GENE EXPRESSIONS OF SREBP-2, PPARδ, MYH7 AND BLOOD LIPID CHANGES INDUCED BY STRETCH-SHORTENING CYCLE EXERCISE

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ABSTRACT

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The present study was to investigate whether the mRNA levels of the transcription factors, sterol regulatory element-binding protein-2 (SREBP-2), peroxisome proliferator-activated receptor-δ (PPARδ) as well as slow type myosin heavy chain (MYH7) gene are modulated after exhaustive exercise and if so, whether such alterations are related to the percentage of MHC isoforms. In particular, this study was undertaken to examine whether the level of selected blood parameters are influenced by fatiguing stretch-shortening cycle (SSC) exercise. Ten healthy men performed unilateral exhaustive repeated jumps on a special sledge. The maximal voluntary contraction (MVC) was examined before, immediately, 3 and 20 hours after exercise. Selected genes expression in vastus lateralis muscle biopsies were measured by real-time polymerase-chain reaction (RT-PCR) 3 h before, immediately and 3 h after exercise. To test the level creatine kinase (CK) activity, blood lactate (B-La), serum cholesterol (CHOL), high density lipoprotein (HDL) and low-density lipoprotein (LDL), blood sample were collected 3 h before, immediately, 3 and 20 h after exercise. The protein myosin heavy chain (MHC) was determined using electrophoretic separation technique (SDS-PAGE).

Declined MVC as well as increased blood lactate and CK activity may imply muscle damage. Significant increase in the level of CHOL, HDL and LDL were observed immediately after exercise that may reflect the synthesis of new cell membranes and healing process of the muscle cells. The expression of PPARδ was decreased significantly after exercise. However, no changes were observed in expressions of SREBP-2 transcripts and MYH7 gene. The expression of PPARδ transcripts was found to be associated with MYH7 mRNA before and after exercise. PPARδ mRNA expression was positively associated with the proportion of the MHCI isoform. Conversely, negative relationship was shown with the proportion of the MHC IIA.

These data suggests a single bout of exhaustive exercise provides molecular responses at early stage after exercise. The decreased mRNA concentration of PPARδ may imply that fatiguing exercise bout can initiate a sequence of events towards more glycolytic muscle phenotype. In addition, the association of PPARδ mRNA level with MHCI isoform and MYH7 gene may support the close relationship between indicator of the oxidative fiber types and the regulatory factor of oxidative metabolism. However, the molecular mechanisms underlying these associations are poorly understood. The results concerning the short-term response of PPARδ mRNA after fatiguing exercise that generate challenging questions for further investigation.

Key words: stretch-shortening cycle exercise; PPARδ; SREBP-2; MYH7; creatine kinase; serum lipoproteins; myosin heavy chain

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Armaghan Khalil pour

LIST OF ABBREVIATIONS

AST	aspartae amino transferase
AU	arbitrary units
BCP	bromocholoropropne
CK	creatine kinase
DBD	DNA-binding domain
DJ	drop jumping
EMG	electromyography
ER	endoplasmic reticulum
FFM	fat–free mass
FM	fat mass
GAPDH	glyceraldehyde- 3-phosphate dehydrogenase
HDL	high density lipid cholesterol
LBD	the Ligand-binding domain
LDH	lactate dehydrogenase
LDL	low-density of lipoprotein
LDLR	low–density lipoprotein receptor
LFF	low frequency fatigue
MDJ	maximal drop jumps
MHC	myosin heavy chain
mRNA	messenger RNA
MVC	maximum voluntary contraction
MYH7	Slow type Myosin heavy chain gene
NEFA	non-esterified fatty acids
NRs	nuclear receptors
NTD	amino-terminal domain
B-La	blood lactate
PPARδ	peroxisome proliferator-activated receptor delta
PPRE	peroxisome proliferator response element
RT-PCR	polymerase chain reaction
RXR	retinoid X receptors
SCAP	SREBPs cleavage activating protein
CHOL	serum cholesterol
SDS-PAGE	sodiumdodecyl sulfate-polyacrylamide gel electrophoretic
SREBP	sterol regulatory element-binding protein
SSC	stretch-shortening cycle

CONTENTS

1 INTRODUCTION

Stretch-shortening cycle (SSC) exercise in the form of repeated jumps provides a high mechanical load on target muscle which increases the risk of muscle damage. Exhaustive exercise is known to elicit a number of molecular responses that may result in metabolic changes. Proske and Morgan (2001) indicated that after an eccentric contraction the sarcomeres can be damaged, and as the area of disruption gets larger the damages advance to membrane. In their study, Nikolaidis et al. (2008) found that eccentric exercise modifies the levels of the lipids and lipoproteins. The induced favorable and prolonged changes of lipids and lipoproteins return toward the baseline 2 to 3 days after exercise.

To date, little is known about the effect of eccentric exercise on muscle fiber action associated with genes like slow type I myosin heavy chain (MYH7) in human skeletal muscle. It is well published that endurance training results in fast to slow alterations in MHC isoforms (Baumann et al. 1987; Schaub et al. 1989). The major MHC isoform in slow type fibers is encoded by MYH7 gene. The question, whether SSC exercise affects MYH7 mRNA expression level is not entirely clear. It is of interest to test whether SSC exercise affects MYH7. In addition, this raises question about whether there is an association between the mRNA expression of PPARδ and MYH7.

Several studies (e.g., Chen et al. 2002; Barash, et al. 2004; Mahoney et al. 2005) have shown that SSC exercise is a strong transcriptional stimulus that alters the expression of a large number of genes. It is suggested that there is a link between exercise and regulation of peroxisome proliferator-activated receptor- δ (PPARδ) and its cofactor (i.e., PGC1α) in mature muscle (Lin, et al. 2002). PPARδ activation can cause to an increase of energy expenditure through regulating of the genes involved in fatty acid oxidation (Dressel et al. 2003) and increasing of exercise tolerance (Wang et al.2004). PPARδ also acts as the master regulator of the slow oxidative type I muscle phenotype in rodents, leading to muscle remodeling by increasing the oxidative fibers (Fredenrich

and Grimaldi 2005). Moreover, the study by Mahoney et al. (2008) has shown that eccentric exercise induced the expression of a number of genes involved in cholesterol and lipid homeostasis. It also showed that sterol regulatory element-binding protein (SREBP) family was one of those genes involved in cholesterol and lipid homeostasis that reacted to eccentric exercise. A real-time PCR (RT-PCR) analysis confirmed that exercise induced muscle damage led to a rapid increase in SREBP-2. It also confirmed that transcriptional responses related to SREBP-2 could regulate the synthesis of cholesterol, fatty acids, triglycerides, phospholipids, and the low-density lipoprotein (LDL) receptors.

In their study on transcriptional regulation of phagocytes, Castoreno et al. (2005) found that SREBP family is central regulators of the membrane biosynthesis in mammalian cells. Eccentric exercise induces membrane damage and it is a strong stimulus for muscle cell growth. That is, SREBP-2 activation may be engaged in a transcriptional program of de novo membrane biosynthesis in skeletal muscle. Gene expression profiles represent a picture of cellular metabolism or activity in the molecular scale. In this regard, one of the purposes the present study was to measure the gene expression, as its levels were assumed to be related to the levels of protein. In addition, any change in the expression of gene may be an explanation of the changes in the levels of protein.

2 REVIEW OF THE LITRETURE

This chapter reviews the theoretical issues and relevant constructions concerning the hypotheses I tested in this study. This chapter mainly contains an overview on the approaches testing the effects of diverse exercises on indicators of the muscle damage and lipoprotein parameters in addition to transcription factors involved in fat and cholesterol metabolism.

2.1 Structural and cellular components of skeletal muscle

Skeletal muscle fibers are specialized in many ways for their function in producing force. The connective tissue of a muscle is almost as important as the muscle fibers. It has three anatomical parts. 1) epimysium, which is tough and thick: it separates a muscle from the other ones. 2) Perimysium, which is divided into fascicles of fibers and provides pathways for blood vessels and nerves through the muscle belly. 3) Endomysium, which develops around each muscle fiber, is composed of the collagen fibrils (McIntosh et al. 2006, p.8). It is likely that the endomysium also makes connections between the basement membrane and a glycoprotein layer that lies on the outside of the muscle fiber membrane.

Sarcolemma is composed of the serumlemma which is about 7.5 μm thick, surrounds a solution of inorganic ions, sugars, amino acids, peptides, and proteins termed cytosol. The cytosol and filaments and other organelles form the cytoplasm. The cell membrane is a lipid bilayer which has fluidic properties. There are important components in muscle as energy sources such as glycogen and lipids. Biochemical and ultra-structural investigations confirm that sarcolemma is largely composed of the phospholipid molecules arranged to the surface of the fiber and forming two layers. The tails consist of fatty acid chains. The serumlemma also contains a large amount of the cholesterol, which causes stiffening of the membrane. Muscle fibers have a diameter between 10-

100 μm but up to several centimeters long. Inside the fiber there are protein filaments that help to produce the contractile elements, which are grouped together in bundles called myofibrils (McIntosh et al. 2006 p.11-12). Myofibrils are composed of many individual sarcomeres that are arranged in series. A sarcomere is a contractile unit of the skeletal muscle. Two primary types of the protein filaments are actin and myosin that they are components of the protein molecules. An 'I band' corresponds to the presence of actin and an 'A band' consists of myosin. The lighter area in the middle of A band, where the thin filaments do not reach, is H zone. The area between Z lines is called sarcomeres (Figure1).

The muscle fiber contains many nuclei which are located along the inner surface of the serumlemma in muscle fiber. Each nucleus is bounded by two membranes: the nuclear membrane and sarcolemma. The functions of the nucleus are to prepare and to send instructions for protein synthesis in the cytoplasm. Expression of gene in skeletal muscle cell is similar to other cells. However, skeletal muscles are unique and multinucleated, containing hundred myonuclei along their length. In terms of their functions, the genes and proteins that are expressed may differ among myonuclei (Mejat et al. 2003).

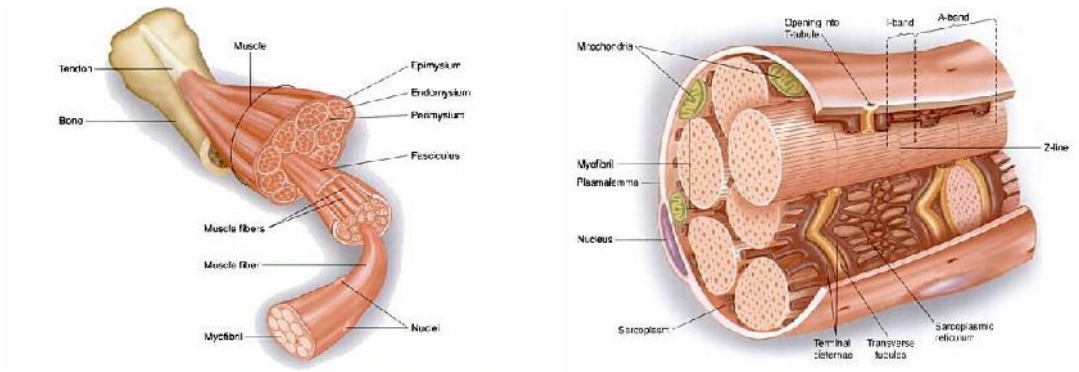


FIGURE 1.The basic structure of muscle belly that are comprised of many components such as, epimysium, perimysium, endomysium. Muscle fiber consists of cell full of filament called myofibrils, which are in turn made up of contractile unit called sarcomere. Two filamentary proteins are as key components of sarcomere that are actin and myosin. Sarcolemma is membrane of muscle fiber. Sarcoplasm is cytoplasm of muscle fibers. Nuclei are detached all long the surface of the fibers. Sarcoplasmic reticulum surrounds the fibers. Transverse tubules are located over surface of sarcoplasmic reticulum and serves as junctions of the A and I bands (Wilmore et al. 2008, p.27-28).

2.1.1 Regulation of gene expression by transcription factor

When the information in a gene is transformed to the final gene product, it is called gene expression. The two strands of DNA serve as a template. They are held together by binding of the complementary base pairs. DNA encodes genetic information in the sequence long one strand. The portion of DNA molecule that is single gene or coding region is bounded by termination of promoter sites. Transcription is the first step of the gene expression. The transformation of the DNA sequence into RNA take places in the nucleus of the cell. The transcription begins with the attendance of necessary transcription factor that binds to specific DNA segment elements.

A molecule of RNA polymerase which serves as enzyme binds to the promoter site and it moves along the DNA sequence by separating the double strand of DNA. The complex of RNA polymerase II and general transcription factors are sufficient to initiate gene transcription at minimum rate. it can be increased by positive or negative regulatory elements. Each unpaired basepair will bind to the nucleotide which has suitable complementary base. In the synthesis of RNA the process stops when RNA reaches the termination site. Synthesized RNA is a copy of the DNA template. The post transcriptional modification step consists of removing intron sequences (slicing RNA) and rejoing of the exon segments (Mooren and Völker 2005. p.40).

Nuclear receptors (NRs) form the largest known family of eukaryotic transcription factors. There is an essential role for NRs in regulation of the wide range of physiological processes involved in growth, development, and homeostasis (Mangelsdorf et al. 1995; Aranda and Pascual 2001; Germain et al.2006). Nuclear receptor is composed of four independent albeits interacted functional domains (Figure2). (1) the amino-terminal domain (NTD), (2) the DNA-binding domain (DBD), (3) the hinge region, and (4) the ligand-binding domain (LBD). The primary functions of DBD and LBD are to recognize specific DNA sequences and ligands, respectively. The main function of the hinge region (H) is to serve as a connector between the DBD and LBD (Glass et al. 1994). NRs generally have two transcription activation functions:

AF-1 and AF-2, which are located in the NTD and LBD, respectively. The NTD's function is to mediate protein-protein interactions with other transcription factors. This region is also involved in forming the three-dimensional structure of the receptor by interaction with other domains, such as LBD (Zhou et al. 1995). In order to activate gene transcription, NRs must be transported to the nucleus. NRs are rapidly and continuously traveling between the nucleus and cytoplasm.

FIGURE 2. Schematic representation of nuclear receptors structure. The main structural and functional domains are amino terminal domain (NTD), DNA-binding domain (DBD), hinge region (H), ligand-binding domain (LBD). The activation function 1 (AF-1) and activation function 2 (AF-2) are located in the NTD and LBD, respectively (Wärnmark *et al.* 2003).

2.1.2 Skeletal muscle fiber types

A muscle consists of thousands of fibers that contract to generate movement. There are different fiber types in terms of contractile, biochemical, and metabolic properties. Muscle group is generally composed of a mixture of the myofibers that differ in their biochemical and physiological feature (Williams and Neufer 1996). The histological diversity of the myofiber is comprised into slow and fast twitch fibers. Myofibers are classified in to type I, type IIa, type IId/x and type IIb. The type I and IIa contain greater amount of myogolobin as well as higher capillary density and exhibiting oxidative metabolism in comparison with type IIx and IIb fibers. Type I and IIa have maximum ability to produce energy (Lieber 2002, P.88). Type IIa fibers are less glycolytic than type IIb and stands in-between type I and type IIb. It also contains high capacity of mitochondria (Bassel-Duby et al. 2006). The IIX and IIb type fibers, which are much quicker, are named fast twitch myofibers and exert quick contractions, then exhaust quick. The fastest muscle type in humans is type IIb, which contain low levels of both mitochondria and myogolobin. This type of fiber can contract much quicker (i.e., with a greater amount of force) than oxidative muscle, but persevere for a short amount of time (Smerdu et al. 1994).

2.1.3 Myosin heavy chain isoform

The great diversity of skeletal muscle fibers is due to polymorphic expression of different myosin heavy chain (MHC). MHC is encoded by multi gene family (Buckingham et al. 1986). Single muscle fibers contain a specific MHC isoforms (type I, IIa, IIx and IIb). Myosin is formed from two heavy chains and two pairs of light chains. MHC-I is preferentially expressed in the slow twitch oxidative type I fibers, whereas MHC-IIa, IIx, and IIb are preferentially expressed in the fast twitch type II fibers. It is well established that MHC expression is altered during muscle development or in response to physical exercise, hormonal treatment, and ageing (Pette and Staron 1997; Pette 1998).

Concerning physical exercise, it is generally accepted that endurance training results in fast to slow alterations in MHC isoforms (Baumann et al. 1987; Schaub et al. 1989). The functional and phenotypic characteristics of fiber types are closely related to the myosin heavy chain (MHC) isoforms (Schiaffino et al. 1996). Although the human genome contains at least 10 genes for myosin heavy chains, only 3 of them are expressed in adult human limb muscles (Pette and Staron 1997; Fry et al. 1994). The myosin heavy chain isoforms can be identified by immunohistochemical analysis using antimyosin antibodies or by sodium dodecyl sulfate-polyacrylamide gel electrophoretic (SDS-PAGE) separation.

Electrophoretic separation allows determining for the relative concentrations of different myosin heavy chain isoforms in a mixed fiber (Pette 1998). However, RT-PCR analysis used in the previous study has shown that high muscle activity leads to an increase in the percentage of type IIx fibers and a decrease in type I fibers (Jones et al. 2001). In addition, several studies have found that the alteration of muscle fiber from type IIb to type IIa and type I can be probably mediated by a calcium signaling pathway that involves the transcriptional of peroxisome proliferator-activated receptor gamma coactivator 1a (PGC-1a) (Naya et al. 2000; Olson and Williams 2000; Lin et al.2002; Wu et al. 2002).

2.2 MYH7, Transcription factor SREBP-2 and PPAR δ

2.2.1 Slow type myosin heavy chain (MYH7)

In adult skeletal muscle fibers, the major MHC isoform in slow type I (i.e., ATPase type 1) fibers is encoded by the MYH7 gene on chromosome 14, which is also the main isoform of the cardiac muscle. In the fast type IIA fibers, the corresponding MHC isoform, IIA is encoded by the MYH2 gene on chromosome 17 (Weiss et al. 1999). In the very fast glycolytic type II B fibers, the corresponding MHC IIX is expressed by the MYH1 gene on chromosome 17 (Weiss et al. 1999). Myh7 is considered a molecular marker and inducer of cardiac growth (Wagner et al. 2009). The significant increased mRNA concentrations of fast IIA (MYH2) and slow type I (MYH7) have been found with 6 weeks of endurance training (Schmutz et al. 2006). However, little is known about the effect of eccentric exercise on genes like MYH7 in human skeletal muscle. The transcriptional alteration of MYH7 can be molecular sign of the shift towards a slower contractile phenotype and the loss of type IIB muscle fibers after several weeks continues exercises (Howald et al. 1985).

2.2.2 The peroxisome proliferators activated receptor- PPAR - family

Peroxisome proliferator-activated receptors are nuclear receptors consisting of the designated subtypes, such as PPARα (NR1C1), PPAR δ (NR1C2), and PPARγ (NR1C3) which serve as transcription factors (Willson et al. 2000; Berger and Moller 2002). PPARs are one of the most important factors in fatty acid metabolism. They are ligand-dependent transcription factors that regulate target genes by binding to peroxisome proliferator response element (PPRE). As is shown in Figure 3, the Retinoid X Receptor binds to its PPRE (Yasui et al. 2008). Retinoid X receptors (RXR) and retinoic acid receptors (RAR) are nuclear receptors that exert their action by binding to specific sequences in the promoters of the target genes and regulating their

transcription. They serve as partners for several of the nuclear receptors (Forman et al. 1995).

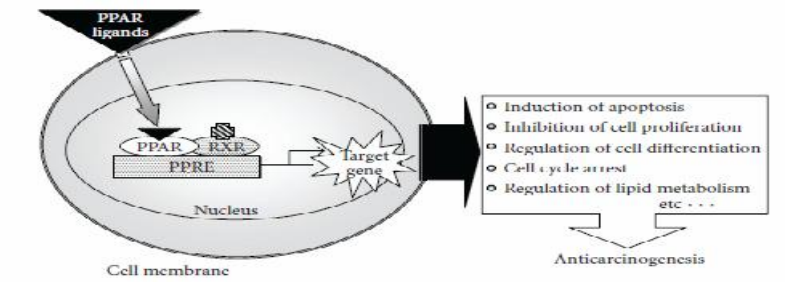


FIGURE 3. Activation pathway of PPAR and its target genes (Yasui et al. 2008).

Isoform PPARδ. PPARδ isoform is involved in the regulation of muscle development and metabolism (Grimaldi 2005). The expression of PPARδ is at 10 to 50 fold higher levels than PPARα and PPARγ in skeletal muscle (Braissant et al. 1996; Muoio et al. 2002). PPARδ over expression and/or its activation increase fatty acid catabolism by up regulating genes that control fatty acid transport (Dressel et al. 2003; Holst et al. 2003; Tanaka et al. 2003). The isoform PPARδ are mostly expressed in the skeletal muscle, especially in the more fatigue-resistant and oxidative type I fibers (Wang et al. 2004). Figure 4 illustrates how over expression of PPARδ in skeletal muscle leads to increase in the metabolic oxidative capability by elevating the amount of oxidative myofiber (type IIa) and reduces body fat (Luquet et al. 2003).

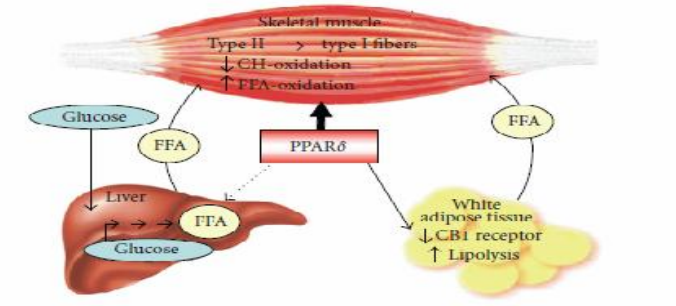


FIGURE 4. The Central fuel-switching mechanisms by which PPAR δ increases the use of fatty acids in skeletal muscle without provoke of insulin resistance. Dotted arrow: indirect effect (De Lange 2008).

2.2.2.1 Exercise-mediated regulation of PPARδ

It is reported that PPARα and PPARδ mRNA are increased following by an acute 3 hours exercise bout (Watt et al. 2004 and Luquet et al. 2003). PPARδ and cofactor PGC1α are among the key factors that are implicated in the regulation of muscle fiber-type. They can be regulated by exercise-training in mature muscle (Lin et al. 2002). Activated PPARδ expression increases the proportion of type I fibers in mice, as a result of transforming the contractile and metabolic properties of skeletal muscle to a slow-twitch oxidative phenotype (Luquet et al. 2003; Wang et al. 2004). Also, mRNA expression of PPARδ was found to be related to physiological and pathological variations in skeletal muscle fiber type in humans (Krämer et al. 2006).

Moreover, it was explained that activation of PPARδ increased expression of molecular marker of cardiac growth (Wang et al. 2004). PPARδ increased in the number of oxidative myofiber (Gaudel et al. 2008). Therefore, PPARδ considered as the strongest marker for oxidative fiber types (Wagner et al. 2009). Mechanism of PPAR δ action on redistribution of the non-esterified fatty acids (NEFA) flux induced by exercise leads to increase oxidative capability rather than to be stored in adipocyte. This, as shown in Figure 5, the decrease in adiposity, enhanced lipolysis and increased secretion of the main insulin-sensitizing cytokine adiponectin (Fredenrich and Grimaldi 2005).

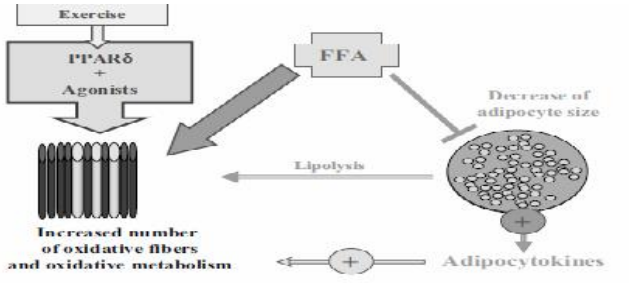


FIGURE 5. Integrated overview of the connecting action of PPAR δ between muscle and adipose tissue. Activation of PPAR δ either by ligands or exercise increases the amount of oxidative fibers in the muscle. This leads to a redistribution of the NEFA flux, then directed to the muscle to be oxidized rather than stored by adipocytes resulting in decrease of the adipocyte size promotes secretion of the main anti-atherogenic cytokine, adiponectin. NEFA: nonesterified fatty acids (Fredenrich and Grimaldi 2005).

2.2.3 Sterol regulatory element binding protein -SREBP-family

Sterol Regulatory Element Binding Proteins family (SREBPs) is transcription factors of the basic *helix–loop–helix leucinezipper* family that control the transcription of genes for fatty acid and cholesterol homeostasis (Brown and Goldstein 1997). Three members of the SREBPs have been identified by cDNA cloning (Hua et al. 1993).The structure of SREBPs share a similar tripartite structure, consisting of (1) an NH_2-terminal transcription factor domain of ~480 amino acids. The N-termini contain DNA-binding sequences (2) a middle hydrophobic region of ~80 amino acids containing two hydrophobic transmembrane segments, and (3) a COOH-terminal regulatory domain of ~590 amino acids.

In humans, hamsters and mice, two members of the SREBPs, designated SREBP-1a and SREBP-1c are produced from a single gene. The third member of the SREBPs designated SREBP-2 is encoded by a separate gene (Hua et al. 1993; Miserez et al. 1997). From the three isoforms of SREBPs, SREBP-2 is thought to activate primarily the cholesterol biosynthesis genes and the low density lipoprotein (LDL) receptor gene (Horton et al. 1998; 2002). The control center and regulation of cholesterol homeostasis is in the endoplasmic reticulum (ER) (Balasubramaniam et al. 1978). The C-terminal domain of SREBPs precursors mediates the formation of complexes with SREBPs cleavage-activating protein (SCAP) (Sakai et al. 1997; 1998a), SCAP is a polytopic membrane protein that plays a essential role in SREBP stability and regulation (Nohturfft et al. 2000, Radhakrishnan et al. 2004) (Figure 6).

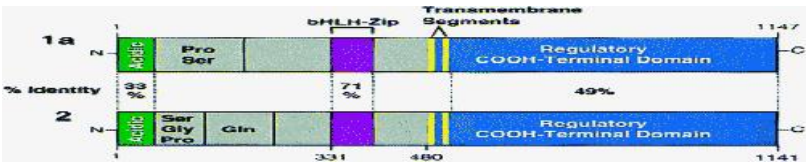


FIGURE 6. Domain Structures of Human SREBP-1a and SREBP-2, The sequence of SREBP-1c (not shown) is identical to that of SREBP-1a except for a shortened NH_2-terminal acidic domain (24 amino acids in SREBP-1c versus 42 amino acids in SREBP-1a) (Brown and Goldstein 1997).

While the cholesterol level falls, HMG-CoA reductase1 and SCAP no longer bind to one of two homologous polytopic ER membrane proteins termed insulin-induced gene (Insig-1) and (Insig-2). SREBPs bind to SCAP, which is an escort protein that carries SREBPs from the ER to the Golgi apparatus. In the Golgi apparatus, SREBPs are sliced from the immature 125 KD form to produce the mature and active 68 KD transcription factors that translocate to the nucleus (Brown and Goldstein 1999; Sakai et al. 1998; Loewen and Levine 2002; Sakai and Rawson 2001). The mature SREBPs bind the sterol response element (SRE) and activate transcription of all necessary genes for sterol synthesis (Brown and Goldstein 1997; Horton et al. 2002), control and uptake of cholesterol as well as unsaturated fatty acids (Gorlich and Annu 1999; Weis 2003).

While the level of cholesterol reaches sufficient, Insig-1 helps to restore the ER complex of Insig, SCAP, and SREBP (Adams et al. 2003). The reduction in SREBP-2 had a significant influence on the expressivity of hypercholesterolaemia (Miserez et al. 2002). It causes to reduce releasing the mature form of SREBPs, declines transcription and reduces cholesterol synthesis (Brown et al. 1999; 2000). hypercholesterolaemia reduces the release of the mature form of SREBPs, inhibits transcription and reduces cholesterol synthesis (Brown et al. 1999; 2000). Increased Insig levels can block SREBPs maturation (Yabe et al. 2002, Yang et al. 2002).

Consequently, cholesterol synthesis is not stimulated and the SREBPs do not transfer to Golgi. The activation of SREBP-2 is regulated by the levels of membrane cholesterol (Desvergne et al. 2006). In other words, the SREBP-SCAP complex is maintained in the endoplasmic reticulum when there is high cholesterol level (Loewen and Levine 2002). A small portion of the total cellular cholesterol, 0.5%, is contained in the ER membrane (Lange et al. 1999; Ikonen 2006). Slight changes in serum membrane cholesterol levels stimulate rapid changes in ER cholesterol regulatory proteins, specifically, in the presence of sufficient sterol levels (Radhakrishnan et al. 2008; Lange 1991; Lange and Steck 1997).

[1] HMG-CoA reductase is the rate-limiting step in cholesterol synthesis. Regulation of HMG-CoA reductase is achieved at several levels: transcription, translation, degradation and phosphorylation(Arnaud et al. 2005)

2.2.3.1 SREBPs genes expression in response to muscle damage

SREBP proteins are expressed in human skeletal muscle (Hua et al. 1993; Guillet-Deniau et al. 2001). SREBPs react to eccentric exercise. This suggests that transcriptional responses centered on SREBP-2 can regulate the synthesis of cholesterol and phospholipids modification (Mahoney et al. 2008). This novel set of genes is involved in cholesterol and lipid homeostasis after damaging eccentric exercise by an increase in expression. Damaging exercise is a strong transcriptional stimulus that alters the expression of a large number of genes (Chen et al. 2002; Barash et al. 2004; Mahoney et al. 2005). Acute exercise induces several physiological responses that are necessary for protein synthesis. As shown in Figure 7, the "damage–responsive" genes are involved in two major processes: recovery from and adaptation to the damage (Tiidus 2008, p.93). Based on his study, Mahoney et al. (2007) have presented real-time RT-PCR data for expressed genes in the vastus lateralis muscle during recovery from acute damage. The number of genes in which, expression level was elevated is much greater than the number of genes with reduced expression. This suggests that the skeletal muscle recovery generates an active process which involves gene activation.

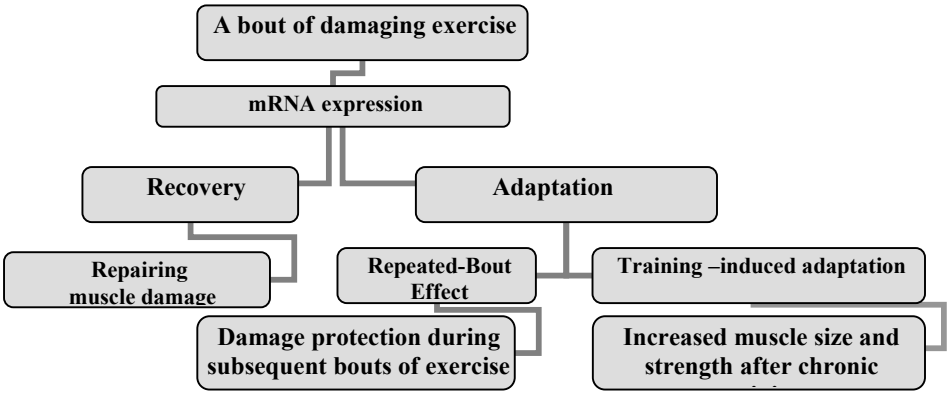


FIGURE 7. The transcriptional response to muscle damage; single bout exercise changes mRNA expression in skeletal muscle, primarily during the recovery period. Expression changes may contribute to the recovery process by encoding for proteins that are involved in repairing damaged skeletal muscle and return muscle homeostasis. As well, expression changes may encode for proteins that participate in protecting muscle from subsequent damaging stimuli (Repeated-Bout effect), and for proteins that are involved in the strength and grow that occur after weeks to month of resistance training (Tiidus 2008.p.93).

A global gene expression study by Mahoney et al. (2008) on the vastus lateralis muscle following by a bout of high intensity eccentric contraction in humans showed stimulation of the related genes involved in cholesterol and lipid metabolism. This study showed that eccentric exercise induces rapid increase in expression of SREBP-2, which was followed by a delayed increase in a number of SREBP-2 gene targets, including the LDL receptor. Mahoney et al. (2008) indicated that 235 genes altered after damaging resistant exercise. The authors speculated that biological role of an SREBP-2 transcriptional response after damaging exercise was to induce a membrane biosynthetic program in response to muscle damage. Mahoney et al. (2008) demonstrate that SREBP family transcription factors are central regulators of membrane biosynthesis in mammalian cells, given that eccentric exercise induces membrane damage, and it is a strong stimulus for muscle cell growth. They suggested that SREBP-2 activation may engage a transcriptional program for de novo membrane biosynthesis in skeletal muscle. In other words, the expression of gene involved in cholesterol and lipid synthesis are subject to contribute in membrane biosynthesis (Mahoney et al. 2008) (Figure 8).

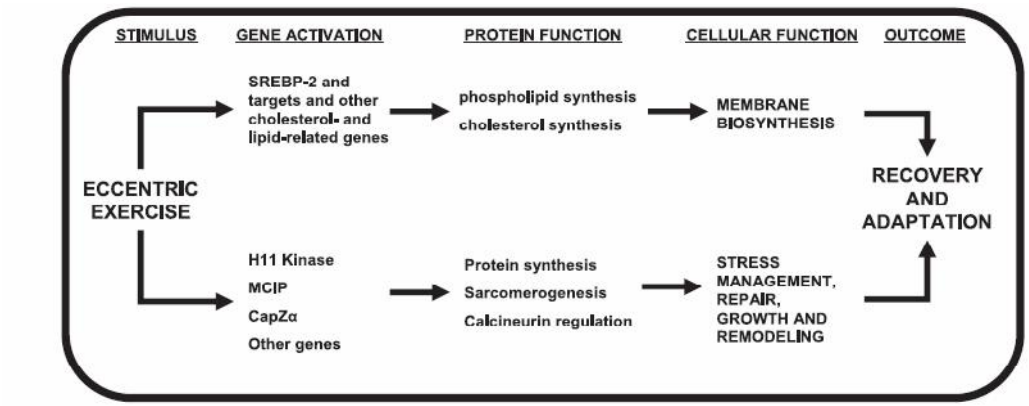


FIGURE 8**.** Schematic representation of the potential biological significance of the gene expression changes observed after eccentric exercise. Mahoney and Tarnopolsky (2007) proposed a model for de novo sarcolemmal synthesis in response to muscle damage .suggesting a transcriptional stimulus for muscle cell stress management and repair, as well as growth and remodeling. Collectively, these programs may be involved in recovery from and adaptation to the eccentric exercise bout. Upper arm: eccentric exercise activated SREBP-2 that involved in cholesterol and lipid regulation. Collectively, these expression changes suggest a transcriptional program geared toward increasing cholesterol and lipid synthesis and regulation in skeletal muscle. Lower arm: eccentric exercise activated a large number of genes involved in protein content regulation, sarcomere synthesis, and stress regulation (Tiidus 2008, p.99)

2.3 Lipids profile

2.3.1 Cholesterol, LDL, HDL

Cellular membranes contain of cholesterol and phospholipids, at a ratio slightly less than 1:1 (Lange et al. 1991). Cholesterol is a lipidic, waxy alcohol. It sets up proper membrane permeability and fluidity (Pearson et al. 2003). Cholesterol composes 13% of muscle membranes (Gurr et al. 2003). This differential distribution is not restricted in the plasma membrane, although it is also found between membranes of the cell. The majority of cellular cholesterol, 65-90%, is found in the plasma membrane (Lange et al. 1989).

Cholesterol also changes the functions of the membrane proteins and participates in several membrane signaling processes (Cherezov et al. 2007; Ikonen 2006; Maxfield and Tabas 2005). Cholesterol is also distributed in membranes of the endocytic pathway and endoplasmic reticulum (0.5% of total cholesterol) (Maxfield and Wustner 2002).Cholesterol and triglycerides are transported to tissue cells via bound to lipid-protein complex called lipoprotein. The lipoproteins contain triglycerides, phospholipids and cholesterol besides protein. In general, there are high density lipoprotein (HDL), Low-density lipoprotein (LDL) and the very low density lipoprotein (VLDL).

VLDL transports triglycerides from the liver to tissues but mostly the adipose tissue. One time triacyglycerol (TG) is unloaded, VLDL is converted to LDL which is cholesterol-rich. LDL is to transport cholesterol to peripheral tissues and regulate cholesterol synthesis in the tissue cells as well. HDL is particularly rich in phospholipids and cholesterol. The major function of HDL is to take out and transport excess cholesterol from peripheral tissues to liver (Elaine and Hoehn 2007, p.980). Nikolaidis, 2008 reported that, eccentric exercise affects the concentration of all lipid and lipoproteins at several time points after eccentric contractions on a dynamometer or downhill running exercise sessions.

2.4 Muscle contractions and muscle damage

Muscle movement generally is classified as three types of contractions; concentric, isometric and eccentric. In many activities, such as running and jumping, all three types of contraction may occur. The muscle shortening is referred to concentric contraction. It is the most familiar type of contraction to understand the mechanism how the thin and thick filaments slide across each other. In a concentric contraction, the thin filaments are pulled toward center of sarcomeres. During isometric contraction muscles act without moving. Therefore, the muscle in this situation can produce force, but the length of muscle remains unchanged.

Muscle can also exert force while lengthening which is called eccentric contraction. In this case, the thin filaments are pulled farther away from the center of the sarcomere and stretch the sarcomere. Therefore, the distance between Z lines will be lengthened as developing contractile force (Wilmore et al. 2008. p.29; Mougios 2006, p.111). The normal movement is a result of the cycling of eccentric and concentric muscle actions. This combination of eccentric and concentric actions forms a natural type of muscle function called the stretch-shortening cycle (SSC) (Norman and Komi 1979; Komi and Nicol 2000). The SSC functions are to save energy and decrease the metabolic cost of physical activity (Komi 1984).

The SSC is a natural form of muscle function that happens during activities such as walking, running, or hopping. Its particular characteristic is that muscle force and power generation are enhanced during the final (concentric) phase compared to a simple concentric contraction (Komi 2000). In lengthening phase, the muscle acts eccentrically, and then a concentric (shortening) action follows. The true definition of eccentric action indicates that the muscles must be active during stretch. SSC exercise in the form of repeated jumps provides a high mechanical load on target muscle which increases the risk of muscle damage.

2.4.1 Physiology of skeletal muscle damage

Muscle damage is defined as the loss of muscle function caused by the physical disruption of muscle structure. Morgan and Allen (1999) have defined contraction-induced injury as the total changes that take place in muscle after a bout of eccentric contractions. Unaccustomed exercise causes feeling of pain and loss of ability to perform daily tasks. It has been reported that resistance overload exercise causes muscle damage and soreness frequently (Friden et al. 1983) which is mostly due to the eccentric (muscle lengthening) part of the exercise (Fielding et al. 1991; Friden et al. 1983; McCully and Faulkner 1985). The time course of the histological, biochemical and functional changes induced by damaging exercise is showed in Table 1.

TABLE 1.Time course of changes in Histological, Bio- Histochemical and functional indices after eccentric exercise and their values as indicators of muscle damage (Tiidus 2008, p.66)

Time post exercise	1-12h	24h	48h	3-5days	5-7days	7+	Values measure
Histological							
Ultra structural changes	++	+++	+++	++	+	++	**Low/Moderate**
Bio- and Histochemical							
CK		+	++	+++	++	+	**Low**
Inflammation (tissue)	+++	+	+				**Low/Moderate**
Inflammation(blood)	+++	+					**Low**
Myogolobin	+	+	++	++	+	++	**Low**
LDH		+	++	++	+	++	**Low**
MHC			+	+++	++	+++	**Low**
Functional							
Strength	---	---	--	--	-	-	**High**
Low frequency fatigue	+++	+++	++	++	++	+	**High**
Soreness		++	+++	++	+		**Low**
Swelling		+	++	+++	++	+	**Low**

2.4.1.1 Histological signs of muscle damage

The histological signs of muscle damage can be examined by light microscopy. More detailed examination of cellular and sub-cellular changes in myofibrils study as screening the Z-line streaming are used by an electron microscope (Yu et al. 2004). The extra cellular disruption and capillary disturbances after a single bout of the maximal

resisted lengthening of the elbow flexors by 48 h post exercise have been reported by Stauber et al. (1990). Although, after eccentric exercise myofibrillar disruption appears in both type I and type II fibers, it mostly occurs in the type II fibers (Friden et al. 1988, 1996).

2.4.1.2 Biochemical evidence for membrane disruption

Early studies on exercise-induced muscle injury utilized an increase in serum levels of intracellular muscle proteins as markers of muscle damage. Myogolobin and the muscle isoform of CK are located fully inside muscle. In resistance exercise, due to muscle cell disruption, myoglobin and CK leak into the circulation. Therefore, circulating concentrations of myogolobin and CK are frequently used as markers of the exercise-induced muscle damage (Driessen-Kletter et al. 1990; Rodenburg et al. 1993; Overgaard et al. 2002).

The muscle soreness is neither the reason of loss of function, nor the cause of elevation of CK activity (Tiidus 2008, p.66). Recently blood level and enzymatic activity of different muscle proteins are examined after acute exercise. It was found increase in serum lactate dehydrogenase (LDH), aspartate amino transferase (AST), aldolase. The bio-histochemical analyses of tissue have recently revealed that the proteins; troponin-1 and myosin heavy chain (MHC) was increased after strenuous exercise (Tiidus 2008, p.40). They are also shown to be linked to altered calcium homeostasis and impaired glucose metabolism in damaged fibers. The impairment of excitation-contraction coupling would reduce the calcium activation of myofibril soon after the deficit of force production (Warren et al. 1993). In addition, eccentric contraction is found to alter the structure of T- tubules. Such structural changes may be responsible for the impairment in excitation-contraction coupling (Takekura et al. 2001; Yeung et al. 2002).

2.4.1.3 Functional evidence of muscle damage

The other common indirect indicators of muscle damage can be listed as follows: loss of strength and recovery, low frequency fatigue, neuromuscular disturbances, modifying in range of motion and stiffness of muscle and joint, swelling, and soreness. *Loss of strength* is considered as one of the most valid and reliable indirect indicator of muscle damage (Warren et al. 1999). *Low frequency fatigue (LFF)* is one of functional changes that demonstrated subsequent of eccentric exercise. LFF is thought to be a sign of the impairment of the excitation–contraction coupling system (Skurvydas et al. 2006). Recent studies (e.g., Butterfield and Herzog 2006) suggested that, the shift in the length-tension relation may be the result of damage to sarcomeres and post exercise fatigue. Neuromuscular disturbance and a decrease in voluntary activation can also impair muscle function through central fatigue (Kent-Braun and LeBlanc 1996; Löscher and Nordlund 2002). In addition, decreased motor neuron, discharge rate or decreased motor unit recruitment can lead to force loss. Some studies (e.g., Warren et al. 1999) documented that decreases in the voluntary range of motion in joints is due to eccentric exercise.

2.4.1.4 Mechanical factors in muscle damage

Mechanical stress induces in deceased cellular integrity and structural changes in the serum membrane that can be enclosed by training (Yalcin et al. 2000). Mechanical factors appear to be associated with the loss of muscle force, disruption of Z lines, disruption of muscle structures and also loss of calcium homeostasis occur in muscle damage (Friden et al. 1983). There are also evidences on disrupting sarcomeres, other skeletal element, impairment of cell membrane, and impairment of excitation–contraction coupling process as well as loss of calcium homeostasis by eccentric contraction (Tiidus 2008, P.5). The focal myofibrillar damage showed that sarcomeres might be injured immediately following eccentric contraction. Proske and Morgan (2001) illustrated that when the area of damage is large, the disruption proceed to membrane damage. This can be seen as a two-stage process, beginning with the tearing

of the t-tubules. Disruption can also be associated with damage in the sarcoplasmic reticulum, and uncontrolled Ca^{2+} release from its stores, as well as be linked to the generation of a local injury contracture. If the damage is wide, parts of the fiber or the whole fiber will die. Breakdown products of dead and dying cells would lead to a local inflammatory response, which is associated with tissue or edema and soreness. Loss of calcium homeostasis caused by disruption of cell membrane leads to increased calcium concentration in the cell. Disruption of membrane boundary on the sarcoplasmic reticulum would also result in an influx of calcium into the cells. A number of studies (e.g., Proske and Morgan 2001) revealed that calcium concentration in skeletal muscle cell is increased following the eccentric contraction.

3 PURPOSES OF THE STUDY and HYPOTHESES

Damaging exercise led to induce the changes in genes involved in cholesterol and fat metabolism (Chen et al. 2002; Barash et al. 2004; Mahoney and Tarnapolsky 2005). PPARδ is known to be expressed in the oxidative type I fibers (Wang et al. 2004) that were shown rapid increase following by endurance exercise (Watt et al. 2004 and Luquet et al. 2003). In addition, an acute eccentric exercise caused to increase the expression of SREBP-2 (Mahoney et al. 2008). The expression of MYH7 as a molecular sign to shift the contractile phenotype of the fibers towards slow type (Howald et al. 1985) was influenced by endurance training (i.e. Schmutz et al. 2005 and 2006). However, transcripnal responses of MYH7 after fatiguing eccentric exercise are still unknown. Since previous studies (e.g. Nikolaidis 2008) have shown favorable effect of eccentric exercise on concentration of all lipid and lipoproteins. However, little is known whether an exhaustive SSC exercise shows similar responses to the level of blood lipid parameters.

The purpose of current study was to assess whether the mRNA expression of SREBP-2, PPARδ and MYH7 gene are modulated after exhaustive SSC exercise and if so, whether such alterations are related to the percentage of MHC isoforms. In particular, this study was undertaken to examine whether the level of selected blood lipids parameters are influenced by fatiguing SSC exercise.

From this perspective, the study questions were:

(1) Are the PPARδ and SREBP-2 transcription factors involved in cholesterol and fat metabolism activated during SSC exercise?

(2) Is the MYH7 gene activated after SSC exercise?

(3) Is there any association between PPAR δ mRNA level with expression of MYH7 and MHC isoform percentages?

(4) Does the SSC exercise affect the blood lipid profiles?

The several hypotheses were proposed based on the findings of the previous studies as discussed comprehensively in chapter 2:

H1. It was hypothesized that SREBP-2 and PPARδ transcription factors are activated at the level of gene expressions following SSC exercise.

H2. MYH7 gene expression is affected following exercise and there is relationship between activation of MYH7 and PPAR-δ.

H3. It is hypothesized that there is a relationship between amount of PPARδ gene expression and proportions of types I, IIA and IIB MHCs

H4. Following the SSC exercise the concentrations of serum cholesterol and HDL decrease. It is assumed that while cell membrane is damaged, the serum cholesterol concentration decreases, because of the biosynthesis of membrane.

4 METHODS

Subjects. Ten young men of the heterogeneous groups ranging from 18 to 36 years of age were recruited to the study. The participants were informed of the risks and benefits associated with the experiments. They signed written consents prior to the measurements started. They were not under pharmacological treatments and followed a normal diet. The study protocol was approved by the ethics committee of the Healthcare District of Central Finland.

TABLE 2. The physical characteristics of the participants, the baseline measurements were done with body composition measuring (Inbody 720 Seoul, Korea). Values are Mean± SD.

Physical characteristics of participants					
Subject	Age (yr)	Body mass (kg)	Height (cm)	BMI ($kg/m^{2)}$	BF (%)
10	26±6	77.8±11.2	180±8	24±2.1	12.1±4.6

Body mass index (BMI), Body fat (BF).

4.1 Study design

Baseline measurements consisted of anthropometric measurements, blood test and biopsy 3 hours before exercise protocol. The experimental measurements comprised of Pre-Test, fatiguing sledge jump (DJs), and two Post-Test exercise protocols. Exercise protocol were performed with Pre-Test (right before DJs) and Post-Test protocols (immediately, 3 and 20 hours after DJs) that were included of maximal voluntary contraction (MVC); maximal drop jumps (MDJ). The subjects had been in over night fasting while the first blood sample was taken. The blood samples were collected from the antecubital vein at four different time points (i.e., -3 h, 0 h, +3 h, and +20h). The first muscle biopsies were taken from the control leg (left leg) ~3 hours prior to the fatiguing exercise. The next two biopsies were taken from the exercised leg (right leg) immediately and 3 hours after the exercise (Table 3).Caloric restriction was performed

during measurements by in taking only two chocolate bars (180 kcal/ 100g) before and after fatiguing exercise protocol.

TABLE 3. The experimental design of study including anthropometric measurement, blood sample, muscle biopsy, maximal voluntary contraction (MVC), maximal drop jump (Max DJ), drop jumps (DJs) test.

Measurements	Baseline Measurement	Experimental measurement			
	~ - 3h	0h	Fatiguing drop jump	0h	+3h	+20h
Anthropometric measurement	X					
MVC		X		X	X	X
MDJ		X		X	X	X
Blood sample	X			X	X	X
Muscle biopsy	X			X	X	

4.2 Exercise protocol

This study utilized the SSC exercise that is known to induce muscle damage (Kyröläinen et al. 1998). The one-leg jumping exercise was performed by using the right leg (in one subject with left leg) on the sledge jumping device (Kyröläinen and Komi 1995). Subjects warmed-up for 5-10 minutes on a bicycle ergometer. They were fixed to the sledge apparatus by using straps in order to avoid unwanted forward sliding of the body during contraction. The sledge was inclined 23° from the horizontal level. In order to perform a maximal jump, the subjects' knee angle was set to 107° which provided the lowest position.

The exercise protocol started with MVC of each leg. Muscle function characteristics were measured prior to and right after, as well as 3.5 h and 20 h after the fatiguing jump protocol. The MVC was performed unilaterally by pressing the foot plate with 3 attempts to reach the maximal force level. Resting time between the sets was 1 to 2

minutes. Then, MDJ was determined. In order to determine the optimal dropping height, the sledge was released by a examiner from 40 cm above each subjects' optimum drop height. Subjects were instructed to jump with maximal effort to get the highest rebound. Each subject performed 10 maximal unilateral drop jumps at a frequency of one every 5 second (Kyröläinen and Komi 1995).

Fatigue was induced by performing an exhausting SSC exercise on a sledge apparatus by continuous drop jumping (DJ) to a certain submaximal height (50% of individual optimal jump) until complete exhaustion. Exhaustive SSC exercise was performed by the right leg, while the left leg served as a control. The post loading was carried out with 10 repetitions of MDJ with the exercised leg from the optimal dropping height. In addition, MVC was measured followed by the MDJ test. The post tests were performed at three and twenty hours after the fatiguing exercise. At this point the total numbers of jump for each subject were recorded. The obtained range was between 60-1280 jumps among them.

4.3 Measurements and Analyses

MVC analysis. MVC of the knee extensors unilaterally was measured. The surface electrodes (Mini Skin Electrode, Nihon Kohden, Japan), were placed at the middle region of the rectus femoris (RF) and vastus medialis (VM) muscles. In order to keep the inter-electrode resistance low (<5 kΩ), the locations for electrode placement were prepared by cleaning the skin. The maximum tension of the three attempts was determined as the maximal isometric force value. The EMG signals were assessed during maximal isometric unilateral leg contractions on the force plate (Department of Biology of Physical Activity, Jyväskylä, Finland). All EMG activity related to maximal force was transmitted to the computer telemetrically. The amplification of the EMG signal (Glonner electronic GMBH, D-82152-planegg-matinsried, Germany) was 1000 times with a sampling frequency of 1 KHz. The *ACODAS* method was used in

recording of the MVC data of each muscle, following they were analyzed with the *Voima* program.

Blood analysis. The subjects had been in over night fasting until the first blood sample was taken. Serum levels of the serum cholesterol , LDL, HDL, Blood lactate and CK activity were analyzed using a commercial test kit (Konelab ^{TM}/ Series), manufactured by Thermo Fisher Scientific Oy, Clinical Diagnostics, Finland**.**

Muscle analysis. The muscle biopsies were taken by the Bergström biopsy needle from the vastus lateralis muscle approximately 15 cm above the patella tendon and 2 cm away from the fascia in middle region of vastus lateralis. In the beginning the area for muscle biopsy was numbed with injection of local anesthetic (i.e., Lidocain 20 mg/ml c. adrenalin). The sample for RNA and protein analyses purposes were wrapped in aluminum foils and kept into liquid nitrogen at -80^{o} C. Finally, the frozen samples were stored at -80ºC for analyses.

Analysis of messenger RNA. Total cellular RNA was isolated with Trizol Reagent following the instructions from the manufacturer (Invitrogen, Carlsbad, CA). The sample was homogenized with 1 ml TRI Regent Solution in Q-Biogene tube by centrifuging at 6 rpm/10s (Fastprp FP120). The homogenate was separated into aqueous phase and organic phases by 200 µl Bromocholoropropne (BCP) during incubating 10-15 min and centrifuging at 11700 rpm 10 min (centrifuge machine; 5424). RNA partitions to the aqueous phase, was collected. Isopropanol was added to aquenous phase and centrifuged at 11700rpm in 8 min. The pellet was washed with 1ml 75% ethanol and again centrifuged for 5 min at 9000 rpm. After removing the ethanol, RNA was dissolved with 50 µl of DEPC water, and warmed for 10 min in multi block box in 55ºC. Total RNA concentration and purity were determined by measuring absorbences at 260 nm and 280nm by using the spectrophotometer ND-1000 (Nano Drop, USA). The A280/A260 nm value was used to verify the purity of the RNA. The values of A280/A260 nm from total RNAs were between 1.8 and 2.1.

cDNA Reaction Preparation. Total RNA was reverse transcribed to cDNA, by using a high-capacity cDNA archive kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. The ABI Prism 7300 sequence detection system was used to perform TaqMan probe-based real-time PCR reactions (Applied Biosystems). The primer and probe sequences which were pre-designed transcripts set for SREBP-2(HS01081778-ml), PPARδ (HS006602622-ml), MYH7 (HS01110632- ml). In order to compensate the variations in mRNA quantity and RT efficiency, the results were normalized to glyceraldehyde- 3-phosphate dehydrogenase (GAPDH) mRNA. GAPDH is considered as the most stable internal control in exercise studies (Jemiolo and Trappe 2004). The gene specific standard curves were produced by serial dilutions of pooled samples. The $2^{-\Delta\Delta Ct}$ using the comparative C_t method was used to analyze the related changes in gene expression from real-time quantitative PCR experiments.

Gel electrophoresis analysis. The distribution of myosin heavy chain (MHC) isoforms of the vastus lateralis muscle was analyzed by sodium dodecylsulfate polymerase gel electrophoresis (SDS-PAGE). 20 slices (10 mm thick) from each biopsy sample were cut. Each fiber was transferred to the tubes containing approximately 20 µl of a denaturing solution (10% glycerol, 5% b-mercaptoethanol, 2.3% SDS, 62.5 mM Tris-HCl, pH 6.8). Fibers were stored in the temperature at −20 °C until further analysis.

The SDS-polyacrylamide gels used for MHC separation consisted of 6% acrylamide (wt/vol), 0.08% bisacrylamide (wt/vol), 0.1% SDS, 375 mM Tris-base (pH 8.8), and 37.5% glycerol. The loading gel consisted of 3% acrylamide (wt/vol), 0.16% bisacrylamide (wt/vol), 0.1% SDS, and 125 mM Tris base (pH 6.8). In both gels, polymerization was initiated by APS/TEMED. Gels were run overnight at 70 V (Anderson et al. 1994). After electrophoresis, gels were silver-stained, and densitometric analysis determined the relative percentage of 3 separate bonds of the MHC isoform containing MHC I, IIA, and IIX.

4.4 Statistical analysis

Descriptive statistics of the subject characteristics were performed using SPSS statistical package (version 18.0). At the first stage, the data was rested for normality using (Kolmogorov-Smirnov). Due to small size of samples the assumptions of homogeneity and normality (Mauchly's Test of Sphericity) were violated. Greenhouse-Geisser estimator revealed that mRNA and blood parameters data were not normally distributed and were therefore analyzed using Friedman's non-parametric test for related samples. Correlations were calculated between mRNA and SDS-PAGE data using Pearson's correlation coefficient test. The Friedman's test was particularly used to determine the changes in different time points of SSC exercise by examining the differences between target genes expression and blood variables. Between group and within group differences for the maximal voluntary contraction variable were determined using a general linear model with repeated measures ANOVA. Myosin heavy chain composition in muscle fiber with target gene expression results were correlated by calculating Pearson coefficient correlation. The level of statistical significance chosen for the analyses was <0.05.

5. RESULTS

5.1 MVC, CK activity and blood lactate

The repeated SSC exercise led to a significant decrease in MVC (57 ± 48 %) right after the fatiguing exercise as compared to the respective control value. MVC shows increase (81±87%) at 3 hours and (89±76%) at 20 hours post exercise as compare to the pre-test value. (See more details about mean values of the individual variations in MVC in appendix 1 and 2). The pair wise comparison analysis showed significant differences in MVC between the time-points -0h and +0h ($p<0.01$) and the time-points +0h and +20h ($P<0.01$) (Figure 9). The changes in MVC at different time points indicated significant fluctuation. However, no significant changes were observed in MVC during SSC exercise in the control leg. The range of individual changes in MVC relative to baseline is presented in appendix 3.

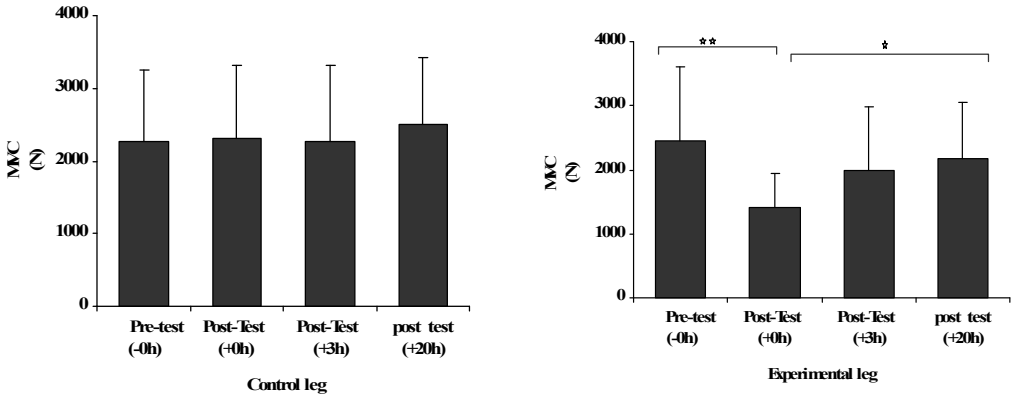


FIGURE 9. The changes in maximal voluntary contraction (N) in the control and exercised legs at different time points. Right before (-0h) immediately after (+0h), post-test (3h and 20h) after fatiguing SSC exercise. Values are Mean ± SD. (ANOVA). *P<0.05 ** P<0.01, *** P<0.001(n =9)

Serum creatine kinase activity. CK activity was significantly changed in different time-points (Figure 10). The analysis also shows significant changes in CK activity followed

by fatiguing SSC in the exercised leg (P<0.001). The increases were observed right after exercise (397±184 U/l, P<0.01), 3 hours after exercise (594±261 U/l, P<0.01) compared to the control value (208±163 U/l) and 20 hours after the exercise (1043±442 U/l, P< 0.001) compared to the right after exercise (Figure 10).

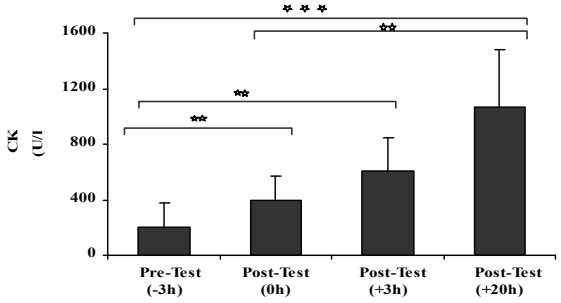


FIGURE 10. Creatine kinase activity (UL before (-3h) immediately after (+0h), post-test (3h and 20h) after fatiguing SSC exercise Mean (± SD). *P<0.05 ** P<0.01, *** P<0.001, N=9.

Blood lactate. The blood lactate accumulation showed a severe increased right after exhaustive jumps relative to the control value (7.78±3.3 mmol/l vs. 0.62±0.14 mmol/l, P<0.001). Subsequently, changes showed significant reduction by 3 hours post exercise as compared to the right after exercise(1.19± 0.7mmol/l, P=0.002). The blood lactate showed lower than the control value after 20 hours (0.56±0.12, P=0.07) (Figure11). The range of individual changes in CK and blood lactate relative to baseline is presented in appendix 4.

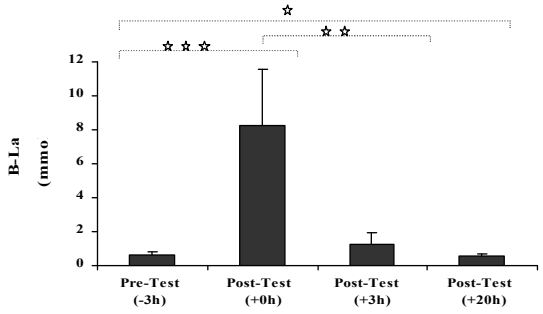


FIGURE 11. Mean (± SD) blood lactate concentration (mmol/l). Before (-3h), immediately, 3 and 20 hours after fatiguing SSC exercise. Time points of the group variations. *P<0.05 ** P<0.01, *** P<0.001, N=9.

5.2 PPARδ, SREBP-2 and MYH7 mRNA expressions

PPARδ. The results indicate a significant decrease in PPAR δ on mRNA expression immediately after the SSC exercise (P=0.008). The value returns back to the baseline by 3 hours after exercise (P=0.008) (Figure 12). Generally, the mean ranks of the variations of PPAR δ mRNA expression at different time points shows a significant changes at 0h and +3h compared to the control value (P<0.001). The level of the changes at different time points with mean ± SD presents before exercise (1.11±0.08 AU), right after (0.79±0.15 AU), and 3 hours after exercise (1.13±0.34 AU).

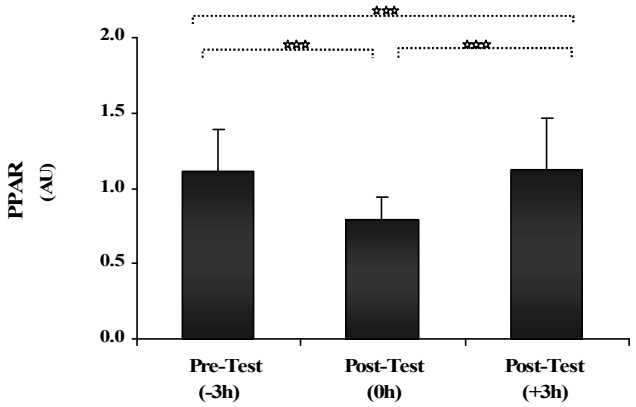


FIGURE 12. Mean (± SD) PPAR δ mRNA expression before (-3h), immediately after, 3 hours post eccentric exercise bouts. Arbitrary units (AU), *P<0.05 ** P<0.01, *** P<0.001, N=8.

It is not observed a significant difference in the level of SREBP-2 mRNA expression after repeated SSC exercise compared to the control value (P>0.05). The level of the changes at different time point was (1.07±0.54 AU) before exercise, (1.10±0.39AU) right after and (0.93±0.42 AU) 3 hours after exercise. MYH7 mRNA expression did not show significant changes following exercise. The level of the changes at different time point was (1.03±0.129AU) before exercise, (1.08±0.129AU) right after and (1.01±0.161 AU) 3 hours after exercise compared to the control value (P>0.05). The mean (± SD) of the changes in MYH7 mRNA expression presents in Figure13. The individual changes

PPARδ, SREBP-2 and MYH7mRNA level relative to the baseline is presented in appendix 5.

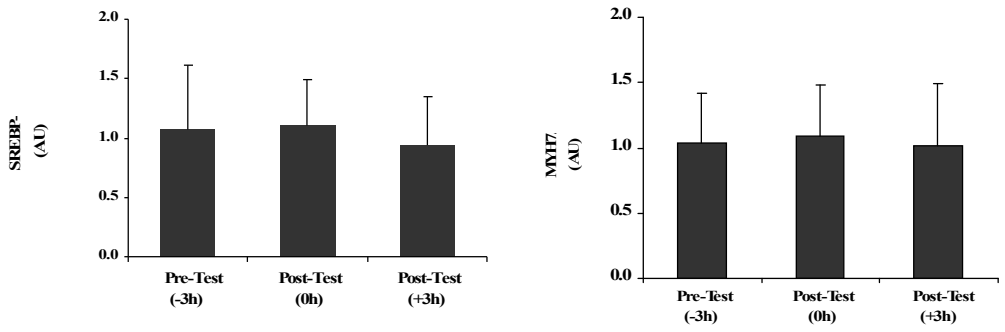
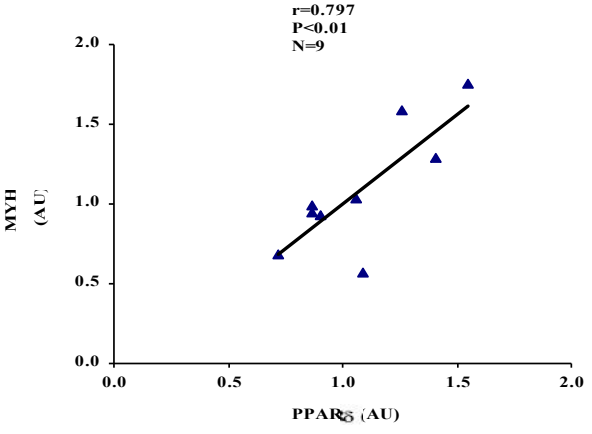


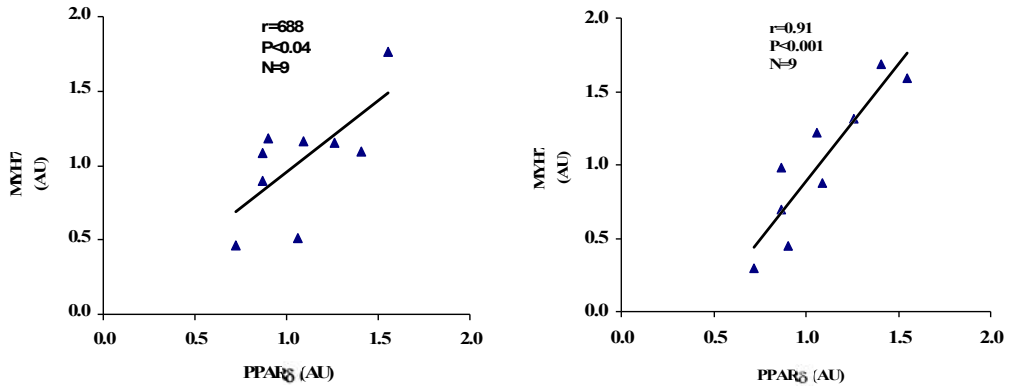
FIGURE 13. Mean (± SD) SREBP-2 and MYH7 mRNA expression before (-3h), immediately after, 3 hours post eccentric exercise bouts. Arbitrary units (AU),*P<0.05 ** P<0.01, *** P<0.001, N=9.

5.2.1 Association with target genes MYH7 and PPARδ

The analysis revealed a significant correlation coefficient between MYH7 and PPARδ in resting state before fatiguing SSC exercise (r= 0.68, p<0.04). Besides, MYH7 immediately after (r=0.79, P<0.01) and 3 hours after exercise(r=0.91, P<0.001) showed an association with PPARδ at the control level (Figure 14).



A



B C

FIGURE 14. The associations between target genes MYH7 and PPARδ mRNA level at different time points. (A) The significant correlations between MYH7and PPARδ mRNA expression at control level (r=0.68, P=0.04). (B) The significant correlations between MYH7 mRNA expression immediately after exercise and PPARδ at control level (r=0.79, P<0.01). (C) The relationship between MYH7 mRNA expression 3 hours post-exercise and PPARδ mRNA expression at control level (r=0.91, P<0.001). Arbitrary units (AU). The line represents the result of the linear regression. *P<0.05 ** P<0.01, *** P<0.001. N=9

5.3 MHC isoform distribution

The myosin isoform of each individual isolated muscle fiber was determined via SDS-PAGE, which has previously been described in detail in chapter 4. The protein myosin heavy chain (MHC) was identified at the control time point. It is determined the vastus lateralis muscle was contained MHCI (43.5±7.98 %), MHC IIA (35.6± 5.44 %) and MHC IIX (20.9±4.28%) of the fibers. A representative MHC gel is shown in Figure 15.

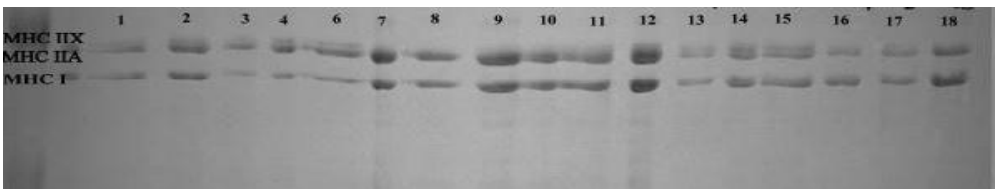


FIGURE 15. Electrophoretic separation of myosin heavy chain (MHC) isoforms in single fiber segments from human vastus lateralis muscle which contain only MHCI , MHCIIA and MHCIIX or MHCI and MHCIIA, respectively.

5.3.1 Relationship between PPARδ expression level and MHC

In order to further test the hypothesis that PPARδ was associated with the oxidative slow-twitch muscle fiber phenotype, the PPARδ mRNA level was correlated with the percentages of MHC isoforms from unexercised muscle fiber of the vastus lateralis. The percentage of MHC I, was positively correlated with mRNA expression of PPARδ (r=0.70, r^2 =*0.49,* P< 0.01) Figure 17A. Whereas Figure 17B shows the negative correlation between expression of PPARδ and type IIA (r=0.62, r^2 =*0.*39, P<0.05).

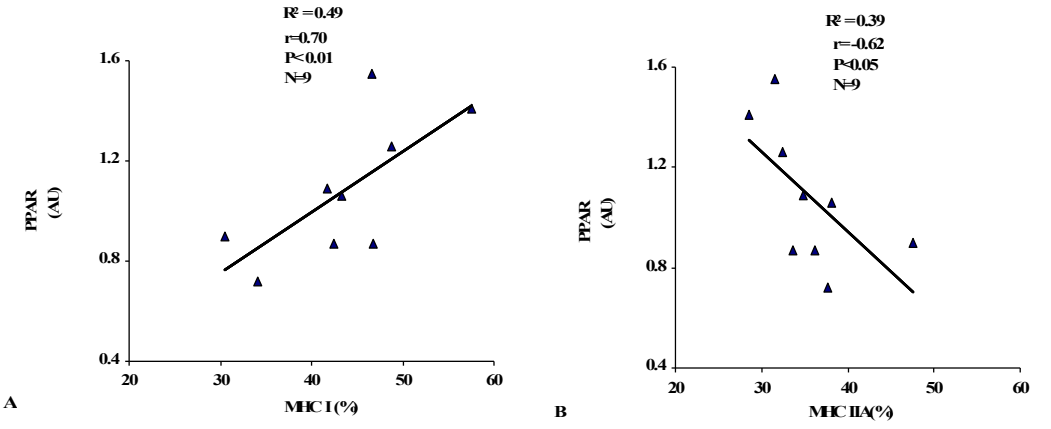


FIGURE 17. (A) Positive relationship between PPARδ expression and the percentage of MHC I (B) Negative relationship between PPARδ expression and the percentage of MHC IIA. AU (arbitrary units). The line represents the result of the linear regression. *P<0.05 ** P<0.01, *** P<0.001. N=9

5.4 Serum cholesterol, HDL and LDL

The serum cholestrol, HDL and LDL concentration levels showed significant fluctuations (P<0.001) before and after SSC exercise. Serum cholesterol was observed to increase significantly (P<0.05) immediately after exercise (0h) and to decrease (P<0.01) in post exercises (+3h, +20h) compared to the control value (-3h). The serum HDL level was significantly higher at 0h (P<0.05) compared to -3h. Subsequently, decreased +3h and +20h (P<0.01) compared to -3h. The LDL level showed significantly increase at 0h (P<0.01) compare -3h. Afterward, slight decrease at +20h (P<0.05) compared to -3h (see Figure18). The range of individual changes in serum cholesterol, HDL and LDL serum level relative to baseline is presented in appendix 6.

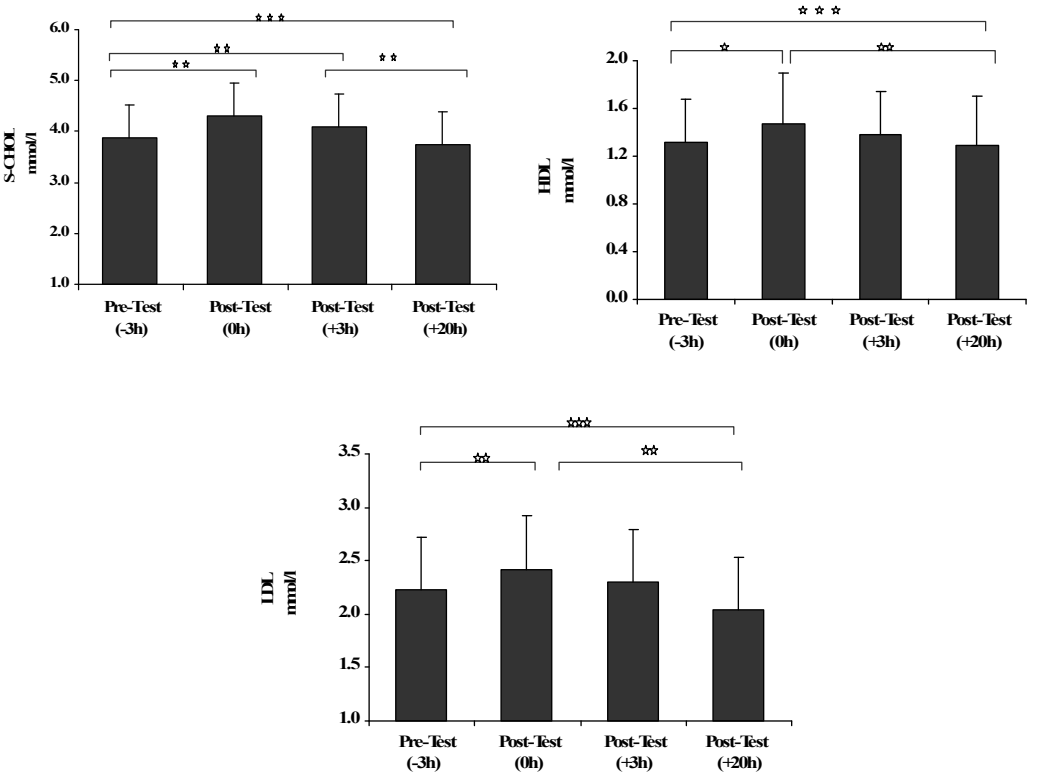


Figure 18. Mean (± SD) Serum levels of total cholesterol (CHOL) ,High density lipid cholesterol(HDL) ,low density lipoprotein cholesterol (LDL) concentrations before(-3h), immediately (0h), and (3 and 20) hours after fatiguing SSC exercise . *P<0.05 ** P<0.01, *** P<0.001, N=9.

6 DISCUSSION

The primary findings from the present study can be summarized as follows:

1) Significant reduction in maximal force (up to 43%) was observed immediately after exhaustive unilateral jumping exercise. The declined MVC force was accompanied by significant increases in blood lactate level and CK activity.

2) It was found out that muscle PPARδ transcript level was reduced immediately after exhaustive SSC exercise. However, no changes observed in the SREBP-2 and MYH7 mRNA level. PPARδ transcript level was correlated with MYH7 mRNA in resting state and after exhaustive SSC exercise.

3) PPARδ mRNA expression was positively associated with the proportion of the MHCI isoform in the vastus lateralis muscle. Conversely, a negative relationship was shown with the proportion of the MHC IIA.

4) Significant increase in the accumulation of serum cholesterol, LDL were observed and accompanied by a parallel elevation of HDL level followed by exhaustive repeated jumps exercise.

6.1 Effects of fatiguing SSC exercise on MVC, CK and blood lactate

The unilateral jumping exercise produced deficit of force, changes in CK activity and serum lactate concentration. According to Pearce et al. (1998), the reduction in the ability of muscle to produce maximal force is caused by the combination of structural and functional changes when damage occurs after eccentric muscle action. The indirect markers (elevated lactate and CK, decreased force) indicate muscle damage at early

stage of the present study. The maximal force immediately after fatiguing jump test was significantly decreased. In addition, the level of MVC increased after the 3 hours time-point of exhaustive SSC exercise. Following the over night rest of 20 hours the increase in MVC was observed. Considering the fact that reduced MVC depends on the presence of factors such as individual differences and the intensity of the exercise (Byrne et al. 2004), the fatiguing SSC protocol was effective in provoking significant changes in biochemical markers of muscle damage in the participants. The study identified significant differences in the CK activity between the time points; the levels of CK right after exercise and following an overnight rest increased over twofold compared to the control level.

The level of CK in the blood reaches maximum level in 3-5 days after eccentric exercise (Tiidus 2008, p.66). As it was expected, the present study showed that there was an increase in CK activity. It is worth mentioning that the release of this molecule into the serum is an indicator of serum membrane disruption, therefore, providing an indirect method to evaluate the level of muscle damage. In some instance, elevated CK goes along with structural protein damage (Martinez-Amat et al. 2005). Blood lactate concentration increases as exercise intensity increases. The concentration of lactate in the blood is directly associated with exercise fatigue and has been long argued to be a contributing factor (Sahlin and Henriksson 1984; Sahlin et al. 1976; 1987; 1998).

6.2 PPARδ gene expression changes after SSC exercise

In this study, the acute effect of exhaustive SSC exercise on the activation of PPARδ was examined. In the healthy men, PPARδ mRNA expression was significantly decreased following exercise. In addition, the values returned to the baseline by 3 hours. Most of the recent reports indicate that expression of PPARδ is increased under physiological condition such as fasting (Holst et al. 2003) and moderate exercise (Luquet e al. 2003 and Takahashi et al. 2006). The results did not support the hypothesis in which the PPARδ protein abundance may be increased after SSC exercise. The

activation of PPAR transcription factors is known to be associated with the regulation of muscular oxidative capacity (Lin et al. 2002 and Wang et al. 2004). Therefore, the reduction in muscular oxidative capacity is high after an exhaustive exercise bout (Newcomer et al. 2005) which was found to be associated with muscle fatigue (Swallow et al. 2007). The present study reports for the first time that PPARδ transcript level was reduced in the skeletal muscle after fatiguing repeated jump exercise under the condition of calorie restriction over 20 hours. The present data suggest that muscle PPARδ transcript downregulation can be provoked by very high muscle activity. Another related point is that eccentric exercise due to high-velocity contraction causes to increase in the percentage of type fast-twitch glycolytic fibers (Jones et al. 2001).

It is important to mention that recruitment of fast-twitch glycolytic fibers during high speeds of muscle contractions is more efficient in doing external work than slow-twitch muscles (Awan and Goldspink 1970). The increases in lactate accumulation found in this study indicate itself also the glycolytic nature of the exercise. This suggests that muscle PPARδ transcript down regulation can be provoked by very high muscle activity. Therefore, such acute damaging contractions affect the reduction of abundance of PPARδ mRNA expression due to utilizing the fast-twitch glycolytic fibers.

6.3 SREBP-2 mRNA level after SSC exercise

In the present study, only a minor increase at the transcription level of SREBP-2 was observed immediately following SSC exercise. The SREBP-2 transcript is returned to the baseline in 3 hours. However, these changes were not statistically significant. The central role of SREBP-2 isoform is the regulation of cholesterol homeostasis (Balasubramaniam et al. 1978). In addition, the findings by Miserez et al. (2002) identified an association between elevated serum cholesterol concentrations and decrease of SREBP-2. Hypercholesterolaemia causes to inhibit SREBPs transcription and reduces cholesterol synthesis (Brown et al. 1999; 2000). Therefore, observed minor

increase in SREBP-2 may be due to an enlarged level of serum cholesterol after exercise.

6.4 MYH7 mRNA level and its association with PPARδ transcript

Little is known about the effect of eccentric exercise on genes like slow type I myosin heavy chain (MYH7) in human skeletal muscle. Most of our knowledge is based on research emphasizing the influence of endurance training on MYH7 mRNA expression (i.e. Schmutz et al. 2005 and 2006). It was of interest to test whether SSC exercise affects on MYH7. The present study showed slight upregulation of MYH7 expression immediately after exercise. A tendency of decreased expression was 3 hours after exercise. However, MYH7 mRNA level was not affected significantly by one session of fatiguing jump exercise. However, MYH7 and PPARδ mRNA expressions were significantly correlated with each other. Considering the decreased level of PPARδ mRNA in this study, this suggests that fatiguing exercise bout can initiate a sequence of events towards more glycolytic muscle phenotype.

6.5 Association between PPARδ and MHC isoform distribution

It was hypothesized that MHC isoforms are coupled to mRNA expression of the gene in controlling skeletal muscle fiber type transformation and metabolism. The simple linear regression showed that percentage of MHCI isoform positively correlated to mRNA expression of PPARδ. Furthermore, PPARδ mRNA expression was negatively correlated with MHC IIA content. These findings are in line with the other findings emerged from studies using human subjects (e.g., Krämer et al. 2006). The studies by Rittenhouse (2008) and Krämer et al (2007) have shown the clear role of PPARδ in the regulation of lipid and glucose metabolism in human skeletal muscle. In addition, the high expression of PPARδ in oxidative (Wang et al. 2004) and type I fibers (Lin et al.

2002) rather than glycolytic myofibers, suggests the clear role of PPARδ as an important stimulus to induce fiber shifts. Furthermore, it has been shown that 48-hours of starvation decreased the PPARδ mRNA levels (Tsintzas et al. 2006). Moreover, non-activated PPARδ expression did not persuade the formation of type I fibers in the human vastus lateralis muscle (Tsintzas et al. 2006). These results together support the idea that PPARδ has a crucial role in the formation of the MHC isoforms.

6.6 SSC exercise and serum cholestrol, LDL, HDL concentrations

One of the main structural disruptions associated with exercise-induced muscle damage is the loss of sarcolemma (Friden and Lieber 2001). Cholesterol is a structural component of the sarcolemma and makes up to 20% of the lipid bilayer level (Tortora and Derrickson 2006). Intracellular cholesterol synthesis is provided by SREBP-2 to protect the skeletal muscle tissue during times of repair (Mahoney et al. 2008). The assumption that the blood lipids (i.e. CHOL) decrease as a result of the cell membrane damage, was tested. However, the result revealed that serum cholesterol concentration was significantly increased immediately after exhaustive exercise. It remains to be shown what was the source of increased serum cholesterol after SSC exercise.

HDL and LDL concentrations were paralleled with total cholesterol during the current study. Earlier studies have shown that exercise increased HDL immediately after the high intensity resistance exercise bout (Hill et al. 2005 and Wallace et al. 1991). The obtained results allowed suggesting that a bout of exhaustive repeated jumping exercise significantly modified the serum lipoprotein levels. As cholesterol is required to the synthesis of new cell membranes, it is suggested that a day after fatiguing SSC exercise, the reduction in serum lipoprotein may reflect muscle cell healing process after the SSC induced damage (Friden et al. 1983). However, the factors modulating the level of lipid parameters in damaging SSC exercise remain to be investigated.

6.7 Study limitations

Several cautions should to be considered in the interpretation of the observed results. One is the small sample size that limited us to test the variables utilizing non-parametric statistical techniques. The result could have gone much further if the sample size increased by at least 30 subjects so that a set of parametric techniques would let to conduct follow up analysis. The second limitation is the lack of muscle sampling on the day after exercise. That did not allow us to follow up the transcriptional responses of target genes. The third aspect to be considered is the lack of the blood sampling right before the fatiguing exercise. In our experiments we measured the baseline blood profile at 3 hours before the exhaustive SSC exercise. That might have underestimated some of the acute effects of fatiguing exercise.

7 CONCLUSIONS

In the present study, the transcriptional responses of specific genes and level of selected blood parameters induced by exhaustive stretch-shortening cycle (SSC) exercise were examined. According to the results and theoretical background, the following conclusion may be derived:

1) Declined MVC force as well as increased lactate and CK activity implies SSC exercise induced muscle damage.

2) PPARδ transcript level was reduced immediately after exhaustive SSC exercise. Despite, the SREBP-2 transcript level changes were not significant. This suggests that muscle PPARδ transcript downregulation can be provoked by very high muscle activity.

3) PPARδ transcripts level showed a significant relationship with MYH7 mRNA in resting state and following fatiguing jumping exercise. These findings may support the close relationship between indicator of the oxidative fiber types and the regulatory factor of oxidative metabolism in skeletal muscle before and after exercise.

4) PPARδ mRNA expression was positively associated with proportion of the MHCI isoform. Conversely, it was shown negative relationship with MHC IIA composition in the vastus lateralis muscle. These results support the observation that activation of PPARδ mRNA level has a crucial role in formation of the MHC isoforms phenotype.

5) Significant increases in serum lipoproteins were seen. The results suggest that a bout of exhaustive repeated jumping exercise significantly modifies the serum lipoproteins levels. The observed changes in lipoprotein accumulations may reflect the synthesis of new cell membranes and healing process of the muscle cells.

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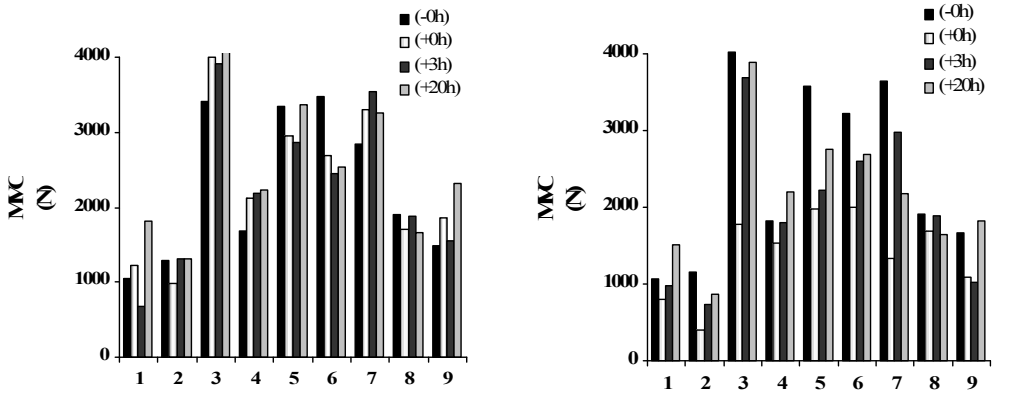
9 APPANDICES

APENDIX1. Displays mean value and percentage of the changes in MVC in control Leg. Right before, immediately, 3 and 20 hours after fatiguing SSC exercise. N = 9.

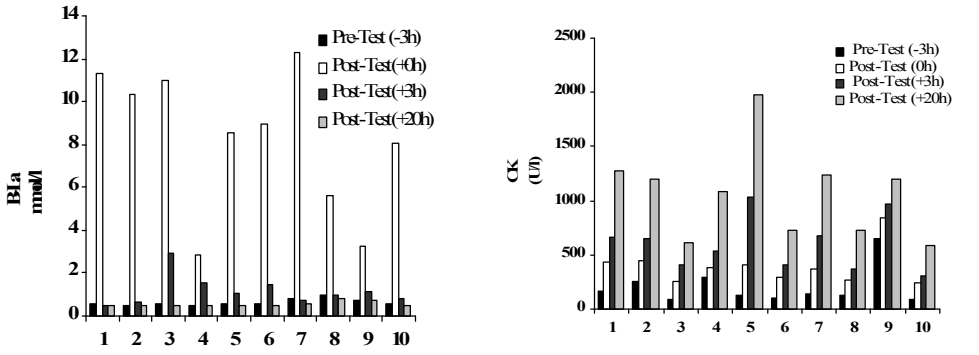
Maximal Force (N)					**percentage of changes (%)**			
	Before exercise	Time after exercise			Before exercise	Time after exercise		
Subject	Pre-test	0h	+3h	+20h	Pre-test	0h	+3h	+20h
1	1055	1214	668	1809	100	115	63	171
2	1283	986	1321	1306	100	77	103	102
3	3399	3992	3906	4093	100	117	115	120
4	1680	2125	2175	2226	100	126	130	133
5	3344	2944	2865	3372	100	88	86	101
6	3471	2690	2457	2527	100	77	71	73
7	2842	3299	3540	3252	100	116	125	114
8	1907	1695	1890	1651	100	89	99	87
9	1485	1849	1560	2317	100	124	105	156
mean	2274	2310	2265	2506	100%	102%	100%	110%
SD	983	995	1049	907	100	101	107	92

APENDIX 2. Displays mean value and percentage of the changes in MVC in exercised Leg. Right before, immediately, 3 and 20 hours after fatiguing SSC exercise. N = 9.

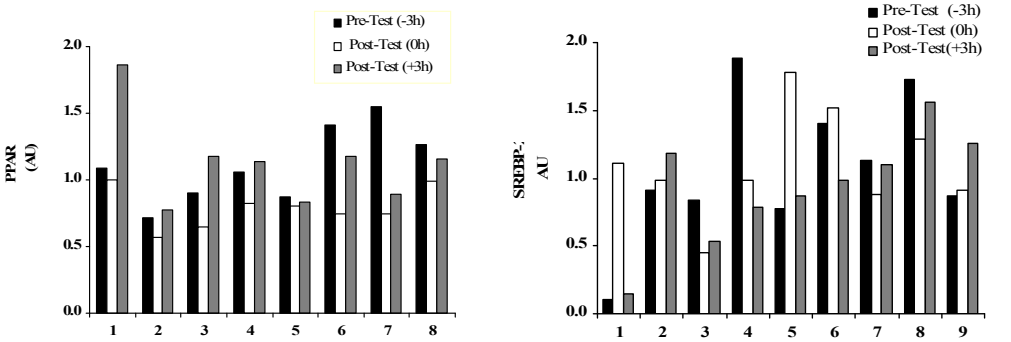
Maximal Force (N)					**Percentage of changes (%)**			
	Before exercise	Time after exercise			Before exercise	Time after exercise		
Subject	Pre-test	0h	+3h	+20h	Pre-test	0h	+3h	+20h
1	1077	793	969	1510	100	74	90	140
2	1147	404	722	860	100	35	63	75
3	4017	1771	3690	3894	100	44	92	97
4	1819	1540	1796	2202	100	85	99	121
5	3584	1975	2213	2760	100	55	62	77
6	3211	2010	2599	2695	100	63	81	84
7	3640	1330	2984	2181	100	37	82	60
8	1907	1695	1890	1651	100	89	99	87
9	1669	1086	1011	1823	100	65	61	109
Mean	2452	1401	1986	2175	100%	57%	81%	89%
SD	1152	549	997	875	100	48	87	76

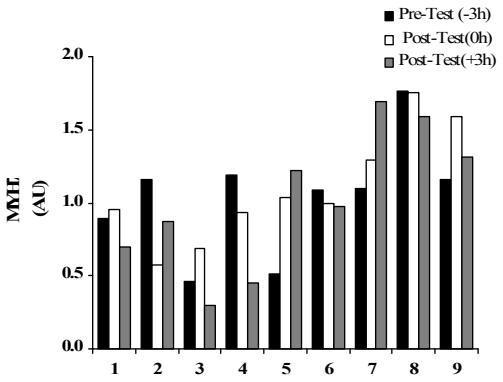


APENDIX 3. Display the mean value of the individual changes in maximal voluntary contraction (N) at different time points. Before (-3h), immediately, 3 and 20 hours after fatiguing SSC exercise. $N = 9$.

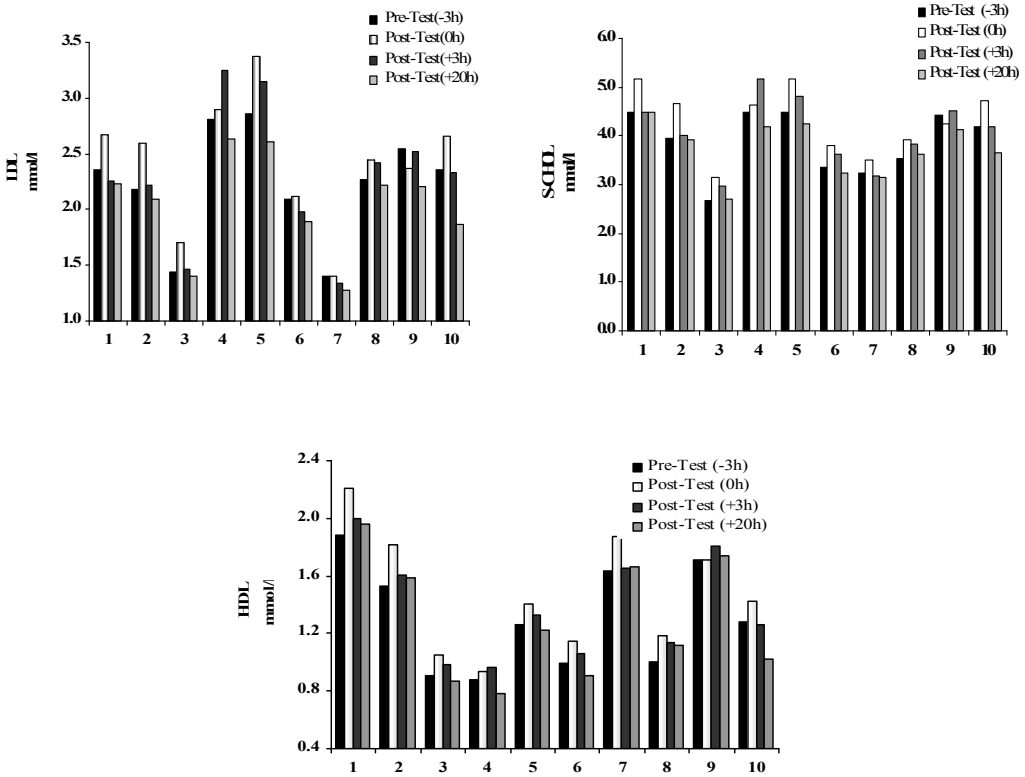


APENDIX 4. Display the mean value of the individual changes in CK and blood lactate relative to the baseline. Before (-3h), immediately, 3 and 20 hours after fatiguing SSC exercise (n=10)





APENDIX 5. Display the mean value of the individual changes in PPARδ, SREBP-2 and MYH7 mRNA expressions relative to baseline. Before (-3h), immediately, 3 hours after fatiguing SSC exercise. Results are expressed as arbitrary units (n=8 and 9).



APENDIX 6. Display the mean value of the individual changes in serum levels of total cholesterol (CHOL), high density lipid cholesterol (HDL), and low density lipoprotein (LDL) concentrations. Before (-3h), immediately (0h), 3 and 20 hours after fatiguing SSC exercise. Results are expressed as arbitrary units (n=10).