, N.P. (2012). The BASES expert statement on...
exercise, immunity, and infection. *J Sports Sci*, 30(3), 321-324.

Gleeson, M. (1991). Immune function in sport and exercise. *J Appl Physiol*, 103, 693-699.

Green, R.L., Kaplan, S.S., Rabin, B.S., Stanitski, C.L. & Zdzianski, U. (1981). Immune function in marathon runners. *Ann Allergy*, 47(2), 73-75.

Helenius, I., Lumme, A. & Haahleta, T. (2005). Asthma, airway inflammation and treatment in elite athletes. *Sports Med*, 35, 565-574.

Hu, M., Finiti, T., Secllak, M., Zhou, W., Alen, M. & Cheung, S. (2008). Seasonal variation of red blood cell variables in physically inactive men: effects of strength training. *Int J Sports Med*, 29, 564-568.

Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., Yoshioka, H., Morí, Y. & Suzuki, K. (2013). Eccentric exercise-induced delayed-onset muscle soreness and changes in markers of muscle damage and inflammation. *Exerc Immunol Rev*, 19, 72-85.

Kehat, I., Shupak, A., Goldenberg, I. & Shoshani, O. (2003). Long-term hematological effects in Special Forces trainees. *Mil Med*, 168, 111-119.

Knechtle, B., Duff, B., Schulze, I. & Holger, G. (2008). A multi-stage ultra-endurance run over 1,200 km leads to a continuous accumulation of total body water. *J Sports Med*, 7(3), 357-364.

Knechtle, B., Knechtle, P., Rosemann, T. & Lepers, R. (2010). Predictor variables for a 100-km race time in male ultra-marathoners. *Percept Mot Skills*, 111, 681-693.

Kratz, A., Lewandowski, K.B., Siegel, A.J., Chany, K.Y., Flood, J.G., Van Cott, E.M. & Lee-Lewandowski, E. (2002). Effect of marathon running on hematologic and biochemical laboratory parameters, including cardiac markers. *Am J Clin Pathol*, 118, 856-863.

Kratz, A., Wood, M.J., Siegel, A.J., Hiers, J.R. & Van Cott, E.M. (2006). Effects of marathon running on platelet activation markers: direct evidence for in vivo platelet activation. *Am J Clin Pathol*, 125, 296-300.

Lamprecht, M. & Frauwallner, A. (2012) Exercise, intestinal barrier dysfunction and probiotic supplementation. *Med Sci Sports Exerc*, 59, 47-56.

Lehmann, M., Wieland, H. & Gastmann, U. (1997). Influence of an unaccustomed increase in training volume vs intensity on performance, haematological and blood-chemical parameters in distance runners. *J Sports Med Phys Fitness*, 37, 110-116.

Lombardi, G., Ricci, C. & Bani, G. (2011). Effect of Winter swimming on hematological parameters. *Biochem Med (Zagreb)*, 21(1), 71-78.

Malcovati, L., Pascutto, C. & Cazzola, M. (2003). Hematologic passport for athletes competing in endurance sports: a feasibility study. *Haematologica*, 88(5), 570-581.

Mastaloudis, A., Traber, M.G., Carnesens, K. & Widrick, J.J. (2006). Antioxidants did not prevent muscle damage in response to an ultramarathon run. *Med Sci Sports Exerc*, 38(1), 72-80.

Morgado, J.M., Ramal, L., Silva, L. & Jesus Inácio, M., Henriques, A., Lombardi, G., Ricci, C. & Bani, G. (2011). Cytokine production by monocytes, neutrophils, and dendritic cells is hampered by probiotic supplementation. *Drug Test Anal*, 2, 469-474.

Rietjens, G.J., Kuipers, H., Adam, J.J., Saris, W.H., Van Breda, E., Van Hamont, D. & Keizer, H.A. (2005). Physiological, biochemical and psychological markers of strenuous training-induced fatigue. *Int J Sports Med*, 26, 16-26.

Rietjens, G.J., Kuipers, H., Hartgens, F. & Keizer, H.A. (2002). Red blood cell profile of elite Olympic distance triathletes. A three-year follow-up. *Int J Sports Med*, 23, 391-396.

Rodrigues dos Santos, J.A., Candeias, J. & Magalhães, M.C. (2006). Immunological and anthropometric alterations induced by a kayaking ultramarathon. A case study. *Rev Port Cien Desp*, 6, 143-153.

Santhiago, V., Silva, A.S.A., Papoti, M. & Gobatto, C.A. (2009). Responses of hematologic parameters and aerobic performance of elite men and women swimmers during a 14-week training program. *J Strength Cond Res*, 23, 1097-1100.

Sawka, M.N., Convertino, V.A., Eichner, R.E., Schneider, S.M. & Young, A.J. (2000). Blood volume: importance and adaptations to exercise training, environmental stresses, and trauma/sickness. *Med Sci Sports Exerc*, 32, 332-348.

Schumacher, Y.O., Sahn, D., Baumstark, M.W. & Pottgiesser, T. (2010). Reticulocytes in athletes: Longitudinal aspects and the influence of long- and short-term exercise. *Drug Test Anal*, 2, 469-474.

Shaskey, D.J. & Green, G.A. (2000). Sports haematology. *Sports Med*, 29, 27-38.

Shimizu, K., Suzuki, N., Imai, T., Aizawa, K., Nanba, H., Hanaoka, Y., Kunio, S., Mesaki, N., Kono, I. & Akama, T. (2011). Monocyte- and T-cell responses to exercise training in elderly subjects. *J Strength Cond Res*, 25(9), 2565-2572.

Smith, J.A., Martin, D.T., Telford, R.D. & Ballas, S.K. (1999). Greater erythrocyte deformability in world-class endurance athletes. *Am J Physiol 276 (Heart Circ Physiol 45)*, 2188-2193.

Starck, R.L., Rolland, J., Angus, D.J., Anderson, M.J. & Febbraio, M.A. (2001). Circulating monocytes are not the source of elevations in plasma IL-6 and TNF-alpha levels after prolonged running. *Am J Physiol Cell Physiol*, 280, 769-774.

Suy, G.D., Chen, H.I. & Jen, C.J. (2012). Differential effects of acute and chronic exercise on human neutrophil functions. *Med Sci Sports Exerc*, 44(6), 1021-1027.

Telford, R.D., Sly, G.J., Hahn, A.G., Cunningham, R.B., Bryant, C. & Smith, J.A. (2003). Footstrike is the major cause of hemolysis during running. *J Appl Physiol*, 94, 38-42.

Vergès, S., Devouassoux, G., Flore, P., Rossini, E., Fior-Gozlan, M., Levy, P. & Wuyam, B. (2005). Bronchial hyperresponsiveness, airway inflammation, and airflow limitation in endurance athletes. *Chest*, 127, 1935-1941.

Wahl, P., Zinner, C., Achtzehn, S., Bloch, W. & Mester, J. (2010). Effect of high- and low-intensity exercise and metabolic acidosis on levels of GH, IGF-1, IGFBP-3 and cortisol. *Growth Horm IGF Res*, 20, 380-385.

Watson, R.R., Morisuchi, S., Jackson, J.C., Turner, L., Wilmore, J. & Freund, B.J. (1988). Modification of cellular immune functions in humans by endurance exercise training during beta-adrenergic blockade with atenolol or propranolol. *Med Sci Sports Exerc*, 18, 95-100.

Wu, H.J., Chen, K.T., Shee, B.W., Chang, H.C., Huang, Y.J. & Yang, R.S. (2004). Effects of 24 h ultra-marathon on biochemical and haematological parameters. *World J Gastroenterol*, 10, 2711-2714.