Chapter 88
Research on ACE Gene I/D Polymorphism of Men and Effects of HiHiLo on SPO_2

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Abstract *Purposes* To explore the association between Insertion/Deletion polymorphism of the angiotensin converting enzyme (ACE) gene and the effects of Living High-Exercise High-Training Low (HiHiLo) on arterial oxygen saturation (SPO_2), so that sports practitioners comprehend better individual hypoxia training effects, coach can constitute individual training program. *Procedures and Methods* 71 healthy men of Han nationality in northern China underwent HiHiLo training for 4 weeks Training programme was as follows: exposure in hypoxic environment (14.5–14.8 % O_2, 10 h/day), three times hypoxic training per week (15.4 % O_2), and training at sea level. Resting, exercising and resuming SPO_2 were measured before and after the protocol, and the ACE gene I/D polymorphism was detected by PCR. *Results* There was no significant differences were in the baseline SPO_2 among groups before HiHiLo. Exercising and resuming SPO_2 both increased significantly in total and II, ID after training than those before the training ($P < 0.01$), but no significant changes showed in DD ($P > 0.05$). *Conclusions* HiHiLo could be helpful for developing individual exercising and resuming SPO_2. Moreover, SPO_2 of men carrying 2 and ID probably were more sensitive to the hypoxic training than those carrying DD.

Keywords ACE gene polymorphism • Living high-exercise high-training • Arterial oxygen saturation

88.1 Introduction

The human renin–angiotensin–aldosterone system (RAS) maintains circulatory homeostasis and melody some organs development as cardiac muscle with fat. As part of the RAS, ACE degrades vasodilator kinins and generates angiotensin II.

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A polymorphism in intron 16 of the human ACE gene has been identified in which the presence (insertion, I allele) rather than the absence (deletion, D allele) of a 287 bp fragment is associated with lower serum and tissue ACE activity [1]. George thanked I allele could help reduce ACE and depress ACE activity, leading to alleviate blood vessel resistance, enhance cardiac output and capillary vessel, increase blood supply, improve physical performance [2]. There were significant differences between subjects reside at high altitude and resident lowlanders about ACE genotypes, the subjects reside at high altitude had significantly greater numbers of the II homozygotes and the ID heterozygotes than the DD homozygotes, the II genotype could be associated with altitude adaptation [3]. Woods [4] and Bigham [5] found that I allele of ACE could help individuals maintain high Arterial Oxygen Saturation ascending to High Altitude. But there were significant differences between Chinese Han population and those in Europe and America in genotype and allele frequencies about ACE gene I/D polymorphism [6].

We explored the association between Insertion/Deletion polymorphism of the ACE gene and the effects of HiHiLo on SPO_2 by observing SPO_2 changes before and after four weeks HiHiLo protocol. This would helpful for forecasting Hypoxia training effect and exploring individual Hypoxia training project.

88.2 Participants and Methods

88.2.1 Participants

The sample consisted of 71 biologically unrelated healthy male volunteers from northern China, who had no experience of hypoxia exposure. The mean (standard deviation (SD) age was 21.10 (0.9) years, the height was 177.93 (5.26) cm and the weight was 69.80 (7.80) kg. Written informed consent was obtained from each participant.

88.2.2 HiHiLo Training Protocol

Subjects had rested in hypoxia condition (O_2:14.5–14.8 %, about 3,000 m altitude) for 10 h per day. Hypoxia training were carried out (O_2:15.4 %, about 2,500 m altitude) 3 times per week for 4 weeks. The participants exercised on Monark 845E bicycle with an intensity of 75 % VO_2 max for 30 min, ordinary aerobic training was carried out in plain.

88.2.3 Arterial Oxygen Saturation Testing

SPO_2 was measured before and after HiHiLo by NONIN 8,500 (America). The procedure was as follows: first, subjects had been rested on cycle ergometer (Monark 845E, Swden) until their HR, SPO_2 level off in hypoxia condition (O_2:15.4 %). Then, resting

SPO_2 were measured every minute for 5 min. Nest, subjects warmed up, and after their HR were in 90–100 b/min, subjects exercised on cycle ergometer with an intensity of 75 % VO_2 max with 60 cycle/min for 15 min. At last, subjects recovered on cycle for 5 min. Their SPO_2 every minute during exercise and recovery were recorded.

88.2.4 Blood Index Testing

Elbow vein blood was selected one day before HiHiLo and the last date. The RBC, HB and HCT were measured by automatic blood corpuscle instrument (Bayer ADVIA120, Germany).

88.2.5 Genotype Analysis

DNA was extracted from blood cells by a protocol recommended by the kit manufacturer (Promega, Madison, Wisconsin, USA). Primer pairs for polymerase chain reaction (PCR) in accord with Rigat [7] were synthesized. Forward primer: CTG GAG ACC ACT CCA TCC TTT CT, reverse primer: GAT GTG GCC ATC ACA TTC GTC A. The amplification protocol was (1) one cycle of denaturation at 94 °C for 5 min; (2) 30 cycles of denaturation at 94 °C for 40 s, annealing at 50 °C for 40 s and extension at 72 °C for 60 s; and (3) one final elongation cycle at 72 °C for 5 min. Preventive contamination measures were taken by the inclusion of PCR mixture without DNA (negative control). The resulting fragments were separated by horizontal electrophoresis on 2 % agarose gels. Each gel was run for 45 min at 100 V, stained with ethidium bromide and photographed under ultraviolet light.

88.2.6 Statistical Analysis

Pearson's x2 test was used to determine whether the observed genotype frequencies were in the Hardy–Weinberg equilibrium. The paired t test was used to examine the differences in variables before and after training. Differences in variables between the three genotypes were tested by one-way analysis of variance and retrospective multiple comparisons. These tests were performed with SPSS software for Windows 11.5 package. P Values < 0.05 were considered significant. The results are presented as mean (standard difference).

88.3 Results

The observed genotypic frequencies of ACE in men of Han nationality in northern China were 39.4 (II), 50.7 (ID) and 9.9 % (DD), in agreement ($p > 0.05$) with those expected under the Hardy–Weinberg equilibrium.

Table 88.1 The variables before and after HiHiLo

Variables	Before	After	P
Resting SPO_2 (%)	94.97 ± 1.57	94.91 ± 1.33	NS
Exercising SPO_2 (%)	90.46 ± 2.37	91.74 ± 1.91[a]	0.000
Resuming SPO_2 (%)	92.42 ± 1.44	93.52 ± 1.95[a]	0.000
RBC (×1012 cell/L)	4.89 ± 0.03	5.02 ± 0.03[a]	0.000
HB (g/L)	152.72 ± 1.02	157.69 ± 1.05[a]	0.000
HCT (%)	43.56 ± 0.23	44.92 ± 0.27[a]	0.000

[a] Means $p < 0.05$

88.3.1 Arterial Oxygen Saturation Change After HiHiLo

Table 88.1 shows that exercising SPO_2, resuming SPO_2, RBC, CHB and HCT increased significantly after training ($p < 0.05$), but no significant difference was found in resting SPO_2 ($p > 0.05$).

88.3.2 Arterial Oxygen Saturation Change After HiHiLo Among ACE Genotypes

Table 88.2 summarises the association between the ACE genotype and SPO_2 variables. No significant differences were observed in baseline resting, exercising and resuming SPO_2 and RBC, CHB, HCT among three genotypes before training ($P > 0.05$). After HiHiLo, the exercising and resuming SPO_2 and RBC, CHB, HCT all increased significantly in II and ID genotypes ($p < 0.05$), but no significant difference was found about those in DD genotype ($p > 0.05$). Meanwhile, no significant difference was found about resting SPO_2 among ACE genotypes ($p > 0.05$).

88.3.3 Effects of HiHiLo on Arterial Oxygen Saturation

Arterial oxygen saturation is arterial oxygen arterial oxygen percentage of arterial oxygen capacity, which reflects state of physiological oxygen supply. Study reported that individual arterial oxygen pressure and arterial oxygen saturation depressed gradually meanwhile increase of intensity [8, 9]. Furthermore, individual VO_2 max is association with his SPO_2. VO_2max decreased by 4.4 % during SPO_2 depressed by 4 % [10]. SPO_2 is an important factor which limit performance. Thus, individual SPO_2 during exercise in hypoxia condition could reflect how subject adapt to hypoxia environment.

We found that subjects' exercising and resuming SPO_2 in hypoxia condition increased significantly after HiHiLo, which implied that HiHiLo could increase

Table 88.2 The variables before and after HiHiLo among the genotypes

Variables	Genotypes			P
	II (28)	ID (36)	DD (7)	
Before resting SPO_2 (%)	95.17 ± 1.71	94.89 ± 1.54	94.57 ± 1.25	NS
Exercising SPO_2 (%)	90.49 ± 2.56	90.34 ± 2.37	90.96 ± 1.37	NS
Resuming SPO_2 (%)	92.26 ± 1.67	92.39 ± 1.25	93.23 ± 1.37	NS
RBC (×1012 cell/L)	4.96 ± 0.05	4.87 ± 0.04	4.76 ± 0.07	NS
HB (g/L)	153.93 ± 1.93	152.39 ± 1.27	149.57 ± 2.35	NS
HCT (%)	43.82 ± 0.44	43.47 ± 0.28	42.99 ± 0.64	NS
After resting SPO_2 (%)	94.79 ± 1.43	94.91 ± 1.24	95.38 ± 1.41	NS
Exercising SPO_2 (%)	91.58 ± 2.14[a]	91.79 ± 1.79[a]	92.15 ± 1.75	NS
Resuming SPO_2 (%)	93.42 ± 1.01[a]	93.51 ± 1.28[a]	94.00 ± 1.43	NS
RBC (×1012 cell/L)	5.13 ± 0.06[b]	4.97 ± 0.04[a]	4.85 ± 0.08	0.026
HB (g/L)	159.79 ± 1.85[a]	156.81 ± 1.38[a]	153.86 ± 2.16	NS
HCT (%)	45.42 ± 0.46[a]	44.61 ± 0.38[a]	44.50 ± 0.57[a]	NS

[a]Means $p < 0.05$
[b]Means $p \geq 0.05$

individual oxygen supply and their performance. Studies [11, 12] reported resting and exercising SPO_2 increased significantly after intermitted hypoxia training. Lvshaojun found that subjects' resting, exercising and resuming SPO_2 increased significantly after HiHiLo [13]. Niejing approved that HiHiLo was helpful for athlete improve their exercising and resuming SPO_2 in hypoxia condition [14]. Those showed that training combined with hypoxia exposure could increase individual hypoxia load, which made athlete adapt to hypoxia environment and improve their performance.

88.3.4 Association Between ACE Gene Polymorphism and the Effects of HiHiLo on SPO_2

Study reported that there was significant difference about effects of hypoxia training between athletes [15]. Moreover, heredity paly an important role during the individuals adapt to hypoxia environment [16, 17]. Some gene polymorphisms could make clear these differences [18–20].

We found that subjects' exercising and resuming SPO_2 in hypoxia condition increased significantly after HiHiLo in II and ID groups, but no significant difference was found in DD genotype. This implied that ACE gene I/D polymorphism may be association with individual SPO_2 adjustability to HiHiLo. Those with II and ID genotypes may be more helpful individuals for maintenance SPO_2 during exercising in hypoxia environment and resuming SPO_2. Woods found that individual SPO_2 change when athletes climbed rapidly mountain was association with ACE gene I/D polymorphism, association of I allele with the maintenance of SPO_2 at high altitude [4]. Bigham reported that individual resting and exercising SPO_2 was association close with ACE II genotype ($P = 0.008$).

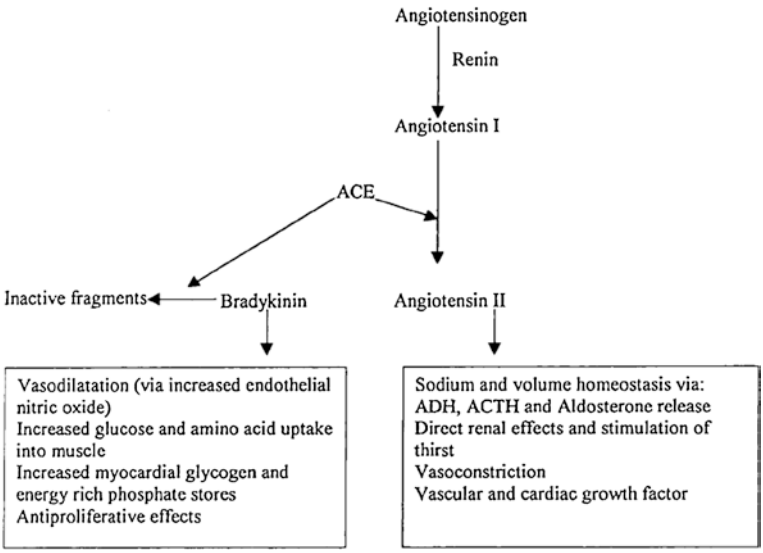

Fig. 88.1 Summary of the RAS. (Cited by Woods, 9)

Subjects' SPO_2 with II genotype were high by 2.3 % than other genotypes [5]. The mechanism of subjects with I allele maintenance SPO_2 during exercising in hypoxia environment and resuming SPO_2 was as follow. One, I allele was association close with less ACE activity blood serum and tissue [1], lower ACE activity could accelerated capillary hyperplasia and increased oxygen reserves (Fig. 88.1). Thus, less arterial oxygen has been absorbed when subject exercised at some a load leading to decreasing SPO_2 descend [21]. The other, subjects' RBC, CHB, HCT with II and ID genotypes had been improved significantly after HiHiLo, which was helpful for maintenance SPO_2 during exercising at some a load in hypoxia environment and resuming SPO_2.

88.4 Conclusion

We found that HiHiLo lasting for 4 weeks could improve individuals adapt to hypoxia environment. Effects of HiHiLo on SPO_2 may be association with ACE gene I/D polymorphism. Subjects with II and ID genotypes may be helpful for maintenance SPO_2 during exercising at some a load in hypoxia environment and resuming SPO_2.

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References

1. Rigat B, Huberi C, lhene GF A et al (1990) An insertion/deletion polymorphism in the angiotensin-1-converting enzyme levels. J Clin Invest 86:1343–1346
2. George G, Bing Y, Brett H et al (1998) Elite endurance athletes and ACE I allele the role of genes in athletic performance. Hum Genet 103:48–50
3. Qadar Pasha MA, Khan AP, Kumar R et al (2001) Angiotensin converting enzyme insertion allele in relation to high altitude adaptation. Ann Hum Genet 65:531–536
4. Woods DR, Pollard AJ, Collier DJ et al (2002) Insertion/Deletion polymorphism of the angiotensin i-converting enzyme gene and arterial oxygen saturation at high altitude. Am J Respir Crit Care Med 166:362–366
5. Bigham A, Kiyamu M, Leon-Vearde F et al (2008) Angiotensin-converting enzyme genotype and arterial oxygen saturation at high altitude in Peruvian Quechua. High Alt Med Biol vol 9:167–178
6. Xi Yi, Zhang Xiuli, Yang Hu et al (2006) The frequency distribution characteristics of the ACE Gene I/D polymorphism of northern han nationality youth. J Tianjin Univ Sport 21:205–208
7. Rigat B, Huberi C, Corvol P et al (1992) PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene. Nucleic Acid Res 20:1433–1436
8. Rice AJ, Scroop GC, Gore CJ (1999) Exercise—induced hypoxaemia in highly trained cyclists at 40 % peak oxygen uptake. Eur J Appl Physiol Occup Physiol 79:353–359
9. Pedersen PK, Mandoe H, Jensen K (1996) Reduced arterial O_2 saturation during supine exercise in highly trained cyclists. Acta Physiol Scand 158:325–331
10. Su Z, Wanmei X, Fang X (2004) Initial exploration into lower arterial oxygen in Hongkong athletes and improved method. The 7th sports science conference
11. Ricart A, Casas H, Casas M et al (2000) Acclimatization near home? Early respiratory changes after short-term intermittent exposure to simulated altitude. Wilderness Environ Med 11:84–88
12. Muza SR, Young AJ, Sawka MN et al (2004) Ventilation after supplemental oxygen administration at high altitude. Wilderness Environ Med 15:18–24
13. Lv S, Chen G, Zhuo Y et al (2010) Research on the effects of HiHiLo on body functions of elite wushu sanda athletes. J Beijing Sport Univ 33:56–59
14. Nie J, Hu Y, Yi L et al (2010) The correlation between the VEGFR2 Gene A+18487T polymorphism and changes of SpO_2 after HiHiLo. J Beijing Sport Univ 33:49–52
15. Chapman RF, Stray-Gundersen J, Levine BD (1998) Individual variation in response to altitude training. J Appl Physiol 85:1448–1456
16. Rajput C (2006) Endothelin-1 gene variants and levels associate with adaptation to hypobaric hypoxia in high-altitude natives. Biochem Biophys Res Commun 341:1218–1224
17. Ramirez G, Bittle PA, Rosen R et al (1997) High altitude living: genetic and environmental adaptation. Aviat Space Environ Med 70:73–81
18. Ahsan A, Norboo T, Baig MA et al (2005) Simultaneous selections of the wild-type genotypes of the G894T and 4B/4A polymorphisms of NOS3 associate with high-altitude adaptation. Ann Hum Genet 69:260–267
19. Jedlickova K, Stockton DW, Chen H et al (2003) Search for genetic determinants of individual variability of the erythropoietin response to high altitude. Blood Cells Mol Dis 31:175–182
20. Suzuki K, Kizaki T, Hitmi Y et al (2003) Genetic variation inhypoxia-inducible factor lalpha and its possible association with high altitude adaption in Sherpas. Med Hypotheses 61(3):385–389
21. Woods DR, Montgomery HE (2001) Angiotensin-converting enzyme and genetics at high altitude. High Alt Med Biol 2:201–210