Dissertation for the Degree of Doctor of Philosophy

The Effects of Endurance Exercise on Expression of Myokines and Tumor Tissue in Colon Cancer-induced Mice

장기간의 운동이 대장암 유발쥐의 마이오카인의 발현과 종양조직에 미치는 영향

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December 2013

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ABSTRACT

The Effects of Endurance Exercise on Expression of Myokines and Tumor Tissue in Colon Cancer-induced Mice

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A few studies have demonstrated that exercise induces the release of cytokines from contracting muscles. These muscle-derived cytokines are collectively named myokines. Skeletal muscle is the largest organ in the human body, as an endocrine organ, muscle tissue has had a central role in orchestrating metabolism of other organs. The researchers have found a link between muscle contraction and these myokines in the form of an ‘exercise factor’.

Recently accumulating evidence indicated that myokines may also play a role in cancer protection. It was shown that the contracting muscle by an acute exercise release humoral factors such as OSM, IL-10, SPARC that can inhibit cancer cell proliferation and induce cancer cell apoptosis.

Base on the prevailing literature, the purpose of the present study was to investigate the impact of long-term exercise on expression of cytokines in muscle, blood and tumor tissue, thus to examine whether exercise mediates independent protective effect against cancer through the release of anti-proliferative proteins from contracting muscles according to exercise intensity.

First, to identify the therapeutic effect of exercise, all mice at 6-wk old were injected AOM and were treated 3 cycles of DSS solution in drinking water to induce colon cancer. Then at 17-wk of old,

mice of exercise groups performed treadmill exercise at different intensity (Low, Moderate, High) for 30 minutes, 5 days per week, during 12 weeks. Twenty-four hours after the last training session, all tissues were removed and blood samples were obtained. To analysis the effect of exercise, OSM and SPARC were evaluated in muscle, serum, and tumor tissue. Also related pro-apoptosis factor, caspase-3 in tumor tissue were analyzed. OSM was significantly overexpressed in exercise groups compare to controls; in gastrocnemius (p=.000), serum (p=.005), and tumor tissue (p=.000). Also, there was significant differences among exercise groups by exercise intensity on expression of OSM in colon-induced mice. The expression of OSM tends to increase in higher intensity in gastrocnemius (p=.000); LIE (p= .014), MIE (p=.000), HIE (p=.000), in tumor tissue (p=.000); LIE (p= .000), MIE (p=.000), HIE (p=.000), but only in serum of LIE (p=.000). The level of SPARC was significantly increased by exercise in muscle (p=.000), serum (p=.002), tumor tissue (p=.001) compare to controls. Also the expression of SPARC tends to increase in higher intensity in gastrocnemius (p=.001); LIE (p= .035), MIE (p=.001), HIE (p=.007), in tumor tissue (p=.000); MIE (p=.000), HIE (p=.000). The expression of caspase-3 increased significantly in MIE (p=.045).

Secondly, to identify the preventive effect of exercise on colon cancer-induced mice, mice of exercise groups, at 6-wk of old, performed exercise at different intensity (Low, Moderate, High) for 30 minutes, 5 days per week, during 12 weeks. At 17-wk of old, all mice were injected AOM and were given 3 cycles of DSS solution in drinking water to induce colon cancer. Then they kept doing exercise for another 12 weeks. The level of OSM in muscle was significantly overexpressed in exercise groups compare to controls, however, not in blood and tumor tissue (p>.05). Also, there was significant differences among exercise groups by exercise intensity on expression of OSM in muscle. The expression of LIE (p=.001) and MIE (p=.000) were significantly increased than controls. However, the expression of OSM in blood was not increased by exercise intensity (p>.05). In the same time, the level of OSM in tumor tissue tends to increase by exercise intensity, but there was no statistical difference (p>.05). The level of SPARC was significantly increased by exercise in blood (p=.002), tumor tissue (p=.001) compare with controls, however, there was no significant difference in muscle (p>.05). However,

the expression of SPARC in MIE was significantly higher than CON in gastrocnemius (p=.010). Also the expression of SPARC in tumor tissue tends to increase significantly by exercise intensity; MIE (p=.001), HIE (p=.000). Even though the expression of cleaved caspase-3 in tumor tissue tends to increase by exercise intensity, there was no statistical difference (p>.05).

In conclusion, this study showed that exercise induced the expression of OSM and SPARC in several tissues by exercise stimulus, which is associated with inducing apoptosis in tumor cells. And the magnitude of OSM and SPARC induction in muscle and tumor tissue was relatively higher in moderate and high intensity exercise groups than low intensity exercise group. Moreover, moderate and high intensity exercise enhanced anti-apoptotic pathway in skeletal muscle, and increased gastrocnemius muscle weight. In addition, it is observed that the number of polyp generation in moderate and high intensity exercise group was significantly decreased than controls. Thus, the results of this study possibly suggest that moderate and high intensity exercise have more protective effect for colon cancer induced mice.

Keywords **: Myokine, Exercise, Cancer, SPARC, OSM, Apoptosis.**

Student Number **: 2009-31035**

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LIST OF ABBREVIATIONS

OSM	**Oncostatin M**
SPARC	**Secreted protein acidic and rich in cysteine**
CRC	**Colorectal cancer**
AOM	**Azoxymethane**
DSS	**Dextran sodium sulfate**
IBD	**Inflammatory bowel**
Bcl-2	**B cell lymphoma 2**
ELISA	**Enzyme-linked immnosorbent assay kit**
LIE	**Low intensity exercise**
MIE	**Moderate intensity exercise**
HIE	**High intensity exercise**
CCON	**Normal mice, wild-type ICR**

I. INTRODUCTION

Cancer is the leading cause of death, and colorectal cancer is the third most common causes of cancer-related death in Korea (Statistics Korea, 2013). The incidence of colon cancer has increased during the last 10 years in Korea, possibly due to a shift toward westernized lifestyles, which has been implicated as a factor in increased risk of colorectal cancer (Potter, 1999).

There is overwhelming evidence that behavioral changes such as reducing tobacco use, increasing physical activity, controlling weight and, limiting alcohol impact cancer risk. Colorectal cancer is thought to be one of the most preventable cancers through lifestyle changes and screenings (Giovannucci, 2002; Quadrilatero & Hoffman-Goetz, 2003). Studies seeking to identify causal factors for colorectal cancer have focused on specific components of western lifestyles such as diet, physical activity and obesity (Slattery, Edwards, Ma, Friedman, & Potter, 1997; Slattery, Potter, et al., 1997).

Accumulating evidence indicate that regular physical activity can significantly reduce the incidence of cancer, and the extent of prevention or retardation of tumor growth can be as much as 50% for a variety of cancers (Radak et al., 2002; Thompson, Westerlind, Snedden, Briggs, & Singh, 1995). The observational data are clearest for colon and breast cancer, with case-control and cohort studies supporting an inverse relation between physical activity and the development of these cancers. The data suggest that active people have approximate reductions in colon and breast cancer risks by 30%~40% ("Physical Activity Guidelines Advisory Committee report, 2008. To the Secretary of Health and Human Services. Part A: executive summary," 2009)

Even though the protective effects of physical activity are undisputed, there have been conflicting reports of the therapeutic effect of physical activity on colon cancer. In other words, it is known that physical activity may play an important role in preventing, attenuating, or rehabilitating late and long-term effects of cancer treatment but we do not know much about the exact mechanisms through which exercise mediates the favorable effect. The list of proposed mechanisms is includes changes in endogenous hormones, immune function, DNA repair, low-

grade inflammation, and energy balance (Grivennikov, Greten, & Karin, 2010; McTiernan, 2008; Neilson, Friedenreich, Brockton, & Millikan, 2009).

The establishment of cancer is dependent on several factors, including immune reactivity toward neoplastic cells and growth factors. Tumor initiation, promotion, and progression are stimulated by systemic elevation of pro-inflammatory cytokines (Handschin & Spiegelman, 2008). Macrophage and neutrophil secretion of Interleukin-6 (IL-6) and TNF-α contribute to elevated cyclooxygnease-2(COX-2) expression, activity and subsequent colon polyp growth (Franchimont et al., 1999). IL-6 is normally not present or is expressed at low levels, unless trauma infection or other stress occurs (Ershler & Keller, 2000). Serum interleukin (IL-6) levels are correlated with larger tumor size and have been found to be higher in colon cancer patients (Chung & Chang, 2003). Therefore, anti-inflammatory drugs that inhibit COX-2 activity as well as, IL-6 and TNF-α production are used for prevention of tumor development and colon cancer progression in human (Rigas & Williams, 2002). It is well known that regular exercise protects against a number of chronic diseases associated with chronic inflammation. This might be due to an anti-inflammatory effect of regular exercise. Moderate physical activity induces stress hormone release and can decrease monocyte and macrophage IL-6, TNF-α and IL-1β production(Franchimont et al., 1999). Epidemiological evidence suggests that physical activity is inversely correlated with serum IL-6 (Pischon, Hankinson, Hotamisligil, Rifai, & Rimm, 2003). Plasma IL-6 and polyp size were decreased with the exercise group in a colon cancer model (Mehl et al., 2005). Physical activity was found to alter both the systemic and intestinal mucosa inflammatory state (Mehl et al., 2005). Improved immune system function is a potential exercise-induced mechanism for colon cancer prevention (Quadrilatero & Hoffman-Goetz, 2003).

Recently a few studies have demonstrated that exercise induces the release of cytokines from contracting muscles (Steensberg et al., 2000). These muscle-derived cytokines are collectively named myokines (Ostrowski, Rohde, Zacho, Asp, & Pedersen, 1998; Rohde, MacLean, Richter, Kiens, & Pedersen, 1997). Examples of myokines are: as IL-6, IL-8, IL-15, BDNF, LIF, FGF21, Follistatin-like-1 (Broholm et al., 2008; Hojman et al., 2009; Izumiya et al., 2008; Ouchi et al., 2008), OSM (Oncostatin M), GDF2, CSF2, GDF5, IL-11, IL-10 (Hojman et

al., 2011). Skeletal muscle is the largest organ in the human body, and as an endocrine organ, muscle tissue plays a central role in orchestrating metabolism of other organs (B. K. Pedersen & Febbraio, 2008).

Myokines may also play a role in cancer protection. Some studies have demonstrated that conditioned media from electrostimulated C_2C_{12} muscle cells inhibit cancer cell proliferation, and serum obtained from subjects who did exercise inhibit cancer cell growth in vitro (Barnard, Gonzalez, Liva, & Ngo, 2006; Soliman, Aronson, & Barnard, 2009). It was found that exercise-induced humoral factors such as OSM and IL-10 which inhibit breast cancer cell proliferation. Furthermore, secreted protein acidic and rich in cysteine (SPARC), a novel myokine, induces apoptosis in colon tumor cell by increasing caspase activity (Tai & Tang, 2008), which is overexpressed by exercise stimulus (Aoi et al., 2013). Therefore, it has been suggested that exercise-induced elevation of OSM and SPARC could inhibit colon tumorigenesis.

However, there are few studies relating to myokines and exercise, or to exercise-induced myokines and cancers. It is known that the expression of cytokine varies by exercise intensity, but there is no study comparing the expression pattern of myokines to exercise intensity.

The purpose of the present study is to investigate the impact of exercise on cytokine expression in muscle, blood and tumor cells, and in the process examine whether exercise mediates an independent protective effect against cancer through the release of anti-proliferative proteins from contracting muscles according to exercise intensity.

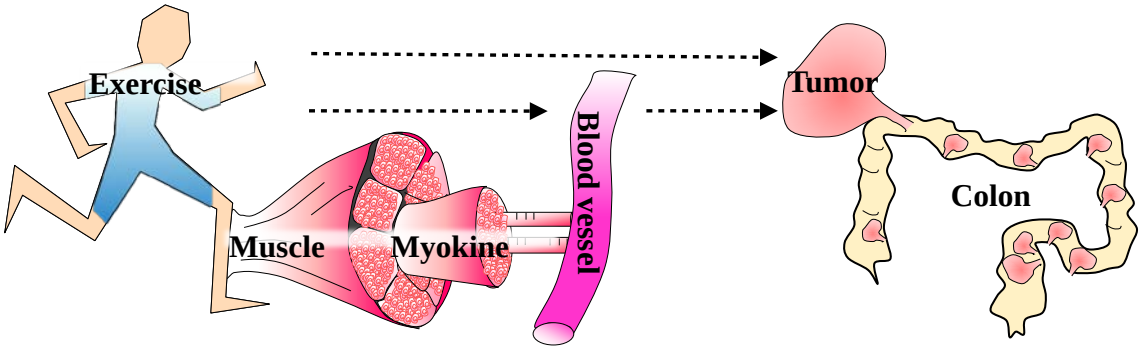

Figure 1. Hypothesis of the present study
(Pedersen B K, 2011 and modified by June Hong Kim)

II. LITERATURE REVIES

2.1. Cancer and physical activity

2.1.1. The incidence of cancer in Korea

The burden of cancer is increasing worldwide. Cancer is the one of the major causes of death, and its incidence and mortality rates are expected to continue to rise in the future. In particular, colon cancer is the second most common cause of all cancer deaths and its incidence has been increasing more rapidly than any other type of cancer in recent years (WHO, 2011). In Korea, due to an increasing westernized lifestyle and aging society, not only the total number of cancer is expected to increase, but also the rate of colon cancer is increased (Ministry of Health and Welfare, 2012). Thus, it is vital to consider colon cancer prevention as an important part of any care program.

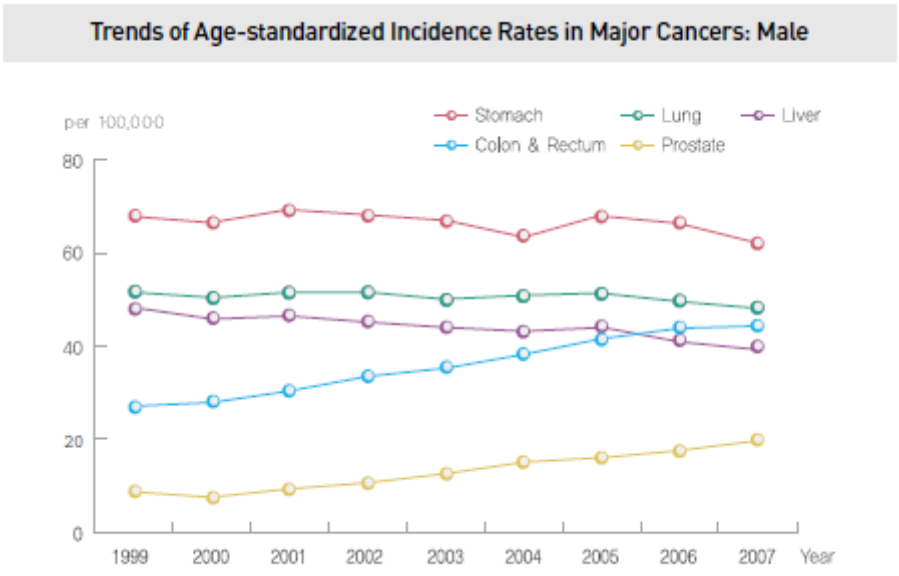

Figure 2. Trends rate of major cancer in Korea

2.1.2. Physical activity and colon cancer

Based on a large body of evidence, the WHO (World Health Organization) reported that one-third of cancers could be prevented by changing lifestyle and vaccination. Another one-third could be cured by early detection. The remaining one-third has yet to be overcome through research and other means, such as clinical advances.

Behavior change include reducing tobacco use, increasing physical activity, controlling weight, improving diet, utilizing safer sex practices, getting routine cancer screening tests, and avoiding excess sun exposure, limiting alcohol is possible and offers great potential for cancer prevention (Stein & Colditz, 2004).

The impact on colon cancer risk is especially prominent; high levels of physical activity may reduce the risk of colon cancer by as much as 50%, on the contrary, sedentary lifestyle has been linked to 5% of heaths from cancer (Colditz, Cannuscio, & Frazier, 1997). Thus a lot of studies seeking to identify causal factors for colorectal cancer have focused on specific components such as physical activity, diet and obesity (Slattery, Edwards, et al., 1997; Slattery, Potter, et al., 1997). Despite difficulties in accurately measuring physical activity, physical activity has been identified consistently as a lifestyle factor that reduces risk of colon cancer (Wiseman, 2008).

The associations between exercise and colon cancer have been documented across levels of obesity, suggesting that physical activity acts on cancer risk independent of its effects on body weight. Excess weight alter levels of hormones and growth factors and causes severe health problems such as colorectal, postmenopausal breast, endometrial, renal cancers (Stein & Colditz, 2004).

Several mechanisms have been proposed to explain the dose-response relationship observed between cancer risk and physical activity. Physical activity may alter prostaglandin level and improve immune function (Martinez et al., 1999) and also reduce circulating levels of insulin, hormones, and other growth factors (Giovannucci et al., 1995; McKeown-Eyssen, 1994). It has been hypothesized that physical activity can also minimize contact time between the colonic

mucosa and potential carcinogens in the stool by decreasing gastrointestinal transit time (McTiernan, Ulrich, Slate, & Potter, 1998)

Several epidemiological studies performed in Europe, the United States and Japan have shown that regular exercise reduces the incidence of colon cancer (Friedenreich et al., 2006; Lee et al., 2007; Slattery & Potter, 2002). The result of the studies has been replicated in case-control and cohort studies showing approximately a 30-40% reduction in risk among the most active relative to those who are sedentary.

The antitumor effect of exercise has also been identified in experimental studies (Buehlmeyer et al., 2008; Ju et al., 2008; Reddy, Sugie, & Lowenfels, 1988), which potential mechanisms such as activation of the immune system, metabolic improvement, and exercise-induced increase in gastrointestinal transit speed have been suggested. However, the exact mechanism involved is not entirely understood, but the protective effects are believed to depend on the intensity, type, frequency, and duration of the activities.

2.2. Chronic inflammation-derived colon tumor mice

Colorectal cancer (CRC)—a frequent malignant tumor and a major cause of death in the Western hemisphere—develops spontaneously or as a long-term complication of chronic bowel inflammation such as in Crohn’s disease and ulcerative colitis. The risk for CRC is influenced by a genetic predisposition, which is especially high for somatic mutations of the tumor suppressor gene adenomatosis polyposis coli (APC) causing familial adenomatosis coli.

Experimental rodent models can mimic many features of CRC, and they are important for studying the mechanisms underlying the initiation and progression of CRC. Additionally, animal models are essential tools for preclinical testing of new therapeutic options. Whereas naturally mutant or genetically modified animals, such as APC in mice, are primarily used for studies addressing hereditary aspects of CRC, inducible tumor models, based on chemical carcinogens, have been developed to mimic non-hereditary tumor development.

AOM and its derivatives are the chemical agents that have been most successfully used in

rodent models of CRC. In contrast to other models of CRC, tumors induced by AOM are very frequent in the distal part of the colon, which resembles the predominant localization of spontaneous CRC in man. They often start with polypoid growth, and frequently exhibit histopathological features similar to CRC in man.

The protocol presented here for highly efficient induction of colon tumors in mice is based on the intraperitoneal administration of AOM. The first option of the protocol comprises repeated injections of AOM, and it is especially useful for the study of factors that drive spontaneous tumor progression. The alternative option, which includes the pro-inflammatory reagent dextran sodium sulfate (DSS), is a model for colitis associated tumor development. It is particularly applicable when the focus of the study is tumor progression driven by chronic colitis, as seen in ulcerative colitis or Crohn's disease.

DSS dissolved in drinking water is very toxic to the epithelial lining of the murine colon, resulting in severe colitis characterized by bloody diarrhea (Okayasu et al., 1990). A two-stage colon tumor model that mimics colitis-driven tumor development has been described (Tanaka et al., 2003). It is based on a single injection of AOM and a single cycle of DSS, and it has demonstrated the development of numerous colon tumors in different mouse strains within a period of 20 weeks (Suzuki, Kohno, Sugie, Nakagama, & Tanaka, 2006; Tanaka et al., 2003). Chemoprevention studies have been successfully performed with that model (Kohno et al., 2006). Whereas colitis-driven tumor progression in the two-stage model is promoted by a short period of acute inflammation, the four-stage protocol we provide here has been developed to model chronic inflammation (including flares of increased activity), which is believed to be the driving force in tumor progression of colitis-associated CRC, as seen in ulcerative colitis or Crohn's disease (Becker et al., 2004; Greten et al., 2004; Okayasu, Ohkusa, Kajiura, Kanno, & Sakamoto, 1996). Strikingly, the four-stage model is based on three cycles of DSS administration, which cause chronic colitis. In combination with the preceding single AOM injection, tumor growth is even accelerated in comparison to the two-stage model, resulting in multiple large tumors already after 10 weeks. The AOM/DSS has become an outstanding model for studying colon carcinogenesis and a powerful platform for chemopreventive intervention studies (Becker et al., 2004; De Robertis et al., 2011; Greten et al., 2004).

In this study, we use One AOM, three cycle of DSS method, which causes rapid growth of multiple colon tumors per mouse within 10 weeks. This method is thought to model closely tumor progression driven by chronic colitis, as seen in IBD (Inflammatory bowel disease).

Table 1. Models of AOM-induced tumors

Brief description of the model	Thought to model Closely	closely Advantages	Disadvantages
Six AOM injections	Spontaneous tumor progression	Cheap and efficient mouse model for spontaneous tumor growth of CRC	Multiple AOM injections, long-term model
Two AOM injections (rat model)	Spontaneous tumor progression	Two AOM injections only	Large breeding space needed, long-term
One AOM, three cycles of DSS	Tumor progression driven by chronic colitis, as seen in IBD	Mimics IBD course of humans, most rapid tumor development, one AOM injection for multiple CRC	Several cycles of DSS
One AOM, one cycle of DSS	Colitis-associated tumor progression	Rapid tumor development, one AOM injection for multiple CRC	No chronic colitis, no flares of colitis activity

AOM, azoxymethane; CRC, colorectal cancer; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease (Neufert, Becker, & Neurath, 2007).

2.3. Myokines

The discovery of cytokines (glycoproteins with molecular weights of 15,000 –30,000) (Dinarello & Mier, 1986) and their immunoregulatory roles was demonstrated that these were involved in a complex network of communication between the neuroendocrine and the immune system. In fact, it appeared that cytokines may also modulate the secretion from the hypopituitary-hypothalamus axis and that an important neuroendocrine-immune loop exists (Crofford, 2002; Steensberg, Toft, Schjerling, Halkjaer-Kristensen, & Pedersen, 2001).

For most of the last century, researchers sought a link between muscle contraction and humoral changes in the form of an ‘exercise factor’. Through last 20 years researchers have demonstrated that exercise induces considerable changes in the immune system. The interactions between exercise and the immune system provide a unique opportunity to evaluate

the role of underlying endocrine and cytokine mechanisms (B. K. Pedersen & Hoffman-Goetz, 2000).

During past 10 years, the identification of skeletal muscle as a cytokine-producing organ has led to the discovery that muscle-derived cytokines may account not only for exercise-associated immune changes, but that these muscle-derived cytokines play a role in mediating some of the exercise-associated metabolic changes following training adaptation (Febbraio & Pedersen, 2002). In the year 2000, it became clear that contracting human skeletal muscle releases significant amounts of interleukin (IL)-6 into the circulation during prolonged single-limb exercise. It was the first myokine to be discovered, and it led to the discovery that exercise provokes an increase in a number of cytokines (Febbraio & Pedersen, 2002), belonging to distinctly different families, such as IL-6, IL-8, IL-15, Myostatin, LIF, BNDF, IL-7 (B. K. Pedersen & Febbraio, 2012).

Recently some researchers (B. K. Pedersen, Akerstrom, Nielsen, & Fischer, 2007) have suggested that cytokines and other peptides are produced, expressed and released by muscle fibers and exert either paracrine or endocrine effects should be classified as ‘myokines’.

2.3.1 Interleukin-6

IL-6 belongs to the IL-6 family of cytokines, including IL-11, oncostatin M, leukemia inhibitory factor, cardiotrophin-1, ciliary neurotrophic factor. A marked increase in circulating levels of IL-6 after prolonged exercise is a remarkable consistent finding. The level of circulating IL-6 increases up to 100-fold in response to exercise, and declines in the post-exercise period.

IL-6 is released from working muscle into the circulation, where it exerts its effects on other organs in a hormone-like fashion. IL-6 is most often classified as a proinflammatory cytokine, although it also has anti-inflammatory properties cytokines such as IL-1ra, IL-10.

IL-6 production is modulated by carbohydrate availability in muscles, and IL-6 functions as an energy sensor. It is released from contracting muscles in large amounts and exerts its effect on adipose tissue, and to inhibit low grade TNF-α production, in part may protect against TNF-

induced insulin resistance. Also, IL-6 is an essential regulator of satellite cell-mediated hypertrophic muscle growth (Serrano, Baeza-Raja, Perdiguero, Jardi, & Munoz-Canoves, 2008).

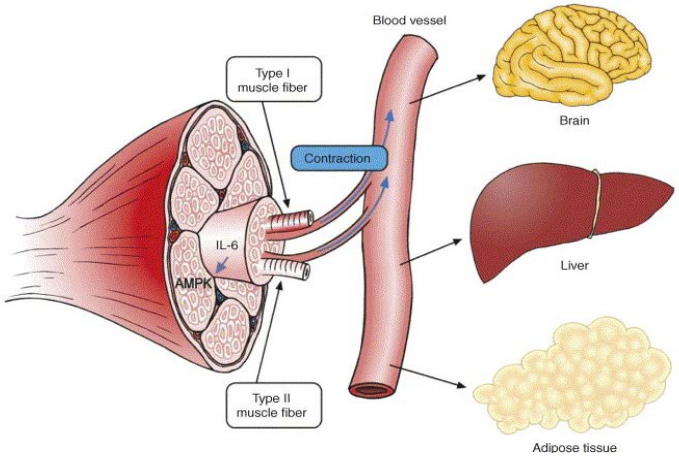

Figure 3. Skeletal muscle is an endocrine organ
Skeletal muscle expresses and releases myokines into the circulation. In response to muscle contractions, both type I and type II muscle fibers express the myokine IL-6, which subsequently exerts its effects both locally within the muscle (e.g. through activation of AMPK) and – when released into the circulation – peripherally in several organs in a hormone-like fashion (B. K. Pedersen & Fischer, 2007)

2.3.2 Interluekin-8

IL-8 belongs to the CXC family of chemokines that attracts primarily neutrophils. The fact that high local IL-8 expression occurs in contracting muscle with only a small and transient net release may indicate that muscle induced IL-8 acts locally and exerts its effect in an autocrine or paracrine fashion (Akerstrom et al., 2005).

However, IL-8 acts as an angiogenic factor. It produces its chemotactic effects through the chemokine receptor CXCR 1, is the receptor responsible for IL-8-induced angiogenesis (Bek, McMillen, Scott, Angus, & Shaftan, 2002). IL-8 signaling promotes angiogenic responses in endothelial cells, increases proliferation and survival of endothelial and cancer cells, and potentiates the migration of cancer cells, endothelial cells and infiltrating neutrophils at the tumor site. Accordingly, IL-8 expression correlates with the angiogenesis, tumorigenicity and

orthotopic in vivo (Waugh & Wilson, 2008).

Therefore, inhibiting the effects of IL-8 signaling may be a significant therapeutic intervention in targeting the tumor microenvironment.

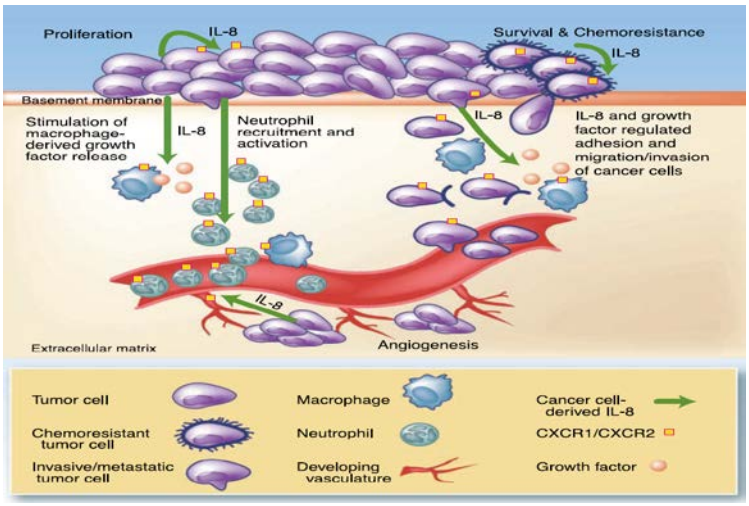

Figure 4. The role of IL-8 signaling in the tumor microenvironment.
Tumor-derived IL-8 has the capacity to exert profound effects on the tumor microenvironment (Waugh & Wilson, 2008).

2.3.3 Interluekin-15

IL-15 is a four α-helix cytokine with structural similarities to IL-2 (Bamford et al., 1994). IL-15 is highly expressed in skeletal muscle (Grabstein et al., 1994) by regular exercise (Nielsen et al., 2007). IL-15 has an anabolic effect on muscle and may support muscle cell differentiation(Quinn, Haugk, & Grabstein, 1995). Moreover IL-15 has effects on fully differentiated myoblasts (Quinn, Anderson, Drivdahl, Alvarez, & Argiles, 2002). It stimulates myocyte and muscle fibers to accumulate contractile protein and is able to slow muscle-wasting in rats with cancer-related cachexia (Figueras et al., 2004).

Interestingly, IL-15 also take part in reducing adipose tissue mass (B. K. Pedersen et al., 2007). Therefore, IL-15 is suggested to be involved in muscle-fat crosstalk. As a potent proinflammatory cytokine, IL-15 plays an important and complex role in autoimmune disease

and inflammation. IL-15 stimulates the proliferation and maintenance of NK cells, B and T lymphocytes, it is probable that IL-15 could play a role in some hematological malignancies (Steel, Waldmann, & Morris, 2012). Indeed, IL-15 may offer protective effects against the development of cancer. Thus, IL-15 has emerged as a candidate immunomodulator for the treatment of cancer.

2.4. Exercise-induced myokines in cancer cell

Recently several researchers have indicated that myokines also play a role in cancer protection. It was shown that conditioned media from electrostimulated C_2C_{12} muscle cells could inhibit cancer cell proliferation (Davis et al., 2009), suggesting that the contracting myotubes release humoral factors that can inhibit cancer cell growth. It has demonstrated that serum obtained from subjects who did exercise and diet, could inhibit mammary cancer cell growth in vitro (Barnard et al., 2006).

Exercise-conditioned serum inhibits mammary cancer cell proliferation and induces apoptosis through caspase activation (Hojman et al., 2011). 1 hour of swimming exercise upregulated seven genes, including Oncostatin M (OSM), IL-6, IL-10, IL-11, GDF-2, GDF-5 and CSF-2.

2.4.1. Oncostatin M

In particularly, Oncostatin M (OSM) proved to be of interest because this cytokine significantly inhibited MCF-7 cell proliferation and induced apoptosis. OSM is pleiotropic IL-6 family cytokine important in inflammation and other cellular processes such as development, hematopoiesis, liver function, neurogenesis, and bone homeostasis (Heinrich et al., 2003). During oncogenesis, OSM often acts as an antiproligerative factor for multiful types of cancers including multiple myolema, lung cancer, and breast cancer and studies suggest that OSM is

expressed in 66% of breast tumors, with the prevalence being even higher inflammatory breast cancers (Garcia-Tunon et al., 2008).

Recently, it is shown that myokines, in addition to the reduction of low-grade inflammation, also play a direct role in the tumor-suppressing effect of exercise (Hojman et al., 2011). Breast cancer cells incubating with serum taken immediately after exercise, could reduce cancer cell vitality and induce apoptosis through caspase activation. This study identified OSM as an exercise-induced myokine with anti-proliferative effects on the breast cancer cell (L. Pedersen & Hojman, 2012).

2.4.2. SPARC (Secreted protein acidic and rich in cysteine)

The level of circulating secreted protein acidic and rich in cysteine (SPARC) was measured in mice and humans that performed a single bout of exercise. Furthermore, regular exercise enhance apoptosis in colon mucosal cells and increased the cleaved forms of caspase-3 and caspase-8 in wild-type mice but not in SPARC-null model (Aoi et al., 2013).

2.5. Apoptosis in colon cancer-induced mice

2.5.1. Mechanism of apoptosis

Apoptosis, or type I cell death, is a tightly regulated process that allows multicellular organisms to maintain tissue homeostasis (Krysko, Vanden Berghe, D'Herde, & Vandenabeele, 2008) and disease-associated processes (Quadrilatero, Alway, & Dupont-Versteegden, 2011).

One of the major regulators of apoptotic signaling is a set of cysteine-dependent aspartate-directed proteases known as caspases (Taylor, Cullen, & Martin, 2008). Several key pathways of apoptosis can lead to caspase activation. The endoplasmic-sarcoplasmic reticulum(ER-SR) stress pathway can be initiated through the accumulation of unfolded and misfolded proteins, which lead to the activation of caspase (Rasheva & Domingos, 2009).

The mitochondrial pathway is currently considered the central mediator of apoptosis because it contains critical apoptotic signaling factors. One of the most important mediators of apoptosis is cytochrome *c*, which is released from the mitochondria upon proapoptotic stimuli. In cytosol, cytochrome *c* associates with several apoptotic proteins, which is ultimately responsible for the degradation of cellular content and for DNA fragmentation (a hall mark of apoptosis) (Taylor et al., 2008). Critical for the release of mitochondrial proteins is mitochondrial outer membrane permeabilization (MOMP), which is highly regulated by a series of proteins belonging to the B cell lymphoma 2 (Bcl-2) family (Kroemer, Galluzzi, & Brenner, 2007). Bcl-2 and Bax are key family proteins with anti- and proapoptotic functions, which regulate apoptogenic protein release at the mitochondria (Wolter et al., 1997; Yang et al., 1997). Finally, in response to DNA damage and cellular stress, p53 can accumulate at the nucleus and lead to the transcription of several proapoptotic proteins such as Bax. Moreover, cytosolic p53 accumulation can result in Bax activation (Galluzzi, Morselli, Kepp, Tajeddine, & Kroemer, 2008; Quadrilatero et al., 2011)

2.5.2. Caspase-3 in apoptosis

The caspase family of cysteine proteases occupies critical positions in signal transduction cascades associated with immune responses. In many of these situations, caspase activation culminates in the apoptosis of cell in which these proteases become activated (Creagh, Conroy, & Martin, 2003).

At the molecular level, the evolving descriptions of mitochondrial dysfunction and caspase activation as key elements of apoptosis have provided significant insight into the understanding of the molecular basis of cancer (Hunter, LaCasse, & Korneluk, 2007)

Caspase-3 is one of effector caspases (caspase-6 and caspase-7), is thought to be responsible for the actual demolition/dismantling of the cell during apoptosis and tens to have short or absent prodomains (Creagh et al., 2003). Also caspase-3, one of a key enzyme which mediate the final stage of cell death in apoptosis (Janicke, Ng, Sprengart, & Porter, 1998).

2.5.3. Muscle apoptosis in cancer model

In humans, the mortality rate and pathogenesis of many age-related diseases are associated with the functional status, metabolic demand, and mass of skeletal muscle (Metter, Talbot, Schrager, & Conwit, 2002; Nair, 2005; Ruiz, Moran, Arenas, & Lucia, 2011).

In addition to exercise and muscle function, muscle mass also an important predictor of mortality, especially in diseased individuals, and can influence the progression and outcome of age-related diseases in humans (Wisloff et al., 2005).

Cachexia is characterized as an overall state of ill health, accompanied by a loss of lean body mass and fat mass, weakness, fatigue, anemia, metabolic abnormalities, inflammations, and impaired immune function (Ardies, 2002; Argiles, Busquets, & Lopez-Soriano, 2003). Cancer patients can lose up to 30% of their original body weight, and cachexia accounts for 20~30% of cancer deaths. Decreased muscle mass and depressed muscle function could eventually impair respiratory muscle function, lead to mortality (Giordano et al., 2003). Strikingly, reducing muscle wasting during cancer cachexia increases the survival of tumor-bearing mice, even if tumor growth is not affected (Zhou et al., 2010).

2.5.4. Exercise effects on apoptosis in muscle

Physical activity is a powerful physiological stimulus that can alter numerous extracellular and intracellular signaling pathways, thus physical activity may directly or indirectly influence cell death-related signaling processes such as apoptosis. For instance, exercise have been shown to influence circulation hormone, cytokine levels (B. K. Pedersen et al., 2001; Steinacker, Lormes, Reissnecker, & Liu, 2004), alter the production of reactive oxygen species (ROS) and reactive nitrogen species (Powers & Jackson, 2008), and modify a number of intracellular signaling and transcription factors (Kramer & Goodyear, 2007), all of which can influence apoptotic signaling (Quadrilatero et al., 2011).

Regular physical activity has been shown to decrease skeletal muscle apoptotic signaling and DNA fragmentation, effects that may be mediated by several potential mechanisms,

including (1) altering the expression of several apoptotic proteins such as Apaf-1, ARC, Bax, Bcl-2, caspases, calpains, FasL, Hsp70, p53, TNFa, TNF receptor, and XIAP (2) influencing mitochondrial function such as PTP formation, release of apoptogenic proteins, and mitochondrial biogenesis (3) reducing mitochondrial–cellular ROS generation and improving antioxidant levels such as catalase, copper–zinc superoxide dismutase, manganese superoxide dismutase, reduced glutathione, and glutathione peroxidase (Quadrilatero et al., 2011).

Treadmill exercise for 8 weeks decrease DNA fragmentation by 33% in healthy rats (Siu, Bryner, Martyn, & Alway, 2004), 12weeks of treadmill running reduced cleaved caspase-3 protein levels and DNA fragmentation in rat gastrocnemius muscle (Song, Kwak, & Lawler, 2006). 7 days of chronic stimulation was successful at decreasing DNA fragmentation by 45% in muscle(Ljubicic et al., 2009), also 4 weeks of treadmill running reduced cleaved caspase-3 and caspase-8 content levels and DNA fragmentation in EDL muscle (Marzetti et al., 2008).

Exercise is associated with a number of whole-body and cellular adaptations that may provide protection against apoptotic signaling in skeletal muscle, include apoptosis-associated regulatory proteins, improved mitochondrial function, and decreased oxidative stress (Quadrilatero et al., 2011).

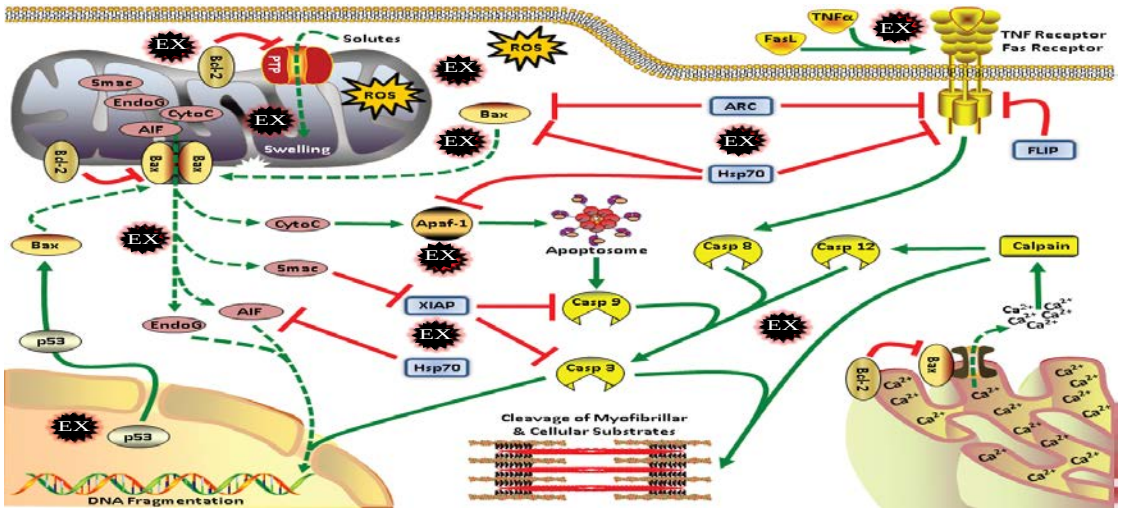

Figure 5. Apoptosis pathways in skeletal muscle
Apoptosis can be initiated by events occurring at the mitochondria, death receptors, endoplasmic–sarcoplasmic reticulum, and nucleus. Regular physical activity has been shown to decrease skeletal muscle apoptotic signaling and DNA fragmentation by several potential mechanisms. Black stars indicate sites, proteins, and mechanisms which could be influenced by physical activity (Quadrilatero et al., 2011)

III. Material and Method

3.1. Animals and treatments

Male ICR mice were used in this experiment (ORIENT). The experimental procedures were performed in accordance with the animal care guidelines of National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. Mice were housed under the controlled temperature (20 ± 2℃) and the lighting (08:00-20:00 h) conditions. Food and water were available ad libitum.

All mice were injected of AOM (Azoxymethane, Sigma, cat. No. A2853) working solution (10mg per kg body weight) in a laminar airflow hood. After 21, 42, 63 days, 2.5 % (wt/vol) DSS (Dextran Sodium Sulfate, Biochemicals Inc., Costa Mesa, CA) in drinking fluid was provided for 7days (5ml DSS solution per mouse per day) to induce inflammation-driven tumor progression. 80days was taken to evaluate tumor development for the protocol of chronic inflammation-deriven tumor progression. Then all mice were randomly assigned to one of four groups: CON(Control Group), LIE(Low-Intensity Exercise Group), MIE(Moderate-Intensity Exercise Group), HIE(High-Intensity Exercise Group).

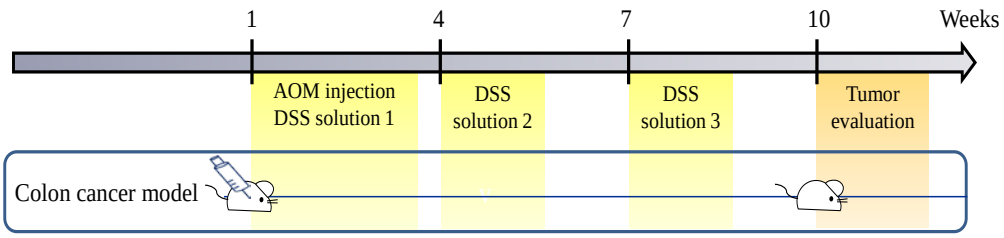

Figure 6. Inflammation-derived tumor progression.
AOM (Azoxymethane) exerts colonotropic carcinogenicity, and injects in mice. DSS(Dextran Sodium Sulfate) is a model for colitis-association tumor development, dissolved in drinking water (Neufert et al., 2007).

3.2. Experimental design

3.2.1. The first experiment

To identify the effect of exercise on expression of myokines in cancer model, we investigate as the following procedures. All mice at 6-wk old, were injected AOM and were treated 3 cycles of DSS solution in drinking water to induce colon cancer (Neufert et al., 2007). It is known that this method mimics IBD course of humans, and result in most rapid tumor development. During the period of tumor development (almost 80 days), a few of them were sacrificed for tumor evaluation. At 17-wk of old, mice of exercise groups performed treadmill exercise at different intensity (Low, Moderate, High) for 30 minutes, 5 days per week, during 12 weeks. 24 hours after the final exercise training, at 30-wk of old, all mice were sacrificed for the analysis.

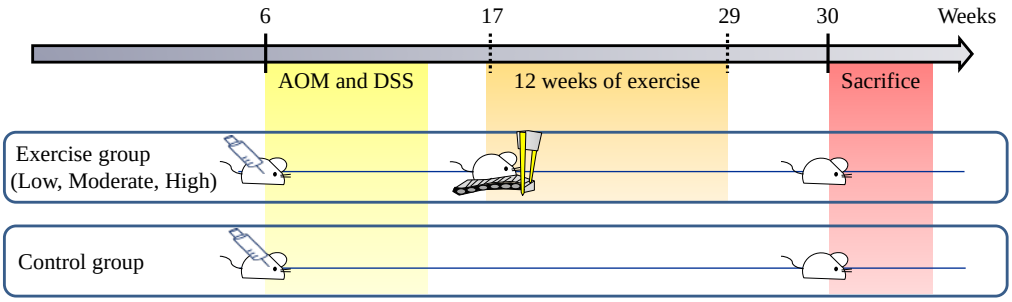

Figure 7. The first experimental design

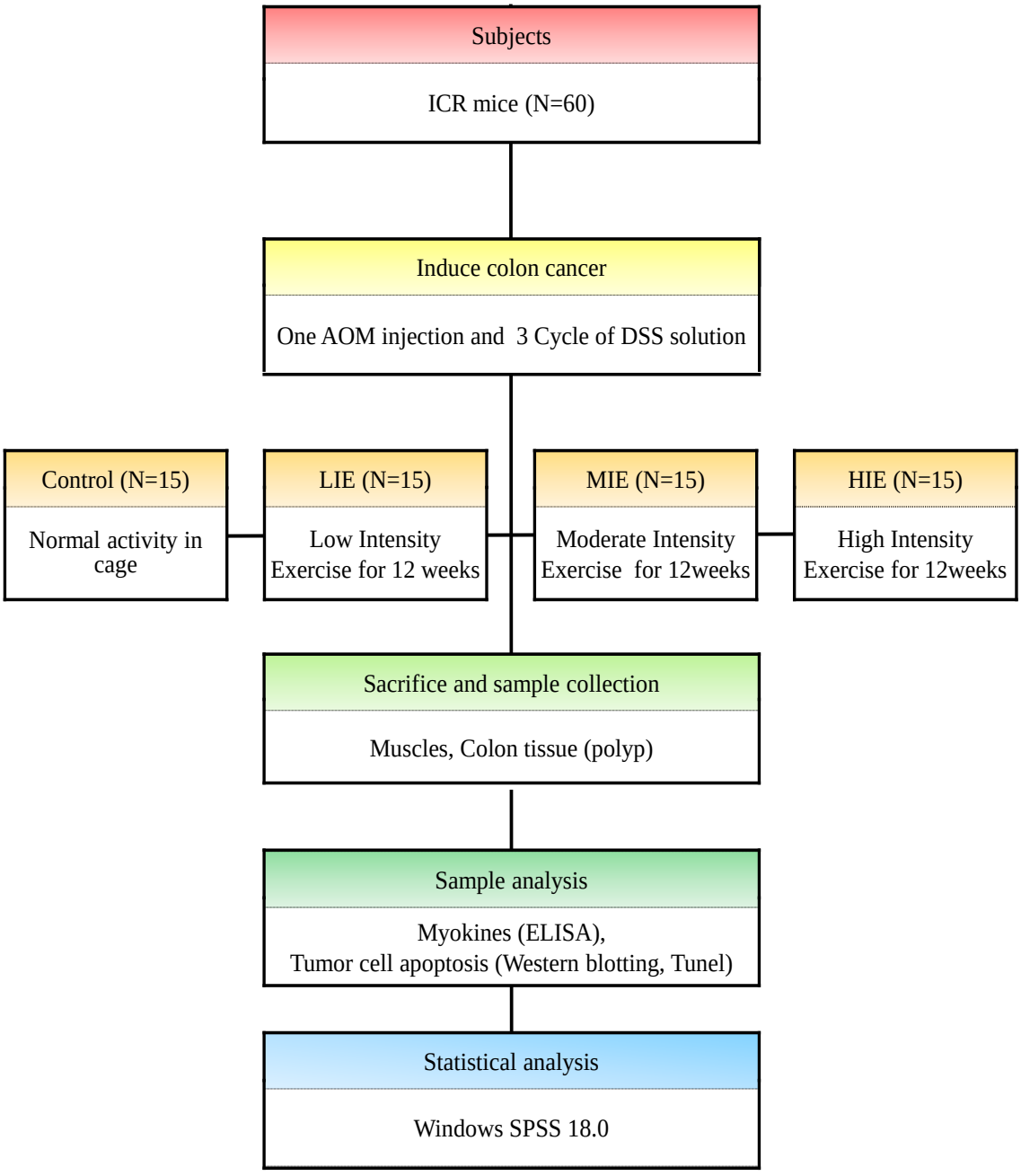

Figure 8. The first experimental procedure.

3.2.2. The second experiment

Mice of exercise groups, at 6-wk of old, performed exercise at different intensity (Low, Moderate, High) for 30 minutes, 5 days per week, during 12 weeks. At 17-wk of old, all mice were injected AOM and were given 3 cycles of DSS solution in drinking water to induce colon cancer. Then they kept doing exercise for another 12 weeks. During the period of tumor development, a few of them were sacrificed for tumor evaluation. 24 hours after the final exercise training, at 30-wk of old, all mice were sacrificed for the analysis.

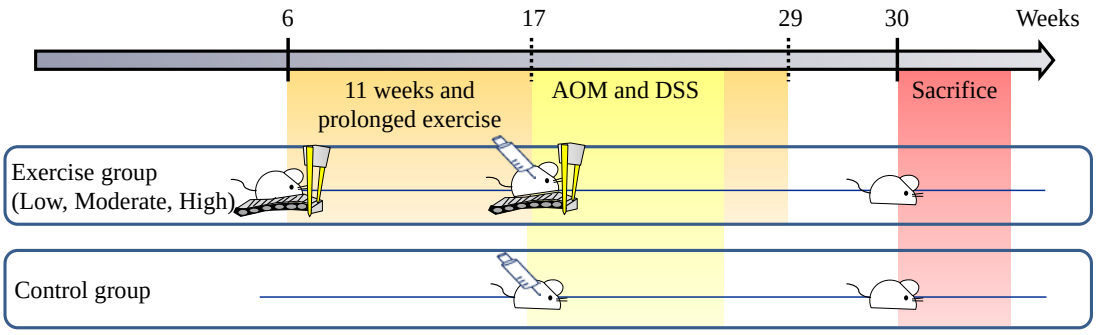

Figure 9. The second experimental design

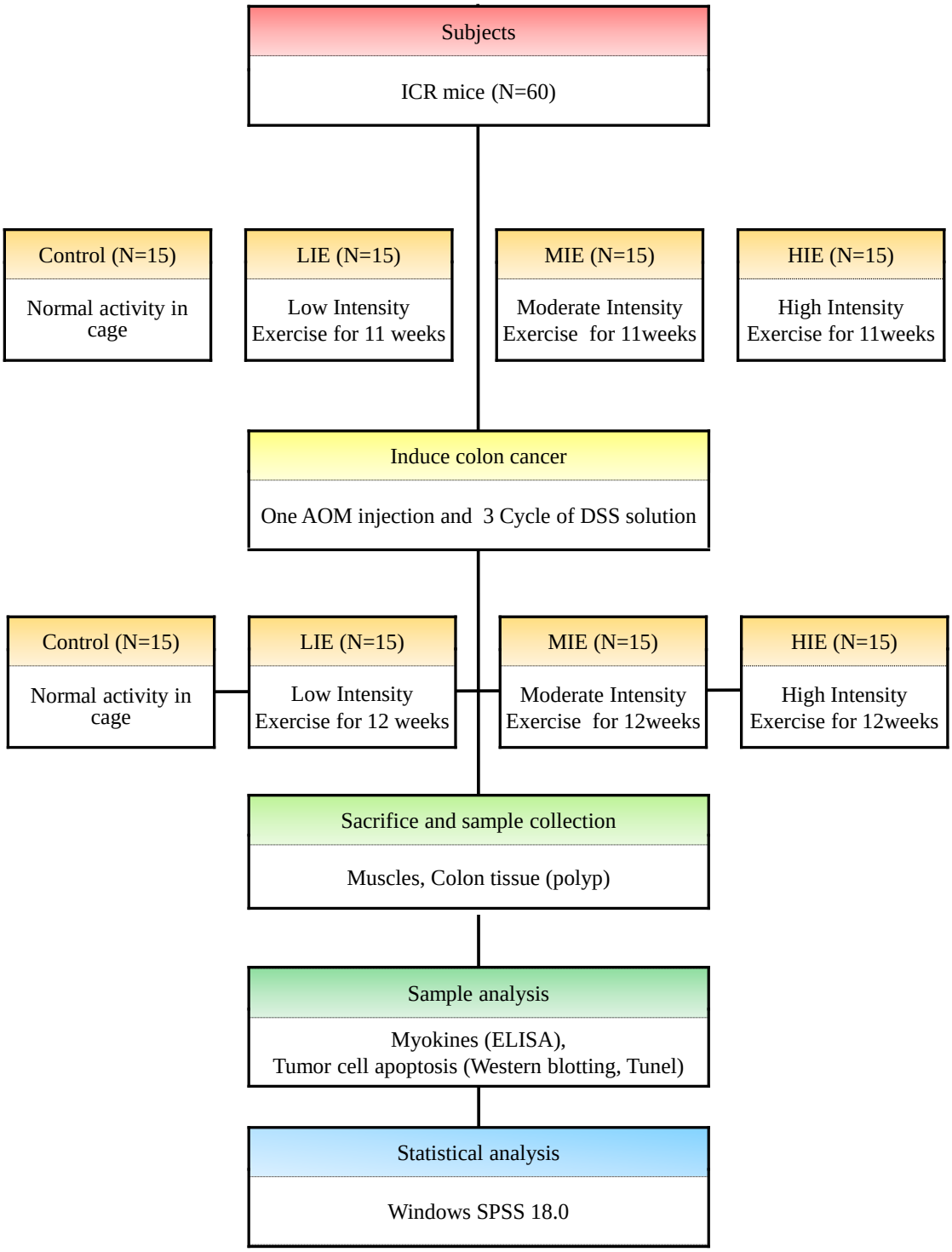

Figure 10. The second experimental procedure

3.3. Exercise protocol

The mice in exercise group were forced to run on a treadmill for 30 min once a day five times a week for 12 weeks. Mice in exercise groups were acclimated to treadmill exercise before training. Initial treadmill speed of each group is set to 6m/min (Low intensity exercise), 10m/min (Moderate intensity exercise), 24m/min (High intensity exercise) respectively and added 2m/min every two weeks, considering the intensity and effect of exercise training. Thus, treadmill speed during last two weeks was about 10m/min, 18m/min, 28m/min respectively. All group had 5mintes of warming-up session in 30minutes and additional 2minutes 30 seconds of cooling-down session.

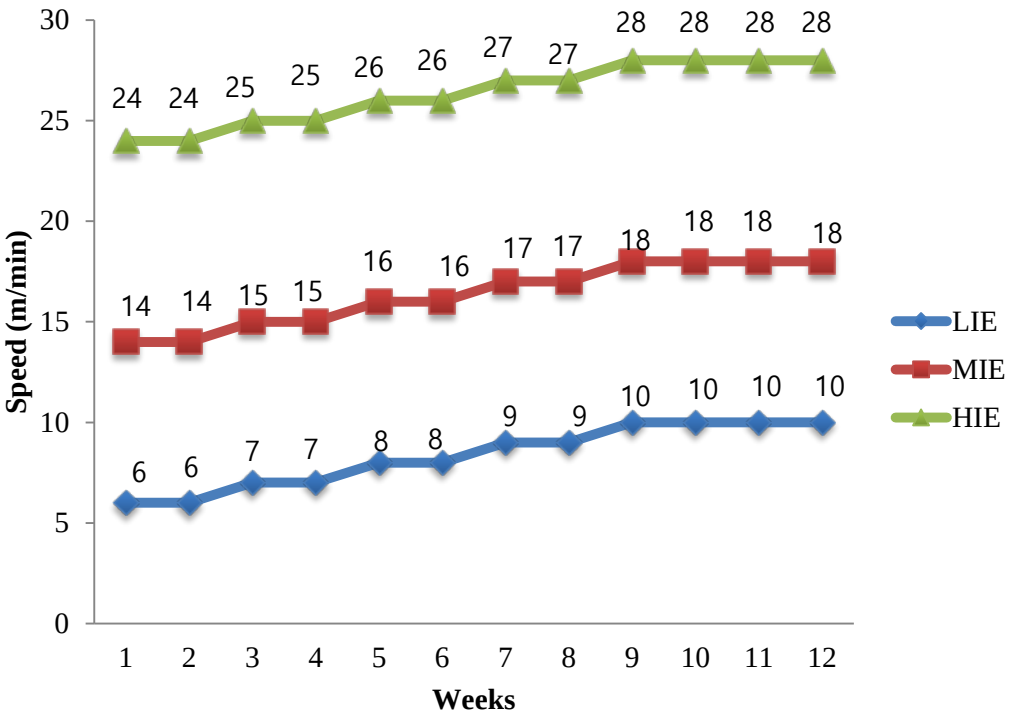

Figure 11. Exercise protocol

3.4. Tissue Collection

24 hours after final exercise, animals were sacrificed by CO_2 asphyxiation. At necropsy, muscles and colons were removed, washed with ice-cold PBS. Muscles and visible colon tumors were grinded using a mortar and rapidly frozen in liquid nitrogen, and were stored at -70°C until further analysis.

3.5. Polyp counts

To count polyps, polyps were counted under a dissecting microscope, using tweezers to pick through the intestinal villi and identify polyps.

3.6. Western blot

After harvest, whole cell extracts will be disrupted with lysis buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 2 mM $MgCl_2$, 1 mM EDTA, and 1% Triton X-100] containing protease Inhibitors cocktail (Roche), 10 mM NaF, 10 mM sodium pyrophosphate, and 100 mM sodium vanadate. After vortexing for 10 sec, cells will be incubated in 4℃ for 40 min. The supernatants were collected after centrifugation with 12000 rpm for 15 min at 4℃. After quantitation with Bradford assay, same amount of samples will be mixed with protein dye and boiled for 2 min. Denatured samples were run on 12 % SDS-PAGE gel and transferred to membrane (Milipore, Milford, MA). Membranes will be preincubated with 5% non-fat milk for 30 min and incubated with appropriate primary antibodies at a 1:1,000 to 1:2,000 dilution for overnight. Anti bcl-2 (cell signaling), anti bax (cell signaling), anti-cleaved caspase-3 (ASP 175, cell signaling), and anti-β-actin (AC-15, Sigma) will be purchased commercially. After 4-times washing for 5 min with TBS-T buffer, the appropriate HRP-tagged secondary antibodies were added at a 1:5,000

dilution and incubated for 30 min at room temperature. LAS-image3000 will be used for detection of ECL after washing 5-times for 5 min.

3.7. ELISA

Enzyme-linked immnosorbent assay kit will be used for the quantitative measurement of mouse myokines; Oncostatin M, SPARC according to the manufacturer's protocol.

3.8. Statistical analysis

Data analysis will be performed using the SPSS 19.0 for Windows statistical software. All data will be expressed as means ± SEM. Two-way ANOVA with Tukey post-hoc will be conducted to determine the existence of mean differences for among groups. The level of significance was set at $p < .05$.

IV. RESULTS

4.1. The first experiment

The first experiment was performed to identify the therapeutic effect of exercise on colon cancer-induced mice. All mice was induced colon cancer by the AOM and DSS method, then the mice of three exercise groups (LIE, MIE, HIE) were trained treadmill running for 30 min, 5 times · $weeks^{-1}$ for 12 weeks.

4.1.1. The change of body weight

Table 2 and figure 12 show the change of body weight during the whole period in the first experiment in CON, LIE, MIE, and HIE. In this study, there was no significant differences among groups during the whole period ($p>.05$), except that the body weight of mice at 29-wk of old in LIE was significantly higher than those in CON ($p<.05$).

Interestingly, the weight of all the mice in the first experiment weighted less than CCON (normal mice, those who was not induced colon cancer), at the same week of old. Especially, the weight of mice in CON was significantly lower than the mice in CCON since they were 21-wk of old ($p<.05$). Also the weight of mice in HIE at 21-27-wk of old (the first half of exercise period) was significantly lower than CCON, but the attenuation of weight loss did not appear in the last half of exercise period, eventually there was no significant differences at the last week of the experiment.

Strikingly, the weight of mice in exercise groups decreased after last bout of exercise, although the mice in CON also decreased dramatically at the same week.

Table 2. The change of body weight during the first experiment

weeks	20	21	23	25	27	29	31	33
Ex	1	2	4	6	8	10	12	
CCON	44.4 ±3.54	45.60 ±4.15	45.86 ±4.69	47.38 ±5.54	46.94 ±5.21	47.80 ±5.42	49.34 ±4.72	46.70 ±5.67
CON	41.08 ±0.51	41.65 ±0.76*	41.48 ±1.10*	42.30 ±1.83*	42.18 ±2.13*	42.60 ±2.50*	42.40 ±3.41*	37.50 ±2.45*
LIE	42.22 ±1.84	43.47 ±1.33	43.63 ±1.21	44.68 ±1.19	45.07 ±140	45.83 ±1.66∫	45.90 ±1.64	37.92 ±0.80
MIE	43.33 ±3.89	43.97 ±3.34	43.93 ±3.41	44.95 ±3.60	43.07 ±3.22	44.80 ±1.40	43.77 ±1.33	39.75 ±1.70*
HIE	43.15 ±1.31	41.77 ±1.52*	41.25 ±1.67*	42.07 ±1.63*	43.35 ±2.32*	43.82 ±1.51	43.78 ±2.30	39.83 ±1.69

Ex: Treadmill exercise, CCON: Normal mice group(ICR), CON: Control group(no exercise), LIE: Low Intensity Exercise group, MIE: Moderate Intensity Exercise group, HIE: High Intensity Exercise group. * = p<.05, Compare CCON and CON, LIE, MIE, HIE. ∫= p<.05, compare CON and LIE

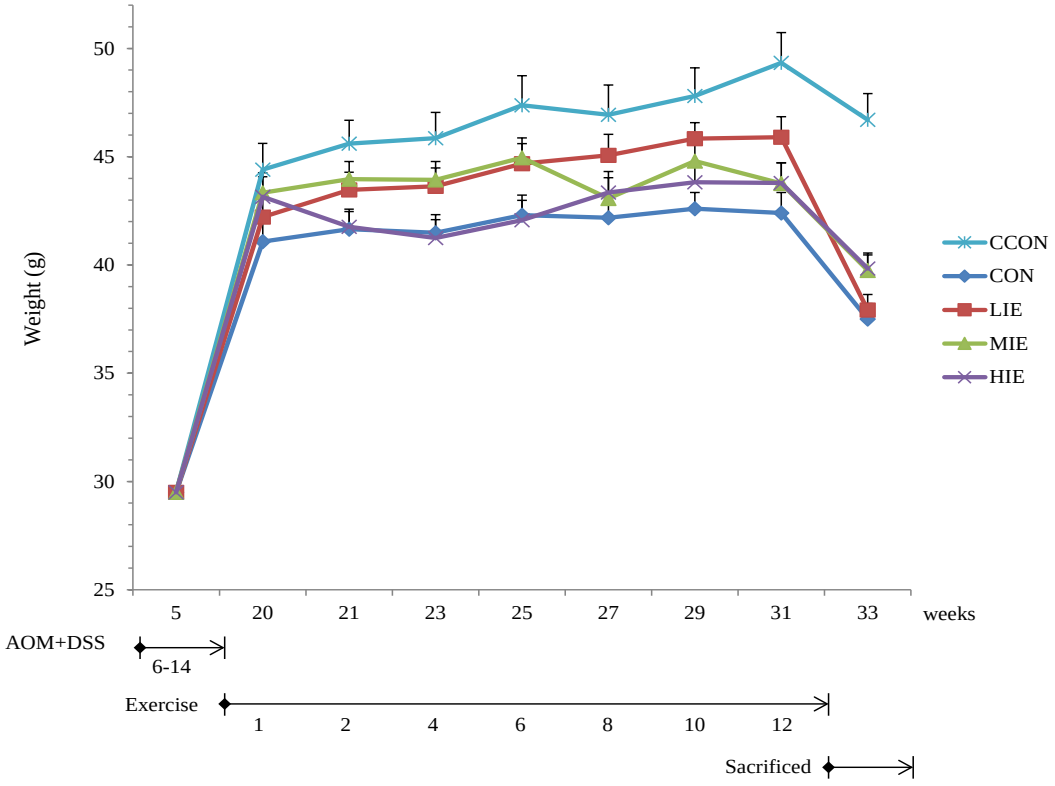

Figure 12. The change of body weight during the first experiment

4.1.2. Muscle weight

In this study, gastrocnemius and quadriceps extracted in all groups, and measured weight. Each muscle weight was calculated by divide one of CON to normalize.

Firstly, to identify the effect of exercise on cancer induced mice, the weight of muscle includes those of all exercise groups. As shown in Figure 13, the weight of gastrocnemius in EXE (include all exercise groups) significantly higher than those of CON (p=.004). However, there is no significant difference between CON and EXE in quadriceps muscle (Figure 14).

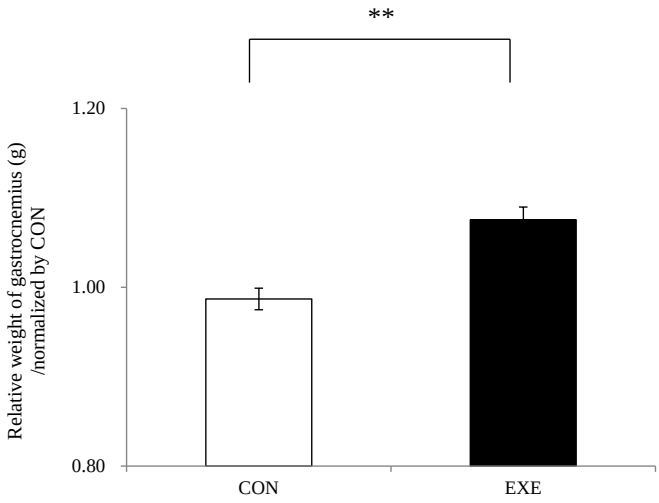

Figure 13. Relative weight of gastrocnemius

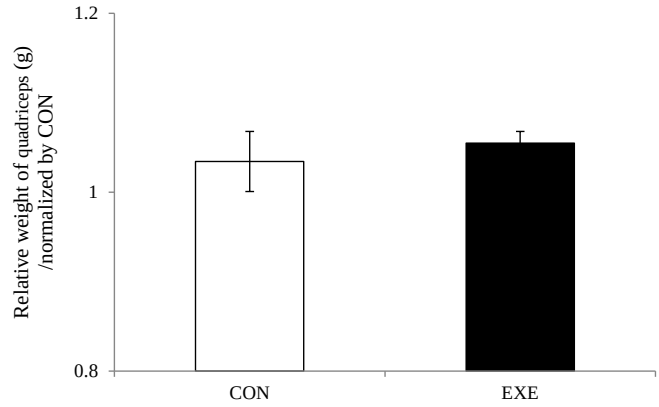

Figure 14. Relative weight of quadriceps

As shown in Figure 15 and Figure 16, to investigate the effect of exercise intensity, the exercise groups divided by 3 groups; LIE, MIE, HIE. There is significant differences among groups in gastrocnemius muscle (p=.005), and the gastrocnemius weight of CON is significantly lower than those of MIE (p=.002), but there was no significant difference among groups in quadriceps muscle (p>.05).

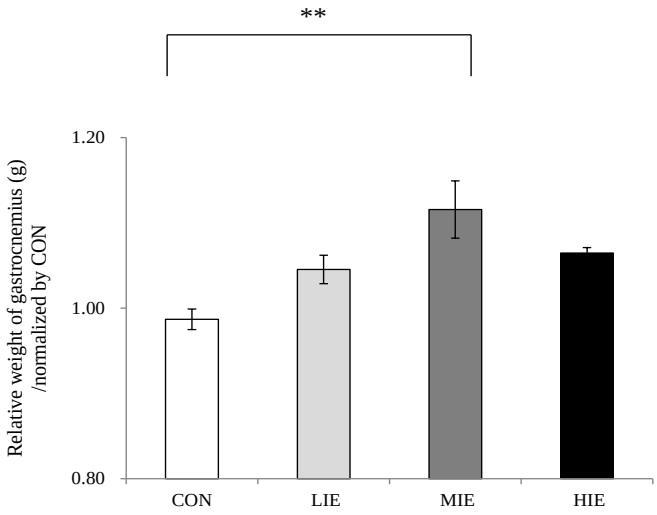

Figure 15. Relative weight of gastrocnemius by exercise intensity

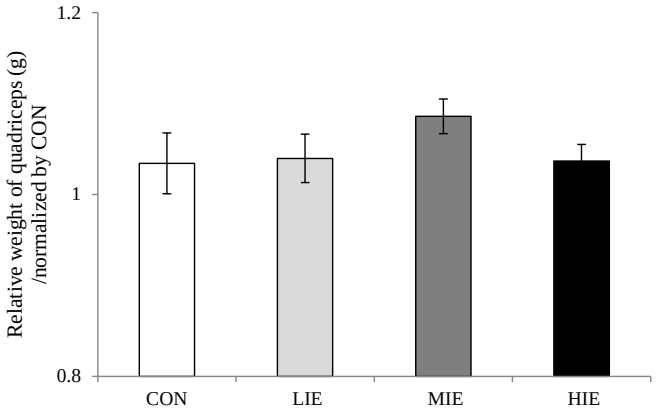

Figure 16. Relative weight of quadriceps by exercise intensity

4.1.3. Colon weight

In this study, all mice was induced colon cancer by the method of AOM and DSS. When they were sacrificed, their colorectal parts were isolated and the weight of colon was measured. Figure 17 shows the weight of colon tissue of all exercise groups was significantly lower than CON (p=.045). Also Figure 18 shows significant differences of colon weight among groups (p=.02), and the weight of colon in HIE was significantly lower than CON (p=.023).

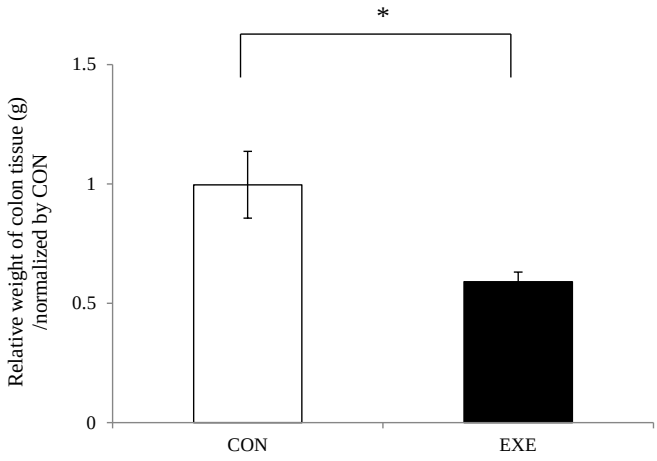

Figure 17. Relative weight of colon tissue

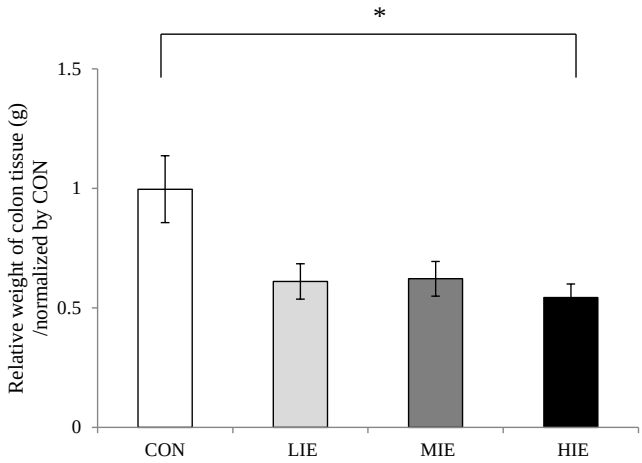

Figure 18. Relative weight of colon tissue by exercise intensity

4.1.4. Polyp incidence

24 hours after final exercise, animals were sacrificed by CO_2 asphyxiation. At necropsy, colons were removed, washed with ice-cold PBS. Polyps were counted under a dissecting microscope, using tweezers to pick through the intestinal villi and identify polyps.

Treadmill exercise significantly decreases incidence of polyp in exercise group compare to control group (p=0.03), and the effect of exercise intensity was founded in MIE (p=.011) and HIE (p=.022) compare to controls.

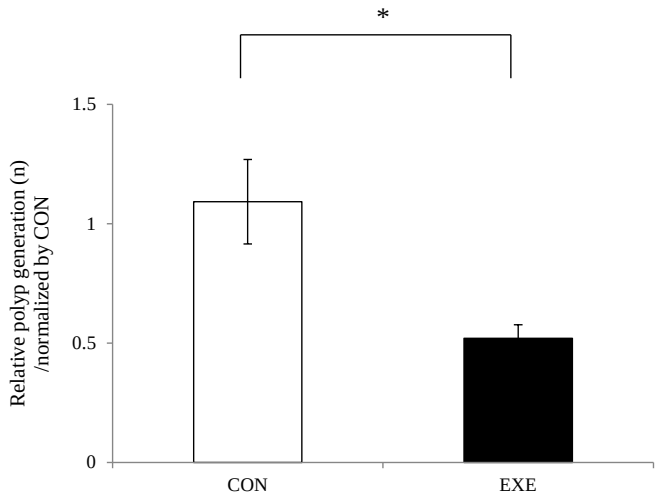

Figure 19. Polyp incidence in CON and EXE

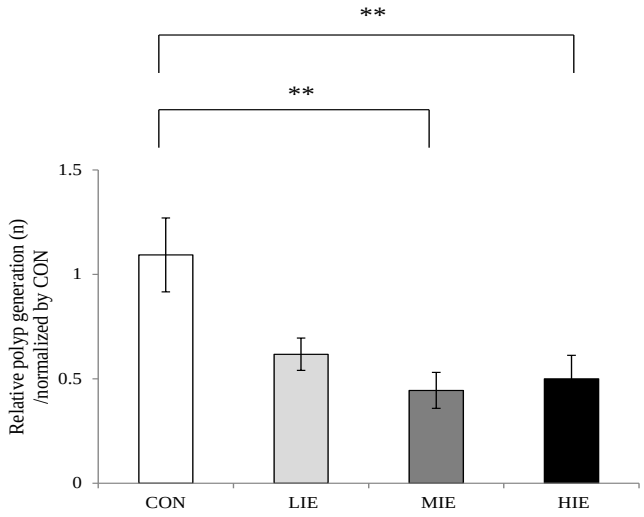

Figure 20. Polyp incidence by exercise intensity

4.1.5. Correlation of muscle weight and polyp number

As shown in Figure 15 and 16, the weight of gastrocnemius in exercise groups significantly higher than controls. One of the feature of exercise is the muscle gain, results in several positive biological improvement of human body.

As the weight of gastrocnemius increases, the number of polyps decrease, thus there was a negative correlation between gastrocnemius weight and polyp number (r=-438; p=.032).

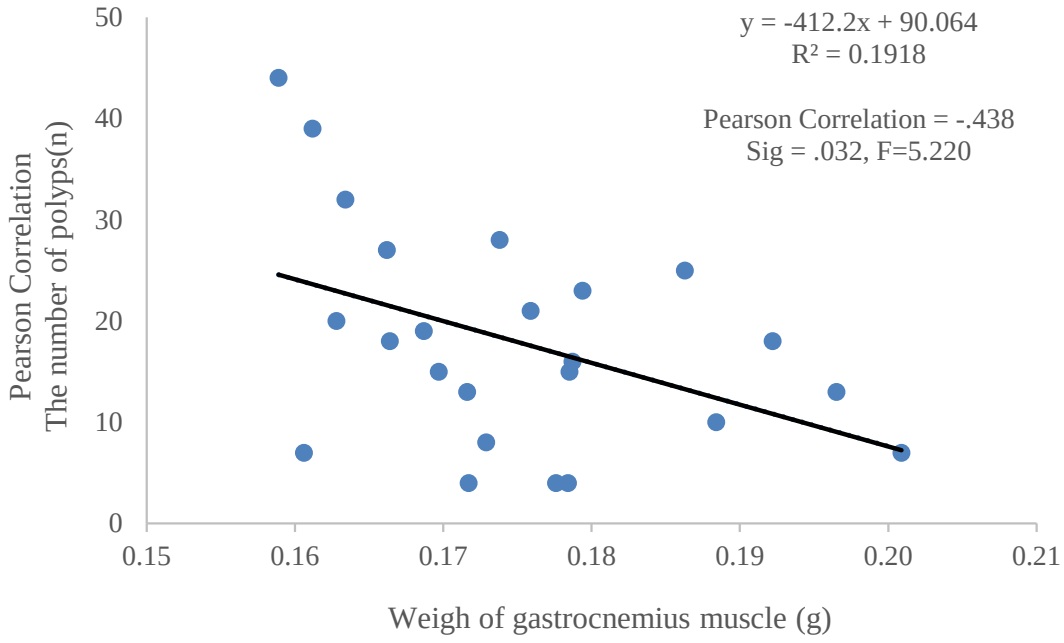

Figure 21. Correlation of muscle weight and polyp number.

4.1.6. Muscle apoptosis

Cancer patients can lose up to 30% of their original body weight, and cachexia, which accompanied by a loss of lean body mass, accounts for 20~30% of cancer deaths. Decreased muscle mass and depressed muscle function could eventually impair respiratory muscle function, lead to mortality.

Exercise is associated with a number of whole-body and cellular adaptations that may provide protection against apoptotic signaling in skeletal muscle, include apoptosis-associated regulatory proteins, improved mitochondrial function, and decreased oxidative stress. In this study, to identify the effect of exercise on muscle wasting in cancer induced model, bcl-2(anti-apoptotic protein) and bax (pro-apoptotic protein) are measured in gastrocnemius.

As shown in Figure 22 and Figure 23, bcl-2 level is significantly overexpressed in HIE compare to controls (p=.002), however the level of bax tends to decrease in exercise groups but there is no significant differences.

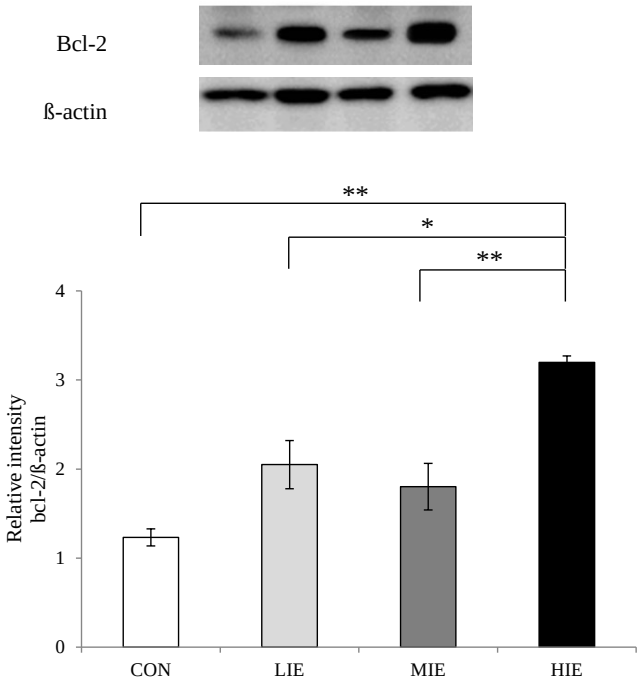

Figure 22. Bcl-2 level in gastrocnemius by exercise intensity
Western blot analysis was conducted to measure the levels of bcl-2. β-actin levels were measured to ensure equal amount of protein loading. The data were quantified by using image densitometric analysis. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

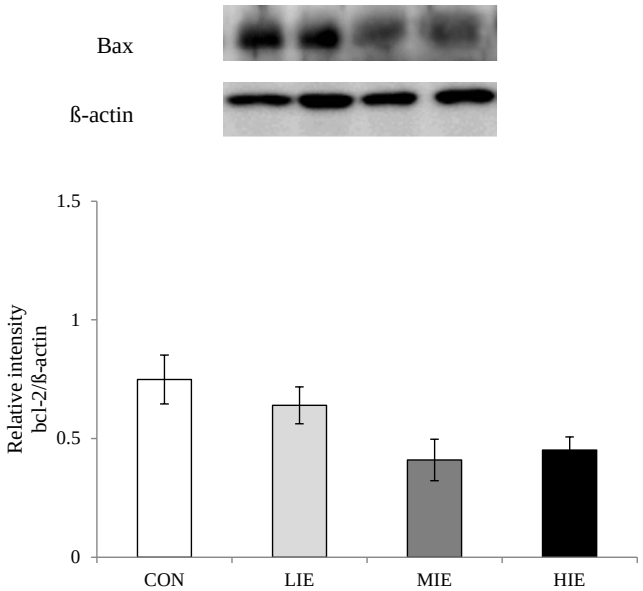

Figure 23. Bax levels in gastrocnemius by exercise intensity
Western blot analysis was conducted to measure the levels of bcl-2. β-actin levels were measured to ensure equal amount of protein loading. The data were quantified by using image densitometric analysis. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

4.1.7. The expression of OSM

Oncostatin M (OSM) is pleiotropic IL-6 family cytokine important in inflammation and other cellular processes. During oncogenesis, OSM often acts as an anti-proliferative factor for multiful type of cancers, and induce apoptosis through caspase activation.

Several studies indicate that OSM is overexpressed by exercise in several tissue including muscle, blood, spleen and it affects apoptosis pathway in breast cancer cell, but there is no study for colon cancer-induced model. Thus the present study is to investigate the effect of exercise on the expression of OSM in colon induced mice.

In this study, the expression of OSM measured in three different location; muscle, blood, and tumor tissue to identify the circulation of exercise-induced OSM. As shown in Figure 24, 25, and 26, in gastrocnemius (p=.000), blood (p=.005), and tumor tissue (p=.000), OSM is significant overexpressed in exercise group compare to controls.

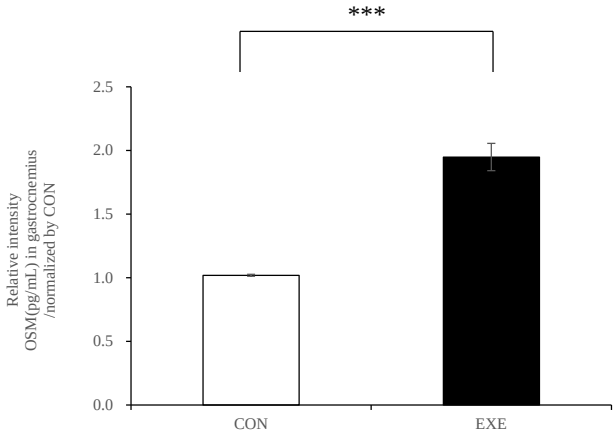

Figure 24. The expression of OSM in muscle
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

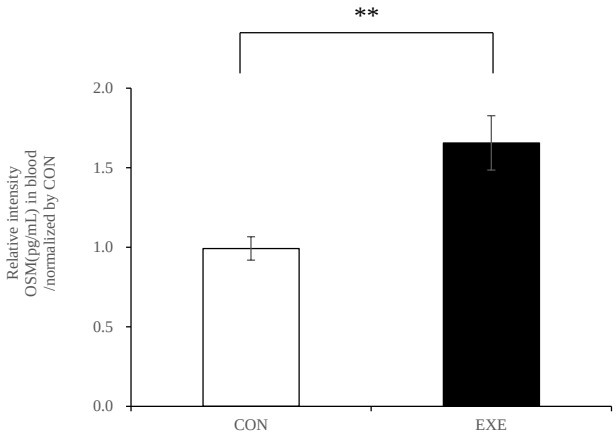

Figure 25. The expression of OSM in blood
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

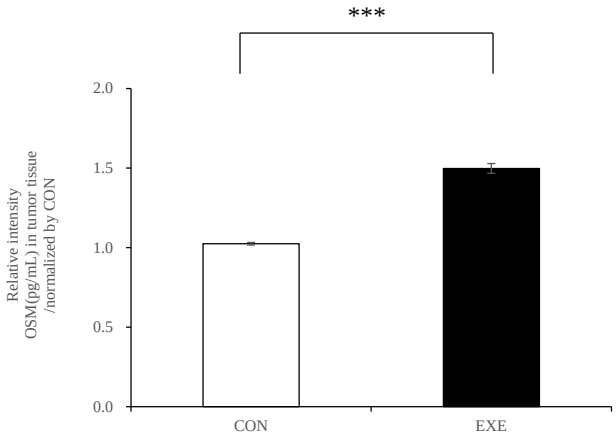

Figure 26. The expression of OSM in tumor tissue
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

Also, there is significant differences among exercise groups by exercise intensity on expression of OSM in colon-induced mice. The expression of OSM tends to increase in higher intensity in gastrocnemius (p=.000); LIE (p= .014), MIE (p=.000), HIE (p=.000), and in tumor tissue (p=.000); LIE (p= .000), MIE (p=.000), HIE (p=.000), but there is only significant difference in expression of OSM in blood of LIE (p=.000) compare to controls.

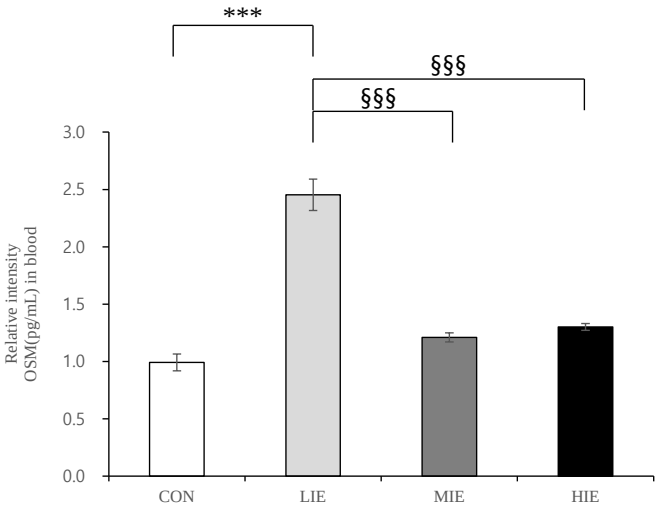

Figure 27. The expression of OSM in blood by exercise intensity
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). ***p<.001: CON vs LIE, §§§p<.001: LIE vs MIE and HIE.

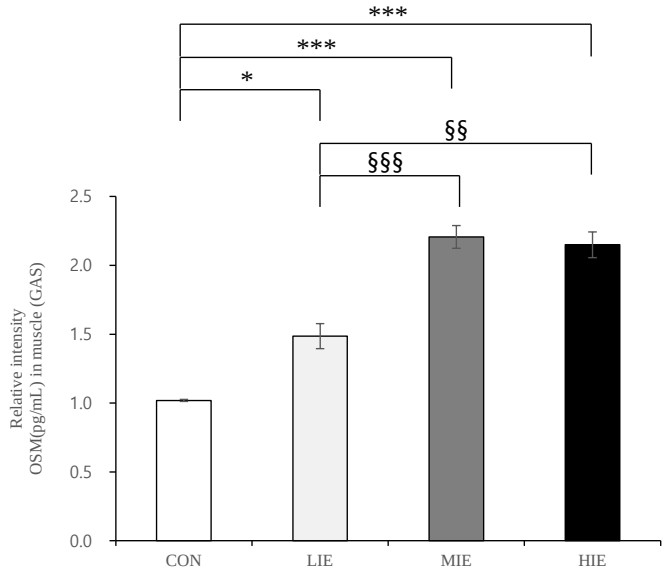

Figure 28. The expression of OSM in muscle by exercise intensity
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. ***p<.001 : CON vs LIE, MIE and HIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE.

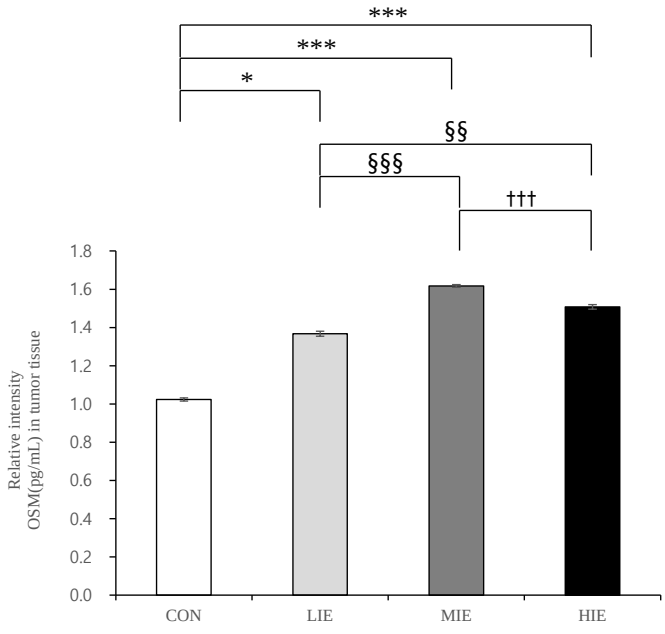

Figure 29. The expression of OSM in tumor tissue by exercise intensity
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. ***p<.001 : CON vs LIE, MIE and HIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE, ††† p<.001: MIE vs HIE.

4.1.8. The expression of SPARC

Recently, the level of secreted protein and rich in cysteine (SPARC) was increased in blood of mice by a single bout of exercise. Furthermore, it is founded that SPARC is associated with apoptosis in colon mucosal cells though the activation of caspase-3. Thus it is suggested that exercise-induced elevation of SPARC could inhibits colon tumorigenesis.

Thus the present study is to investigate the long term effect of exercise on the expression of SPARC in colon induced mice, the level of OSM measured in three different location; muscle, blood, and tumor tissue to identify the circulation of exercise-induced SPARC.

In the present study, the level of SPARC was significantly increased by exercise in muscle ($p=.000$), blood ($p=.002$), tumor tissue ($p=.001$) compare to controls.

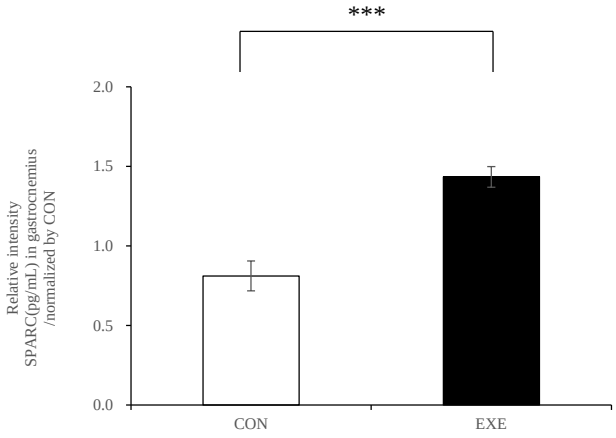

Figure 30. The expression of SPARC in muscle
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

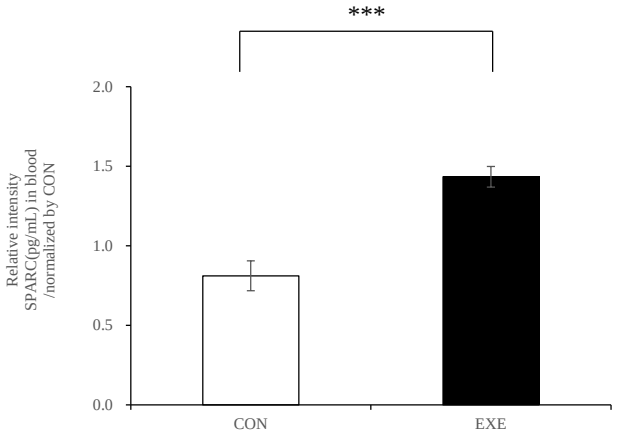

Figure 31. The expression of SPARC in blood
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

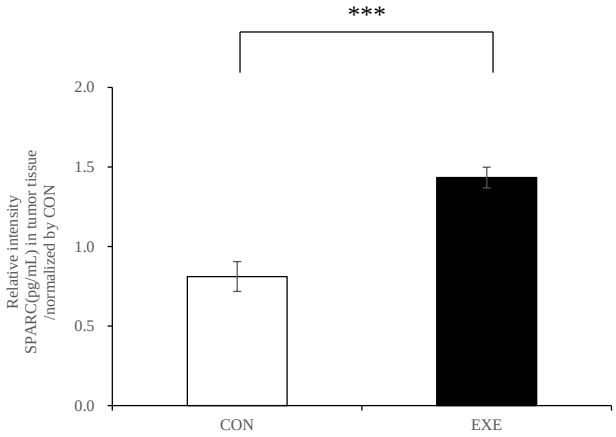

Figure 32. The expression of SPARC in tumor tissue
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

Also, there is significant differences among exercise groups by exercise intensity on expression of SPARC in colon-induced mice. The expression of SPARC tends to increase in higher intensity in gastrocnemius (p=.001); LIE (p= .035), MIE (p=.001), HIE (p=.007), in tumor tissue (p=.000); MIE (p=.000), HIE (p=.000), but there is no significant difference in expression of SPARC in blood among exercise groups (p=.055) compare to controls.

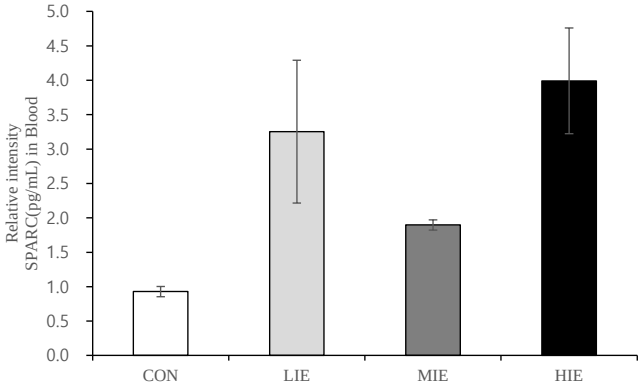

Figure 33. The expression of SPARC in blood by exercise intensity
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. ***p<.001 : CON vs LIE, MIE and HIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE, ††† p<.001: MIE vs HIE.

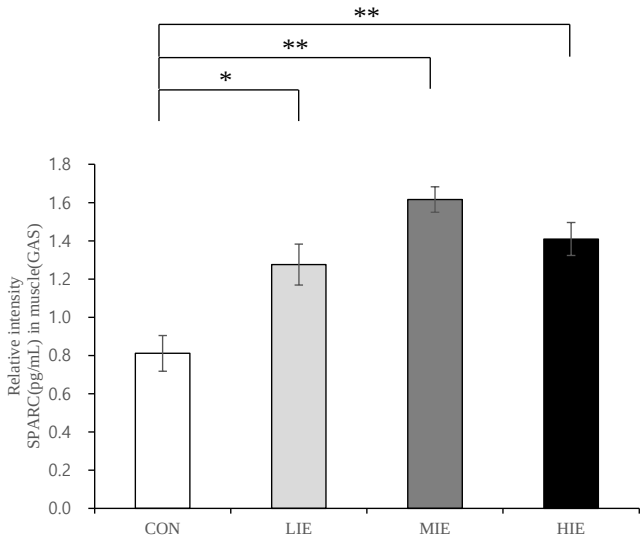

Figure 34. The expression of SPARC in muscle by exercise intensity

SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. ***p<.001 : CON vs LIE, MIE and HIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE, ††† p<.001: MIE vs HIE.

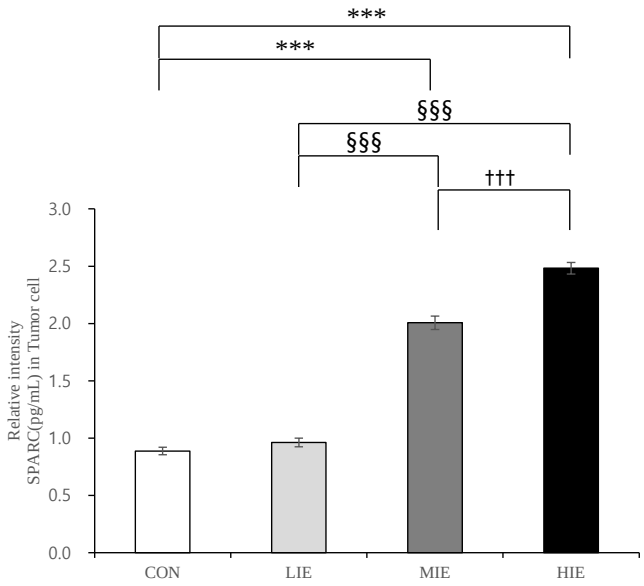

Figure 35. The expression of SPARC in tumor tissue by exercise intensity

SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. ***p<.001 : CON vs LIE, MIE and HIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE, ††† p<.001: MIE vs HIE.

4.1.9. The expression of caspase-3 in tumor tissue

Caspase-3 has been identified as a key mediator of apoptosis in tumor cells. Also myokines such as SPARC and OSM is known that which induce tumor cell apoptosis through elevation of caspase-3 activity.

In the present study, the expression of caspase-3 increased significantly in MIE (p=.045), but not in LIE and HIE (p>.05)

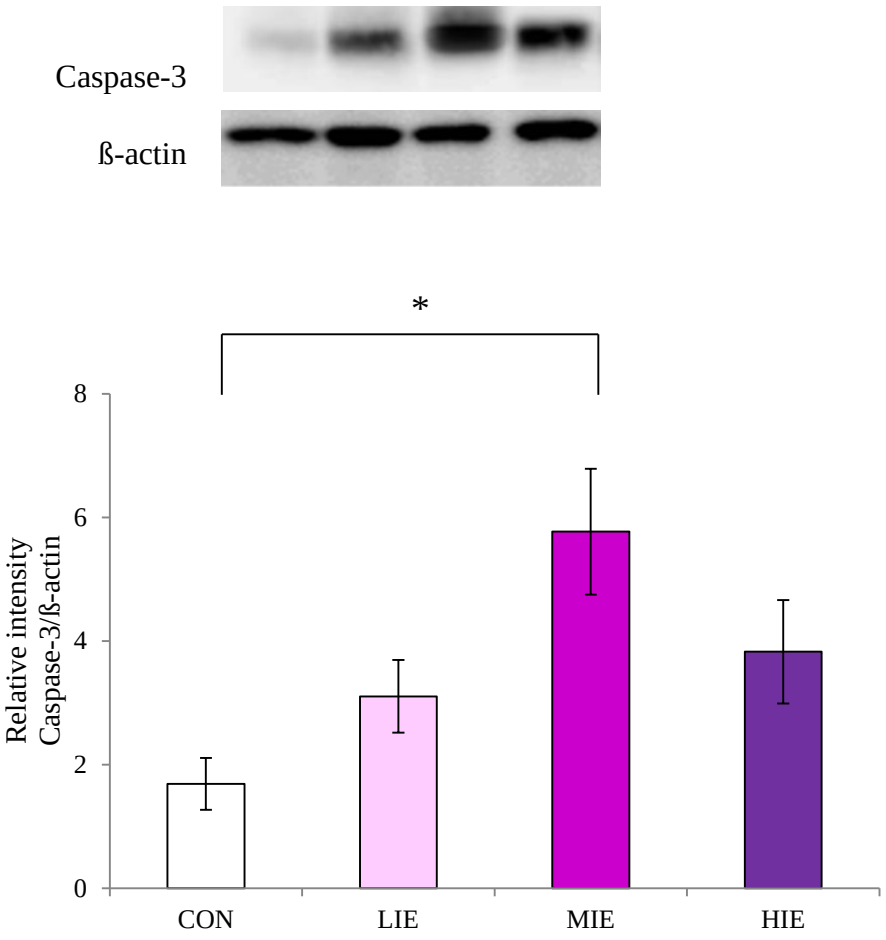

Figure 36. The expression of cleaved caspase-3 in tumor tissue
Western blot analysis was conducted to measure the levels of caspase-3. β-actin levels were measured to ensure equal amount of protein loading. The data were quantified by using image densitometric analysis. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

4.2. The second experiment

The second experiment was performed to identify the preventive effect of exercise on colon cancer-induced mice. Mice of exercise groups, at 6-wk of old, performed exercise at different intensity (Low, Moderate, High) for 30 minutes, 5 days per week, during 12 weeks. At 17-wk of old, all mice were injected AOM and were given 3 cycles of DSS solution in drinking water to induce colon cancer. Then they kept doing exercise for another 12 weeks. During the period of tumor development, a few of them were sacrificed for tumor evaluation. 24 hours after the final exercise training, at 30-wk of old, all mice were sacrificed for the analysis.

4.2.1. Relative risk of premature mortality

In the present study, there is a preventive effect of exercise on reducing premature mortality.

As shown in Figure 36, regular treadmill exercise attenuated the mortality of exercise group compare to controls (RR = 0.96). Also, there is an intensity effect of exercise on premature mortality, relative risk of MIE was lower than other groups (Figure 37).

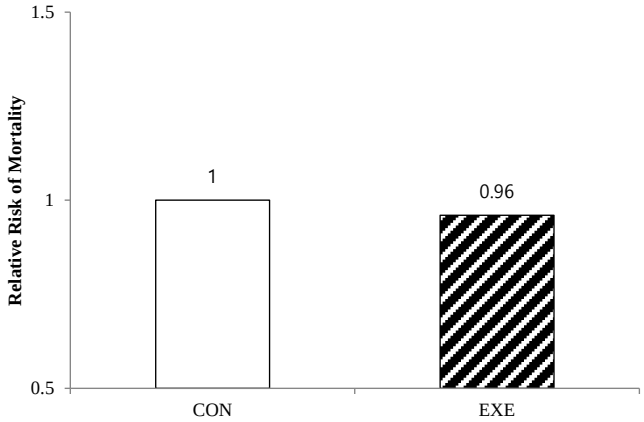

Figure 37. Relative risk of premature mortality

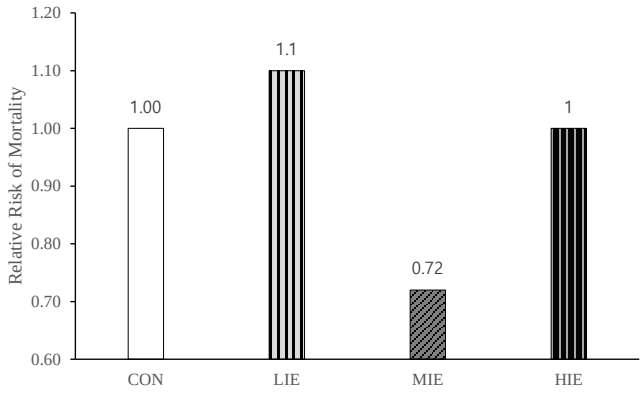

Figure 38. Relative risk of premature mortality by exercise intensity

4.2.2. The change of body weight

As shown in Table 3 and Figure 38, there was no significant differences among groups during whole period (p>.05). Interestingly, the weight of all mice decreased in the period of inducing colon cancer (9-wk of old to 15-wk of old), but as time goes by only the mice of MIE and HIE increased weight gain compare to CON and LIE. However, there is no statistical differences among groups (p>.05)

Table 3. The change of body weight during the second expreiment

weeks	6	8	10	12	14	16	20	24	28
Ex	1	3	5	7	9	11	15	19	23
						1 AOM	4 DSS	8	12
CON	33.83 ± 1.69	37.33 ± 1.60	38.52 ± 2.53	40.35 ± 2.52	40.47 ± 2.50	40.18 ± 3.39	43.48 ± 1.39	43.60 ± 1.39	40.60 ± 3.66
LIE	33.10 ± 1.56	36.80 ± 1.89	38.10 ± 1.35	39.16 ± 1.13	40.40 ± 1.82	38.40 ± 2.05	43.42 ± 1.80	39.60 ± 5.00	37.91 ± 4.60
MIE	32.33 ± 2.07	37.83 ± 2.48	39.90 ±2.62	42.20 ± 3.16	42.50 ± 2.72	37.02 ± 5.12	42.95 ± 2.77	41.33 ± 4.70	42.83 ± 4.89
HIE	33.33 ± 1.72	37.40 ± 2.25	41.13 ± 2.38	42.28 ± 2.50	42.07 ± 2.64	40.28 ± 2.34	42.18 ± 2.78	45.05 ± 2.67	44.83 ± 3.92

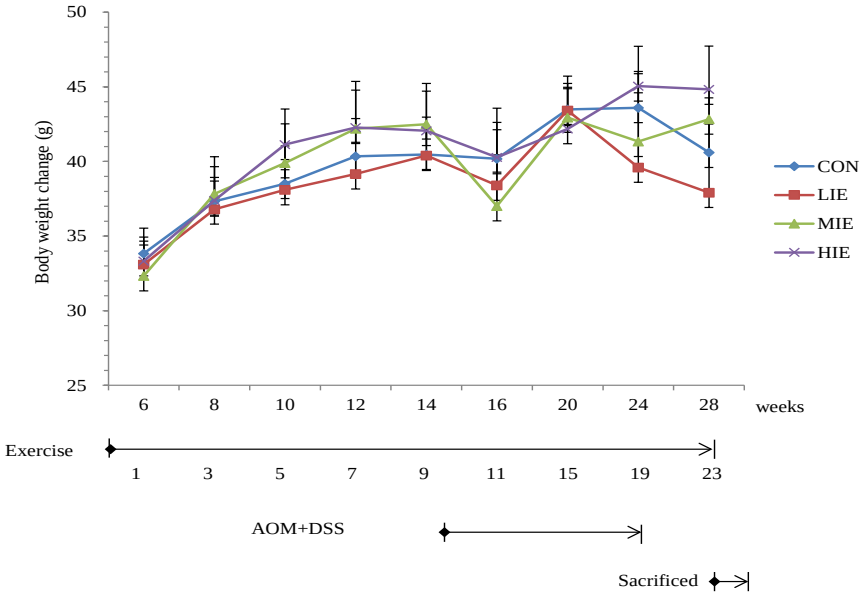

Figure 39. The change of body weight during the second experiment
Ex: Treadmill exercise, CON: Control group(no exercise), LIE: Low Intensity Exercise group, MIE: Moderate Intensity Exercise group, HIE: High Intensity Exercise group.

4.2.3. Muscle weight

In this study, gastrocnemius and quadriceps extracted in all groups, and measured weight. Each muscle weight was calculated by divide one of CON to normalize.

Firstly, to identify the preventive effect of exercise on cancer induced mice, the weight of muscle includes those of all exercise groups. As shown in Figure 13 and 14, there is no significant differences between CON and EXE in gastrocnemius and quadriceps muscle.

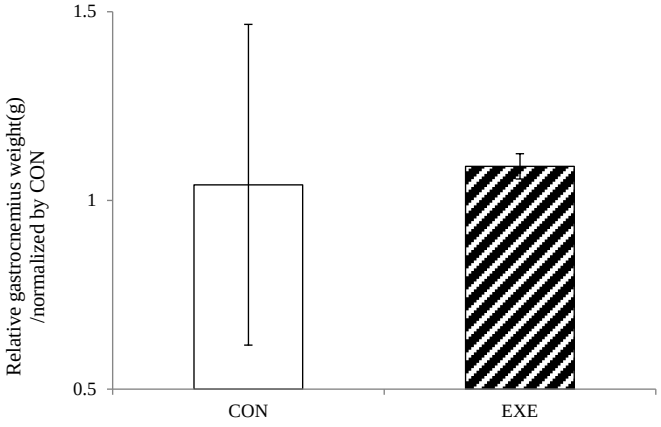

Figure 40. Relative weight of gastrocnemius

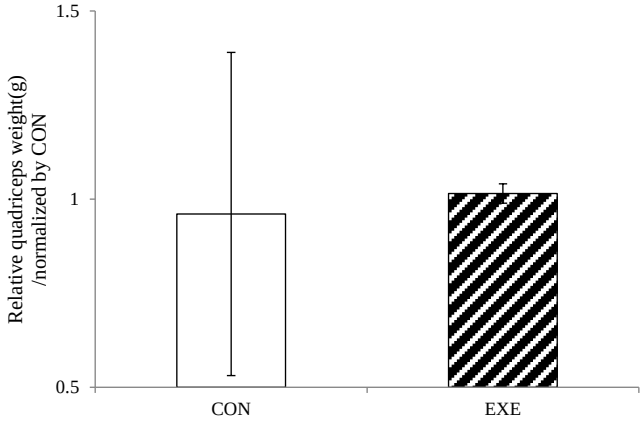

Figure 41. Relative weight of quadriceps

As shown in Figure 41 and 42, to investigate the effect of exercise intensity, the exercise groups divided by 3 groups; LIE, MIE, HIE. There is significant differences among groups in gastrocnemius muscle (p=.011), and the gastrocnemius weight of HIE is significantly higher than those of CON (p=.025), but there was no significant difference among groups in quadriceps muscle (p>.05).

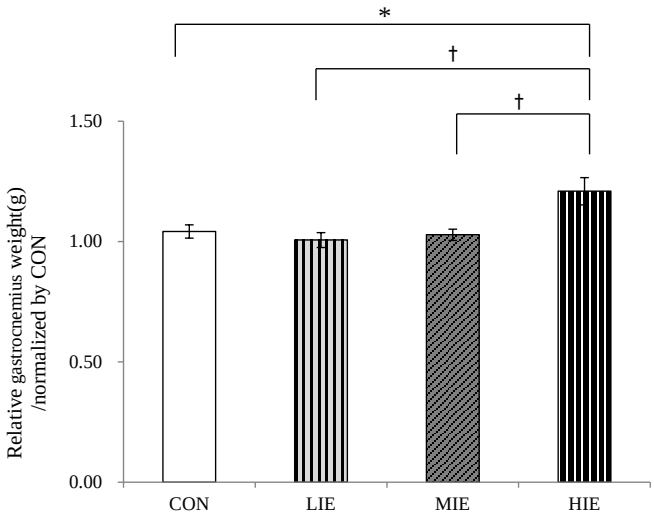

Figure 42. Relative weight of gastrocnemius by exercise intensity
All values are the mean ± SE (standard error of mean). *p<.05: CON vs LIE, MIE and HIE, §p<.05: LIE vs MIE and HIE, †p<.05: HIE vs LIE and MIE.

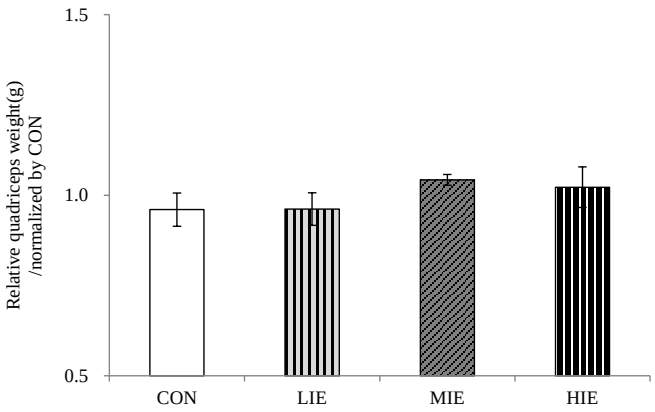

Figure 43. Relative weight of quadriceps by exercise intensity
All values are the mean ± SE (standard error of mean). *p<.05: CON vs LIE, MIE and HIE, §p<.05: LIE vs MIE and HIE, †p<.05: HIE vs LIE and MIE.

4.2.4. Muscle apoptosis

Exercise is associated with a number of whole-body and cellular adaptations that may provide protection against apoptotic signaling in skeletal muscle, include apoptosis-associated regulatory proteins, improved mitochondrial function, and decreased oxidative stress. In this study, to identify the effect of exercise on muscle wasting in cancer induced model, bcl-2(anti-apoptotic protein) and bax (pro-apoptotic protein) are measured in gastrocnemius.

As shown in Figure 43, bcl-2 level is significantly overexpressed in LIE (p=.009) and HIE (p=.015) compare to controls, and the level of bcl-2 in HIE is the highest among exercise groups. However as shown in Figure 44, there is no significant differences in the expression of bax among groups.

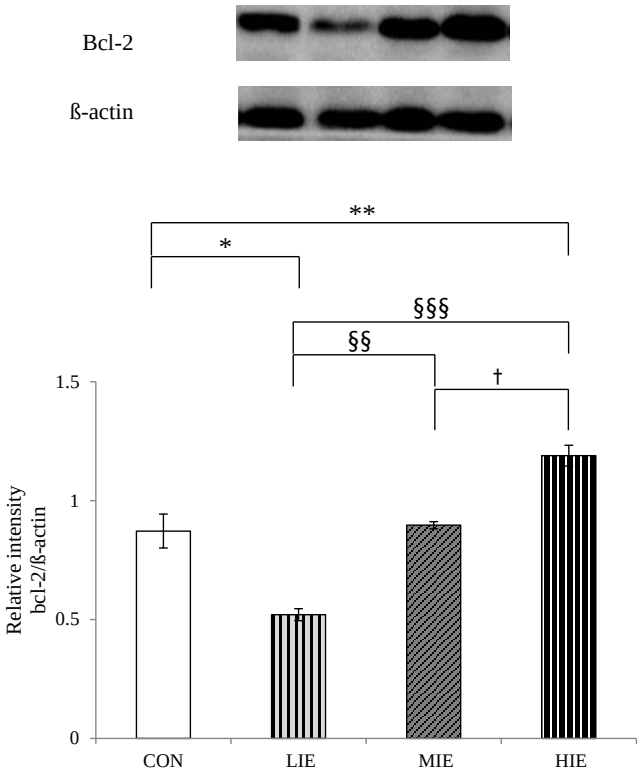

Figure 44. Bcl-2 level in gastrocnemius by exercise intensity
Western blot analysis was conducted to measure the levels of bcl-2. β-actin levels were measured to ensure equal amount of protein loading. The data were quantified by using image densitometric analysis. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

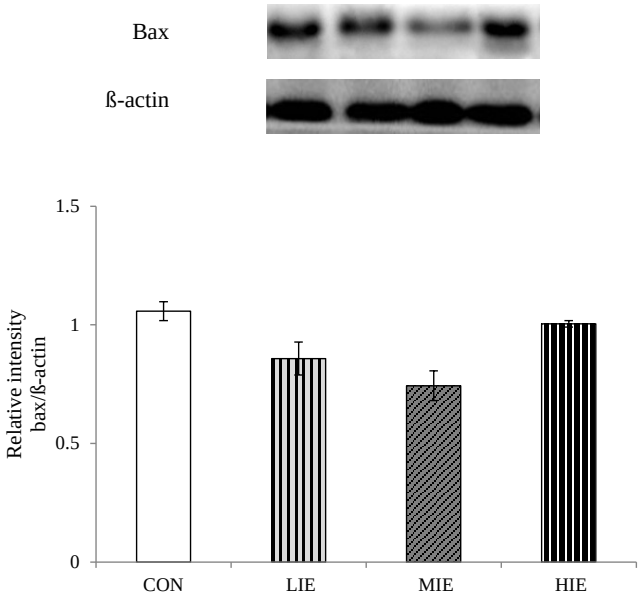

Figure 45. bax level in quadriceps by exercise intensity
Western blot analysis was conducted to measure the levels of bcl-2. β-actin levels were measured to ensure equal amount of protein loading. The data were quantified by using image densitometric analysis. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

4.2.5. The expression of OSM

Oncostatin M (OSM) is pleiotropic IL-6 family cytokine important in inflammation and other cellular processes. During oncogenesis, OSM often acts as an anti-proliferative factor for multiful type of cancers, and induce apoptosis through caspase activation.

Several studies indicate that OSM is overexpressed by exercise in several tissue including muscle, blood, spleen and it affects apoptosis pathway in breast cancer cell, but there is no study for colon cancer-induced model. Thus the present study is to investigate the effect of exercise on the expression of OSM in colon induced mice.

In this study, the expression of OSM measured in three different location; muscle, blood, and tumor tissue to identify the circulation of exercise-induced OSM. As shown in Figure 45, 46, and 47, the level of OSM in muscle is significantly overexpressed in exercise group compare to controls, however, not in blood and tumor tissue ($p>.05$).

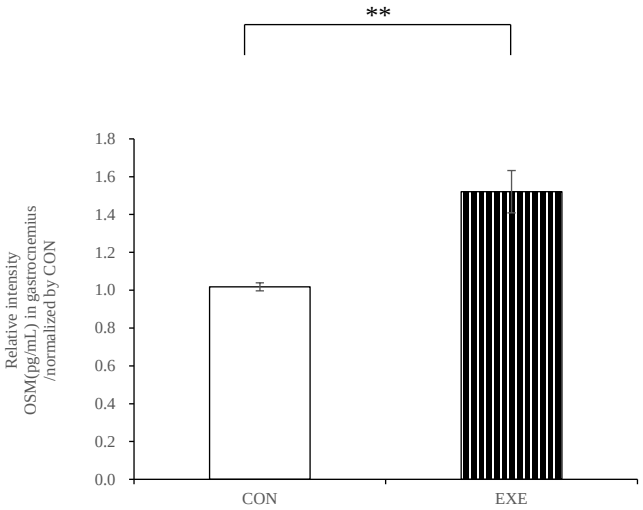

Figure 46. The expression of OSM in muscle
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

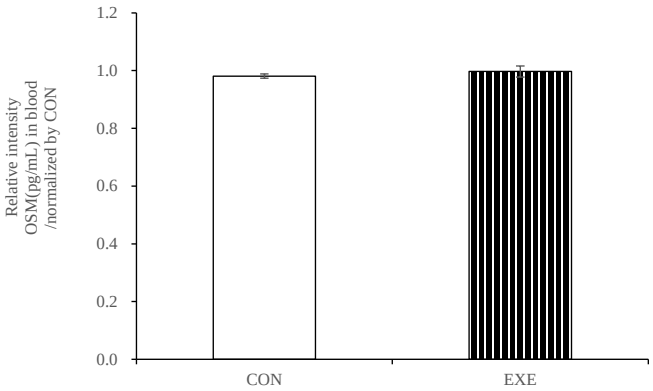

Figure 47. The expression of OSM in blood
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

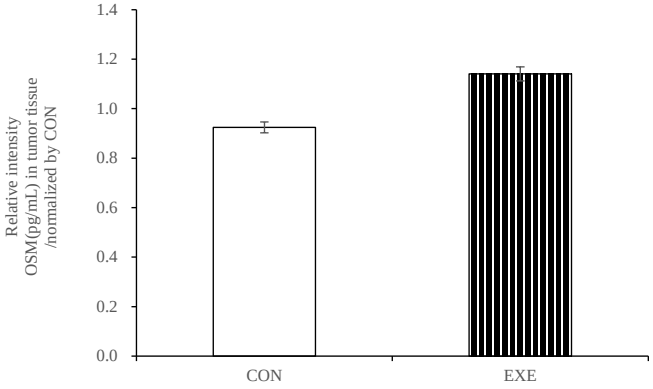

Figure 48. The expression of OSM in tumor tissue
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

Also, there is significant differences among exercise groups by exercise intensity on expression of OSM in muscle. The expression of OSM in muscle of LIE (p=.001) and MIE (p=.000) are significantly increased compare to controls. There is no significant difference between CON and HIE(p>.05). However, the expression of OSM in blood (Figure 49), was not increased by exercise intensity (p<.05), also levels of OSM in tumor tissue (Figure 50) tends to increase by exercise intensity, but there is no statistical difference (p>.05).

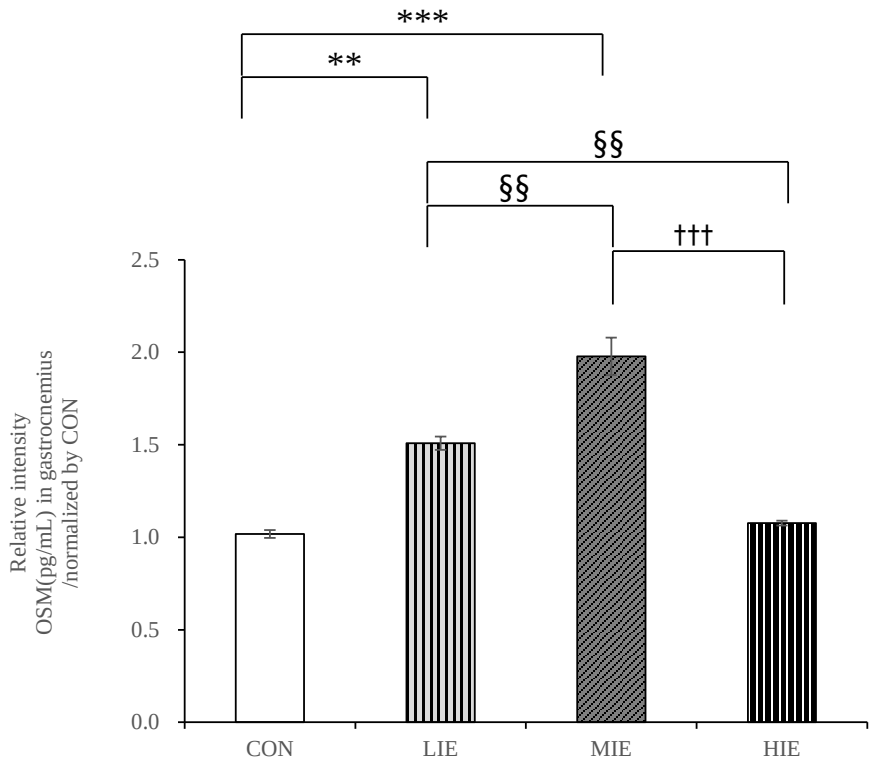

Figure 49. The expression of OSM in muscle by exercise intensity
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). **p<.01, ***p<.001: CON vs LIE, MIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE. †††p<.001: MIE vs HIE.

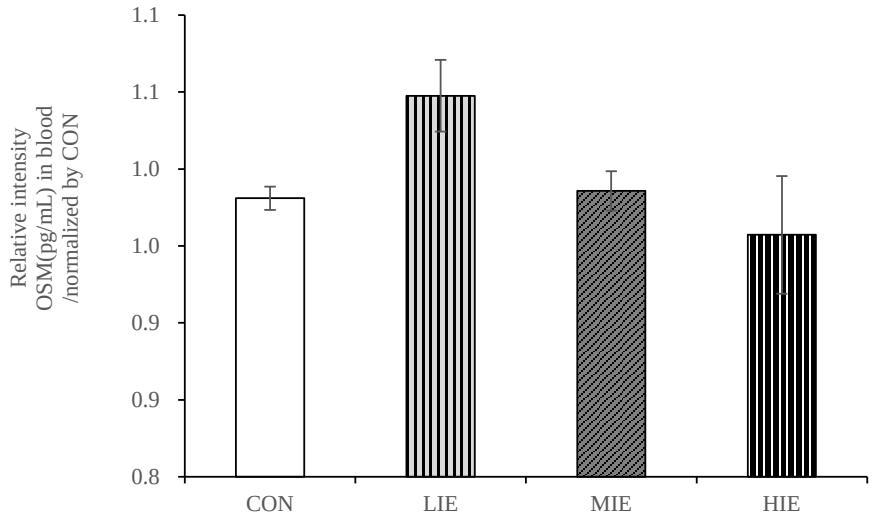

Figure 50. The expression of OSM in blood by exercise intensity
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean).

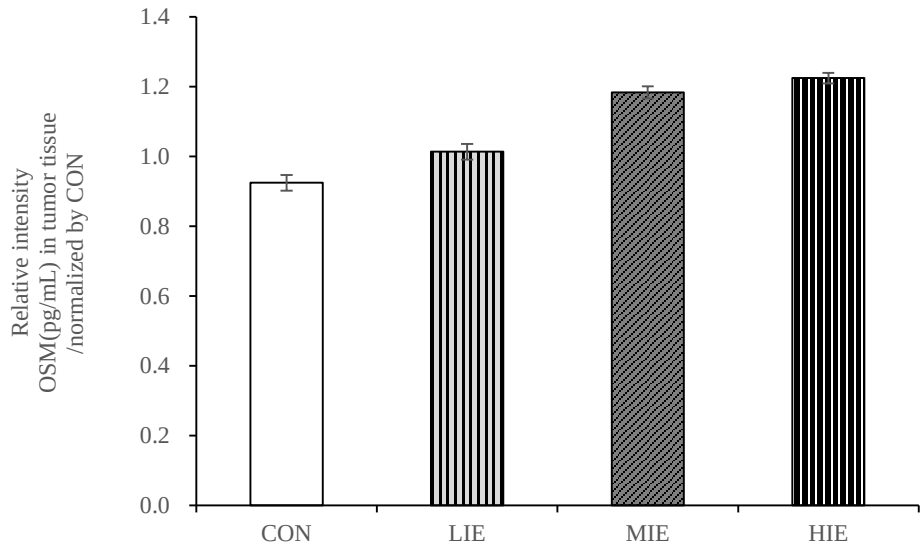

Figure 51. The expression of OSM in tumor tissue by exercise intensity
OSM activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean).

4.2.6. The expression of SPARC

Recently, the level of secreted protein and rich in cysteine (SPARC) was increased in blood of mice by a single bout of exercise. Furthermore, it is founded that SPARC is associated with apoptosis in colon mucosal cells though the activation of caspase-3. Thus it is suggested that exercise-induced elevation of SPARC could inhibits colon tumorigenesis.

Thus the present study is to investigate the long term effect of exercise on the expression of SPARC in colon induced mice, the level of OSM measured in three different location; muscle, blood, and tumor tissue to identify the circulation of exercise-induced SPARC.

In the present study, the level of SPARC was significantly increased by exercise in blood ($p=.002$), tumor tissue ($p=.001$) compare with controls, however, there is no significant difference in muscle ($p<.05$).

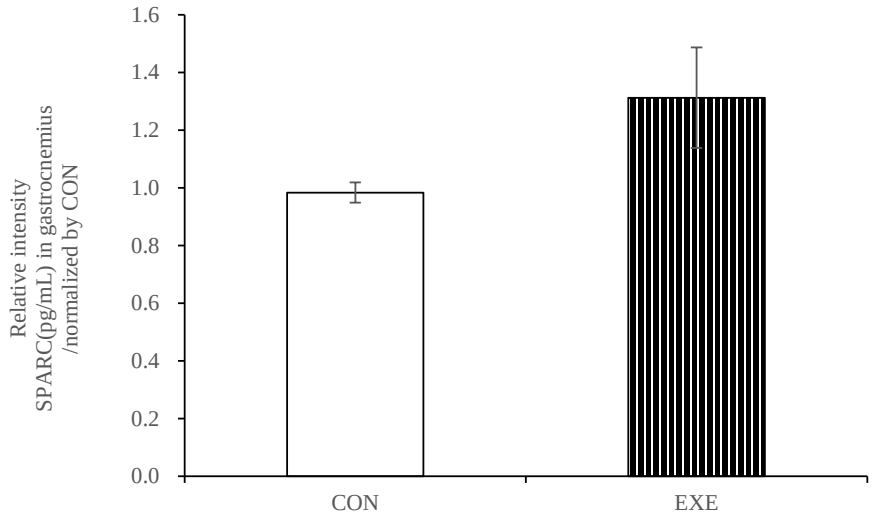

Figure 52. The expression of SPARC in muscle
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

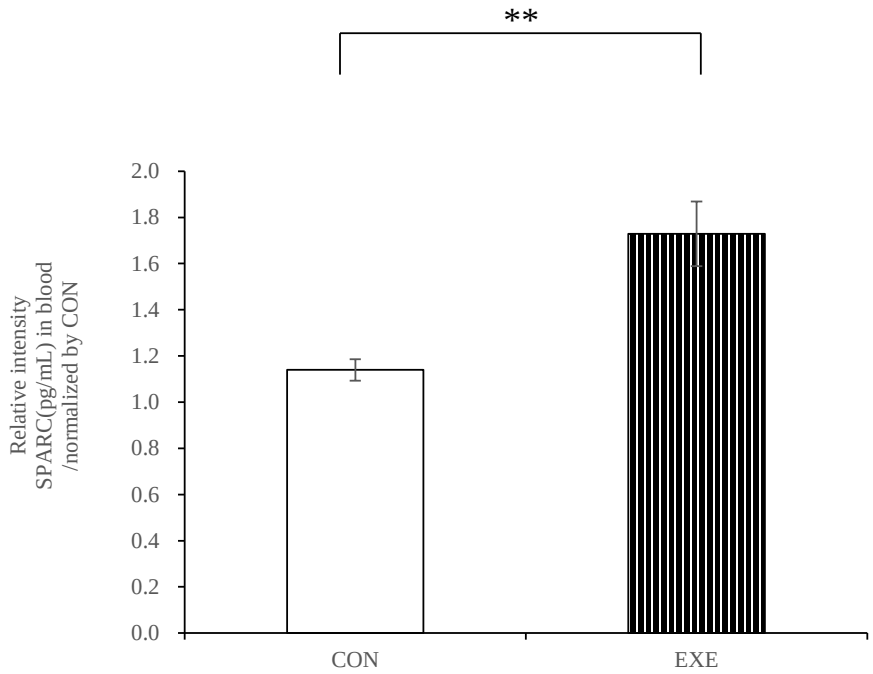

Figure 53. The expression of SPARC in blood
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

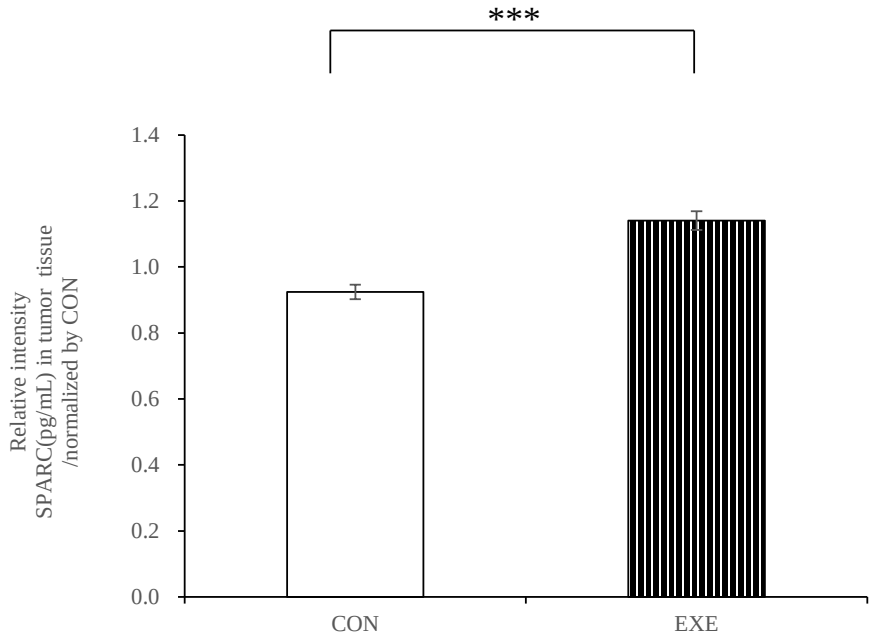

Figure 54. The expression of SPARC in tumor tissue
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

Also, there is significant differences among exercise groups by exercise intensity on expression of SPARC in colon-induced mice. The expression of SPARC in MIE is significantly higher than CON in gastrocnemius (p=.010), also the expression of SPARC in tumor tissue tends to increase significantly by exercise intensity; MIE (p= .001), HIE (p=.000). However, However, there is no significant difference in expression of SPARC in blood among exercise groups (p=.096) compare with controls.

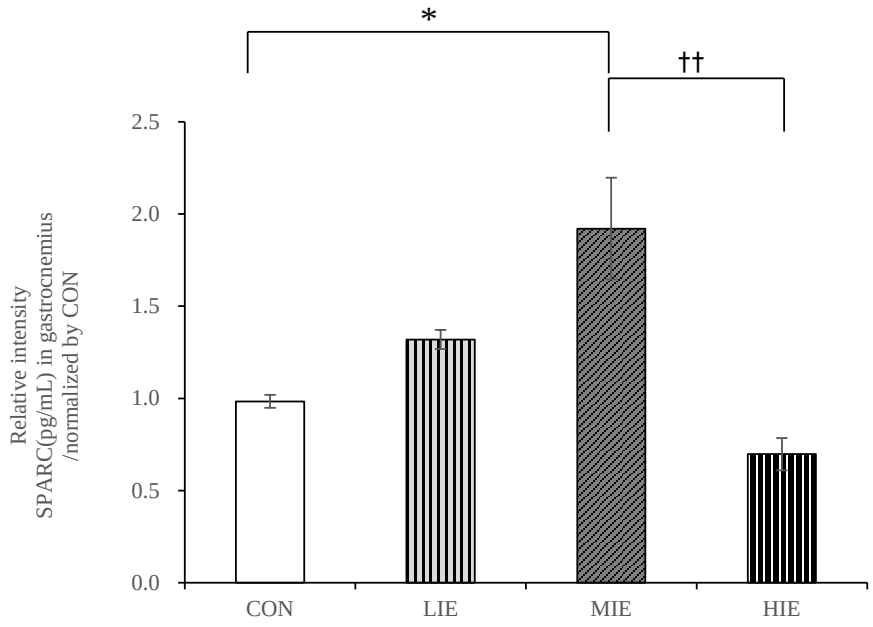

Figure 55. The expression of SPARC in muscle by exercise intensity
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05, **p<.01, ***p<.001 : CON vs LIE, MIE and HIE; §p<.05, §§p<.01, §§§p<.001: LIE vs MIE and HIE; †p<.05, ††p<.01, †††p<.001: MIE vs HIE.

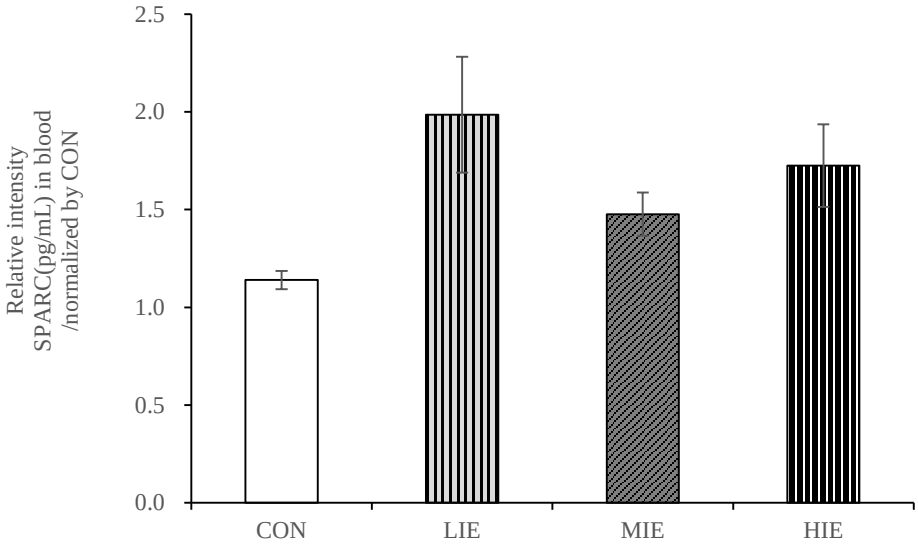

Figure 56. The expression of SPARC in blood by exercise intensity
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05, **p<.01, ***p<.001 : CON vs LIE, MIE and HIE; §p<.05, §§p<.01, §§§p<.001: LIE vs MIE and HIE; †p<.05, ††p<.01, †††p<.001: MIE vs HIE.

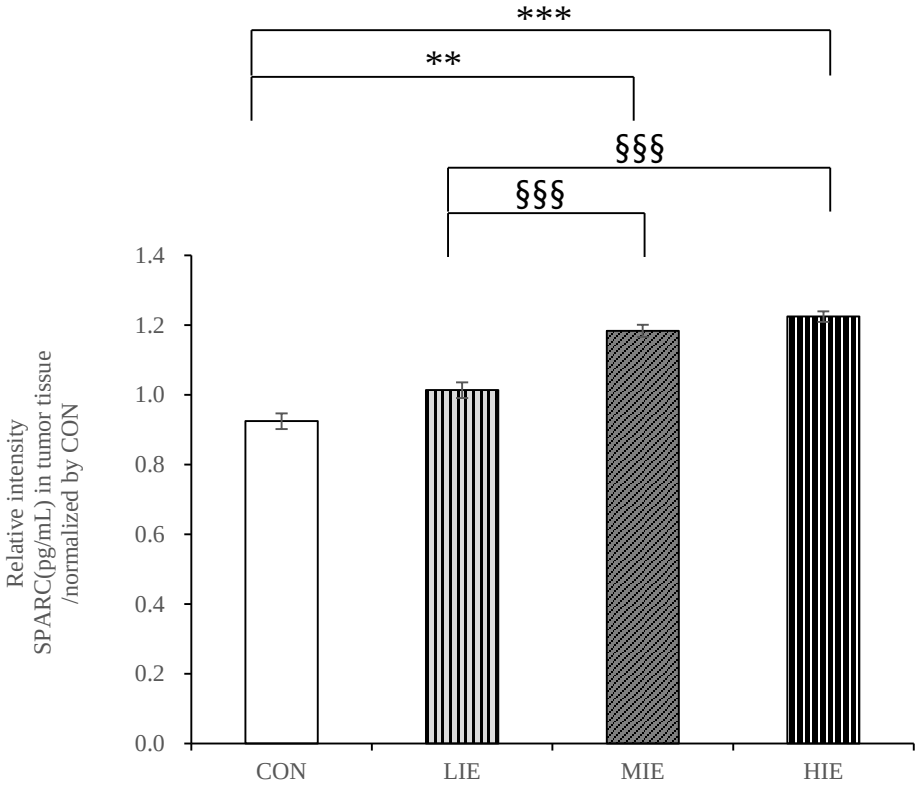

Figure 57. The expression of SPARC in tumor tissue by exercise intensity
SPARC activity as a percent of controls was quantified via sensitive ELISA. All values are the mean ± SE (standard error of mean). *p<.05. ***p<.001 : CON vs LIE, MIE and HIE, §§p<.01, §§§p<.001: LIE vs MIE and HIE, ††† p<.001: MIE vs HIE.

4.2.7. The expression of cleaved caspase-3

Caspase-3 has been identified as a key mediator of apoptosis in tumor cells. Also myokines such as SPARC and OSM is known that which induce tumor cell apoptosis through elevation of caspase-3 activity.

In this study, the expression of cleaved caspase-3 in tumor tissue tends to increase by exercise intensity, but there is no statistical difference (p>.05).

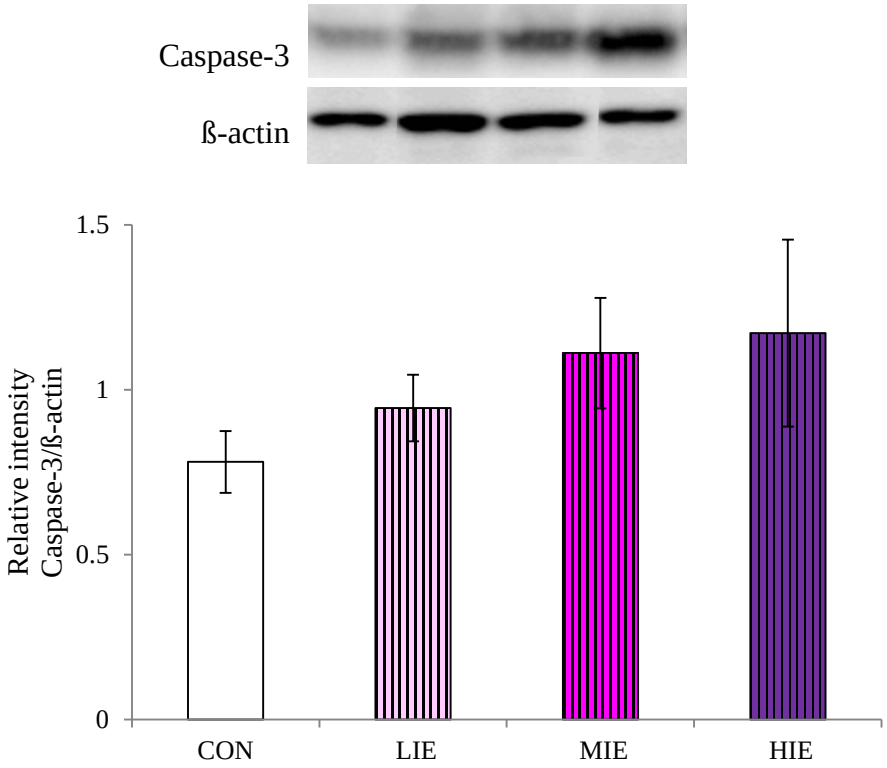

Figure 58. The expression of cleaved caspase-3 in tumor tissue.
Western blot analysis was conducted to measure the levels of caspase-3. β-actin levels were measured to ensure equal amount of protein loading. The data were quantified by using image densitometric analysis. All values are the mean ± SE (standard error of mean). *p<.05. **p<.01, ***p<.001.

V. Discussion & Conclusion

Despite the astonishing advancement in science and the modern medical system, cancer is one of the major causes of death, and its incidence and mortality rates are expected to continue to rise in the future. Based on a large body of evidence, one-third of cancers could be prevented by lifestyle change such as increase physical activity, and habitual dietary intake. Regular physical activity can significantly reduce the incidence of cancer by as much as 30% (Radak et al., 2002).

In particular, the protective effect of physical activity against colon cancer is consistent. High levels of physical activity may reduce the risk of colon cancer by as much as 50% (Colditz et al., 1997). Long-term physical activity or over 60 minutes of vigorous physical activity were associated with decreased risk of colon cancer (Slattery, Edwards, et al., 1997). A few animal studies have indicated that voluntary wheel running or treadmill exercise decreased the incidence of tumors in mice, and treadmill exercise for five days per week lowered tumor incidence and multiplicity in cancer-induced rats (Thorling, Jacobsen, & Overvad, 1994).

Several studies have also reported the therapeutic effects of exercise for cancer patients. A randomized clinical trial study showed that exercise reduced fatigue in cancer patients and improved the quality of life for patients with colon cancer. In particular, patients who engaged in at least 18MET-hours per week had lower mortality (Meyerhardt et al., 2009). Furthermore, regular treadmill exercise and anaerobic exercise can prevent tumorigenesis and reduce total polyp number and multiplicity in tumor-bearing rats (de Lima et al., 2008). However, the effect of exercise is complicated and the exact mechanism of exercise on cancer is not entirely understood.

In spite of that several mechanisms such as activation of the immune system, metabolic improvement, and exercise-induced increase in gastrointestinal transit speed have been proposed to explain the dose-response relationship observed between exercise and colon cancer risk. Inter alia, immune reactivity toward neoplastic cells is important for tumor initiation, promotion and progression. Researchers have demonstrated that exercise induces considerable

changes in the immune system by producing anti-inflammatory cytokines and reducing pro-inflammatory cytokines.

Recently some studies have demonstrated that cytokines are produced, expressed and released by skeletal muscle contraction, and these muscle-derived cytokines are collectively named myokines. Myokines act in a hormone-like fashion, exerting specific endocrine effects on distant organs. Skeletal muscle is therefore considered to be part of an endocrine organ. There are a large number of myokines including but not limited to, IL-6, IL-8, IL-10, OSM, SPARC, BDNF, Follistati-like-1. In addition, some researchers have suggested that myokines may play direct roles as tumor suppressors (Kos & Wilding, 2010). Secreted protein acidic and rich in cysteine (SPARC) is one of myokine, and plays multiple roles in cell adhesion, angiogenesis interaction with growth factors and cell differentiation. Furthermore, it was founded that SPARC is associated with antitumor effects via the activation of the apoptosis pathway (Tang & Tai, 2007).

In the present study, SPARC expression in colon cancer-induced mice was significantly increased by 12 weeks of treadmill exercise in muscle tissue (p=.000) and serum (p=.001). In line with the result, a relevant study has shown that a single bout of exercise increase SPARC expression in skeletal muscle and in human and mice serum (Aoi et al., 2013). At the initial stage of the study, the authors hypothesized that expression of myokine would differ by the intensity of exercise. There was indeed significant differences in SPARC expression in colon cancer-induced mice by exercise intensity.

The expression of SPARC tends to be higher in skeletal muscle (p=.001); LIE (p= .035), MIE (p=.001), HIE (p=.007), and the difference in SPARC expression in muscle was greater in moderate intensity exercise groups. However, there was no significant difference on SPARC expression in serum among exercise groups compared to a control group (p=.055). One explanation for the result was that the amount of some cytokines released from muscle is directly proportional to the amount of muscle being used; the more muscle contracted, the greater the response (Ostrowski et al., 1998; Ostrowski, Schjerling, & Pedersen, 2000). For instance, by increasing exercise intensity full-body muscle contraction enhances IL-6 release in muscle and serum from 20-to 100-fold over resting levels. The released IL-6 begins to exert

influence from the muscle to adipose tissue or immune cells, acting like a hormone rather than a cytokine (Ostrowski et al., 2000). Thus the amount of exercise stimulus is important for expression of myokines, and it may be the reason for varying expression of SPARC with different exercise intensities in the present study.

It is also interesting to note that the level of SPARC in tumor tissue was significantly increased by exercise as well as exercise intensity (p=.000); MIE (p=.000), HIE (p=.000) in the present study. This result was in contrast to a previous study showing that the expression of SPARC in other tissue (colon, liver, adipose tissue) was unaffected by exercise stimulus (Aoi et al., 2013). However, in the study, the mice performed just a single bout of exercise for 30 minutes, whereas the mice in the present study performed 12 weeks of treadmill exercise. A possible explanation for these inconsistent results is that repetition increases the SPARC expression in muscle during long-term exercise period. This could lead to the elevation of SPARC in colon tumor tissue as an endocrine activation. Another possible explanation is that the tumors produce SPARC in response to exercise stimulus. Many previous studies demonstrated that exercise improves the expression of proteins that mediate apoptosis in tumor tissue such as p53, caspase-3 and caspase-8 (Leung, Aronson, Ngo, Golding, & Barnard, 2004). Long-term exercise may therefore induce SPARC production in tumor cells, but the possible mechanisms were not evaluated in this study. There is no previous study focusing on the effect of long-term exercise on SPARC expression in tumor tissue, and this remains a topic for future study.

It has also been demonstrated that SPARC can act as a tumor suppressor by suppressing the formation of ACF in colon cells (Aoi et al., 2013; Tang & Tai, 2007). In previous studies, inactivation of SPARC was associated with tumorigenesis of colon, ovarian and pancreatic cancer (Puolakkainen, Brekken, Muneer, & Sage, 2004; N. Said & Motamed, 2005; Yiu et al., 2001). Increasing SPARC expression is a treatment method employed in cancer therapy (Cheetham et al., 2008). In addition, regular exercise significantly reduced the number of ACF and aberrant crypts (AC) in the colons of AOM-treated mice although exercise did not suppress the formation of ACF in AOM-treated SPARC-null mice. It is proposed that SPARC is activated by exercise stimulus and subsquently inhibits tumorigenesis. This suggestion is in agreement

with the results of the present experiment, where 12 weeks of treadmill exercise significantly decreased the incidence of polyp in the exercise group compared to the control group (p=0.03). The effect of exercise intensity was evaluated in MIE (p=.011) and HIE (p=.022), and compared to the control group. Furthermore, the expression of SPARC in tumor tissue was observed to increase with higher exercise intensity; MIE (p=.000), HIE (p=.000). This result suggests that higher exercise intensity induced more expression of SPARC in tumor tissue, which can lead to antitumorigenesis. The result is consistent with a previous study where recombinant SPARC was injected into AOM-treated mice, higher doses of SPARC injection suppressed more AC and ACF (Aoi et al., 2013).

In the present experiment, moderate intensity treadmill exercise increased the expression of cleaved caspase-3 in tumor tissue (p=.045). Caspase-3 has been identified as a key enzyme that mediates the final stage of cell death in apoptosis (Janicke et al., 1998). In a previous study, the levels of cleaved caspase-3 and caspase-8 were higher in the colon of AOM-treated mice than in AOM-treated SPARC-null mice (Aoi et al., 2013). Several studies have also indicated that SPARC is a key regulator of proliferation and apoptosis in cancer (Brekken et al., 2003; Puolakkainen et al., 2004; N. Said & Motamed, 2005; Tai & Tang, 2008; Tang & Tai, 2007). These findings are consistent with a tumor suppressor function of SPARC.

There is another myokine which mediates caspase-3 activity in tumor cells based on exercise stimulus. Oncostatin M (OSM) is a pleiotropic IL-6 family member that plays a role in inflammation and other cellular processes, and often acts as an antiproliferative factor for cancers (Bolin et al., 2012; Liu et al., 1997). It was reported that OSM derived from a variety of tissues inhibit proliferation of tumor cells (Grant & Begley, 1999; Hutt & DeWille, 2002; Liu et al., 2000). Similarly, another previous study indicated that OSM was overexpressed with exercise in several tissues including muscle, blood, and spleen. The study also reported that injection of the OSM into breast cancer cells decreased cancer cell proliferation and induced apoptosis through caspase activation (Hojman et al., 2011). The results are supported in the present study, where OSM was significantly overexpressed in the muscle (p=.000), blood (p=.005), and tumor tissue (p=.000) of the exercise groups compared to the control group. Also, the expression of OSM appears differently response to exercise intenstiy in muscle (p=.000);

LIE (p= .014), MIE (p=.000), HIE (p=.000), and in tumor tissue (p=.000); LIE (p= .000), MIE (p=.000), HIE (p=.000). There was a significant difference in the expression of OSM in blood of LIE (p=.000) compared to the control group. In other words, OSM was overexpressed by exercise stimulus in the muscle and tumor tissues of colon cancer induced mice and the expression of OSM was mediated by exercise intensity. Interestingly, the study also observed significant improvement in the expression of OSM in muscle and tumor tissue within the moderate intensity exercise group. The expression of caspase-3 in tumor tissue was highest in MIE. Thus, it is proposed that the amount of OSM could mediate caspase activity in tumor cells.

On a similar note, previous studies have shown a dose-dependent inhibition of breast cancer cells with OSM (Douglas et al., 1998; Liu et al., 1999). This supports the idea that SPARC and OSM were up-regulated by moderated exercise in the colon cancer induced mice, which led to higher expression of caspase-3. Although several studies have investigated the correlation of OSM with tumor tissue, they reported that overexpression of OSM in response to exercise or other stimulus was observed only in breast, ovarian, and lung cancer. The current study was the first to identify the expression of OSM in muscle and colon tumor tissue in response to exercise stimulus. The exact mechanisms of OSM and SPARC in tumor tissue remain to be identified in future studies.

The elevation of OSM and SPARC by exercise intensity might be influenced by the exercise protocol. According to several studies, exercise can induce the systemic and intestinal mucosa inflammations (Mehl et al., 2005). Moderate exercise induces stress hormone release and can decrease monocyte and anti-inflammatory cytokines such as, IL-6, TNF-α, and IL-1β (Franchimont et al., 1999). OSM is a pleiotropic cytokine belonging to the IL-6 superfamily, and SPARC can regulate inflammatory activity thorough IL-6 and prostanoids (N. A. Said, Elmarakby, Imig, Fulton, & Motamed, 2008) or IL-8 (Podhajcer et al., 2008). Altered inflammatory cytokines such as IL-6 and IL-8 could affect the expression of OSM and SPARC in muscle and tumor tissue. Furthermore, elevation of pro-inflammatory cytokines stimulates tumor initiation, promotion, and progression (Handschin & Spiegelman, 2008). Therfore, improved immune system function is also a potential exercise-induced mechanism for colon cancer prevention (Quadrilatero & Hoffman-Goetz, 2003). In the present study, other cytokines

such as IL-6, IL-8 and, IL-10 were not analyzed. The detailed mechanism underlying the effect of exercise on expression OSM and SPARC should be investigated in future studies.

Interestingly, the apoptosis pathway of muscle tissue was different from tumor tissue in colon induced mice. In the present study, Bcl-2 level was significantly overexpressed in the high intensity exercise group (p=.015) compared to the control group, but expression of Bax was not affected by exercise. This result is supported by other studies showing that 14 weeks of endurance training significantly elevated expression of Bax in muscle, but had no effect on Bcl-2 protein levels (Adhihetty, Taivassalo, Haller, Walkinshaw, & Hood, 2007). Eight weeks of treadmill running significantly increased Bcl-2 mRNA and protein, and decreased Bax and Apaf-1 protein in soleus muscle (Siu et al., 2004; Siu, Bryner, Murlasits, & Alway, 2005). Similarly 12weeks of treadmill running reduced cleaved caspase-3 protein levels and DNA fragmentation in rat gastrocnemius muscle (Song et al., 2006). Chronic exercise has been reported to decrease proapoptotic p53 potein levels in muscle (Qi, He, Zhang, Shao, & Ding, 2011). One possible explanation for this is that exercise is associated with a number of whole-body and cellular adaptations that may provide protection against apoptotic signaling in skeletal muscle, such as apoptosis-associated regulatory proteins, improved mitochondrial function, and decreased oxidative stress (Quadrilatero et al., 2011). Another possible explanation is that long-term exercise reduces ROS generation, improves antioxidant status, and decreases oxidative stress in skeletal muscle. These adaptations in redox status should serve to attenuate any ROS-induced activation of apoptosis in skeletal muscle. That is, regular exercise may represent an effective strategy to reduce or prevent apoptotic signaling during aging and disease(Quadrilatero et al., 2011).

In line with the previous results, regular exercise increased the weight of skeletal muscle in colon cancer-induced mice in the present study. The weight of the gastrocnemius muscle in the MIE (p=.002) of experiment 1, and in HIE (p=.025) of experiment 2, were significantly higher than the control group. One possible explanation of this result is that long-term treadmill exercise attenuated inhibition of skeletal muscle protein synthesis in cancer bearing mice via altered IGF-I, MuRF-1 and inflammatory cytokines (Lecker et al., 2004; Mehl et al., 2005). As noted, tumor-derived reactive oxygen species, inflammatory cytokines and elevated apoptotic

signaling in skeletal muscle cause muscle dysfunction, degradation, and atrophy. However, the protective effect of exercise on muscle wasting was shown by altered apoptotic protein expression in colon cancer-induced mice in the present study. Interestingly experiment 1 showed a negative correlation between gastrocnemius weight and polyp number (r=-438; p=.032). In general, cancer patients can lose up to 30% of their body weight, which causes 20%-33% of cancer deaths (Giordano et al., 2003). Muscle wasting is also associated with many other symptoms, such as chronic inflammation, fatigue, and metabolic disturbances in cancer patients (Ardies, 2002; Baltgalvis, Berger, Pena, Davis, & Carson, 2009). Maintaining body weight is therefore very important for cancer patients. In the present study, the mice with more muscle weight showed a lower number of polyps, suggesting that maintaining muscle weight by regular exercise might be very important for effective treatment in cancer patients.

The strength of this study was that it was the first study to test the effects of long-term exercise on the expression of OSM and SPARC in several tissues of colon cancer-induced mice. However, there are some limitations to the present study. Other cytokines or detail mechanism which might relate to OSM and SPARC were not estimated in this study. Therefore, future studies should investigate the mechanism through which exercise induces myokine or cytokine expression in cancer models.

In conclusion, the findings of this study provided strong support for the use of long-term exercise training as an approach to increasing OSM and SPARC in cancer models. The study showed that exercise induced overexpression of OSM and SPARC in the muscle, blood, and tumor tissue of colon cancer-induced mice. It is known that OSM and SPARC inhibit tumorigenesis, therefore, exercise may have a positive effect on the cancer model in this study. The magnitude of OSM and SPARC expression in muscle and tumor tissue was higher in the moderate and high intensity exercise groups than in the low intensity exercise group. Additionally, moderate and high intensity exercise enhanced the anti-apoptotic pathway in skeletal muscle, and increased gastrocnemius muscle weight. Finally, it was observed that the amount of polyp generation in moderate and high intensity exercise group was significantly decreased compared to the low exercise and control groups. The results of this study suggest that moderate and high intensity exercise have a protective effect on colon cancer-induced mice.

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ABSTRACT IN KOREAN

장기간의 운동이 대장암 유발 쥐의 마이오카인의 발현과 종양조직에 미치는 영향

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최근 많은 연구들에서 운동 자극에 의해 근육이 수축활동을 하면서, 골격근의 근섬유에서 사이토카인(cytokine)이 분비된다는 것이 밝혀졌다. 이러한 사이토카인은 골격근에서 생성 및 분비가 된다고 하여 마이오카인(myokine)으로 불린다. 인체 기관 중 가장 넓은 분포를 차지하는 근육은 이러한 마이오카인의 발현으로 다른 기관의 대사작용을 조절하는 내분비 기관으로써의 역할을 수행할 수 있을 것으로 제안되고 있다. 또한 운동에 의해 과발현이 되는 마이오카인은 운동의 효과를 규명할 수 있는 기전으로 여겨지고 있다.

마이오카인은 일반적으로 사이토카인의 역할로 알려진 면역체계나 대사작용을 조절하는 역할 뿐 아니라 최근 암을 예방하는 데에도 직접적인 중요한 역할을 하는 것으로 밝혀지고 있다. 일회성 운동 후 근육에서 생성된 마이오카인인 IL-6, IL-8, 1L-10, OSM, SPARC 등을 암 세포에 투ㅌ여한 결과 암 세포의 증식을 직접적으로 억제하거나 암 세포의 세포사멸을 촉진시키는 기전에 작용함으로써, 암에 대한 보호효과를 나타내는 것으로 보고되었다.

이러한 결과들을 바탕으로 본 연구의 목적은 장기간 다양한 강도의 운동을 통해 근육, 혈액, 종양 조직에서의 사이토카인의 발현 양상을 확인하고 종양 조직에서의 세포사멸을 확인함으로써, 이들 운동 유발성 사이토카인이 암에 대해 보호적인 역할을 할 수 있는 기전으로 작용하는 지를 규명하는 데 있다.

첫째, 암을 유발한 후 운동을 실시함으로써, 운동의 치료적 효과를 검증하기 위한 Experiment 1은 다음과 같이 진행되었다. 6주령의 ICR 마우스의 복강에 AOM을 1회 주입하고 3주에 걸쳐 DSS 희석 용액을 제공하는 방법을 통해 대장암을 유발한

후, 17주령에 저강도 (LIE, 10m/min), 중강도 (MIE, 18m/min), 고강도 (HIE, 27m/min) 운동군과 통제군으로 무선 배정한 후, 운동군들은 각각 하루 30분, 주 5회, 12주간에 걸쳐 트레드밀 러닝을 실시하였다. 마지막 운동 세션이 끝나고 24시간이 지난 후 분석을 위해 근육과 대장조직을 적출하였으며 혈액을 채취하였다. ELISA kit을 이용하여 근육, 혈액, 대장조직에서 OSM과 SPARC의 발현을 확인하였으며, 대장 종양조직에서 세포자멸을 촉진하는 것으로 알려진 cleaved Caspase-3의 발현을 western blotting을 통해 측정하였다.

Experiment 1의 결과는 다음과 같다. 장기간의 운동에 의해 근육 (p=.000), 혈액 (p=.005), 그리고 종양조직 (p=.000)에서 OSM의 발현이 유의하게 증가하였다 (p=.000). 운동 강도에 따른 차이를 살펴보면 근육에서의 발현은 LIE (p=.014), MIE (p=.000), HIE (p=.000), 종양조직에서는 LIE (p=.000), MIE (p=.000), HIE (p=.000)에서 과발현이 되었으며, 특히 중강도 운동군에서 그 발현량이 더욱 증가하는 것을 확인할 수 있었다. 하지만 혈액에서는 LIE (p=.000)에서만 유의하게 증가하였다. 또 다른 마이오카인인 SPARC 역시 운동 자극에 의해 근육 (p=.000), 혈액 (p=.002), 종양조직 (p=.001) 모두에서 과발현이 되었다. 운동 강도에 따른 차이를 살펴보면 근육에서는 LIE (p=.035), MIE (p=.001), HIE (p=.007)에서 과발현이 되었으며, 저강도보다는 중강도와 고강도 운동에서 유의한 증가 양상을 보였다. 종양조직에서는 MIE (p=.000), HIE (p=.000)에서 유의하게 과발현이 되었으며 고강도에서 더 유의한 증가 양상을 확인하였다. 한편 SPARC와 OSM에 의해 촉진된다고 알려진 세포자멸유도인자인 cleaved caspase-3의 발현은 MIE (p=.045)에서 유의하게 증가하는 것으로 확인되었다.

둘째, 장기간 운동을 실시하고 이 후 암을 유발시킴으로써 암에 대한 운동의 예방적 효과를 확인하기 위한 Experiment 2는 다음과 같이 진행되었다. 6주령의 ICR 마우스를 저강도 (LIE, 10m/min), 중강도 (MIE, 18m/min), 고강도 (HIE, 27m/min) 운동군과 통제군으로 무선 배정한 후, 운동군들은 각각 하루 30분, 주 5회, 12주간에 걸쳐 트레드밀 러닝을 실시하였다. 이 후 17주령에 모든 쥐들을 AOM과 DSS 방법으로 대장암을 유발시키고 암이 유발되는 과정 중에도 12주 동안 추가적으로 운동을 지속적으로 실시하였다. 마지막 운동세션이 끝나고 24시간 후, 근육과 대장조직을 적출하고 혈액을 채취하여 OSM과 SPARC 그리고 cleaved Caspase-3의 발현을 분석하였다.

Experiment 2의 결과는 다음과 같다. 장기간 운동을 통해 근육에서의 OSM 발현은 유의하게 증가하는 것으로 나타났으나, 혈액과 종양조직에는 유의한 차이가 나타나지 않았다. 운동 강도에 따른 발현 차이를 살펴보면 근육에서는 LIE (p=.001), MIE (p=.000)에서 유의하게 과발현이 되었으며, 특히 중강도 운동군에서 그 발현량이 가장 큰 것으로 나타났다. 하지만 혈액과 종양조직에서는 운동 강도에 따른 OSM의 발현 차이가 나타나지 않았다. SPARC의 발현은 운동 자극에 의해 혈액

(p=.002)과 종양조직 (p=.001)에서 유의하게 증가하는 양상을 보였다. 운동 강도에 따른 차이를 살펴보면 근육에서는 MIE (p=.01)에서 종양조직에서는 MIE (p=.001), HIE (p=.000)에서 유의하게 증가하는 것을 확인하였으며, 특히 고강도 운동군에서 보다 높은 발현량을 확인할 수 있었다. 하지만 혈액에서는 운동강도에 따른 SPARC의 발현차이를 확인할 수 없었다. 종양 조직의 세포자멸을 확인한 결과 cleaved Caspase-3의 발현은 운동강도에 따라 증가하는 경향을 보였으나 통계적인 차이는 나타나지 않았다 (p>.05)

결론적으로, 치료적 효과의 목적으로 실시된 운동은 근육과 종양조직에서 OSM과 SPARC의 발현을 중강도와 고강도 운동에 의해 더욱 증가 시켰으며, 또한 종양 세포자멸인자인 cleaved caspase-3의 발현은 중강도 운동군에서 유의하게 증가하였다. 한편 폴립의 생성 수를 확인 한 결과 중강도 (p=.011)와 고강도 (p=.022) 운동군에서 현저하게 감소하였으며, 암의 영향으로 감소한다고 알려진 근육양도 중강도 (p=.002) 운동군에서 근육의 세포자멸을 억제하는 인자인 Bcl-2의 발현도 고강도 (p=.002) 운동군에서 유의하게 높게 발현이 되는 것을 확인할 수 있었다. 본 실험에서 얻어진 결과를 통해 유추해보면, 암에 대한 치료적 효과를 위해 실시한 운동은 중강도와 고강도의 운동이 저강도 운동보다 효과적이었음을 제안할 수 있다.

반면, 암에 대한 예방적 효과를 검증하기 위해 실시된 두 번째 실험에서는, OSM은 저강도와 중강도 운동에 과발현이 되었으나 SPARC는 중강도와 고강도 운동 자극에 발현량이 더욱 증가되었다. 한편 암을 유발한 후 31주령에 분석을 위해 희생되기 전까지의 조기사망률을 확인한 결과 운동은 암에 의해 조기사망률을 낮추었으며 (RR=0.96), 특히 중강도 (RR=0.72) 운동군에서 가장 낮은 사망률을 보였다. 이와 같은 결과를 통해 암을 예방하고자 실시하는 운동은 다른 강도에 비해 중강도 운동이 보다 효과적이었음을 제시할 수 있으나, OSM과 SPARC이 치료적 효과와는 다른 메커니즘으로 작용한 것으로 사료된다.

따라서 운동은 다양한 조직에서 종양조직의 세포자멸을 촉진하는 OSM과 SPARC의 발현을 증가시킴으로써 암에 대한 보호적 역할을 할 수 있을 것으로 사료된다.

주요어 : 마이오카인, 운동, 암, SPARC, OSM, Apoptosis.
학 번 : 2009-31035