

Endurance Training Enhances Circulating Plasma VEGF And b-FGF in Open Water Swimmers

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Abstract: This study aimed to investigate the changes in circulating plasma VEGF and b-FGF in response to acute endurance exercise. Methods: 8 open water swimmers (16 ± 1 years) exercised for 5 kilometers. Antecubital vein plasma was collected at rest and at 0, 2 and 4 h post exercise. Plasma VEGF was measured by ELISA analysis. b-FGF levels has been measured in serum by ELISA analysis. Results: Acute endurance exercise significantly increased VEGF and b-FGF at 0, 2h and 4 h post exercise in open water swimmers. Conclusions: Endurance exercise can greatly increase plasma vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in open water swimmers. That physical activity enriched the bFGF response is consistent with the hypothesis that hemodynamic factors are important contributors to collateral vessel enlargement. The use of either plasma or serum for the measurement of VEGF and bFGF should yield similar conclusions on circulating VEGF. In addition, increases of both VEGF and bFGF enhance open water swimmers performance.

Key words: VEGF • B-FGF • Open water • Endurance training

INTRODUCTION

Maglischo [1] believes that athletes should use three levels of endurance training to achieve their goal of improving endurance:

- Basic endurance training or Endurance 1 (End-1) training.
- Threshold endurance training or Endurance 2 (End-2) training.
- Overload training or Endurance 3 (End-3) training.

Holloszy *et al.* [2] reported that fat can account for 50% to 75% of the total amount of energy expended during basic endurance swimming depending on the length and the average speed of the swimming sets. Ivy *et al.* [3] stated that the most important differences between threshold endurance training and basic endurance training is that threshold training extends the adaptations that improve oxygen utilization and lactate removal to the fast-twitch group, particularly FTa fibers, to rotate in and become involved in the work.

Treffene *et al.* [4] reported that the maximum rate of lactate removal from muscles to blood occurred at swimming speeds 6% to 14% faster than anaerobic threshold speed, due to additional lactate removed from FTb fibers and high threshold Fta fibers once those fibers

began contracting. Guyton and Hall [5] explained that deficiency of tissue oxygen or other nutrients, or both, that leads to formation of the vascular growth factors, which are, (eg) vascular endothelial growth factor (VEGF), fibroblast growth factor (b.FGF) and angiogenin. They also added that all vascular growth factors promote new vessel growth in the same way. They cause new vessels to sprout from other small vessels, by dissolution of the basement membrane until forming new arterioles or venules or perhaps even larger vessels.

Gomez *et al.* [6] stated that b-FGF mRNA and protein levels also increased in the hippocampus after 4 nights of running and then return to control levels after 7 nights of running. The time course suggests that exercise could be beneficial at the early stages of a training program. The point is that hippocampus is important for cognitive function (atrophic factor involvement in cognition). b-FGF, a known angiogenic factor, may also be involved with the angiogenesis associated with exercise [7]. In many cases growth factors are likely to be mediators for the positive effects of exercise on the brain.

Growth factors may offer a new avenue to treat muscle injuries. Recently, *in vitro* and *in vivo* experiments with growth factors have shown improved healing, especially with basic fibroblast growth factors (b-FGF), nerve growth factor and insulin-like growth factor (IGF1) [8, 9].

The aim of this study: To investigate the change in circulating blood, VEGF and b-FGF in response to endurance training.

MATERIALS AND METHODS

Research Protocol: The protocol was approved by the university’s ethical committee on the protection of human subjects and a written informed consent was obtained from all participants. Each participant underwent a medical history and physical examination; none had hypertension or any other chronic medical illness. Blood pressure and heart rate were measured in the nondominant arm under standardized conditions by an automated oscillometric method.

Methodology: A group of 8 open water swimmers, volunteered in this study, signed an informed consent for talking blood samples. Swimmers age (16±1 years), Height 173±2 (m), Weight 69± 3(Kg) and years of training 7±1 (years) exercised for 5 kilometers. Antecubital vein blood was collected into two polyethyline tubes with covers, one of them for plasma collection, EDTA added and the other tube for serum collection after blood centrifugation.

The blood collection was at rest and immediately after, after 2 hours and after 4 hour post exercise, plasma VEGF was measured by Elisa analysis and b-FGF in serum was also measured by Elisa analysis. Vascular endothelial growth factor (VEGF) was estimated using a sandwich enzyme immunoassay technique [10], Quantikine, R and D system USA.

As for (b-FGF) fibroblast growth factor was estimated using the method of Bender Med System GmbH, Austria [11].

Statistical Analysis: Data analysis and tabulation were done using SPSS computer software, statistical data were expressed as Mean ± standard deviation. 5% level was used as cut off point for significance.

RESULTS AND DISCUSSION

Table 1 shows VEGF and b-FGF at rest and immediately after, after 2 hours and after 4 hours. post exercise. Fig. 1 and 2 show the differences in the mean at rest, immediately after, after 2 hours and after 4 hours post exercise for the variables. VEGF and b-FGF were all significantly increased after swimming 5 kilometers.

DISCUSSION

Results indicated (Tables 1-3, Fig. 1 and 2) a significantly increased VEGF and b-FGF at 2 hours and 4 hours post exercise in open water swimmers. These results are in compatible with previous studies [8, 12, 13]. They stated that VEGF is a survival factor for endothelial cells. It can remodel extracellular matrix (ECM) components necessary for angiogenesis.

Neufeld *et al.* [8] stated that hypoxia is a major stimulator of VEGF expression, several growth factors expressed during cerebral ischemia, including b-FGF and others can upregulate VEGF mRNA expression, suggesting that their paracrine or autocrine release could cooperate with local hypoxia in regulating VEGF release. Guyton and Hall [5] reported that a dozen of factors that increase growth of new blood vessels have been found, almost all of which are small peptides, three of those that have been best characterized are (VEGF), (b-FGF) and angiogenin, each of which has been isolated from tissues,

Table 1: VEGF and b-FGF concentrations at rest, immediately after, after 2 hours and 4 hours after endurance training (N=8)

Variables	At rest	Immediately after	After 2 hours	After 4 hours
VEGF (pg/ml)	117	119	141	175
b-FGF (ng/ml)	7.08	7.28	7.46	8.3

Table 2: VEGF and b-FGF concentrations at rest, immediately after, after 2 hours and 4 hours after endurance training, ANOVA (N=8)

Variables		Sum of squares	df	Mean square	F	Sig
VEGF	Between groups	17440	3	5813.333	1892.713*	.000
	Within groups	86	28	3.071		
	Total	17526	31			
b-FGF	Between groups	9.462	3	3.154	202.095*	.000
	Within groups	0.437	28	.016		
	Total	9.899	31			

Table 3: VEGF and b-FGF concentrations at rest, immediately after, after 2 hours, 4 hours after endurance training, Tukey HSD (N=8)

VEGF	Mean	At rest	Immediately after	After 2 hours	After 4 hours
At rest	117	-----	2	24*	58*
Immediately after	119	-2	-----	22*	56*
After 2 hours	141	-24*	-22*	-----	34*
After 4 hours	175	-58*	-56*	-34*	-----
b-FGF					
At rest	7.08	-----	-0.20*	0.38*	1.22*
Immediately after	7.28	0.20*	-----	0.58*	1.42*
After 2 hours	7.46	-0.38*	-0.58*	-----	0.84*
After 4 hours	8.30	-1.22*	-1.42*	-0.84*	-----

Significant differences as: P<0.05

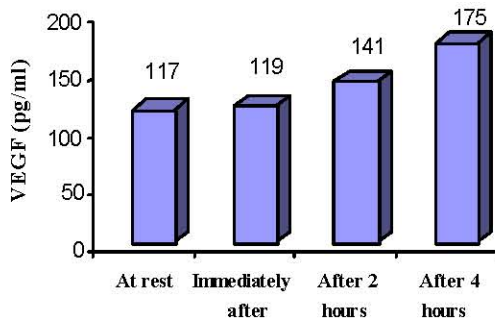


Fig. 1: VEGF concentration at rest immediately after after 2 hours, and 4 hours after endurance training

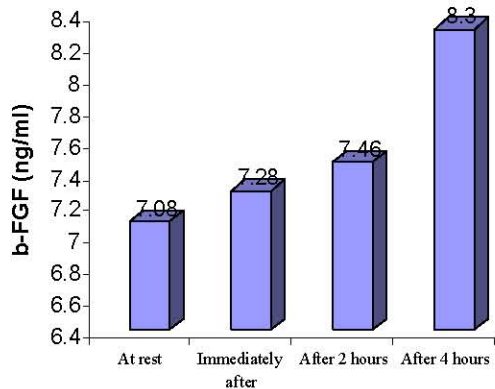


Fig. 2: b-FGF concentration at rest, immediately after, after 2 hours and 4 hours after endurance training

that have inadequate blood supply. They also added that if the blood flow is great enough, smooth muscle cells eventually invade the wall, so that some of the new vessel eventually grows to be new arterioles or venules or perhaps even larger vessels. Thus, angiogenesis explains the manner in which metabolic factors in local tissues can cause growth of new vessels.

Harder *et al.* [14] stated that the extra blood vessels normally remain mainly vasoconstricted, opening to allow extra flow only when appropriate local stimuli such as oxygen lack, nerve vasodilatory stimuli or other stimuli call forth the required extra flow.

Berne and Levy [15] reported that hemodynamics or the dynamics of blood circulation are related to laws. Most laws of hemodynamics assume that blood flow is laminar. Laminar flow is characterized by the fluid having a uniform velocity throughout the radial dimensions of the vessels. Anderson and Saltin [16] added that during exercise total blood flow is directed to the working skeletal muscle, consequently, blood flow to skeletal muscle can increase to more than 80% of maximal cardiac output.

Endurance training increases the volume of plasma in blood. Simultaneous increases in Red blood corpuscle counts and hemoglobin. The increase in plasma volume can be reached after just one session of intense intermittent cycle ergometer exercise, which induced a 10% increase in plasma volume after 24 hours [17]. The increased plasma volume leads to an increased venous return to the heart, as well as increased ventricular preload and stroke volume [18, 19].

Anderson and Henriksson [20] indicated that training revealed a 20% increase in capillary density and 16% increase in $\dot{V}O_2$ max, also an increased maximal blood flow capacities and leading to better performance.

Exercise increases the rate of blood flow through the tissue, leading to active hyperemia. The increase in local metabolism causes the cells to devour tissue fluid nutrients extremely rapidly and also to release large quantities of vasodilator substances. The result is to dilate the local blood vessels and, therefore, to increase local blood flow.

In this way, the active tissue receives the additional nutrients required to sustain its new level of function. Active hyperemia in skeletal muscle can increase local muscle flow as much as 20- fold during intense exercise [21].

Tipton [22] reported that the higher number of capillaries after a period of endurance training leads to a greater capillary volume, which in turn results in a longer capillary mean transit time (MTT = capillary blood volume / muscle blood flow) at a given muscle blood flow.

Also the capillary surface area is enlarged. Also the highest number of capillaries contributes to greater fat oxidation, lowered glycogen utilization and movement economy; these are the main factors that determine endurance performance.

Research has shown that endurance training will increase the number of capillaries around alvolic and muscle fibers [23]. The increase of capillaries around individual muscle fibers has the greater effect on improving endurance. The improvement was between 15% to 50% after long term endurance training [24, 25]. An increase in the number of capillaries around muscle fibers can significantly increase the amount of oxygen that diffuses from the blood into the muscle fibers can significantly increase the amount of oxygen that diffuses from the blood into the muscles [26].

Maglischo [1] added that swimmers should do most of their aerobic training in the pool to ensure that they increase the number of capillaries around the muscle fibers they will use in races.

CONCLUSION

Endurance exercise can greatly increase plasma vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) in open water swimmers and that physical activity enriched the (b-FGF) response is consistent with the hypothesis that hemodynamics factors are important contributors to collateral vessel enlargement. The use of either plasma or serum for the measurement of VEGF and b-FGF should yield similar conclusions on circulating VEGF. In addition, increase of both VEGF and b-FGF enhance water swimmers performance.

REFERENCES

1. Maglischo, E., 2003. Swimming fastest. Human Kinetics, USA.
2. Holloszy, J., P. Dalsky and B. Nemeth, 1986. Utilization of fat as substrate during exercise effect of training. *Biochemistry of Exercised*. Human Kinetics, USA.
3. Ivy, J., Y. Chi and O. Lowry, 1987. Muscle fiber recruitment during a lactate threshold test. *Med. and Sci. in Sports and Exercise*, 19: 35.
4. Treffene, R., C. Dickson and K Hobbs, 1980. Lactic acid accumulation during constant speed swimming of controlled relative intensities. *J. of Sports Medicine*, 20: 244-254.
5. Guyton, A. and H. Hall, 2006. *Medical Physiology*. El Sevier, USA.
6. Gomez, F., L. Daol and V. So, 1997. Physical exercise induces FGF and its mRNA in the hippocampus. *Brain Res.*, 764: 1-8.
7. Black, J., K. Isaacs and B. Anderson, 1990. Learning causes synaptogenesis whereas motor activity causes angiogenesis in cerebellar cortex of adult rats. *Proc. Nat. Acad. Sci.*, 87: 5568-5572.
8. Kawkab, E., M. Eman and H. Passant, 2005. Role of vascular endothelial growth factors and transforming growth factor B1 in both transient and permanent cerebral ischemia in rats. *EJJBMB*, 23: 1-10.
9. Neufeld, G., T. Cohen and Z. Poltorak, 1999. Vascular endothelial growth factor and its receptors. *FASEB J.*, 13: 9-22.
10. Slevin, M., S. Krupinski and A. Slowik, 2000. Serial measurement of vascular endothelial growth factor in serum of patients with acute ischamia stroke. *Storke*, 31: 1663-1670.
11. Kang, R., T. Marui and S. Ghivizani, 1997. Vivo gene transfer to chondrocytes in full thickness articular cartilage defects. *Osteoarthritis Cartilag*, 5: 139-143.
12. Testa, M., P. Ennezat and T. Le Jemtel, 2000. Modulation of vascular endothelial gene expression by physical training in patients with chronic heart failure. *Ital. J., Heart J.*, 6: 426-430.
13. Kropf, J., J. Schurek and A. Wollner, 1997. Immunological measurement of b-FGF fibroblast growth factor in blood. *Clin. Chem.*, 43: 1965-1974.
14. Harder, D., C.Zhang and D. Gebremedhlin, 2005. Astrocyte function in matching blood flow to metabolic activity. *New Physiol. Sc.*, 17: 27.
15. Berne, R. and M. Levy, 2007. *Physiology*. Mosby, 3rd Ed., St. Louis, USA.
16. Anderson, P. and B. Saltin, 1995. Maximal perfusion of skeletal muscle in man. *J. Physiol.*, 366: 233-249.
17. Gillen, C., R.Lee and G. Mark, 1999. Plasma volume expansion in humans after a single intense exercise protocol. *J. Appl. Physiol.*, 71: 1914-1920.
18. Coyle, E., M. Hemmett and A. Coggan, 1996. Effect of detraining on cardiovascular responses to exercise. *J. Appl. Physiol.*, 60: 95-99.
19. Hopper, M., A. Coggan and E. Loyle, 1998. Exercise stroke volume relative to plasma volume expansion. *J. Appl. Physiol.*, 64: 404-408.
20. Andreson, P. and J. Henrikson, 1997. Capillary supply of the quadriceps femoris muscle of man. *J. Physiol.*, 270: 677-690.

21. Davis, M. and M. Hill, 1999. Signaling mechanisms underlying the vascular myogenic response. *Physiol. Rev.*, 79: 387.
22. Tipton, C., 2006. *ACSM's advanced exercise physiology*. Lippincott Williams and Wilkins, USA.
23. Brodal, P., F. Ingjer and L. Hermansen, 1977. Capillaries supply of skeletal muscle fibers in untrained and endurance trained men. *Am. J. Physiol.*, 232: 705-712.
24. Rosler, K., K. Hoppeler and H. Howald, 2005. The effect of long term endurance training on capillaries density in skeletal muscle. *Eur. J. of Appl. Physiol.*, 54: 355-362.
25. Linkhart, T., S. Mohan and D. Baylink, 1996. Growth factors for bones growth and repair. *Bone.*, 19: 1-12.
26. Saltin, B., E. Henrikson and P. Andersen, 1997. Fiber types and metabolic potentials of skeletal muscles in sedentary men and endurance runners. *Annals of New York Academy of Sci.*, 301: 3-29.