International Journal of Applied Sports Sciences
2014, Vol. 26, No. 2, 76-88.
ⓒ Korea Institute of Sport Science
ISSN 2233-7946 (Online)
ISSN 1598-2939 (Print)
http://www.sports.re.kr/eng/05publication/Callforpaper.jsp

Review on Molecular Biological Approaches to Investigate the Effect of Endurance Training

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Abstract

The purpose of this study is to examine details of domestic and foreign papers that verified the effect of endurance training using molecular biological approaches and to investigate the directivity of molecular biological study, analytical technology and analytical factors for the analysis of training effects. Existing domestic and foreign papers on endurance training that investigated the effect of training based on molecular biological analysis of human beings were reviewed. In order to increase reliability of the selected papers and their compatibility with the purpose of this study, the papers were reclassified by a group of experts that consisted of physical education professors and doctors of exercise physiology. The final papers selected were studied for the change in hormones and enzymes through blood and muscular tissues based on study topic and trend analysis from a macroscopic perspective. Analytical methods used include western blot, RT-PCR, ELISA and immunohistochemistry of molecular biology. Analytical variables investigated / analyzed were ACE, ACTN, PPARs, UCP, mitochondrial DNA variant, creatine kinase, renin-angiotensin, AMPK for blood and COX, myosin binding protein C fast-type, glycogen phosphorylase, pyruvate kinase, GLUT, PPARs, and AMPK for muscle. Such molecular biological analytical techniques and variables should be widely used to provide 1) conditioning of professional athletes and life sports athletes, 2) analysis of training effect, and 3) scientific and future-oriented methods for health enhancement.

Key words: Mucosal Endurance Training, Biochemical Analysis, Protein, Hormone, Enzyme

Introduction

For improvement of athletic performance of professional athletes, it is most important to develop and apply scientific and highly efficient training methods so as to improve personal performance, maintain homeostasis of the body, and maximize capabilities. The effect of a training program can be maximized when it considers basic principles such as incremental overload, specificity, individuality, diversity, change and cyclisation (Kim, 2011). Also, such principles need to be supported by field experience and the results of studies in different areas of sports science.

Looking at the status of support for sports science by the Korea Institute of Sport Science between 2000 and 2010, there have been 488 cases of on-site support including 293 cases in the field of exercise physiology,

Submitted : 16 October 2014, revised : 22 November 2014, accepted : 25 November 2014.
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114 cases in the field of sports biomechanics, and 81 cases in the field of sports psychology. Under such scientific support, the national team of Korea demonstrated its status as a powerful sports nation by ranking 12th at the 27th Sydney Olympic Games, 9th at the 28th Athens Olympic Games, 7th at the 29th Beijing Olympic Games, and 5th at the 30th London Olympic Games. As a result of analyzing research trends in sports science of KCI (Korea Citation Index), 177 papers showed a distribution of 65.53% on exercise physiology, 14.12% on sports nutrition, 10.16% on training method, and 4.52% on special sports. Also, they can be classified according to subjects into 33.90% on adults, 24.86% on professional athletes and 12.99% on animals. In addition, 86 of 177 papers are biochemical monitoring papers that provide scientific evidence, and they mainly analyzed energy metabolism, oxidation stress, hormone, inflammation, immunity and blood lipid through blood sampling.

Among previously reported academic studies in Korea, training studies presented for the purpose of increasing athletic performance were mostly about verification of cardiorespiratory endurance and anaerobic power related to mobilization of energy metabolism. Important indicators of cardiorespiratory endurance evaluation in development of training programs include maximum oxygen consumption using exercise stress test equipment, anaerobic threshold, buffering of fatigue substance, and restoration ability (Kim, 2003; Jung et al., 2012). Also, physiological indicators widely used in training intensity analysis are heart rate, perceived awareness, and blood lactate concentration (Park et al., 2006; Byeon & Kang, 2003). The most representative method of anaerobic exercise capacity evaluation is the Wingate Test, power at each hour, blood lactate concentration, blood ammonia concentration, enzyme activity, and various blood hormone concentrations exhibited during the test are analyzed together (Yoon et al., 2010). On one hand, foreign journal articles are analyzing training effects in connection with the latest molecular biological analysis technologies (western blot, RT-PCR, proteomix, microarray, SNP, etc.) using blood, saliva and muscular tissues, as well as cardiorespiratory endurance and anaerobic power. In particular, samples are extracted by muscle biopsy after application of training programs to biologically analyze adaptation of skeletal muscles on the molecular level, immunity, change in muscular fiber type, recreation of mitochondria, change in mRNA on the level of gene expression, and protein expression (Reznick & ShuThe, 2006; Malm et al., 2000; Tegtbur et al., 2009). Summarizing the results of such studies, various domestic studies on sports science limit the scope of endurance training effect to factors related to athletic performance, and there is a need to actively review new methods for training effect that include molecular biological analysis. Future studies must be carried out in line with the global research trend.

The purpose of this study is to classify domestic and foreign papers that analyzed training effect using molecular biological analysis methods after application of endurance training programs into study topic, subject, method of use, analytical technique (western blot, RT-PCR, histology, gene, proteomix, microarray, SNP, hormone assay, in vitro (cell line, primary cell culture), analytical factor (protein, mRNA, gene, histological change), to examine details of such papers, and to investigate molecular biological approach for analysis of training effects.

Methods

Selection of papers for analysis

Domestic and foreign papers reported between 2000 and 2013 that used molecular biological analysis after application of endurance training were selected. Papers were mostly searched on websites of the Korea Education and Research Information Service (www.riss.kr) and the US National Library of Medicine National Institutes of Health (www.ncbi.nlm.nih.gov/pubmed), and about 130 domestic and foreign research papers

were selected using search keywords such as endurance training, human, protein, muscle, hormone and mRNA. The selected papers were re-verified by a group of experts (physical education professors, national team coaches, and experts of exercise physiology) to reselect studies that are highly related to the purpose of this study. In specific, selection criteria for the level of papers were limited to SCI and SCIE. Acute exercise instead of training programs and studies conducted on children, adolescents, seniors and patients were excluded to analyze 13 domestic papers and 21 foreign papers. Details of analysis were classified into study topics, trends, molecular biological analysis variables and analytical techniques.

Study method

Studies that verified the effect of trainings using molecular biological analysis were analyzed through classification of analytical factors. Hormone and enzyme were analyzed for blood, and protein and enzyme for muscle.

Results

Effects of endurance training

The human body shows physiological, biochemical, molecular biological, dynamic and psychological adjustments under repetitive exercise, showing improvement on specific motion performance. The aspect and size of adjustment reaction differ according to intensity of training, period, form, frequency, genetic limitation, and level of individual prior activity. Repetitive exercise overload must be applied in order to induce effective adjustment, and physiological and metabolic adjustments on training generally occur specific to the aspect of exercise overload (Coggan et al., 1993; Coggan & William, 1995).

Endurance training quickly adjusts the body to metabolic demands from stable state to steady state, reducing dependence on limited liver and sugar sources. It helps maintain homeostasis through control of the cardiovascular system and temperature. This is because early metabolic adjustment caused by endurance training occurs as an adjustment of nerve or neurohormone receptor, which leads to a structural change. Increase of motor performance by endurance training seems to result from great biochemical and structural changes in the trained skeletal muscles, rather than a small increase of maximum oxygen consumption (Green et al., 1991).

Representative effects of endurance training are an increase in the number of mitochondria and increase of capillary density (Holloszy & Coyle, 1984). This is related to an increase of enzymes included in oxidative metabolism (Krebs cycle, fatty acid cycle, electron transport system). Increase in the number of mitochondria reduces ADP concentration needed to maintain given oxygen consumption, and low ADP concentration and PC concentration reduce irritation of PFK, corresponding speed, and lactate production. Low lactate production decreases blood lactate concentration, and mobilization of free fatty acid from fatty tissues becomes smooth. In other words, low ADP concentration reduces irritation of PFK at the start of exercise, and increased use of fat reduces oxidation of carbohydrates during long exercise. Reduced use of carbohydrates will reduce the generation of pyruvic acid. Therefore, an increase in the number of mitochondria increases the opportunity for pyruvic acid to be used by mitochondria for oxidation in the Krebs cycle, rather than to be combined with LDH in the cytoplasm. Such an adjustment decreases concentration of pyruvic acid and lactate production.

NADH created in this process reacts with pyruvic acid or is oxidized in the electron transport system to increase the circulation system in which NADH is transported to mitochondria that generate ATP (Holloszy & Coyle, 1984). NADH generated in this process is quickly transported to the mitochondria, reducing the rate of lactate and H+. This process arouses a change

in the circulation system used to transport NADH formed in the cytoplasm to the electron transport system for the generation of ATP. The shape of the LDH enzyme used to convert pyruvic acid into lactate is also changed.

Endurance training increases the capillary density of active muscles. This means that the distance from capillary to mitochondria is shortened and blood flow velocity is reduced by each capillary. Accordingly, greater oxygen diffusion occurs in mitochondria. As a result, active muscles require smaller blood flow after training at the same submaximal exercise. Muscles can use more oxygen per 1 l of blood in order to accomplish oxygen consumption in the same steady state using small blood flow.

On one hand, regulation of peripheral nerves and central nerves after an endurance training program affects the activity of the sympathetic nervous system, heart rate and ventilation under submaximal exercise. Performance of submaximal exercise fixed for a long period of time depends on how many exercise units are mobilized to satisfy tension requirements through the oxidative phosphorylation process. When there is a small number of mitochondria, many exercise units are required to perform exercise with the given oxygen consumption. This causes increased regulation of central nerves in cardiopulmonary center, as well as a further increase of the sympathetic nervous system, heart rate, and ventilation. Feedback from the chemoreceptors of untrained muscles also stimulates the cardiopulmonary center. However, local tolerance is not changed according to increase in the number of mitochondria after endurance training. Therefore, local stimulation of blood flow is reduced and information input of chemoreceptor in cardiopulmonary center is reduced. When there is a large number of mitochondria, tension can be maintained despite a small number of exercise units, and the decrease of feed-forward information input in such higher brain centers and the decrease of feedback in muscles reduce the information output of the sympathetic nervous system for exercise, heart rate, and ventilation response (Rowell, 1986; Rowell et al., 1990).

Trend of domestic and foreign molecular biological studies on endurance training

Looking at the trend of academic papers registered with the Korea Research Foundation that analyzed the effects of endurance training using molecular biological analysis methods between 2000 and 2013, there were a limited number of studies on molecular biological analysis of endurance training. Studies that performed analysis of training effects using molecular biological analysis methods after application of endurance training programs can largely be classified according to study topic into energy metabolism, blood lipid factors, immunocyte, oxidation stress, hormone and inflammatory factors. Blood sampling is mostly done on blood serum or plasma of the fingertips and brachial vein. The sample included a diverse distribution of subjects including undergraduates, professional athletes, adolescents, and obese middle-aged adults.

Among studies reported between 2000 and 2013, those that applied endurance training to ordinary people reported that the reinforcement of oxygen transfer ability and the endocrine function of that human body, and positive improvement on the composition of blood lipids helped prevent and treat cardiovascular diseases and obesity, and improved metabolic syndrome in terms of insulin resistance and fibrinolytic ability. Kim EJ et al. (2012) implemented 3km running as an endurance exercise for overweight adults to analyze body compositions (height, weight, BMI) and inflammatory factors in the blood (WBC, albumin, neutrophil) of subjects before and after the experiment. In another study, obese but healthy females with sedentary jobs received a 4-week endurance training program to analyze basic body compositions

and blood oral glucose tolerance test (OGTT), insulin and fatty free acid (Baek, 2010). In both studies, the effects of endurance training implemented for a given period were improved with statistical significance. These studies demonstrate that endurance training is a representative form most widely applied to overweight and obese subjects.

Also, inflammatory factors and factors related to oxidation stress can be seen as common variables of experimental studies that apply highly intense endurance training for ordinary people or professional athletes. The cause of cytopathic activity has recently been explained by the theory of oxidation stress from oxygen radicals. There are studies that propose health problems of intensive endurance exercises based on lipid peroxidation, oxidative DNA damage and antioxidative enzyme as evaluation of oxidative stress from exercise, and other studies are reporting substances that regulate oxidative stress. Kim et al. (2009) analyzed the antioxidative effect of rooibos-tea in relation to oxidative stress caused by long-term training through changes in lipid peroxidation (malonedialdehyde: MDA), oxidative NDA damage (8-hydroxyguanine) and antioxidative enzymes superoxide dismutase (SOD) and catalase (CAT) according to application of intensive endurance training and medication of rooibos-tea in professional athletes. A 12-week training regimen was conducted at high intensity of 6km runs at 70~80%HRmax, 40 minutes per session and 3 sessions per week on ordinary students to analyze tissue damage indicators such as MDA, SOD and CAT in blood (Ju, 2006).

Furthermore, experimental studies that classified endurance training into intensity, type and surrounding environment based on variables related to immunity were confirmed. Han (2004) analyzed Hct, RBC and Hb in blood after conducting 12-week's worth of endurance training on ordinary persons by dividing them into high intensity (80% HRR) and medium intensity (60% HRR) groups. The study of Kim DJ (2003) performed exercise stress tests on three healthy middle school cyclists aged between 14 and 16 years and ordinary students to measure leukocyte, erythrocyte, hematocrit, mean hemoblobin of erythrocyte, and mean concentration of erythrocyte haemoglobin in the blood. There are studies that performed an 8-week endurance training program under three climatic conditions to test the effect of endurance training by analyzing leukocyte, neutrophils, lymphocyte, NK cell, catecholamine, epinephrine, and norepinephrine through blood (Lee, 2001) and an 8-week endurance training program with ergometers on bicycles four days a week and 60 minutes a day to verify changes in total leukocyte count, lymphocyte, and monocyte through the blood (Lee, 2001). Studies on immune function and the effects of endurance training have been consistently conducted starting before 2000, but there are limitations in the analytical variables of blood and lack of diversity in the molecular biological approach. For such reasons, domestic studies are only showing phenomenological approaches, which present mostly predictable results. However, there are limitations in examining theoretical causes and analyzing the cause of difference in the results.

Compared to domestic papers, foreign papers published in international journals analyze diverse analytical variables using different methods by extracting muscular tissues based on muscle biopsy. Most studies use western blot, RT-PCR, HPLC and ELISA as analytical methods, and molecular biological studies have recently become active with analysis factors such as microRNA, protein and hormones. Looking at the representative foreign studies reported between 2000 and 2013, study topics under broad context are not much different from domestic studies. Topics can be classified into energy metabolism, blood lipid variables, immune cell, oxidative stress, hormone and inflammatory factors. Differences from domestic studies were found in diversity of variables and segmentation of sample unit from tissues into molecules. Moreover, interesting and complicated studies were found with collection of samples using blood, muscular

tissues and hair. Among studies related to inflammatory factors, Yoe et al. (2008) conducted a study that analyzed the difference between daily bicycles training program and two training sessions a day on alternate days in well-trained professional endurance athletes. Mitochondrial DNA, citrate synthase, β-HAD, COX-2, COX-4, AMPK, and PGC-Iα were analyzed by extracting muscle tissue using muscle biopsies, and variables were analyzed with RT-PCR and western blot techniques. Gibala et al. (2006) studied changes in adaptability of molecules and cells in skeletal muscles after short-term sprinter interval training and endurance training. Their study also extracted muscle tissue to analyze COX-2, COX-4 and β2M using RT-PCR and western blot methods. The study reported temporal efficiency of quick muscle formation and high performance in short-term sprinter interval group. Studies above examined the cause through expression of inflammatory factors with short-term intensive endurance training. Examination of the causes of changes has become more specific on the level of cells with a molecular biological approach, allowing for scientific interpretations on the molecular level.

Further, various causes of fatty acid metabolism have been reported based on endurance training, and a study (Bruce, 2006) reported that endurance training increases glucose tolerance and oxidation of mitochondria fatty acid, and changes fat content in muscles of obese people. The study extracted blood and muscle to analyze citrate synthase, β-HAD, AMPK, ACC and PPARα through western blot and ELISA. Studies on AMPK, a factor frequently analyzed to study obesity, have recently become active. This AMPK controls cell metabolism by inducing ATP production signal transduction pathway and suppressing energy consumption signal transduction pathway. This is the phosphorylase enzyme that controls activity by responding to the energy status (ATP / AMP ratio) of cells changed by nutritional status, exercise and stress of cells (Hardie, 2004; Ruderman et al., 2003), and it is known to affect various physiological activities such has sugar transport, lipogenesis and cholesterol synthesis by controlling phosphorylation of various enzymes that participate in energy metabolism of cells (Christopher et al., 2003; Holmes et al., 1999; Sakamoto et al., 2004).

Mortensen (2013) reported a study that analyzed AMPK signaling after conducting a 12-week endurance training program on people with normal body weight and low birth weight, which results in increased risk of type 2 diabetes. The study analyzed PGC-1α, Glut4, HK2, Sirt1, GS, GS site 2+2a, GS site 3a+b and glycogen on mRNA level, as well as AMPK α1, AMPK α2, AMPK β2, AMPK γ1, AMPK γ3, ACC2, GS, GS site 1B and GS site 2+2a using methods such as RT-PCR, western blot and ELISA. As such, molecular biological studies are being actively conducted using different variables to track signaling of AMPK.

Lundby et al. (2006) reported a study that reported regular endurance training under normoxic condition reduces expression of HIF-1α and HIF-2α in skeletal muscles from exercise. Samples were extracted from femoral muscles using muscle biopsy, and RNA was separated to analyze variables using RT-PCR.

There was a study on miRNAs, an unfamiliar topic of physical education, reported in 2013. The study showed increase of drosha, dicer & exportin-5, as well as miR-1, -133a, -133b and miR-181a, with one-time high-intensity endurance exercise and decrease of miR-9, -23a, -23b and -31. Also, short-term training showed increase of miR-1 and miR-29b and reduction of miR-31. Russell et al. (2013) reported that exercise can quickly and temporarily control various miRNA types that participate in regeneration of skeletal muscles, gene transcription and biosynthesis of mitochondria.

Recent studies reported in international journals have been examining phenomenological changes and discoving the causes for analysis of mechanisms.

Molecular biological variables for analysis of endurance training effects

Characteristics of blood analysis

Games genes for analysis of endurance training effects

ACE, ACTN-3, PPARr-3, UCP, mitochondrial DNA variant, creatine kinase activity, and renin-angiotensin transition system were suggested as candidate genes that can be obtained from blood. Especially among studies on endurance training, studies related to genes in the blood were mostly reported on DNA damage caused by oxidative stress between 2000 and the present. The cause of cytopathic activity is explained by the theory of oxidative stress according to the oxygen radical. When electrons in cells fail to form a pair on the orbit, there is an extremely activated response to absorb electrons from outside. Excessive oxygen radicals formed in this process inflict various harm to the human body. Evaluation of oxidative stress related to genes in the blood can be measured using lipid peroxidation (MDA, malonedialdehyde) and oxidative DNA damage (8-hydroxy guanine). MDA is a resolvent of early products in lipid peroxidation reaction caused by oxygen radicals, which reflects cytopathic activity and functional degradation. 8-hydroxy guanine is a degenerated substance of the hydration process in 8-position of guanine base among four nitrogen bases, which refer to the degree of oxidative DNA damage. In addition, antioxidative enzymes such as SOD (superoxide dismutase), CAT (catalase) and GPX (glutathione peroxidase) are indicators that defend oxidative stress, and various studies are underway in regards to exercise intensity, training, stress and antioxidants along with indicators of oxidative stress. According to Ju YS (2006) in a study report on the effect of 6km endurance training on generation of lipoperoxide and concentrations of SOD and CAT, a 12-week program of 6km runs at intensity of 70~80%HRmax and 40 minutes / session, 3 sessions / week caused an increase of lipoperoxide due to increased MDA and CAT concentrations, but an increase of antioxidative enzymes showed effect protection of cell membrane components. There are many studies in the field of sports nutrition related to food and nutrients that exhibit specific antioxidative effects. In a study about the effect of a 12-week endurance training program and medication of rooibos-tea on oxidative DNA damage, lipid peroxidation and antioxidative enzymes, a 3-month cyclic training program that consists of 3 weeks of preparation, 7 weeks of training and a two week regulation period was applied according to the schedule of the Korea University Football Confederation. As a result of implementing rooibos-tea intake of 500ml before and after meals and exercise, medication of rooibos-tea was found to increase the activity of antioxidative enzymes. Intake of rooibos-tea was reported (Kim JT, 2009) to increase the activity of antioxidative enzymes regardless of training. Studies on the relationship between genes in the blood and relevant exercise abilities are using diverse methods with further vitalization of biochemical and molecular biological techniques.

Blood hormones for analysis of endurance training effects

Hormones are classified according to chemical composition into amino acid derivative, peptide protein and steroid. Chemical structures affects the way in which hormones are transported to blood and reach tissues.

During an exercise, secretion of adrenaline and noradrenaline is increased in the adrenal gland as a response to an increase of metabolic demand. These hormones decompose sugar in the liver and facilitate lipolysis from fatty tissues to help mobilization of glucose and fatty acid to muscles as energy sources. In addition, increased levels of adrenaline and noradrenaline in circulating blood during exercise induce a wide variety of physiological responses such as an increase of heart rate, expansion of bronchial tubes, and

expansion of blood vessels in muscles (Kjaer, 1998). In general, thyroxin, cortisol and growth hormone become slowly active to help the activity of other hormones during exercise, and growth hormone and cortisol are known to have a slow effect on the metabolism of carbohydrates and lipids. Plasma glucose is maintained during exercise through increased mobilization of hepatic glucose, increased use of plasma free fatty acid, increase of gluconeogenesis and reduced intake of glucose by tissues. A reduced level of plasma insulin and increase of plasma epinephrine, norepinephrine, growth hormone, glucagon and cortisol maintain the concentration of glucose during exercise and assist the mobilization of free fatty acid from fatty tissues. Training reduces the responses of epinephrine, norepinephrine, insulin and glucagon to exercise.

Among studies on endurance training, Baek SS (2010) analyzed the change in insulin sensitivity to examine the effect of low intensity endurance training programs on lipid metabolism and insulin sensitivity of obese females. According to the study, 4-week low intensity training increased insulin sensitivity more than the interval training of this study. Such results have drawn the conclusion that continuous low intensity training improves lipid metabolism and is related to increased insulin sensitivity of obese females. Also, according to a study of Bruce et al. (2006), an 8-week ergometer cycle training program was carried out at 60~70%VO2peak for 5 days a week and 60 minutes per session to analyze insulin sensitivity in blood. As a result, insulin sensitivity was substantially increased after training.

Leptin is a 16kDa protein hormone that plays an important role in energy intake and consumption controls including appetite, hunger, metabolism and action. It is one of the most important hormones generated in fat, and leptin is activated by combining with the leptin receptor. A recent study drawing attention in relation to leptin shows that leptin controls the growth of bones in two ways. That is, leptin is known to facilitate the emission of an inhibiting factor on hypothalamus osteoblast, which performs the role of limiting the amount of bone matrix formed by osteoblast. Leptin is a bone reinforcing factor that directly facilitates the growth of bones by inducing osteoblast to generate IGF-1. While it is known to aggravate inflammation in inflammatory bowel diseases and animal models with multiple sclerosis, leptin increases survivability in septicaemia models. On the other hand, leptin deficiency prevents inflammation in inflammatory bowel diseases and animal models with arthritis and increases the fatality of septicaemia. Accordingly, studies on leptin are developing at a fast rate, and strategies to grant various treatment effects of this target are becoming increasingly clear. It is not only used in diabetes and obesity but can also show treatment effects on septicaemia and osteoporosis, and it can be used to alleviate various inflammatory diseases. Nonetheless, studies on sports are still mostly related to fat metabolism and diabetes.

Blood enzymes for analysis of endurance training effects

Enzymes are protein catalysts that mediate chemical reactions in organisms. They combine with substrates to form the enzyme-substrate complex and reduce the activation energy of reactions. Enzymes have specificity for substrates, and they are generally operated at body temperature and neutral pH. Enzymes have different forms of active sites to only interact with specific substrates that can properly combine with their active sites. Therefore, enzymes and substrates are only combined when the structure of the active site of an enzyme can interlock with the structure of the substrate.

There are active studies on enzymes with a particular focus on AMPK, which plays the role of a sensor in maintaining energy homeostasis in cells. AMPK is a type of serine / threonine kinase widely distributed in the body known (Hardie et al., 2001) to function as an energy detector that arbitrates nutritional conditions of cells and adaptation of cells to changes in external

environment. AMPK can be activated (Corton et al., 1995) by reduced levels of ATP in cells or AICAR (5-aminoimidazole-4-carboxamide ribonucleotide), an analogue of adenosine. AMPK is an enzyme that functions as a sensor that maintains energy homeostasis in cells. When energy in cells is reduced by metabolic stress or exercise, or when the ratio of AMP / ATP is increased by depletion of ATP, AMPK is activated to inhibit consumption of ATP and facilitate generation of ATP (Hardie, 2007). The effects on activation of AMPK are involved with target organs (liver, muscle, fat and pancreas) closely related to the control of energy metabolism (Zhang et al., 2009). Once AMPK is activated in the liver, it inhibits the synthesis of fatty acid and cholesterol and facilitates oxidation of fatty acid. Activation of AMPK in skeletal muscles is known to facilitate oxidation of fatty acid and absorption of sugar and to inhibit decomposition and generation of fat in fat cells. Mortensen (2013) reported a study that analyzed AMPK signaling after a 12-week training program on people with ordinary body weight and people with low birth weight, which causes increased risk of type 2 diabetes. The training involved 12-week ergometer cycling at 65%VO2peak intensity, 1 hour per session and 4 sessions a week. Concentration of insulin in the blood was lower after training, and C-peptide in blood was not changed by training.

Characteristics of muscle analysis

Inflammatory factors for analysis of endurance training effects

With the development of muscle biopsy techniques in 1966, analytical methods using the biochemical dyeing technique and the fluorescence microscope were applied to easily obtain muscular tissues of the human body through muscle biopsy. Histo-biochemical and molecular biological techniques for muscular fiber composition, ratio, cross sectional area, enzyme activity, metabolic by-products such as fatigue substances like lactate, electrolytes, and energy sources have been used to measure proteins in muscles and determine the oxygen utilization ability of muscular fibers. With the application of single muscle fiber separation, electrophoresis and electron microscope, diverse studies were presented to analyze exercise ability and the effects and problems of training. However, there was a serious lack of domestic studies using muscle biopsies in the past ten years. Studies on the cellular level using muscle biopsies are being actively conducted in foreign countries. Recent studies related to endurance training include cells related to inflammation, glycolysis and regeneration, fat oxidation and biosynthesis of mitochondria.

Known isoforms of the COX (cyclooxygenases) enzyme include COX-I, COX-II and COX-III. While COX-1 has a constitutive form mostly expressed in normal tissues to perform the function of maintaining homeostasis of cells and tissues, COX-II is known as an inducible form induced by irritations such as inflammatory precursor cytokine, lipopolysaccharide and growth factors in specific cells. COX-III is generated by frame shift mutation as a splice variant of COX-, and it is also referred to as COX-Ib or COX-I variant (COX-Iv) (Chandrasekharan et al., 2002). In human beings, about 60-65% of amino acids in COX-I and COX-II coincide, and they have similar activity of enzymes. However, the COX-II gene is an inducible enzyme synthesized by inflammatory substances like growth factors, cytokines and endotoxins in macrophage and other inflammatory parts and is distinguished from the COX-I enzyme, which is a constitutive enzyme that is always immanent in all tissues such as the gastrointestinal tract, kidney, platelets, and vascular endothelial cells. COX-II participates in synthesis of metalloproteinase and formation of capillaries to affect infiltration and growth of cancer cells, and it generates PGE2 to inhibit immune response of the human body against tumor cells by stimulating synthesis of IL-10 in immunocytes such as lymphocyte and macrophage. Among various studies on inflammation, a study

reported by Yoe et al. (2008) analyzed the difference between a daily bicycle training program and two sessions of training performed on alternate days in trained professional endurance athletes. Intensive training performed twice in a day showed higher potential expression of citrate synthase, β-HAD, COX(cyclooxygenase)-II, COX-IV, AMPK and PGC-I α compared to daily training, and such result was reported to show increase in endurance exercise ability.

Gibala et al. (2006) studied changes in the adaptability of molecules and cells in skeletal muscles after short-term sprinter interval training and typical endurance training. As a study carried out to find the difference caused by different exercises implemented for two weeks, short-term sprinter interval training was performed as a cycling exercise of 30 seconds per session with four minute breaks repeated four times in a session, three sessions a week. Endurance training was carried out at 65%VO2max for 90~120 minutes, three sessions a week. Muscle tissue was extracted to analyze COX-II, COX-IV and β2M as analytical variables for the two groups. As a result, the short-term sprinter interval group showed temporal efficiency of quick muscle growth and exercise performance.

Muscle proteins for analysis of endurance training effects

Yoshioka et al. (2003) presented a study that compared skeletal muscles between professional endurance athletes and ordinary people using SAGE. Muscles were extracted from professional endurance athlete group, which consists of three 5,000 meter runners, two marathoners, one triathloner, and cyclist and eleven ordinary males to perform Serial Analysis of Gene Expression (SAGE). Thirty-three genes were differently expressed between professional endurance athletes and ordinary people. While myosin binding protein C fast-type, glycogen phosphorylase and pyruvate kinase were expressed less in professional athletes, EST crystallin alpha B, EST myosin light chain 2, EST surfactant pulmonary-associated protein A1, EST thrombospondin, EST fructose-bisphosphate aldolase A, EST cytochrome oxidase 1, NADH dehydrogenase 3, and G8 protein showed higher expression. This result was reported using the RT-PCR analysis method.

Glucose transporters are proteins that pass glucose through cell membranes to inject it into cells. Glucose transporters are largely classified into two types. One is the facilitated diffusion transporter that uses the difference in glucose concentration inside and outside cells as power for glucose transport, and it is known to exist in most cells of mammals. Another is an active transporter that controls transport of sodium / glucose that retrogresses the slope of glucose concentration by sharing with Na+ transport. The primary structure of protein is determined by separation of cDNA in each glucose transporter. According to this, there are six known types of facilitated diffusion transporter, which are respectively named as GLUT1~7. GLUT6 is a pseudogene, and GLUT1, GLUT3 and GLUT5 are widely distributed in most tissues. GLUT2 is mainly expressed in the liver and β cell of pancreas, GLUT4 in muscular and fatty tissues, and GLUT7 on top of microsome. GLUT4 and 8 have been widely reported as glucose transporters related to endurance training. Among such studies, a study presented by Seki et al. (2006) analyzed the effect of endurance training on GLUT expression in skeletal muscles. Muscle tissue was extracted from sixteen athletes and fifteen ordinary people who received endurance training after exercise stress testing to classify RNA. GLUT4, 8 and 12 were used as analytical variables to deduce respective mRNA expression using RT-PCR method. As a result of analysis, expression of GLUT12 mRNA was low in professional endurance athletes. There was no difference in GLUT8 between groups, and expression of GLUT4 was higher in professional endurance athletes.

Peroxisome proliferator-activated receptors (PPARs) recognized as one of tne candidate genes for obesity were reported. They are a type of transcription factor that performs various physiological roles by combining

with different natural or synthetic ligands in cell nucleus (Lehmann et al., 1984). In addition, there are 3 known types of PPARs including PPAR-α, PPAR-δ (or β) and PPAR-γ (Auwerx, 1999).

Bruce et al. (2006) reported a study where endurance training increases glucose tolerance and oxidation of mitochondrial fatty acid and changes fat content in muscles of obese subjects. An 8-week ergometer cycle training program was implemented at 65~70%VO2max for 60 minutes per session and five sessions a week on nine male and female subjects with BMI over 30kg/m2. Muscle tissue was extracted to verify training effects by analyzing AMPK, ACC and PPARα through western blot. As a result, there was a trend of decrease in AMPK and ACC and increase in PPARα after training.

Mortensen (2013) reported a study that analyzed AMPK signaling after a 12-week training on people with ordinary body weight and people with low birth weight, which causes increased risk of type 2 diabetes. Variables such as AMPK α1, AMPK α2, AMPK β2, AMPK γ1, AMPK γ3, ACC2, GS, GS site 1B and GS site 2+2a were analyzed on the protein level using western blot method, and a 12-week training was implemented at 65%VO2max for 60 minutes per session and four sessions a week. Each variable was measured before, just after, and again four hours after exercise.

Conclusions

The aim of this study was to analyze domestic and foreign molecular biological studies on endurance training published between 2000 and 2013 based on trends, variables, analytical techniques and analytical factors of molecular biology, providing data for future-oriented directivity of studies on endurance training. Analytical factors of domestic studies were limited to blood, but there are molecular biological attempts to analyze variables related to immune function and oxidative stress as well as various physiological indicators for analysis of mechanisms. Foreign molecular biological studies published on endurance training were mostly examined the mechanisms of immune function, oxidative stress, inflammatory response and metabolic syndrome through changes in genes, mRNA and cells observed in blood and muscular tissues. Analytical techniques used in these studies include western blot, RT-PCR, ELISA and immunohistochemistry of molecular biology and cell biology. These molecular biological techniques have a high reliability of samples and are appropriate for examining the effects of training. However, they have disadvantages such as difficulty in pre-treatment of samples, need for presence of medical staff during sample collection, complexity of analytical procedure, and high difficulty of analysis. Nevertheless, such study methods will be widely used to provide scientific and future-oriented methods of controlling conditions, analyzing training effects, and enhancing health of professional athletes and life sports athletes because of high reliability of results.

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