INVITED REVIEW

Altitude training as a powerful corrective intervention in correcting insulin resistance

Shu-Man Chen¹ and Chia-Hua Kuo^{2*}

¹Committee for General Education, Shih Hsin University, Taipei, Taiwan

²Department of Sports Sciences, Taipei Physical Education College, Taipei, Taiwan

(Received: 2012/05/30, Revised: 2012/06/12, Published online: 2012/06/19)

ABSTRACT

Oxygen is the final acceptor of electron transport from fat and carbohydrate oxidation, which is the rate-limiting factor for cellular ATP production. Under altitude hypoxia condition, energy reliance on anaerobic glycolysis increases to compensate for the shortfall caused by reduced fatty acid oxidation [1]. Therefore, training at altitude is expected to strongly influence the human metabolic system, and has the potential to be designed as a non-pharmacological or recreational intervention regimen for correcting diabetes or related metabolic problems. However, most people cannot accommodate high altitude exposure above 4500 M due to acute mountain sickness (AMS) and insulin resistance corresponding to a increased levels of the stress hormones cortisol and catecholamine [2]. Thus, less stringent conditions were evaluated to determine whether glucose tolerance and insulin sensitivity could be improved by moderate altitude exposure (below 4000 M). In 2003, we and another group in Austria reported that short-term moderate altitude exposure plus endurance-related physical activity significantly improves glucose tolerance (not fasting glucose) in humans [3,4], which is associated with the improvement in the whole-body insulin sensitivity [5]. With daily hiking at an altitude of approximately 4000 M, glucose tolerance can still be improved but fasting glucose was slightly elevated. Individuals vary widely in their response to altitude challenge. In particular, the improvement in glucose tolerance and insulin sensitivity by prolonged altitude hiking activity is not apparent in those individuals with low baseline DHEA-S concentration [6]. In addition, hematopoietic adaptation against altitude hypoxia can also be impaired in individuals with low DHEA-S. In short-lived mammals like rodents, the DHEA-S level is barely detectable since their adrenal cortex does not appear to produce this steroid [7]. In this model, exercise training recovery under prolonged hypoxia exposure (14-15% oxygen, 8 h per day for 6 weeks) can still improve insulin sensitivity, secondary to an effective suppression of adiposity [8]. Genetically obese rats exhibit hyperinsulinemia (sign of insulin resistance) with up-regulated baseline levels of AMP-activated protein kinase and AS160 phosphorylation in skeletal muscle compared to lean rats. After prolonged hypoxia training, this abnormality can be reversed concomitant with an approximately 50% increase in GLUT4 protein expression. Additionally, prolonged moderate hypoxia training results in decreased diffusion distance of muscle fiber (reduced cross-sectional area) without affecting muscle weight. In humans, moderate hypoxia increases postprandial blood distribution towards skeletal muscle during a training recovery. This physiological response plays a role in the redistribution of fuel storage among important energy storage sites and may explain its potent effect on changing body composition.

Conclusion: Prolonged moderate altitude hypoxia (ranging from 1700 to 2400 M), but not acute high altitude hypoxia (above 4000 M), can effectively improve insulin sensitivity and glucose tolerance for humans and antagonizes the obese phenotype in animals with a genetic defect. In humans, the magnitude of the improvement varies widely and correlates with baseline plasma DHEA-S levels. Compared to training at sea-level, training at altitude effectively decreases fat mass in parallel with increased muscle mass. This change may be associated with increased perfusion of insulin and fuel towards skeletal muscle that favors muscle competing postprandial fuel in circulation against adipose tissues.

Keywords: hypoxia, insulin sensitivity, glucose tolerance, metabolic syndromes

INTRODUCTION

To maintain life, cellular ATP is continuously consumed

and must be regenerated at an equal pace. The regeneration of ATP is, for the most part, driven by energy released from oxidation of reducing compounds- fatty acid and glucose.

* Corresponding author: Chia-Hua Kuo, Email. kuochiahua@gmail.com

©2012 The Korean Society for Exercise Nutrition

Oxygen serves as the final electron acceptor, receiving electrons that ultimately come from both of the energy-rich reducing compounds. Without oxygen, ATP regeneration will be compensated for mostly by increasing the rate of anaerobic glycolysis (conversion of glucose to lactate). Therefore, reducing oxygen availability would undoubtedly impact the human metabolic system by increasing the dependence on anaerobic glycolysis. This has been elegantly demonstrated in a study that reported the significant influence of altitude exposure in elevation of the respiratory exchange ratio (RER) and increased lactate accumulation, in parallel with reduced glycerol release (from triglyceride breakdown) from the periphery [1].

In this review paper, we summarize past findings and the current knowledge in the metabolic influence of altitude hypoxia training on carbohydrate metabolism.

STUDIES ON HEALTHY MEN

Insulin resistance is an early sign of type 2 diabetes, coronary heart disease, hypertension, stroke, and cancer [9,10]. These clinical events develop in approximately one out of three normal middle-aged adults who are in the upper tertile of insulin resistance at baseline, during an average follow-up 6 years. Interestingly, both cardiovascular and cancer mortality rates in humans who reside at moderate altitudes are lower than those living at sea-level. Whether the recreational intervention of altitude could help to improve insulin sensitivity is unknown, but would be valuable, as the aforementioned clinical event accounts for the majority of deaths in the modern aging global society [13].

Altitude is characterized by hypoxia, low temperature, and low atmospheric pressure. Many studies have focused on the hypoxia aspect of altitude on the physiological response, since this component potentially affects the human body. Our past experience on hypoxia has shown that an oxygen concentration of 12% can be tolerated by most animals, but still confers death in approximately 6% of cases. This risk should be prevented by lowering altitude when it is utilized for clinical use. Based on this preliminary test, all of our experiments were performed using an oxygen concentration of 14% oxygen or greater.

High altitude at a level above 4500 M can create discomfort and is unsuitable for humans to improve their insulin sensitivity. Larsen *et al.* (1997) reported that acute mountain sickness (AMS) and insulin resistance can result in healthy young men (aged approximately 27 years) after a quick passive ascent to a high altitude of 4559 m (approximately

12% oxygen). Under this condition, insulin-stimulated glucose uptake decreases by about 50%. The decreases in insulin action were associated with the increases in counter-regulatory hormones, such as cortisol and norepinephrine. Even though this acute insulin resistance state would be short (less than one week), the effects of this altitude are too unpleasant to be clinically efficacious.

Based on the foregoing, we designed a study to determine the effect of moderate altitude (3 days at 2400 M, approximately 16% oxygen) on glucose tolerance [3]. Eight subjects 18-43-years-of-age tolerated the conditions well, with only one participant experiencing a 1-day bout of AMS. No vigorous physical training was performed in the study. Glucose tolerance, but not fasting glucose levels, was significantly improved after the 3 days of altitude exposure. We further tested the combined effect of altitude plus hiking activity on the same metabolic parameters in Pamirs confined to a highland area with altitude varying between 2500 and 4000 M (13-16% oxygen). The backpack load was controlled within 0.3-0.33 kg per kg body weight. Glucose concentrations during the oral glucose tolerance test (OGTT) were measured at baseline and 3 days after the high-altitude hiking activities at about 4000 M. Glucose tolerance was significantly improved, yet fasting glucose concentration was slightly elevated from approximately 85 to 102 mg/dL. Both experiments provided consistent evidence that short-term moderate altitude with or without increased physical activity can improve glucose tolerance in healthy humans.

STUDIES ON HUMANS WITH METABOLIC PROBLEMS

A group from Austria investigated the effects of recreational activity at altitude on 22 males with metabolic syndromes [4]. During a 3-week stay at 1,700 m (approximately 17% oxygen) on an Austrian mountain, the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) value and glucose tolerance were significantly improved. Altitude at this level appeared to be well-accommodated in these metabolically abnormal individuals.

Obesity is a major cause of accelerated age-onset insulin resistance and metabolic disorders in adults. We previously investigated the effect of 4-week altitude hiking on obese human subjects. The subjects were individuals with a history of drug abuse (users of heroin, cocaine, or amphetamines). A huge regain in body weight (18-100% above their original body weight) was noted during the abstention period. All subjects exhibited severe hyperinsulinemia (a sign of insulin

resistance) following an oral glucose load. In this study, nine obese males (age 28.7 ± 1.3 years) and 17 control subjects (age 29 ± 1.1 years) voluntarily participated in a 4-week hiking activity (altitude 2200-3800 M, 13-16% oxygen). After the altitude expedition, insulin levels during the OGTT in the ex-addicts were completely normalized to control level. Along with the improvements, a significant reduction in waist-to-hip ratio, with a small increase in lean body mass was observed. Both studies supported the health benefit of altitude activity for metabolically abnormal individuals.

HYPOXIA INCREASES BASAL GLUCOSE UPTAKE IN THE HEART

Hypoxia increases glucose uptake in skeletal muscle [14]. However, we need to aware that most such experiments were performed under ischemic or anoxic conditions (oxygen concentration close to zero), which is apparently lethal to humans and cannot be compared to the high altitude hypoxia condition. Altitude hypoxia places the human heart into a stressful condition since greater cardiac muscle contraction is needed to compensate for reduced peripheral arterial oxygen. Using positron emission tomography (PET) with 2-deoxy-2- ^{18}F fluoro-D-glucose (^{18}F FDG) injection, we found that heart has a higher priority for glucose supply from circulation than skeletal muscle under altitude hypoxia. An increased cardiac glucose uptake (by about 70%) in a simulated altitude hypoxia condition (14-15% oxygen) was observed, whereas no significant change was evident in overall skeletal muscle glucose uptake (fasting sedentary condition). Despite the fact that the heart contributes to small part of the whole body glucose uptake under fasted condition, it is worthy to acknowledge that moderate altitude hypoxia exert strong impact to this vital organ.

DHEA-S UNDERLIES INDIVIDUAL VARIATION

Individual responses in glucose tolerance and erythropoiesis against altitude challenge vary widely in healthy humans [6]. We first found that the serum dehydroepiandrosterone sulfate (DHEA-S) level drops significantly at altitude in subjects with higher baseline DHEA-S level, whereas subjects with a lower baseline DHEA-S level remain stably low. To further elucidate the role of DHEA-S in the adaptation of glucose metabolism to altitude activity, subjects were then divided into lower and upper halves according to their baseline DHEA-S concentrations at sea level. We found that glucose and insulin concentra-

tions on OGTT were significantly decreased by the mountaineering activity confined only to those subjects with initially higher DHEA-S. Similarly, increased hematocrit and hemoglobin concentrations with altitude occurred only in the high DHEA-S group. Intriguingly, the low DHEA-S subjects exhibited a higher erythropoietin (EPO) value at both sea level and altitude than the high DHEA-S group, suggesting a resistance of the hematopoietic system to EPO stimulation for erythropoiesis.

DHEA and its sulfate derivative, DHEA-S, are the most abundant steroids in humans and are mainly produced in the adrenal gland [15-17]. They are also the primary precursors in biosynthesis of many steroid hormones, including androgens, estrogens, and some other steroid hormones. This endogenous steroid generally declines with advancing aging. Baseline plasma DHEA-S concentration is closely associated with human longevity and fitness.

Coping against acute stress generally involves suddenly increased oxygen demand, which can result in transient tissue hypoxia. Successful coping against variable environmental changes, such as muscular work, temperature shift, and mental challenge, is essential to ensure continued wellbeing and survival. We previously reported a prolonged decrease in circulating DHEA-S for 5 days in golfers who experienced negative competition outcome, whereas the overall mood state (measured by Profile of Mood States (POMS) inventory) remained stable. The depression level in the losing golfers recovered quickly demonstrating that the psychological readjustment was successful with a concurrent decline in the DHEA-S level. In contrast, the mood state and DHEA-S were not changed for the golfers who advanced to the last two rounds of the tournaments throughout the entire observation period.

Coping with heat stress is one of the most important survival mechanisms for the human body, since increasing body temperature can cause a permanent change in protein conformation, leading to death. A state of insulin resistance, evidenced by increased area under curve of glucose and values of the insulin resistance indicator HOMA-IR, was manifested in 16 healthy males with a 30-min hot spring immersion at 41 °C. A wide range of individual variations for the heat-induced stress response was also identified. We found that changes in insulin resistance measured after heat stress is associated with baseline DHEA-S level. In particular, hot spring immersion-induced insulin resistance state was restricted only to the low DHEA-S group.

Muscular challenge against various physical work occurs in everyday life. Fast recovery from physical work ensures an active life. We found a persistent reduction in DHEA-S

level concurrent with improvement in insulin sensitivity during the recovery period after resistance exercise [18]. Resistance exercise generates considerable muscle damage and thereafter induces a protracted repair process. The decline of DHEA-S appears to be related to increased consumption for tissue repair during recovery in damaged skeletal muscle. The increased demand on DHEA-S could account for consumption of this steroid during recovery, based on the evidence that exogenous DHEA administration following various conditions causing tissue damage enhances functional recovery [19,20].

Exercise training is generally recommended for the elderly to maintain metabolic health. However, it was also reported that the training effect aimed at improving glycemic control for middle-aged or older individuals is not as effective as for young individuals [21]. We investigated the effect of a 4-month exercise training program on glucose tolerance and insulin sensitivity in a group of elderly individuals over 80-years-of-age in relation to their baseline DHEA-S levels. The result shows that the 4-month exercise training effect on improving insulin sensitivity cannot occur in the elderly with low DHEA-S level [22].

MECHANISM INVOLVED WITH ALTITUDE TRAINING EFFECT

To elucidate the mechanism for the beneficial effect of altitude training on insulin sensitivity and glucose tolerance, we performed both animal and human experiments. Similar to obese humans, genetically obese animals exhibit greater fasting insulin levels, and exaggerated glucose and insulin responses following an oral glucose challenge compared with their lean counterparts. During a 6-week training period, rats performed a swimming exercise progressively increasing in time from 30 to 180 min/day-1, and recovered under hypoxia (14% oxygen for 8 h/day). At week 6, body weight, fasting glucose, fasting insulin, area under curve of glucose (GAUC) and insulin (IAUC) were significantly decreased among the obese rats. Capillaries to fiber ratio, capillary density, and type IIa fiber proportion of the plantaris muscle in the exercise-trained group were significantly increased above control level, but no additive effect of hypoxia and exercise training was observed. Our data also demonstrate that exercise training with prolonged hypoxia recovery offers better metabolic benefits than exercise training alone for genetically obese animals. This advantage was closely associated with the effective weight reduction [8]. Obesity has been recognized as a major contributor of type 2 diabetes and its metabolic

complications [23].

Under insulin-stimulated condition, skeletal muscle accounts for approximately 85% of glucose uptake [24]. Thus, interventions that affect muscle property may also influence the whole-body glucose tolerance and insulin sensitivity. The effects of altitude training on the AMP-activated protein kinase (AMPK)-related glucose transport pathway in skeletal muscle of both lean and obese Zucker rats was studied [8,25]. The skeletal muscle AMPK-related glucose transport pathway is involved in glucose homeostasis. We found that obese animals have abnormal LKB1-AMPK-AS160-GLUT4 signaling system in red gastrocnemius muscle compared to that in lean (normal) animals. We compared the chronic training effects of hypoxia training and exercise training on this pathway in lean and obese rats. The obese rats had higher body weights, elevated fasting insulin and glucose levels, and higher baseline levels of muscle AMPK and AS160 phosphorylation compared with those of lean animals. After hypoxia training, the levels of AMPK, AS160 phosphorylation, fasting insulin, and fasting glucose were reversed toward normal concomitant with an approximately 50% increase in the muscle GLUT4 protein level compared with those of the control group. GLUT4 is the protein expressed in skeletal muscle that is responsible for insulin-stimulated glucose transport, and plays a major role in postprandial glucose disposal.

In animals, the knowledge of prolonged systemic hypoxia exposure (with or without training) reducing body fat mass [26] and increasing muscle mass [27] has been well-established. Since ambient oxygen for fatty acid oxidation is less available during altitude hypoxia, fat burning cannot be the best explanation for the aforementioned body fat-reducing effect. In animals, interventions that increase muscle mass lead to significant reduction in body fat mass [28,29], suggesting that skeletal muscle plays the dominant role in the regulation of fuel storage in fat tissues. Therefore, we hypothesized that greater distribution of circulatory insulin to skeletal muscle can directly alter the deposition of fuel storage [30], influencing body composition. Insulin is the main anabolic hormone that stimulates fuel storage and syntheses of glycogen, triglyceride, and protein in skeletal muscle and adipose tissues [31]. Withdraw of insulin in animals for only 7 days can lead to marked reductions in fat mass and fat cell diameter at all depots [32]. Therefore divergent distribution in circulatory insulin perfusion among tissues would be expected to alter storage among energy-storage tissues. Insulin delivery shift can presumably be maneuvered by increasing blood distribution towards skeletal muscle.

Two sets of human experiments were performed to compare the effect of altitude training on muscle and fat masses. For

the first experiment, the effect of 3-week altitude training on body composition was determined by dual-energy X-ray absorptiometry (DXA) in 10 male swimmers (age, 14.9 ± 0.4 years; BMI, 20.8 ± 0.4 kg/m²) who regularly completed a fixed training distance of 12.3 km per day. After baseline measurements, all subjects moved from sea-level (Singapore) to altitude (2200 m, Kunming, Yunnan, PRC). They maintain the same training distance at sea-level (all were motivated to complete their daily swimming volume in the shortest possible time). An additional 8 male control subjects (age: 13.0 ± 1.6 years, BMI: 20.5 ± 1.2 kg/m²) resided at sea level were recruited to monitor the possible time-to-time variation of DXA measurements on body composition. Altitude exposure rapidly decreased fat mass and increased lean mass for youth swimmers maintaining their body weight. Since the overall dietary intake was not apparently changed, energy balance per se cannot be the best explanation for this significant change.

For the second experiment, the effect of hypoxia (16%) on blood distribution to skeletal muscle was measured under glucose-ingested condition (insulin-stimulated condition) during the training recovery. We hypothesized that the fat-reducing effect is related to increased distribution of insulin and energy substrates towards skeletal muscle for swimmers. Skeletal muscle blood distribution was measured using near infrared spectroscopy (NIRS) to detect changes in hemoglobin concentration under hypoxic (16% oxygen) and normoxic conditions for 90 min after oral glucose ingestion. We evaluated blood distribution under glucose-ingested hyperinsulinemic condition to mimic postprandial state. The increased blood perfusion into skeletal muscle would be expected to result in an increased delivery of glucose and insulin into skeletal muscle.

Our data on hemoglobin increases in skeletal muscle fit well with the finding of reciprocal increase in lean mass and decrease in fat mass for all swimmers after altitude exposure. Increasing insulin distribution into skeletal muscle can promote fuel deposition and muscle growth specifically in the region, which will in turn decrease fuel availability to adipose tissue. Insulin is the major anabolic hormone released after a carbohydrate meal, which is able to stimulate glucose uptake [33], glycogen synthesis [34,35], amino acid uptake [36], net protein synthesis [37], cell proliferation [38], and triglyceride synthesis [31] in insulin-targeted tissues. Therefore, competition between skeletal muscle and adipose tissue for insulin-dependent fuel acquisition plays a key role in regulating body composition.

CONCLUDING REMARKS

1. Altitude exposure increases energy reliance on carbohydrate metabolism.
2. Short-term moderate altitude hypoxia can improve glucose tolerance without affecting fasted glucose (or slight increases).
3. Altitude exposure above 4000 M increases physiological stress, elicits discomfort, and leads to insulin resistance.
4. During training recovery, prolonged moderate hypoxia exposure can significantly reduce fat mass with slight increase in muscle mass without affecting body weight for athletes.
5. During training recovery, moderate altitude exposure increases blood perfusion towards skeletal muscle under glucose ingested hyperinsulinemic state.
6. In genetically obese animals, exercise training with moderate hypoxia recovery can still exert potent effect in correcting obese phenotype.
7. Skeletal muscle from genetically obese animals has abnormal LKB1-AMPK-AS160-GLUT4 signaling pathway, which can be reversed by exercise training with moderate hypoxia recovery.

REFERENCES

- [1] K Katayama, K Goto, K Ishida, F Ogita. Substrate utilization during exercise and recovery at moderate altitude. *Metabolism*. 2010;59:959-966.
- [2] JJ Larsen, JM Hansen, NV Olsen, H Galbo, F Dela. The effect of altitude hypoxia on glucose homeostasis in men. *Journal of Physiology*. 1997;504:241-249.
- [3] WC Lee, JJ Chen, HY Ho, CW Hou, MP Liang, YW Shen, CH Kuo. Short-term altitude mountain living improves glycemic control. *High Alt Med Biol*. 2003;4: 81-91.
- [4] W Schobersberger, P Schmid, M Lechleitner, S von Duvillard, H Hörtnagl, H-C Gunga, A Klingler, D Fries, K Kirsch, R Spiesberger, R Pokan, P Hofmann, F Hoppechler, G Riedmann, H Baumgartner, E Humpeler. Austrian Moderate Altitude Study 2000 (AMAS 2000). The effects of moderate altitude (1,700 m) on cardiovascular and metabolic variables in patients with metabolic syndrome. *Eu J Appl Physiol*. 2003;88:506-514.
- [5] W-C Lee, J-J Chen, DD Hunt, C-W Hou, Y-C Lai, F-C Lin, C-Y Chen, C-H Lin, Y-H Liao, C-H Kuo. Effects of hiking at altitude on body composition and insulin sensitivity in recovering drug addicts. *Prev Med*. 2004;

- 39:681-688.
- [6] WC Lee, SM Chen, MC Wu, CW Hou, YC Lai, YH Laio, CH Lin, CH Kuo. The role of dehydroepiandrosterone levels on physiologic acclimatization to chronic mountaineering activity. *High Alt Med Biol.* 2006;7: 228-236.
 - [7] WM van Weerden, HG Bierings, GJ van Steenbrugge, FH de Jong, FH Schröder. Adrenal glands of mouse and rat do not synthesize androgens. *Life Sciences.* 1992;50: 857-861.
 - [8] MC Wu, YL Tsai, CY Huang, CL Kao, CW Hou, CY Chen, M Korivi, WH Jean, SD Lee, CH Kuo. Hyperinsulinemia and overweight in obese Zucker rats effectively suppressed by exercise training with hypoxia recovery. *Eu J Sport Sci.* 2012;1-10.
 - [9] FS Facchini, N Hua, F Abbasi, GM Reaven. Insulin resistance as a predictor of age-related diseases. *J Clin Endocrinol Metab.* 2001;86:3574-3578.
 - [10] NP Nunez, W-J Oh, J Rozenberg, C Perella, M Anver, JC Barrett, SN Perkins, D Berrigan, J Moitra, L Varticovski, SD Hursting, C Vinson. Accelerated tumor formation in a fatless mouse with type 2 diabetes and inflammation. *Cancer Res.* 2006;66:5469-5476.
 - [11] J Amsel, JW Waterbor, J Oler, I Rosenwaike, K Marshall. Relationship of site-specific cancer mortality rates to altitude. *Carcinogenesis.* 1982;3:461-465.
 - [12] EA Mortimer, RR Monson, B MacMahon. Reduction in mortality from coronary heart disease in men residing at high altitude. *New Engl J Med.* 1977;296:581-585.
 - [13] DE Alley, EJ Metter, ME Griswold, TB Harris, EM Simonsick, DL Longo, L Ferrucci. Changes in weight at the end of life: Characterizing weight loss by time to death in a cohort study of older men. *Am J Epidemiol.* 2010;172:558-565.
 - [14] GD Cartee, AG Douen, T Ramlal, A Klip, JO Holloszy. Stimulation of glucose transport in skeletal muscle by hypoxia. *J Appl Physiol.* 1991;70:1593-1600.
 - [15] EE Baulieu, P Robel. Dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) as neuroactive neurosteroids. *Proc Natl Acad Sci U S A.* 1998;95: 4089-4091.
 - [16] AW Krug, CG Ziegler, SR Bornstein. DHEA and DHEA-S, and their functions in the brain and adrenal medulla. *Neuroactive Steroids in Brain Function, Behavior and Neuropsychiatric Disorders;* 2008:227-239.
 - [17] IH Zwain, SSC Yen. Dehydroepiandrosterone: biosynthesis and betabolism in the brain. *Endocrinology.* 1999;140: 880-887.
 - [18] YM Tsai, SW Chou, YC Lin, CW Hou, KC Hung, HW Kung, TW Lin, SM Chen, CY Lin, CH Kuo. Effect of resistance exercise on dehydroepiandrosterone sulfate concentrations during a 72-h recovery: Relation to glucose tolerance and insulin response. *Life Sciences.* 2006;79: 1281-1286.
 - [19] J Herbert. Neurosteroids, brain damage, and mental illness. *Exp Gerontol.* 1998;33:713-727.
 - [20] AS Malik, RK Narayan, WW Wendling, RW Cole, LL Pashko, AG Schwartz, KI Strauss. A novel dehydroepiandrosterone analog improves functional recovery in a rat traumatic brain injury model. *J Neurotrauma.* 2003; 20:463-476.
 - [21] KR Short, JL Vittone, ML Bigelow. Impact of aerobic exercise training on age-related changes in insulin sensitivity and muscle oxidative capacity. *Diabetes.* 2003;52: 1888-1896.
 - [22] Y Huang, M Chen, C Fang, W Lee, S Yang, C Kuo. A possible link between exercise-training adaptation and dehydroepiandrosterone sulfate- an oldest-old female study. *Int J Med Sci.* 2006;3:141-147.
 - [23] PG Kopelman. Obesity as a medical problem. *Nature.* 2000;404:635-643.
 - [24] RA DeFronzo, E Jacot, E Jequier, E Maeder, J Wahren, JP Felber. The effect of insulin on the disposal of intravenous glucose. *Diabetes.* 1981;30:1000-1007.
 - [25] YC Chen, SD Lee, CH Kuo, LT Ho. Effects of altitude training on the AMPK-related glucose transport pathway in the red skeletal muscle of both lean and obese zucker rats. *High Alt Med Biol.* 2011;12:371-378.
 - [26] CY Chen, YL Tsai, K C.L., SD Lee, MC Wu, K Mallikarjuna, YH Liao, JL Ivy, CH Kuo. Effect of mild intermittent hypoxia on glucose tolerance, muscle morphology and AMPK-PGC-1 α signaing. *Chin J Physiol.* 2010;53:62-71.
 - [27] D Deveci, JM Marshall, S Egginton. Relationship between capillary angiogenesis, fiber type, and fiber size in chronic systemic hypoxia. *Am J Physiol.* 2001;281: H241-252.
 - [28] M Suzuki, T Doi, SJ Lee, K Okamura, S Shimizu, G Okano, ..., T Fushiki. Effect of meal timing after resistance exercise on hindlimb muscle mass and fat accumulation in trained rats. *J Nutri Sci Vitaminol.* 1999; 45:401-409.
 - [29] JM Argilés, J López-Soriano, V Almendro, S Busquets, FJ López-Soriano. Cross-talk between skeletal muscle and adipose tissue: A link with obesity? *Med Res Rev.* 2005;25:49-65.
 - [30] A Bonen, MG Clark, EJ Henriksen. Experimental approaches in muscle metabolism: hindlimb perfusion

- and isolated muscle incubations. *Am J Physiol.* 1994; 266:E1-E16.
- [31] F Magkos, X Wang, B Mittendorfer. Metabolic actions of insulin in men and women. *Nutrition.* 2010;26: 686-693.
- [32] A Ghorbani, M Varedi, MA Hadjzadeh, GH Omrani. Type-1 diabetes induces depot-specific alterations in adipocyte diameter and mass of adipose tissues in the rat. *Exp Clin Endocrinol Diabetes.* 2010;118:442-448.
- [33] YC Lai, E Zarrinpashneh, J Jensen. Additive effect of contraction and insulin on glucose uptake and glycogen synthase in muscle with different glycogen contents. *J Appl Physiol.* 2010;108:1106-1115.
- [34] CH Kuo, H Hwang, MC Lee, AL Castle, JL Ivy. Role of insulin on exercise-induced GLUT-4 protein expression and glycogen supercompensation in rat skeletal muscle. *J Appl Physiol.* 2004;96:621-627.
- [35] M Bouskila, MF Hirshman, J Jensen, LJ Goodyear, K Sakamoto. Insulin promotes glycogen synthesis in the absence of GSK3 phosphorylation in skeletal muscle. *Am J Physiol.* 2008;294:E28-35.
- [36] G Biolo, BD Williams, RY Fleming, RR Wolfe. Insulin action on muscle protein kinetics and amino acid transport during recovery after resistance exercise. *Diabetes.* 1999; 48:949-957.
- [37] AA Ferrando, DL Chinkes, SE Wolf, S Matin, DN Herndon, RR Wolfe. A submaximal dose of insulin promotes net skeletal muscle protein synthesis in patients with severe burns. *Ann Surg.* 1999;229:11-18.
- [38] W-W Kuo, L-C Chung, C-T Liu, S-P Wu, C-H Kuo, F-J Tsai, C-H Tsai, M-C Lu, C-Y Huang, S-D Lee. Effects of insulin replacement on cardiac apoptotic and survival pathways in streptozotocin-induced diabetic rats. *Cell Biochem Funct.* 2009;27:479-487.