

Review

Skeletal Muscle, Myokines and Health

Halil Duzova

Inonu University School of Medicine, Department of Physiology, Malatya, Turkey

Abstract

Accumulating epidemiological data suggest that a physically active life plays an independent role in the protection against metabolic syndrome, type 2 diabetes, cardiovascular diseases, cancer and dementia. For most of the last century, researchers sought a link between muscle contraction and humoral changes in the form of an 'exercise factor', which could be released from skeletal muscle during contraction and regulate some of the exercise-induced metabolic changes in other organs such as the liver, the adipose tissue and other tissues. Researchers have indicate that cytokines or other peptides that are produced, expressed and released by muscle fibers exert autocrine, paracrine or endocrine effects should be classified as 'myokines'. Given that skeletal muscle is the largest organ in the human body, researchers' discovery that contracting skeletal muscle secretes proteins sets a novel paradigm: skeletal muscle is an endocrine organ producing and releasing myokines, which work in a hormone-like model, exerting specific endocrine effects on other organs. Other myokines work via paracrine mechanisms, exerting local effects on signaling pathways involved in muscle metabolism. It has been shown that skeletal muscle has the capacity to express several myokines including tumor necrosis factor-- α (TNF- α), brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6), IL-8, IL-15 and IL-17. Moreover, myokines contribute to exercise-induced protection against several chronic diseases. Myokines such as IL-6 is likely to show the anti-inflammatory effects of exercise and inhibit low-level TNF- α production. By this ways, TNF- α -induced insulin resistance reveals an important role in mediating the beneficial health effects of exercise.

Keywords: Exercise, IL-6, IL-8, IL-15, IL-17, myokine, TNF- α

(Rec.Date: Agu 15, 2012 - Accept Date: Agu 29, 2012)

Corresponding Author: Halil Düzova, Department of Physiology. School of Medicine İnönü University, 44280 Malatya, Turkey
Tel: + 90 422 341 06 60 / 1274 Fax: + 90 422 341 00 36
E-Mail: halil.duzova@inonu.edu.tr or hduzova@hotmail.com

Introduction

For most of the last century, researchers sought a link between muscle contraction and humoral changes in the form of an 'exercise factor', which could be released from skeletal muscle during contraction and regulate some of the exercise-induced metabolic changes in other organs such as the liver, the adipose tissue and other tissues. Last 25 years witnessed to the considerable exercise induces changes in the immune system [1]. Likewise, Pederson suggested that skeletal muscle has been identified as an endocrine organ that produces and releases cytokines and other peptides, named "myokines." Given that the skeletal muscle is the largest organ in the human body, Pedersen and coworkers recently showed that contracting skeletal muscle as a cytokine-producing organ sets a novel paradigm: skeletal muscle is as an endocrine organ producing and releasing myokines in response to contraction, which can influence metabolism in other tissues and organs [1-4]. Myokines expand our knowledge on how the nervous, endocrine, and immune systems contribute to the maintenance of homeostasis, also when challenged by physiological demands such as exercise condition [3]. The relations between exercise and the immune system provided a unique opportunity to evaluate the role of underlying endocrine and cytokine mechanisms. In an attempt to understand the mechanisms underlying exercise-induced changes in the distribution and concentrations of lymphocyte subpopulations, Pedersen and coworkers besides others focused on cytokines and their possible roles as a link between muscle contractions and cellular immune changes. It has, therefore, been suggested that cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert paracrine, autocrine, or endocrine effects should be classified as "myokines." With the discovery that exercise provokes an increase in a number of cytokines, a possible link among skeletal muscle contractile activity, immune changes and chronic diseases were established [1,4].

The identification of skeletal muscle as a cytokine-producing organ soon led to the discovery that muscle-derived cytokines (which they have named myokines play a role in mediating the exercise-associated metabolic changes), as well as the metabolic changes after training adaptation. Pederson and coworkers identified and quantitatively analyzed 635 secreted proteins, including 35 growth factors, 40 cytokines, and 36 metalloproteinases [4]. It appears that skeletal muscle has the capacity to express several myokines including tumor necrosis factor- α (TNF- α), brain-derived neurotrophic factor (BDNF), interleukin-6 (IL-6), IL-8, IL-15

[4] and IL-17 [5]. The metabolic actions of these myokines are also especially in relationship to energy metabolism and insulin signaling. Contractile activity plays a role in regulating the expression of many of these cytokines in skeletal muscle [4].

Pedersen proposed that muscles produce and release myokines provides a conceptual basis for understanding some of the molecular mechanisms underlying organ cross talk, including muscle–liver and muscle–fat cross talk [4]. The interactions between exercise and the immune system enable the a unique opportunity to evaluate the role of underlying endocrine and cytokine mechanisms. The identification of skeletal muscle as a cytokine-producing organ soon led to the discovery that muscle-derived cytokines could account not only for exercise-associated immune changes, but also played a role in mediating the exercise associated metabolic changes, as well as the metabolic changes following training adaptation. Recent findings demonstrate that physical activity induces an increase in the systemic levels of a number of cytokines with anti-inflammatory properties [2, 6]. In this regard, regular aerobic exercise (training) exerts a suppressing effect on low grade chronic systemic inflammation [7].

Physical inactivity has been identified as a stronger predictor of these chronic diseases than risk factors such as hypertension, hyperlipidemia, diabetes, and obesity for all-cause mortality. Moreover, regular physical activity shown to be protect against premature death independent of obesity. Regular physical activity and weight loss offers protection against, and may be useful as a treatment for a wide variety of chronic diseases associated with low-grade inflammation, as a result of an improved inflammatory profile, that is, a decrease in the pro-inflammatory molecules and an increase in the anti-inflammatory molecule [8]. Gleeson et al [9] reported that mice studies have revealed that the anti-inflammatory effects of exercise also relied on other mechanisms, such as the inhibition of monocyte and macrophage infiltration into adipose tissue and the phenotypic switching of macrophages within adipose tissue. The protective effects of regular exercise against diseases such as cardiovascular disease, type 2 diabetes, colon cancer, inflammatory bowel disease and breast cancer are well-established [1].

IL-6

Typically IL-6 is the first identified cytokine (myokine) to respond to exercise in the year 2000 [1]. It derives predominantly from the contracting skeletal muscle [10-11], although other studies have demonstrated that skeletal muscle is not the sole source of exercise-induced IL-6. Other sources of IL-6 include connective tissue, the brain and adipose tissue [12]. The literature indicates that white adipose tissue is responsible for 30% or more of the circulating IL-6 at rest [9, 13-14]. Those concentrations of IL-6 in the interstitial fluid of adipose tissues are markedly higher than those in the circulation [13], but only about 10% of this can be attributed to the adipocytes with the remainder coming mostly from adipose tissue-resident macrophages [9]. Lira et al reported absence of difference in IL-6 concentration in rat adipose tissue between sedentary and trained animals, and in obese women, long-term exercise was also unable to affect IL-6 gene expression in the adipose tissue [15].

It is well recognized that contracting skeletal muscle may synthesize and release IL-6 into the interstitium as well as into the systemic circulation in response to about of exercise [4] without muscle damage has been a remarkably consistent finding [16]. Steensberg et al [17] showed that IL-6 mRNA increased after only 30 min of knee-extensor exercise and peaked at the cessation of exercise, when values were 100-fold higher compared with rest. Although several sources of IL-6 have been demonstrated, contracting muscles contribute to most of the IL-6 present in the circulation in response to exercise [4]. The magnitude of the exercise-induced IL-6 response is dependent on the exercise intensity, especially duration [4, 16], the mass of muscle recruited, and one's endurance capacity, whereas the mode of exercise has little effect. Eccentric exercise may result in a delayed peak and a slower decrease of plasma IL-6 during recovery in response to exercise [1, 12]. Eccentric exercise is not associated with a larger increase in plasma IL-6 than exercise involving concentric "non damaging" muscle contractions [1]. Regular physical training is reported to attenuate such a response [16]. Namely, several epidemiological studies have reported a negative association between the amount of regular physical activity and the basal plasma IL-6 levels: the more physically active, the lower the basal plasma IL-6. Although plasma IL-6 appears to be down regulated by training, the muscular expression of the IL-6 receptor appears to be up regulated. Accordingly, it is possible that the down regulation of IL-6 is partially counteracted by an enhanced expression of IL-6R. This finding has made us speculate that IL-6 resistance exists

as a biological phenomenon. If the amount of IL-6 receptor on the surface of muscle fibers reflects enhanced IL-6 signaling, it appears that healthy, well-trained humans are more sensitive to IL-6, whereas untrained people have impaired IL-6 signaling and compensatory high circulating IL-6 levels [6].

The exercise-induced increase of plasma IL-6 concentrations is not linear over time; repeated measurements during exercise show an accelerating increase of the IL-6 in plasma in an almost exponential manner [1, 4, 9] with exercise duration [4,9]. Furthermore, the peak IL-6 level is reached at the end of the exercise or shortly thereafter, followed by a rapid decrease toward pre exercise levels [1, 4]. It is well characterized in the literature that after an acute session of physical exercise there is an increase in the concentration of IL-6 (above 100-fold) depends on exercise variables such as intensity, duration, recruited muscle mass and individual aerobic capacity [7]. Pedersen (2012) showed that an increase of the IL-6 mRNA content is detectable in the contracting skeletal muscle after 30 min of exercise and up to 100-fold increases of the IL-6 mRNA content may be present at the end of the exercise bout [4].

Pedersen (2003) et al reported that there is a correlation between the intensity of exercise and the increase in plasma IL-6. Peak plasma IL-6 during exercise has been shown to correlate with plasma lactate. Exercise induces a pronounced increase in the production of IL-6 and that IL-6 infusion also inhibits [10]. In contrast, the IL-6 response is sensitive to the exercise intensity, which again indirectly represents the muscle mass involved in the mechanical work, and the endurance capacity [3]. Since contracting skeletal muscle per se is an important source of IL-6 found in the plasma, muscles of the upper extremities may be insufficient to increase plasma IL-6 above preexercise level. In contrast, running, which involves several large muscle groups, is the mode of exercise where the most dramatic plasma IL-6 increases have been observed. In fact, more than 50% of the variation in plasma IL-6 following exercise can be explained by exercise duration alone [1].

Thus, muscle biopsies obtained before and after exercise in humans and rats demonstrate very low IL-6 mRNA in resting muscle, but up to a 100- fold increase in exercising skeletal muscle [2]. Small amounts of IL-6 protein produce predominantly in type I fibers. In response to muscle contractions, both type I and type II muscle fibers express the myokine IL-6. In response to exercise, an increase of the IL-6 mRNA content in the contracting skeletal

muscle. In addition, assessment of the interstitial IL-6 concentration using microdialysis indicates that the concentration of IL-6 within the contracting skeletal muscle may be 5- to 100-fold higher than the levels found in the circulation [1]. Somewhat paradoxically, however, in our recent study both IL-6 mRNA and protein during prolonged (120 min) moderate intensity (approximately 55% of peak oxygen consumption; $\dot{V}O_{2peak}$) exercise was almost exclusively expressed in type 2 muscle fibers. Because during this type of exercise type I muscle fibers are preferentially recruited, this resulted in an inverse relationship when comparing fiber-specific IL-6 expression and glycogen content. The relationship between glycogen and IL-6 was observed in mixed muscle biopsy samples; therefore, we can interpret these recent data as follows: as glycogen becomes depleted in type I fibers during prolonged exercise, type 2 fibers are serially recruited to maintain force [18]. Both intramuscular IL-6 mRNA expression and protein release are exacerbated when intramuscular glycogen is compromised, suggesting that IL-6 is somehow related to glycogen content [1, 4, 9, 18]. The trained skeletal muscle is less dependent on plasma glucose and muscle glycogen as substrate during exercise. Several epidemiological studies have reported a negative association between the amount of regular physical activity and the basal plasma IL-6 levels: the more physically active, the lower basal plasma IL-6. High plasma levels of IL-6 are closely associated with physical inactivity and metabolic syndrome. Moreover, basal levels of IL-6 are reduced after training [4].

Pedersen (2012) suggested that muscular IL-6 has a role in metabolism rather than in inflammation. Skeletal muscle during physical activity generated renewed interest in the metabolic role of IL-6 because it created a paradox [4]. On one hand, IL-6 is markedly produced and released in the post-exercise period when insulin action is enhanced but, on the other hand, IL-6 has been associated with obesity and reduced insulin action [1]. In vivo, experiments demonstrated that IL-6 may increase basal and insulin-stimulated glucose uptake via an increased GLUT4 translocation. The main effect of IL-6 on insulin-stimulated glucose metabolism is likely to occur in peripheral tissues (e.g., skeletal muscle and adipose tissue), whereas IL-6 does not influence glucose output from the liver [3]. Moreover, IL-6 increased insulin-stimulated glucose uptake in vitro. IL-6 knockout mice develop mature onset obesity and glucose intolerance. IL-6 seemed to play a role in endogenous glucose production during exercise in humans [4]. As these fibers are recruited, they transcribe and ultimately produce IL-6. IL-6 may activate the AMP-activated kinase 5'-AMP-activated protein kinase (AMPK),

is a fuel-sensing enzyme that is activated by changes in the energy state of the cell, as well a number of adipokines and hormones, including adiponectin and leptin [18]. Studies have demonstrated that into humans to obtain physiological concentrations of rhIL-6 infusion or incubation results in lipolysis and fat oxidation both in healthy humans in vivo and in skeletal muscle in vitro [4, 18]. IL-6 alone increases both lipolysis and fat oxidation; identify IL-6 as a lipolytic factor. Conversely, rhIL-6 infusion caused an increase in skeletal muscle unidirectional fatty acid and glycerol release. Acute increase in IL-6 at a normphysiological level primarily stimulates lipolysis in skeletal muscle, whereas adipose tissue is unaffected [4].

Nielsen and Pedersen (2008) suggested that IL-6 induced increased levels of plasma cortisol. Consequently, this effect is an increase in circulating neutrophils and a decrease in the lymphocyte number without effects on plasma epinephrine, body temperature, mean arterial pressure, or heart rate. Both exercise and IL-6 infusion suppress TNF- α production in humans [19]. Though an acute bout of physical activity is accompanied by responses that in many respects are similar to those induced by infection and sepsis, there are some important differences in the cytokine response to exercise from that elicited by severe infection? The striking difference between exercise and sepsis with regard to cytokine responses is that the classical pro-inflammatory cytokines, TNF- α and IL-1 β , in general do not increase with exercise. Interleukin-6 is most often classified as a pro-inflammatory cytokine, although data also suggest that IL-6 regulated acute phase proteins are anti-inflammatory and immunosuppressive, and may negatively regulate the acute phase response [2].

Gleeson (2007) suggested that elevated systemic levels of IL-6 during and following exercise could be one of the mechanisms by which regular exercise provides protection against the development of chronic diseases [20]. Elevations of IL-6 do not occur with short durations of low to moderate intensity exercise despite the known health benefits (for example, reduced risk of heart disease) associated with only very moderate increases in physical activity above that of a sedentary lifestyle [9]. Namely, Gleeson reported that it could be argued that the relative importance of IL-6 in this context is likely to be rather small, as significant health benefits of regular exercise are apparent even when the exercise is of light-moderate intensity and not prolonged (e.g., brisk walking for 1 h/day). Such exercise is not associated with substantial elevations of circulating IL-6. Individuals who are physically active on a regular

basis have a reduction in the levels of biomarkers that are used to assess systemic inflammation [20].

IL-8

Gray and Kamolrat showed that skeletal muscle contractile activity increases the production of the myokines interleukin- 8 (IL-8) [21]. Pedersen and Febbraio reported that a high local IL-8 expression takes place in contracting muscle with only a small and transient release indicates that muscle-derived IL-8 exerts its effect locally [1]. IL-8 from contracting skeletal muscle is regulators of energy metabolism and angiogenesis by promoting angiogenic responses in endothelial cells. The IL-8 produced by the exercising limb might elicit its response by interacting with the CXCR2 receptor present in the endothelia of capillaries. The recent finding that concentric exercise induces CXCR2 mRNA and protein expression in the vascular endothelial cells of the muscle fibers suggests that muscle-derived IL-8 acts locally to stimulate angiogenesis through CXCR2 receptor signaling [1]. Buford et al also showed that increases in the mRNA expression of IL-8 occur in the vastus lateralis as a result of damaging eccentric exercise in young male [22]. Akerstrom et al showed that the small release of IL-8 from muscle did not result in an increase in the systemic plasma concentration of IL-8, suggesting that muscle-derived IL-8 may play a local role, e.g. in angiogenesis [23]. Yoon suggested that IL-8 is a paracrine mediator secreted from contracting skeletal muscle. IL-8 is a chemokine that acts as an attractor for neutrophils and as an angiogenic factor (24). Thus, as anticipated Pedersen and Febbraio suggested that muscle-derived IL-8 should be classified as a myokine [1].

IL-15

The cytokine IL-15 is a recently discovered anabolic factor that is constitutively highly expressed by skeletal muscle [1, 13, 21, and 25] and regulated by strength training. Therefore, Pederson and Febbraio (2008) suggest that muscle-derived IL-15 should be classified as a myokine [1]. Riechman et al. showed that skeletal muscle levels of IL-15 are among the highest of any tissue. IL-15 is one of the most abundant cytokines in skeletal muscle [26].

While IL-15 has solid anabolic effects, it also seems to play a role in reducing adipose tissue mass [1, 27], lipolysis and suppress lipogenesis [13] via a muscle-to-fat endocrine pathway, it is therefore suggested that IL-15 may play a role in muscle-fat cross-talk. Quinn further demonstrated a negative association in humans between plasma IL-15 concentration and trunk fat mass, but not limb fat mass. In support of this finding [27], Pedersen also demonstrated a decrease in visceral fat mass, but not subcutaneous fat mass, when IL-15 was overexpressed in murine muscle [12]. Interestingly, for the first time, Gangemi et al demonstrated that ultralongeal subjects displayed significantly higher circulating IL-15 compared to each of the two age control populations. Thus, they suggested that it has relationship between IL-15 and ultralongeal [28]. IL-15 plays an autocrine or paracrine role in modulation of skeletal muscle metabolism, growth, and (or) adaptation. Muscle-derived IL-15 can cell culture and short-term in vivo experiments have indicated that IL-15 inhibits skeletal muscle protein degradation. IL-15 has direct actions on cultured adipocytes and decreases fat deposition in vivo in rodent models. IL-15 exhibited greater rates of protein synthesis and lower rates of protein degradation, resulting in markedly increased accretion of myofibrillar proteins and a hypertrophic morphology. Some in vitro evidence indicates that the activated IL-15R α directly inhibits TNF- α signaling by competing with the type-1 TNF- α receptor for a specific adaptor protein stimulating skeletal muscle proteolysis and apoptosis. Quinn (2008) reported injections of recombinant human IL-15 into laboratory rats, observed that IL-15 inhibited muscle protein breakdown but did not increase muscle protein synthetic rates. In healthy, growing rats, IL-15 administration induced more than 3-fold decreases in muscle proteolysis rates, associated with a slight depression in muscle protein synthetic rates. In vivo studies indicate IL-15 has limited ability to stimulate muscle growth in healthy animals. Quinn indicated that genetic variability in the human IL-15R α correlated with the degree of muscle hypertrophy developed in response to a 10-wk program of resistance exercise training. IL-15 may play a role in skeletal muscle hypertrophy in human subjects and possibly in other large mammalian species. Interleukin-15 stimulates lipid oxidation in isolated skeletal muscles and in liver. Muscle IL-15 expression, at least at the mRNA level, is modulated by advanced age and muscle activity [27]. Although recently, Pedersen (2011) reported that IL-15 mRNA levels were upregulated in human skeletal muscle following a bout of strength training, He/She suggested that IL-15 may accumulate within the muscle as a consequence of regular training [12]. Riechman et al (2004) founded that plasma IL-15 protein was significantly

increased immediately after acute resistance exercise but did not change with training and was not associated with variability in muscle responses with training. Resting concentrations, acute and chronic changes in IL-15 were not significantly correlated to muscle mass, strength, or quality at baseline or in response to resistance exercise training [26]. Using strength-trained human subjects, Quinn reported no changes in muscle IL-15 mRNA after 2 h of intensive weight training [27]. However, Quinn (2008) reported plasma IL-15 protein levels from both untrained and 10-wk-trained human subjects were increased acutely by whole-body resistance exercise and speculated that IL-15 was released after exercise via microtears in muscle fibers [27]. Confirmation that the increase in serum [27] and that a bout of resistance exercise induced increased muscular IL-15 mRNA levels 24 h post-exercise [19, 25]. That IL-15 mRNA content increased 24 hours following a bout of resistance exercise IL-15 is constitutively expressed by skeletal muscle and regulated by strength training [19]. It is unclear if the differences among these studies was due to the use of highly trained vs. relatively untrained athletes or to the difference between aerobic vs. resistance exercise [27]. Nielsen and Pedersen (2008) reported that the level of IL-15 mRNA was higher in human skeletal muscles dominated by type II muscle fibres than in type I muscle fibers [19].

Using primary human myoblast cultures, Quinn (2008) suggested that both intracellular and secreted IL-15 protein were dose-dependently stimulated by several inflammatory mediators, including TNF- α [27]. When IL-15 was administered to healthy (non-tumor-bearing) adult rats for 7 days, it resulted in a 33% decrease in white adipose tissue mass, with no change in food intake. The tissue response to IL-15 was related to the amount of IL-15/IL-15 receptor complex expression, suggesting a direct action of IL-15 on adipose tissue [3]. IL-15 dose-dependently stimulated lipolysis and was a much more potent inducer of acute lipolysis than TNF- α , IL-6. IL-15 inhibited preadipocyte differentiation and also dose-dependently stimulated secretion of the insulin sensitizing hormone, adiponectin, from differentiated adipocytes [27].

IL-17

Interleukin-17 (IL-17) is a pro-inflammatory cytokine mainly secreted by activated Th17-cells, based on its ability to induce a wide array of inflammatory effectors in target cells [5],

to induce the production of TNF- α , IL-6, IL-8 [29], Th17 cells are not the only source for IL-17, rather other cells of innate, adaptive immune and other tissue such as skeletal myofibril, brain and adipose tissue can produce IL-17, IL-17F, and IL-22 [5]. Reasons described below, we suggested IL-17 as myokine for a first time. Gaffen (2004) reported that IL-17 cooperates either additively or synergistically with various inflammatory cytokines or agonists [30]. IL-17 is the founding member of the IL-17 family of cytokines, which includes IL-17A (also called IL-17), IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F [31]. IL-17's can act on a broad range of cell types to the expression of pro inflammatory cytokines such as TNF- α and IL-1b from fibroblasts and peritoneal exudate cells and promote neutrophil migration, suggesting that these family members play similar roles in the development of certain acute and chronic autoimmunity and infectious diseases [32].

IL-17RC mRNAs and IL-17RC expression are detected in human prostate, kidney, cartilage, liver, heart, skeletal muscle [33]. IL-2 and IL-15, which is a recently discovered anabolic factor that is constitutively expressed by skeletal muscle and regulated by strength training, have been shown to induce the differentiation of human regulatory T cells into IL-17-producing cells, and this is further enhanced in particular when exogenous IL-1b, IL-23, or IL-21 is present. In the lymphocytic infiltrates in myositis muscle tissue, detection of the 2 key Th1 and Th17 cytokines, IFN γ and IL-17, respectively, suggests the involvement of activated T cells in the pathophysiology of this disease [34]. Tournadre et al. (2010) explain that IL-6 can drive Th17 differentiation through the induction of IL-23 [35]. Kondo et al. [2009] also reported that have detected IL-17-expressing T cells and DCs in inflamed muscle tissues. Tissues are associated with the migration, differentiation, and maturation of inflammatory cells, including dendritic cells (DCs). Th1 and Th17 cytokine expression has been reported and their contribution to DC homing and function has been suggested through chemokine up-regulation [36]. The pro inflammatory properties of IL-17 were demonstrated by the synergistic interaction with TNF- α and IL-1 on IL-6 production by myoblasts, which quiescent myoblast precursors, also known as satellite cells, are present in normal muscle tissue. Conversely, IFN γ has been shown to have an inhibitory effect on the production of IL-17. Fas/FasL interaction between invading dendritic cells and CD4+ T cells induces local production of IL-23 and pro inflammatory cytokines, which can promote the proliferation of Th17 cells and enhance Fas-mediated apoptosis of muscle cells, respectively. IL-17-expressing mice displayed significant body weight loss, caused by depletion of both lean mass

and fat mass [37]. Álvarez-Rodríguez et al showed that a strong negative correlation between age and circulating levels of IL-17 was also found. Some negative correlations were found for limb muscle IL-17 [38]. In addition, we not demonstrated that VO₂max was any correlated with serum IL-17 levels in sedentary and training woman [39]. Lyons et al. (2010) demonstrates that IL-17 levels were elevated in C57BL/6 high fat fed mice compared to controls at all-time points [40].

Hoffman-Goetz et al did not find any significant effects of acute exercise on the expression of the pro-inflammatory cytokine IL-17 in mouse [41] IL whereas Düzova et al (2009) showed that it is higher plasma IL-17 at strenuous treadmill exercise than both moderate and control animals [42]. Hoffman-Goetz et al suggested that differences in tissues (plasma vs. intestinal lymphocytes) used for assessment of IL-17 levels and methods (EIA vs. western blots) may contribute to the differences between these studies [41]. Satarifard et al. (2012) showed that blood samples were collected pre exercise, immediately post exercise, and 2 h post exercise in ten young male athletes in neutral and hot environment. Serum concentration of IL-17 increased significantly after exercise only in hot environment and these values were higher than neutral environment. IL-17 concentrations reduced significantly 2 h after exercise in hot environment [43]. Golzari et al. (2010) founded that IL-17 concentrations were decreased significantly after 8 weeks combined training in multiple sclerosis (MS) patients and their findings suggest that combined training has useful anti-inflammatory effects. Detraining period for 2 weeks was associated with loss of some adaptations after 8 weeks training period. Pro-inflammatory cytokine levels are increased in a variety of tissues with acute physical exercise [44]. Haaland et al. (2008) reported that training was associated with a decrease in circulating pro-inflammatory cytokines and their receptors [45].

TNF- α

Researchers showed that tumor necrosis factor- α (TNF- α) can also be expressed in skeletal muscle as thought a myokine [46]. TNF- α is a proinflammatory cytokine that is also produced and secreted from adipocytes and plays a major role in mediating immune responses [47]. Quinn suggested that this is significant to scientists interested in muscle biology, in light of the strong evidence that TNF- α function to stimulate skeletal muscle proteolysis and apoptosis

[27], and thus is implicated in cachexia and age associated muscle wasting. It is known however that high TNF- α plasma concentration are also associated with low muscle mass and lower muscle strength in frail individuals. TNF- α have been shown to increase immediately after exercise (30 minutes) while IL-6 displays a more delayed response (0-3 hours) [46]. Steensberg (2002) et al reported that elevations in the plasma concentration of TNF- α as a result of exercise have been observed only highly strenuous after marathon running, but not after other forms of strenuous or prolonged exercise [17]. Although in most exercise studies, TNF- α does not change [1], reducing body mass through combined exercise training and dietary restriction decreases plasma TNF- α . Stefanyk and Dyck (2010) et al showed that plasma TNF- α is generally unchanged by a single bout of exercise [11]. However, Marin et al. (2011) showed that at plasma IL-6 levels increased significantly only immediately postgame and normalized at 24 hours in handball game in elite male players, the other unexpected result of their study was the significantly reduction of TNF- α after 24 hours of recovery [48]. Steensberg et al (2002) could not detect any arterial-femoral venous difference for TNF- α therefore, there was no net release of TNF- α either before or during knee-extensor exercise. They also reported that TNF- α gene and protein expression decreased in frail elderly patients after a resistance training program [17]. Lira et al. (2009) shown that the mesenteric adipose tissue of rats subjected to a chronic aerobic training protocol (8 weeks, five times a week, 60% do VO₂max) showed an increase in TNF- α concentration [7].

Thus the cytokine response to exercise is not preceded by an increase in plasma TNF- α . On this condition, Pedersen and Febbraio suggested that exercise is likely to suppress TNF- α also via IL-6 independent pathways [1]. Of note, the cytokine's response to exercise and sepsis differs concerning TNF- α [4].

BDNF

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophic factor family, which plays a key role in regulating survival, growth, and maintenance of neurons, and BDNF plays a role in learning and memory [6]. Expression profiling has shown that BDNF is differentially expressed in skeletal muscle under various physiological and pathological conditions [49]. Interestingly, low levels of circulating BDNF are also found in individuals

with obesity, Type 2 diabetes and the severity of insulin resistance [6]. BDNF appears to play a role in both neurobiology and metabolism. Several studies have demonstrated that physical exercise can increase circulating BDNF levels in both healthy humans [6, 49] and patients with multiple sclerosis [49]. Pedersen et al studied whether skeletal muscle would produce BDNF in response to exercise. It was found that BDNF mRNA and protein exercise; however, muscle-derived BDNF appeared not to be released into the circulation. BDNF mRNA and protein expression were increased in stimulated muscle cells [6]. Swift et al. (2012) showed that serum BDNF was not associated with fitness, body composition, anthropometry, glucose control, or strength measures at baseline. Likewise, serum BDNF measures were not altered by 9 months of aerobic, resistance, or combination training. However, reductions in waist circumference were associated with decreased serum BDNF levels [50]. Interestingly, BDNF increased phosphorylation of AMPK and ACC and enhanced fat oxidation both in vitro and ex vivo. Thus, Pedersen and Edward have been able to identify BDNF as a novel contraction-induced muscle cell-derived protein that may increase fat oxidation in skeletal muscle in an AMPK-dependent fashion. The possibility exists that BDNF may be classified as a myokine, which works in an autocrine or paracrine fashion [6].

Obesity and myokine

Adipose tissue, apart from being a lipid-storing organ, produces and secretes an array of bioactive molecules, also called ‘adipokines’, including adiponectin, leptin, resistin, cytokines TNF- α , and IL-6. Increased adipose mass associated with obesity has been linked with a low-grade, chronic inflammatory response with increased macrophage infiltration and two- to threefold production of pro-inflammatory cytokines such as TNF- α [1, 14]. It is also characterized by altered production of adipokines, and increases in biological markers of inflammation [14]. Jacobi et al. (2006) suggested that excessive adipose accumulation lead to impairment in immune function and disease susceptibility [13]. Adipose tissue and skeletal muscle also act reciprocally affect as endocrine organs, and produce various cytokines that can potentially alter peripheral insulin sensitivity [11].

Growing evidence suggests that TNF- α plays a direct role in metabolic syndrome and obesity, via direct effect of TNF- α on insulin signaling and insulin resistance [1-2, 47]. Patients with

type 2 diabetes and obesity demonstrate high protein expression of TNF- α in plasma [2]. It is likely that adipose tissue, which produces TNF- α , is the main source of the circulating TNF- α [1-2, 47] and whether increased TNF- α concentrations correlate with insulin action [47]. In vivo, experiments demonstrated that IL-6 may increase basal and insulin-stimulated glucose uptake via an increased GLUT4 translocation [11, 19]. Excessive concentrations of TNF- α negatively regulate insulin signaling and whole-body glucose uptake in humans [2]. Chronic treatment with TNF- α is decreased insulin-stimulated glucose uptake in rat skeletal muscle [14]. Interestingly, while IL-6 activates AMPK activity, evidence exists that TNF- α blocks AMPK signaling [19]. Studies examining the direct effect of TNF- α on intact skeletal muscle have yielded negative results [47].

Indeed, whether IL-6 has positive or negative effects on insulin resistance and metabolism in adipocytes and skeletal muscle is the subject of continuing controversy [1, 13]. Both, TNF- α and IL-6 levels have been found to be higher in obese than in lean subjects [14]. Although in humans the role of IL-6 in the etiology of obesity-induced insulin resistance is not resolved [1] and highly controversial, circulating IL-6 levels may or may not be associated with insulin resistance [2]. However, it has been consistently observed that IL-6 levels in adipose tissue and plasma are elevated closely related to inactivity, obesity and insulin resistance [4, 11] and implicating this cytokine as having a negative effect on glucose metabolism [11]. Studies demonstrating that IL-6 is released from skeletal muscle have shed a different light on the role of IL-6 in the etiology of insulin resistance since insulin action is known to be enhanced in the period immediately after exercise [14]. IL-6 is markedly produced and released in the post-exercise period when insulin action and glucose uptake is enhanced independent of obesity status. It has also been observed that the expression of IL-6 and its receptor increase with training, a time when enhanced insulin sensitivity, and glucose uptake and utilization in human is enhanced. With exercise training a decrease in plasma IL-6 levels are observed post exercise and under resting conditions; conversely, muscle IL-6 receptor mRNA content is increased with training, potentially improving the sensitivity of muscle to the positive metabolic effects of IL-6 [11]. Infusion of recombinant human (rh) IL-6 into resting healthy humans does not impair whole body, lower limb, or subcutaneous adipose tissue glucose uptake or endogenous glucose production. When diabetes patients were given an rhIL-6 infusion, plasma concentrations of insulin decreased to levels comparable with that in age and BMI (body mass index)-matched healthy controls, indicating that in rest [2] and exercise-

produced [10]. The muscle produces IL-6 to aid in maintaining metabolic homeostasis during muscular exercise of long duration.

Regarding the pig adipocyte, IL-6 is more highly expressed under basal conditions than is TNF- α [13]. There are data demonstrating that IL-6 can impair increases in TNF- α [17]. After adjustment for multiple confounders, including IL-6, high plasma TNF- α concentration is associated with insulin resistance. In the rodent studies, IL-6 seems to induce insulin resistance via adverse effects on the liver. The IL-6-induced insulin resistance appears to be due to an increase in suppressor of cytokine signaling 3 (SOCS-3) expressions, as SOCS-3 may directly inhibit the insulin receptor [12]. IL-6 knockout (deficient) mice develop mature onset obesity and glucose intolerance, supporting the notion that IL-6 may exert partially beneficial effects on metabolism [4, 12]. In cultured cells, TNF- α induces insulin resistance through increased serine phosphorylation of insulin receptor substrate-1 (IRS-1) [2].

Data suggest that IL-6 exerts inhibitory effects on TNF- α production. It has been suggested that IL-6 promotes insulin resistance due to the observation that plasma IL-6 is often elevated in patients with metabolic disease [2]. Given the different biological profiles of TNF- α and IL-6 and given that TNF- α may trigger IL-6 release, one current theory is that adipose tissue-derived TNF- α is actually the “driver” behind the metabolic syndrome and that increased systemic levels of IL-6 reflect locally produced TNF- α [1].

Jocobi et al. (2006) reported that receptors for both leptin and adiponectin are localized in many peripheral tissues [13]. Leptin and adiponectin on the immune system, pro-inflammatory (e.g. TNF- α and IL-6) cytokines reciprocally affect the adipocyte/ endocrine system [11, 51]. It is now recognized that obesity results in the secretion of not only TNF- α , but many cytokines including resistin, and IL-6 and that these cytokines are secreted not only from adipocytes, but from macrophages within the adipose tissue bed. Obesity is characterized by decreased secretion of adiponectin, which has been shown to exert anti-inflammatory effects on macrophages, whereas the production of the pro-inflammatory adipokine leptin is increased [14]. Several adipokines, including resistin and TNF- α have been implicated in the impairment of insulin sensitivity, while leptin and adiponectin are generally believed to exert an insulin sensitizing effect. Leptin resistance can be modulated by both diet and training in rodents [47] and humans [52]. The effects of these adipokines on metabolism

and insulin sensitivity are generally studied in isolation [47]; in future, ours understand the interaction amongst the adipokines will be a most difficult task. Adipose-derived factors such as leptin, TNF- α , IL-6 and adiponectin have been shown to affect muscle metabolism, protein dynamics, or both, by direct actions. A reciprocal signaling pathway, in which factors secreted by skeletal muscle tissue affect adipose tissue metabolism, has not been definitively identified [27]. In addition, these hormones have direct effects on skeletal muscle mass, maintaining skeletal muscle protein health [13, 27] and physiologic activities [27]. In obesity linked insulin resistance, both leptin and adiponectin [11, 53] and adiponectin receptors are down regulated in tissues such as skeletal muscle. Adiponectin and TNF- α control each other's synthesis and activity, thus, creating a balanced physiologic situation [53]. Stefanyk et al. reported that adiponectin inhibits the production of TNF- α and IL-6 production, whereas TNF- α inhibits the production of adiponectin but stimulates IL-6. IL-6, on the contrary, attenuates TNF- α expression (11). Upregulation of adiponectin/adiponectin receptors or enhancing adiponectin receptor function may represent an interesting therapeutic strategy for obesity-linked insulin resistance. Expression of leptin and resistin, is increased by TNF- α and IL-6. Conversely leptin and resistin up regulate the production of TNF- α and IL-6. Leptin, however, also was reported to suppress the expression of resistin [53].

Conclusion

This review summarized and highlighted the current understanding of the normal distribution and functional role of myokines in plasma and skeletal muscle during acute exercise, training, and disorders. To study whether acute exercise induces a true anti-inflammatory response, a model of "low-grade inflammation". Acute bouts of exercise cause a temporary change of various aspects of immune and endocrine function. That effect is lasts 3–24 h after exercise, depending on the intensity and duration of the exercise bout. Post-exercise immune function depression is most pronounced when the exercise is continuous, prolonged, of moderate to high intensity and performed. Moreover, regular exercise protects against diseases associated with chronic low-grade systemic inflammation [2]. Higher levels of habitual physical activity are associated with lower skeletal muscle inflammatory protein content, lower adipokine production. It is be one of the mechanisms through which regular exercise benefits long-term

health [20]. Thus, muscle contraction- induced factors, so-called myokines, may be involved in mediating the health beneficial effects of exercise [2]. A few researchers suggested that myokines such as IL-6 is likely to the anti-inflammatory effects of exercise and inhibit low-level TNF- α production. Thereby, TNF- α -induced insulin resistance and thus be an important player in mediating the beneficial health effects of exercise. In contrast to the known, it would be interesting to determine whether high-intensity exercise or its combination with resistance exercise training is more beneficial moderate-intensity exercise in reducing risk of chronic cardiovascular and metabolic diseases via its anti-inflammatory effects. But, low intensity physical activity (e.g., walking) reduces symptom severity in patients with quiescent some chronic disease such as inflammatory bowel disease compared to non-exercising controls.

Further detailed studies are needed to clarify the differences between data from healthy sportsmen and patients with chronic diseases.

References

- 1) Pedersen BK, Febbraio MA. Muscle as an Endocrine Organ: Focus on Muscle-Derived Interleukin-6. *Physiol Rev.* 2008;88(4):1379-406.
- 2) Pedersen BK, Akerström TC, Nielsen AR, Fischer CP. Role of myokines in exercise and metabolism. *J Appl Physiol.* 2007; 103(3):1093–8.
- 3) Mathur N, Pedersen BK. Exercise as a Mean to Control Low-Grade Systemic Inflammation. *Mediators Inflamm.* 2008;2008:109502.
- 4) Pedersen BK.. Muscular Interleukin-6 and Its Role as an Energy Sensor. *Med Sci Sports Exerc.* 2012;44(3):392-6.
- 5) Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol.* 2009; 27:485-517.
- 6) Pedersen BK. Edward F. Adolph distinguished lecture: muscle as an endocrine organ: IL-6 and other myokines. *J Appl Physiol.* 2009;107(4):1006-14.
- 7) Lira FS, Rosa JC, Zanchi NE, Yamashita AS, Lopes RD, Lopes AC, Batista ML Jr, Seelaender M. Regulation of inflammation in the adipose tissue in cancer cachexia: effect of exercise. *Cell Biochem Funct.* 2009; 27(2):71-5.
- 8) Forsythe LK, Wallace JM, Livingstone MB. Obesity and inflammation: the effects of weight loss. *Nutr Res Rev.* 2008;21(2):117-33.
- 9) Gleeson M, Bishop NC, Stensel DJ, Lindley MR, Mastana SS, Nimmo MA.. The anti-inflammatory effects of exercise: mechanisms and implications for the prevention and treatment of disease. *Nat Rev Immunol.* 2011;11(9):607-15. doi: 10.1038/nri3041.
- 10) Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, van Hall G, Plomgaard P, Febbraio MA. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. *Pflugers Arch.* 2003;446(1):9-16.

- 11) Stefanyk LE, Dyck DJ. The interaction between adipokines, diet and exercise on muscle insulin sensitivity. *Curr Opin Clin Nutr Metab Care*. 2010;13(3):255-9.
- 12) Pedersen BK. Muscles and their myokines. *J Exp Biol*. 2011; 15;214(Pt 2):337-46.
- 13) Jacobi SK, Gabler NK, Ajuwon KM, Davis JE, Spurlock ME. Adipocytes, myofibers, and cytokine biology: new horizons in the regulation of growth and body composition. *J Anim Sci*. 2006; 84 Suppl:E140-9.
- 14) Galic S, Oakhill JS, Steinberg GR. Adipose tissue as an endocrine organ. *Mol Cell Endocrinol*. 2010;316(2):129-39.
- 15) Lira FS, Rosa JC, Yamashita AS, Koyama CH, Batista ML Jr, Seelaender M. Endurance training induces depot-specific changes in IL-10/TNF- α ratio in rat adipose tissue. *Cytokine*. 2009;45(2):80-5.
- 16) Gokhale R, Chandrashekara S, Vasanthakumar KC. Cytokine response to strenuous exercise in athletes and non-athletes—an adaptive response. *Cytokine*. 2007;40(2):123-7.
- 17) Seensberg A, Keller C, Starkie RL, Osada T, Febbraio MA, Pedersen BK. IL-6 and TNF- α expression in, and release from, contracting human skeletal muscle. *Am J Physiol Endocrinol Metab*. 2002;283(6):E1272-8.
- 18) Febbraio MA, Pedersen BK. Contraction-induced myokine production and release: is skeletal muscle an endocrine organ? *Exerc Sport Sci Rev*. 2005;33(3):114-9.
- 19) Nielsen S, Pedersen BK. Skeletal muscle as an immunogenic organ. *Curr Opin Pharmacol*. 2008;8(3):346-51.
- 20) Gleeson M. Immune function in sport and exercise. *J Appl Physiol*. 2007; 103(2):693-9.
- 21) Gray SR, Kamolrat T. The effect of exercise induced cytokines on insulin stimulated glucose transport in C2C12 cells. *Cytokine*. 2011;55(2):221-8.
- 22) Buford TW, Cooke MB, Shelmadine BD, Hudson GM, Redd L, Willoughby DS. Effects of eccentric treadmill exercise on inflammatory gene expression in human skeletal muscle. *Appl Physiol Nutr Metab*. 2009;34(4):745-53.
- 23) Akerstrom T, Steensberg A, Keller P, Keller C, Penkowa M, Pedersen BK. Exercise induces interleukin-8 expression in human skeletal muscle. *J Physiol*. 2005;563(Pt 2):507-16.
- 24) Yoon JH, Kim J, Song P, Lee TG, Suh PG, Ryu SH. Secretomics for skeletal muscle cells: A discovery of novel regulators? *Adv Biol Regul*. 2012;52(2):340-50.
- 25) Nielsen AR, Mounier R, Plomgaard P, Mortensen OH, Penkowa M, Speerschneider T, Pilegaard H, Pedersen BK. Expression of interleukin-15 in human skeletal muscle effect of exercise and muscle fibre type composition. *J Physiol*. 2007;584(Pt 1):305-12.
- 26) Riechman SE, Balasekaran G, Roth SM, Ferrell RE. Association of interleukin-15 protein and interleukin-15 receptor genetic variation with resistance exercise training responses. *J Appl Physiol*. 2004;97(6):2214-9.
- 27) Quinn LS. Interleukin-15: a muscle-derived cytokine regulating fat-to-lean body composition. *J Anim Sci*. 2008;86(14 Suppl):E75-83.
- 28) Gangemi S, Basile G, Monti D, Merendino RA, Di Pasquale G, Bisignano U, Nicita-Mauro V, Franceschi C. Age-related modifications in circulating IL-15 levels in humans. *Mediators Inflamm*. 2005;31(4):245-7.
- 29) Awasthi A, Murugaiyan G, Kuchroo VK. Interplay between effector Th17 and regulatory T cells. *J Clin Immunol*. 2008;28(6):660-70.
- 30) Gaffen SL. Recent advances in the IL-17 cytokine family. *Curr Opin Immunol*. 2011;23(5):613-9.
- 31) Cooke A. Th17 cells in inflammatory conditions. *Rev Diabet Stud*. 2006; 3(2):72-5.

- 32) Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. *Immunity*. 2011;34(2):149-62.
- 33) Ge D, You Z. Expression of interleukin-17RC protein in normal human tissues. *Int Arch Med*. 2008;1(1):19.
- 34) Honkanen J, Nieminen JK, Gao R, Luopajarvi K, Salo HM, Ilonen J, Knip M, Otonkoski T, Vaarala O. IL-17 immunity in human type 1 diabetes. *Immunol*. 2010;185(3):1959-67.
- 35) Tournadre A, Lenief V, Miossec P. Expression of Toll-like receptor 3 and Toll-like receptor 7 in muscle is characteristic of inflammatory myopathy and is differentially regulated by Th1 and Th17 cytokines. *Arthritis Rheum*. 2010;62(7):2144-51.
- 36) Kondo M, Murakawa Y, Harashima N, Kobayashi S, Yamaguchi S, Harada M. Roles of proinflammatory cytokines and the Fas/Fas ligand interaction in the pathogenesis of inflammatory myopathies. *Immunology*. 2009;128(1 Suppl):e589-99.
- 37) Duzova H. Skeletal Muscle and interleukin-17, In *Skeletal Striated Muscle*, Academy Publish, 2012 (in press).
- 38) Alvarez-Rodríguez L, López-Hoyos M, Muñoz-Cacho P, Martínez-Taboada VM. Aging is associated with circulating cytokine dysregulation. *Cell Immunol*. 2012;273(2):124-32.
- 39) Duzova H, Güllü E, Çicek G, Köksal B, Kayhan B, Güllü A, Şahin İ. Effects of various types aerobic exercise methods on myokines and some other cytokines that secreted from muscle and adipose tissues (Joint Congress of FEPS and Turkish Society of Physiological Sciences 03/09/2011-07/09/2011 Istanbul, Turkey). *Acta Physiologica*. 2011;203:S686.
- 40) Lyons JA, Haring JS, Biga PR. Myostatin expression, lymphocyte population, and potential cytokine production correlate with predisposition to high-fat diet induced obesity in mice. *PLoS One*. 2010;5(9):e12928.
- 41) Hoffman-Goetz L, Pervaiz N, Packer N, Guan J. Freewheel training decreases pro- and increases anti-inflammatory cytokine expression in mouse intestinal lymphocytes. *Brain Behav Immun*. 2010;24(7):1105-15.
- 42) Duzova H, Karakoc Y, Emre MH, Dogan ZY, Kilinc E. Effects of acute moderate and strenuous exercise bouts on IL-17 production and inflammatory response in trained rats. *Journal of Sports Science and Medicine*. 2009;8:219-24.
- 43) Satarifard S, Gaeini AA, Choobineh S, Neek LS. Effects of acute exercise on serum interleukin-17 concentrations in hot and neutral environments in trained males. *Journal of Thermal Biology*. 2012;37(5):402-7.
- 44) Golzari Z, Shabkhiz F, Soudi S, Kordi MR, Hashemi SM. Combined exercise training reduces IFN- γ and IL-17 levels in the plasma and the supernatant of peripheral blood mononuclear cells in women with multiple sclerosis. *Int Immunopharmacol*. 2010;10(11):1415-9.
- 45) Haaland DA, Sabljic TF, Baribeau DA, Mukovozov IM, Hart LE. Is regular exercise a friend or foe of the aging immune system? A systematic review. *Clin J Sport Med*. 2008;18(6):539-48.
- 46) Castellano V. cytokine and neurotrophin response to acute and chronic aerobic exercise in individuals with multiple sclerosis. University of Florida. Thesis. 2006.
- 47) Dyck DJ, Heigenhauser GJ, Bruce CR. The role of adipokines as regulators of skeletal muscle fatty acid metabolism and insulin sensitivity. *Acta Physiol (Oxf)*. 2006;186(1):5-16.
- 48) Marin DP, dos Santos Rde C, Bolin AP, Guerra BA, Hatanaka E, Otton R. Cytokines and oxidative stress status following a handball game in elite male players. *Oxid Med Cell Longev*. 2011;2011:804873.

- 49) Sakuma K, Yamaguchi A. The recent understanding of the neurotrophin's role in skeletal muscle adaptation. *J Biomed Biotechnol.* 2011;2011:201696.
- 50) Swift DL, Johannsen NM, Myers VH, Earnest CP, Smits JA, Blair SN, Church TS. The effect of exercise training modality on serum brain derived neurotrophic factor levels in individuals with type 2 diabetes. *PLoS One.* 2012;7(8):e42785.
- 51) Simons PJ, van den Pangaart PS, van Roomen CP, Aerts JM, Boon L. Cytokine-mediated modulation of leptin and adiponectin secretion during in vitro adipogenesis: evidence that tumor necrosis factor-alpha- and interleukin-1beta-treated human preadipocytes are potent leptin producers. *Cytokine.* 2005;32(2):94-103.
- 52) Sartor F, de Morree HM, Matschke V, Marcora SM, Milousis A, Thom JM, Kubis HP. High-intensity exercise and carbohydrate-reduced energy-restricted diet in obese individuals. *Eur J Appl Physiol.* 2010;110(5):893-903.
- 53) Rabe K, Lehrke M, Parhofer KG, Broedl UC. Adipokines and insulin resistance. *Mol Med.* 2008;14(11-12):741-51.