ENERGY EXPENDITURE AND SUBSTRATE UTILIZATION DURING ECCENTRIC EXERCISE IN OBESE AND DIABETICS

Riikka Kivistö

Master's Thesis
Exercise Physiology
Winter 2008
The Department of
Biology of Physical Activity
University of Jyväskylä
Supervisors: Heikki Kainulainen,
Eino Havas

ABSTRACT

Kivistö, Riikka 2008. Energy expenditure and substrate utilization during eccentric exercise in obese and diabetics. The Department of Biology of Physical Activity, the University of Jyväskylä, 92 p.

The present research was a pilot study examining the effects of diabetes and obesity on energy expenditure (EE) and oxidation of energy substrates (CHO, lipid) during eccentric exercise. Exercise is an important part of treatment of diabetes, but diabetics have often increased risk of heart failure. Eccentric exercise requires lower heart rate and systolic blood pressure compared to concentric mode. Thus, it could provide a safe mode for increasing impaired glucose disposal in the skeletal muscles of diabetic patients.

Subjects were males (n=28, age 52±7), of which 14 were diabetics (DM), 12 obese (OND) and 3 lean non-diabetics (LND). The OND and LND were combined to healthy controls (HC). After anthropometrical measurements, fasting blood sample, maximal VO_{2max} test with a bicycle ergometer and maximal isometric force measurement, the subjects had 4 weeks (2 sessions a week) adaptation period with eccentric ergometer. EE on the eccentric ergometer was measured from the respiratory gases during two intensity levels for 7 min. Muscle biopsy was obtained from the m. vastus lateralis with a Bergström biopsy needle.

There were no differences in EE and substrate utilization between the DM and OND. DM (-5.0±1,3 kcal/min) had higher EE compared to HC (-4,4±1,3; p=0.037) during second intensity level. The DM (53,2±10,4 %) used higher proportion of VO_{2max} during eccentric exercise compared to the HC (41,2±14,7; p=0.023). DM (42,0±10,0 %) had lower fraction of type I fibres compared to OND (49,1±10,1; p=0.048) and HC (50,8±11,5; p=0.020). In the DM, the fraction of type IIb fibres correlated with CHO oxidation (r=-0.661, p=0.027). In the HC, the percentage of type IIab fibres correlated with CHO oxidation (r=0.661, p=0.038) and EE (r=-0.758, p=0.011). Also, the fraction of type IIb fibres correlated with the lipid oxidation (r=0.648, p=0.043). Obesity influences more on the EE and the oxidation of substrates than diabetes mellitus alone. Lower percentage of type I fibres in DM is showed in the higher oxidation of CHO for energy compared to HC. The diabetics used higher proportion of their oxygen consumption capacity. This could be a sign for increased glycolytic enzyme activity in diabetics compared to non-diabetics, which have been found in many previous studies. These variables could partly explain the subjects' sensitivity to develop type 2 diabetes mellitus.

Key words: energy expenditure, substrate utilization, eccentric, diabetes, obesity

TABLE OF CONTENTS

1 INTRODUCTION

The changes in the working and living environments and availability of excess nutrition have launched a growing epidemic of obesity and type 2 diabetes mellitus all around the world. Particularly, the developed countries have suffered from the economical and health burdens caused by diabetes and its comorbidities. If the current course continues, even millions of people will develop metabolic disorder at some degree. Inherited properties have some influence on the development of obesity and diabetes, the physical inactivity plays even more crucial role. (LaMonte et al. 2005.)

Effect of exercise interventions on substrate utilization and insulin sensitivity of diabetics have been studied but they have mostly concentrated on the concentric exercise. There are less evidence of the influence of eccentric exercise which is considered safer exercise mode, especially, for cardiac patients and older subjects. Type 2 diabetes is associated usually with heart-related health problems, such as high blood pressure and high blood cholesterol. Therefore, eccentric exercise could provide means for increasing the insulin sensitivity of tissues by training, without jeopardizing the individual on cardiac arrest or other heart failure. (Vallejo et al. 2006.)

The aim of this study is to examine whether the energy expenditure and utilization of energy substrates are influenced by type 2 diabetes mellitus and obesity during eccentric exercise. In addition, the relation of muscle fibre distribution to energy metabolism is under examination. The nature of this research is more cross-sectional than longitudinal intervention study, of which measurements were performed in cooperation with LIKES Research Centre and the Department of Biology of Physical Activity, University of Jyväskylä.

2 ECCENTRIC EXERCISE

2.1 Biomechanical properties of eccentric exercise

2.1.1 Eccentric exercise and force production

Human movement is divided into three different muscle actions according to the muscle and load torques (figure 1). During isometric muscle action muscle exerts equivalent amount of force compared to load torque, thus leading to static action without any movement. Dynamic movements require unbalance between load and muscle torques. In concentric action, muscle force exceeds the load torque. On the contrary, during eccentric muscle action, load torque is higher than muscle torque. In eccentric movement, activated muscle is lengthening. (Enoka 1996.)

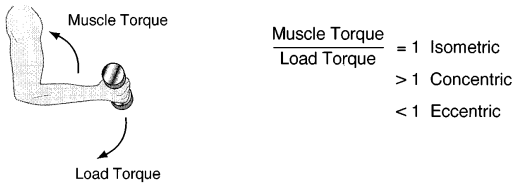


FIGURE 1. Relationship between muscle and load torques in muscle actions (modified from Enoka 1996).

Eccentric actions are part of daily movements. Through eccentric movements high muscle forces can be reached, but also tissue damage may be enhanced which is observed as muscle soreness. In addition, special control strategies by the central nervous system may be required. Mechanical efficiency and energy dissipation of eccentric actions are greater than in concentric ones. (Enoka 1996.) The mechanical efficiency increases, when the mechanical work increases in eccentric muscle actions (Kyröläinen et al. 1990).

Muscle exerts greater torque in eccentric exercise compared with concentric one. In eccentric action, muscle can produce up to 200% of maximal isometric tetanic force. Even relatively small number of high force eccentric actions may lead to reduced muscle performance. (Warren et al. 1993.) The stimuli for muscle hypertrophy and muscle damage are also stronger in eccentric muscle actions (Enoka 2002, 399-401).

Both eccentric and concentric (shortening) actions are used in daily living. Muscle action modes differ in the EMG activity patterns and in the order of motor unit recruitment (figure 2a). (Enoka 2002, 354-356.) When Nardone & Schieppati (1988) measured activation pattern of triceps muscles during breaking a foot-dorsiflexing load, they observed different patterns compared to shortening (concentric) or isometric actions. During concentric and isometric actions, all muscles are activated gradually and in parallel. In eccentric action activation pattern, either the soleus muscle is rapidly relaxed and the fast gastrocnemii activated instead, or the gastrocnemii are recruited less but it produces high-amplitude spikes.

Electrical activity in a muscle is directly proportional to the force of contraction (i.e. tension) it is exerting, when shortening or lengthening of muscle is done at a constant velocity. During lengthening however, the electrical activity is less compared to shortening at similar speed (figure 2b). This means that in order to exert the same force of muscle action, active muscle have more electrical activity in shortening than in lengthening action. Amount of tension is dependent on the number and discharge frequency of active motor units. (Bigland & Lippold 1954.)

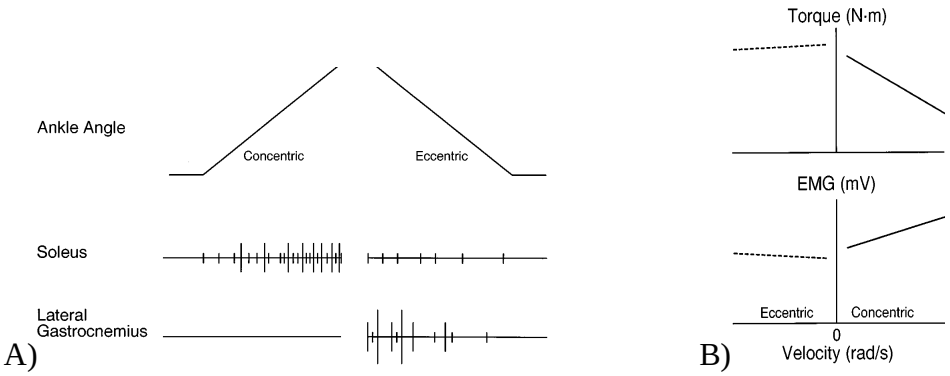


FIGURE 2. Differences in (A) motor unit activity, and (B) in torque and EMG during concentric and eccentric muscle actions (modified from Enoka 1996).

Motor potentials evoked (MEP) by brain stimulation are larger in a voluntary muscle action involving muscle shortening than muscle lengthening. Thus, motor potentials are task dependent. A phenomenon has been seen in magnetic and electrical stimulation of the brachioradialis and the biceps brachii muscles. Because both MEP and H reflex are decreased in the brachioradialis during lengthening and increased during shortening, task dependency of MEP is due to spinal mechanism. Shortening performance does not affect MEP of the biceps brachii so extensively, but this is probably due to different role of the muscle. (Abbruzzese et al. 1994.)

When muscle is lengthening at a constant velocity, tension rises first steeply and then falls briefly before it continues rising throughout the movement. Variation of the tension in the beginning of the stretch is due to strength distribution in the sarcomeres. Continued rise of the tension is due to sarcomeres which “pop” from the weakest to strongest one during the stretch. Tension has to be slightly higher in order to pop the next sarcomere. At low stretching velocities, all the sarcomeres are continually lengthening, but they do not reach their yielding point. During long stretches the tension decreases. Because many sarcomeres have been popped, the density of sarcomere strength distribution is greater than in the beginning of the stretch. Sarcomeres are lengthening slowly, so the decrease in strength is higher than the difference between strengths of popping sarcomeres. Thus, the nature of lengthening is non-uniform. (Morgan 1990.)

When sarcomere reaches its yield point, only mass or passive viscosity limits its lengthening speed. Force velocity curve has no effect on it. The distribution of the yield tension of the sarcomeres determines the tension during the stretch. (Morgan 1990.) Overstretched sarcomeres, which do not return to normal pattern, do not function normally. This leads to reduction of the tension and changes in the tension-length curve and excitation-contraction coupling. (Morgan & Allen 1999.)

2.1.2 Adaptations in muscles and nervous system

Eccentric, lengthening, muscle actions may induce muscle damage and degeneration and replacement of the muscle fibres. Muscle pain and stiffness occur a day or two after eccentric exercise. Because an active muscle is lengthening, it is suggested that in each myofibril few sarcomeres are extended too much and some of them will not return fully to the normal pattern during relaxation. (Morgan 1990.) When sarcomeres reach their yield point in the force-velocity relationship for lengthening, mainly passive structures will control the lengthening (Whitehead et al. 1998). During repeated active lengthening these sarcomeres are quickly stretched and extra tension is passed to neighbouring myofibrils. This results in tearing of the sarcomere which may lead to damages in the sarcoplasmic reticulum or the sarcolemma (figure 3). Damages at these sites would cause a release of intracellular calcium, contractures, clots and even destruction of the fibre during days after eccentric exercise. (Morgan 1990.)

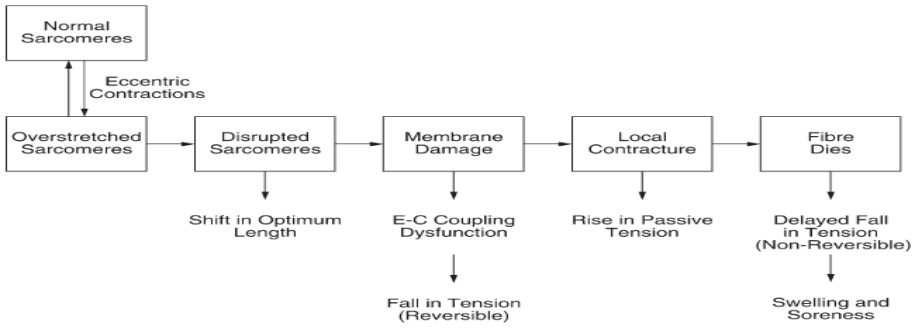


FIGURE 3. Possible series of events leading to muscle adaptation from eccentric exercise (modified from Proske & Morgan 2001).

It is suggested that the number of sarcomeres connected in series and resistance to damage from eccentric action increase after eccentric training compared to concentric one. Thus, lengthening active muscle at long length is distributed non-uniformly. This means that most of the length change is due to few sarcomeres stretched beyond filament overlap in each myofibril. (Lynn et al. 1998.)

Muscle adaptation. An eccentric exercise does not stimulate usual sensations of fatigue (Whitehead et al. 1998). Muscle pain after eccentric exercise occurs several hours later, and it peaks at about 48 h post-exercise. Muscles adapt rapidly to eccentric training. Already after a second eccentric exercise bout, muscles are less stiff and sore. The immediate signs of the muscle damage after eccentric exercise are disrupted sarcomeres in myofibrils and damage to the excitation-contraction (E-C) coupling system. The primary sign, starting the process, remains unknown. Proske & Morgan (2001) suggested that overstretching of the sarcomeres would start the damage process.

Structural changes in E-C coupling system are quite unknown. In the study of Takekura et al. (2001), downhill running resulted in rats' forelimb muscles by ultrastructural abnormalities. The number of longitudinally oriented t-tubule increased in fast-twitch (FT) and slow-twitch (ST) fibres. In addition, the networks of caveolar clusters and the number of multiple interactions of t-tubule segments with terminal cisternae elements in sarcoplasmic reticulum are increased. Changes in the caveolar clusters are seen in ST

fibers immediately after exercise. Increased interaction between t tubules and SR terminal cisternae is only found in FT fibres during recovery 2-3 days post exercise. Furthermore, this suggests that sarcomeric changes start the damage process. Sarcomere damage slides myofibril past one another, finally damaging t-tubules.

Tendency on the structural disruption of membrane system is fibre type-specific. FT fibres are susceptible to muscle damage caused by eccentric exercise, even if there are both fibre types in the same muscle. The difference is probably due to different mechanical and metabolic properties of the fibre types. (Takekura et al. 2001.) In a rat study, fast oxidative glycolytic fibres were damaged the most and slow oxidative fibres the least after eccentric muscle actions. It is possible that fibre phenotype or lower contractile workload might be responsible for damaging first fast-oxidative-glycolytic fibres. (Vijayan et al. 2001.)

A single bout of eccentric exercise induces cellular adaptations in skeletal muscles. Malm et al. (2004) found no muscle inflammation 48h after concentric or eccentric exercise, although serum CK activity, muscle soreness and the number of blood granulocytes was increased. This led to conclusion that physical exercise does not induce muscle inflammation or leukocyte infiltration. Elevated neutrophil infiltration, Z band damage and presence of IL-1β is suggested to be consequences of the muscle biopsies.

However, partly contrary results have been obtained by the study of Stupka et al. (2001). They observed gender-related differences in the secondary responses to eccentric action-induced injury, such as serum CK activity, inflammatory cell infiltration and activation of protein degradation pathways. In females, the CK activity is lower and muscle neutrophil counts are higher compared with males. Proteolytic pathways activate after muscle damage, and consist of extracellular (e.g. inflammatory cells) and intracellular (e.g. ubiquitin) pathways. Independently of gender, ubiquitin-conjugated protein content and muscle macrophages are increased. Repeated exercise bout attenuated the

magnitude of force deficit in both genders. In addition, the muscle action velocity and type (concentric and eccentric) had not influenced on the force deficit. The Z-band streaming was not significantly different after the exercise bouts compared to rest values.

Damages caused by an eccentric exercise induce genes of stress response, specific growth-promotion and antiproliferation. Inflammatory responses (such as chemokine ligand 2 and IL-1 receptor) and vascular remodeling (tenascin C and lipocortin II) have been observed after the eccentric exercise. (Chen et al. 2003.)

During first days after eccentric training, maximal isometric tetanic force is decreased, mainly due to the E-C coupling failure. Primary site of the muscle damage takes place at the interface of t tubule and calcium ion (Ca^{2+}) release channel of the sarcoplasmic reticulum (SR). Eccentric actions may induce allosteric effects of ion or metabolites, physical disruption, and proteolytic degradation to proteins affecting the t tubule voltage sensor and SR Ca^{2+} channel. During recovery, the contribution of E-C coupling declined in relation to decreased maximal isometric tetanic force. (Ingalls et al. 1998.)

After a quick stretch, the incremental tensions are unrelated to age. Force reduction is smaller in eccentric muscle actions compared to isometric and concentric ones in aging. Contractile elements of the muscle cells may contribute to the preservation of tension and eccentric force. (Ochala et al. 2006.) Eccentric actions produce ultrastructural damages, which may increase muscle protein turnover. Muscle damages are more pronounced in older compared with younger subjects. This is due to greater rates of myofibrillar protein breakdown in elderly. (Evans 1992.)

Muscle mass is considered to be the most important determinant of functional capabilities. Isometric, concentric and eccentric exercise modes stimulate muscle hypertrophy but the relative effectiveness of different exercise modes has remained unclear in human

studies. Differences are greatly due to types of testing and effects of individual training mode on the testing in the same mode. Adams et al. (2004) studied the adaptation of skeletal muscle hypertrophy in response to isometric, lengthening and shortening mode exercises in rat muscles.

The integrated torque was highest in eccentric exercise and smallest in concentric mode. Although torque integrals differed significantly, the majority of anabolic changes were similar. Thus, training with the same activation parameters in isometric, concentric and eccentric modes led to equivalent levels of muscle hypertrophy. (Adams et al. 2004.) The level of muscle injury depends on the mechanical factors, such as peak force, initial length, length change and lengthening velocity. Total peak force has the greatest effect on the muscle performance. (Warren et al. 1993.)

Adaptation of the nervous system. Neural mechanisms have greater role in strength gain during eccentric exercise compared to other forms of activity. Altogether, eccentric actions induce structural adaptations in muscle, activate inflammatory responses and alter neural commands for movement control. (Enoka 1996.)

Nervous system controls eccentric muscle actions through different activation strategies compared to isometric and concentric actions. Relative excitability of motoneurons of muscle, its synergists and contralateral homologous muscles may be altered in order to maximize the activity of high-threshold motor units. In daily life, these motor units are not active, but they are required for high levels of muscle power. During maximal eccentric action, the activation of muscles is reduced. In a submaximal level, the recruitment order of motor units is altered. If muscles are stimulated during eccentric exercise by transcranial and peripheral nerves, the size of potentials in the muscle are decreased. In addition, muscles have better ability to resist fatigue during repeated muscle actions. (Enoka 1996.)

Neural activation is limited in lengthening actions. During maximal eccentric actions, the voluntary activation level is significantly lower compared to concentric and isometric ones. However, all fibre types are active during maximal actions in every action mode. Thus in the absence of selective recruitment pattern of muscle fibre types, the degree of voluntary activation does not differ between muscle action modes. (Beltman et al. 2004a.) These suggestions are contrary to the findings of Nardone et al. (1989) who stated that the activity of the motor unit varies according to the muscle action. They found a considerable number of high-threshold fast-twitch motor units being active only during lengthening muscle actions.

Submaximal eccentric training induces more neural adaptations than maximal concentric training when measured as surface EMG activity. Also, after repeated eccentric actions fatigue occurred less than after concentric training. Thus, concentric training causes more fatigue and less strength adaptation compared with eccentric one. It is unclear whether the neural adaptations occur centrally or peripherally. It has been suggested that neural adaptation would be more peripheral because magnitude of fatigue is the same pre- and post-training with both training modes. (Hortobágyi et al. 1996.)

The reduced force production after eccentric exercise may be due to fatigue, death or electrical inexcitability of some fibres in a whole muscle, changes in length-tension curve and reduced excitation-contraction coupling. Each of these variables has different recovery time. (Morgan & Allen 1999.)

2.2 Eccentric training

Submaximal eccentric training improves significantly both eccentric and isometric maximal force more than maximal-effort concentric training improves concentric and isometric maximal strength. Eccentric training induces more muscular adaptations and it may enhance the stiffness of passive elements more than concentric training, thus im-

proving more isometric forces. This mechanism could also partly explain slightly increased concentric force after eccentric training and vice versa. (Hortobágyi et al. 1996.)

After a concentric training period, eccentric exercise causes more muscle damage and delayed-onset muscle soreness (DOMS) compared to untrained state. This is based on a hypothesis that the variation of sarcomere lengths and the non-uniform areas of cross-section in myofibrils causes muscle fibres to lengthen non-uniformly during the eccentric action. When the weak sarcomeres of myofibril lengthen, they will affect the areas surrounding them. If the length of the weak sarcomere corresponds to the plateau of their length-tension relationship, further overextension changes sarcomere to weaker and accelerates lengthening. (Whitehead et al. 1998.)

2.2.1 Eccentric exercise modes

Eccentric muscle actions are used in movements with braking actions in order to control the motions of the body. Walking and running downhill are most common examples of eccentric exercise. (Whitehead et al. 1998.) The quadriceps muscles control the rate of knee extension against the gravity and undergo an eccentric action in each of the steps (Proske & Morgan 2001). In addition, eccentric movements are performed in activities such as horse-riding and skiing (Whitehead et al. 1998).

Eccentric training can also be done with isolated muscle groups, such as the quadriceps or the biceps brachii muscles. When training isolated muscle groups eccentrically, isokinetic or other weight machines, and with free weights can be used. In eccentric movement, for example lowering dumbbell in the biceps curl, same muscles are active during lifting and lowering the load. (Fleck & Kraemer 2004, 40.)

There are special isokinetic dynamometers which are designed for eccentric training mode, or resistance can be increased for the eccentric phase of the movement. The latter

one is called accentuated eccentric training. Also, in many machines such as leg press, load can be lifted (concentric) with two limbs and lowered (eccentric) with only one limb. While training with free weights, assistance or spotters are needed for safety reasons. Especially, when one uses training loads over 1 repetition maximum (1 RM), spotters help lifting the load or release it. They also assist with lowering the load, if it is too heavy and would result in injuries due to wrong position in the trunk during the movement. (Fleck & Kraemer 2004, 40.)

2.2.2 Eccentric training with different subjects

Athletes and body builders use eccentric training methods in order to increase muscle strength (Morgan & Allen 1999). Also weight and power lifters benefit from the exercise mode (Fleck & Kraemer 2004, 40). It is suggested that older patients with cardiopulmonary impairments, such as coronary ischemia or cardiomyopathy, will benefit from the eccentric training. Compared with concentric exercise bouts at a same submaximal workload, eccentric exercise demands lower heart rate, systolic blood pressure, and cardiac index and ventilation in young and old individuals. Thus, subjects with lower exercise tolerance are able to do aerobic or resistance training. (Vallejo et al. 2006.)

3 MUSCLE FIBERS

3.1 Structure of muscle fibre

A skeletal muscle consists of numerous muscle fibres, which vary in diameter from 10 to 80 micrometers. Fibres are composed of several hundreds of myofibrils (figure 4); myosin and actin filaments are polymerized protein molecules composing the myofibrils. Filaments lay adjacent and are responsible for the muscle action. Myosin filaments are thicker and they are fewer in number compared with the actin ones. Titin is a filamentous and springy protein holding the filaments close to each other, thus making the muscle action possible. Myofibrils have darker and lighter bands due to filaments overlapping partially. *I* bands are light, consisting only actin filaments. On the contrary, *A* bands compose both myosin filaments and the ends of actin filaments. (Guyton & Hall 2006, 72-73.)

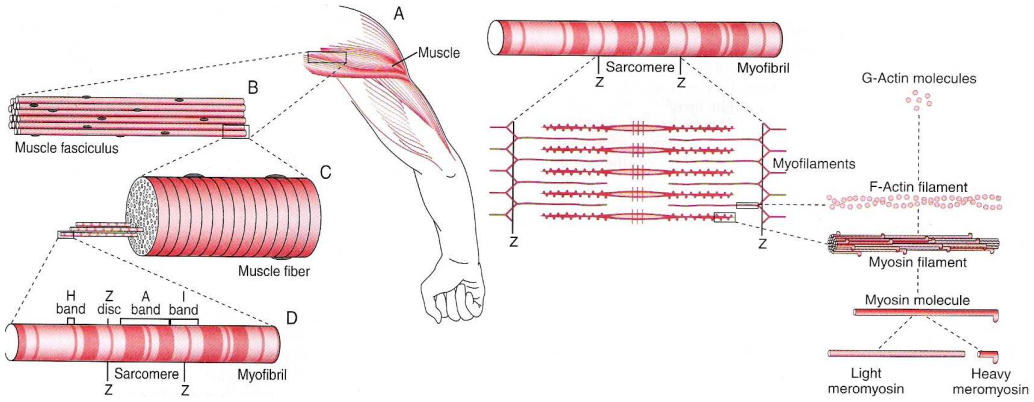


FIGURE 4. Structure of sarcomere (modified from Guyton & Hall 2006, 68).

Actin filaments are attached to *Z* discs composed of filamentous proteins. *Z* discs across myofibrils and attach them to each other in the muscle fibre. Two successive *Z* discs form a sarcomere. During a muscle action greatest force is produced when sarcomere

length is about 2 micrometers. This is due to complete overlap of myosin and actin filaments. In this sarcomere length, the tips of actin filaments start to overlap with each other. (Guyton & Hall 2006, 72-73.)

Myosin filament. A myosin molecule consists of two heavy chains and four light chains. Heavy chains are in a double helix form, wrapped spirally with each other. The double helix part is called the tail of the myosin molecule. One end of each heavy chain forms the head of the myosin molecule by bending into a globular polypeptide structure. Two light chains are also in the head part of each myosin. Myosin head functions also as an ATPase enzyme, thus affecting the use of energy in muscle action. Myosin filament is composed by hundreds of myosin molecules. Myosin tails form the body of filament by bundling together. Part of the body and the myosin head hang in the sides of the myosin filament composing cross-bridges. During the muscle action cross-bridge interacts with the actin filaments. (Guyton & Hall 2006, 75-76.)

3.2 Muscle fibre types

Human skeletal muscle consists of slow and fast fibre types (figure 5). Type I fibres are slow-twitch and have high oxidative enzyme activity. Fast-twitch, type II, fibres have lower oxidative capabilities and higher glycolytic enzyme activity compared to slow fibres. Fast fibres are divided into two subgroups according to the enzyme activity. Type IIa have higher oxidative enzyme activity compared to IIb fibres. Although both type I and IIa are oxidative, slow fibres are more insulin sensitive than fast oxidative fibres. (He et al. 2001.)

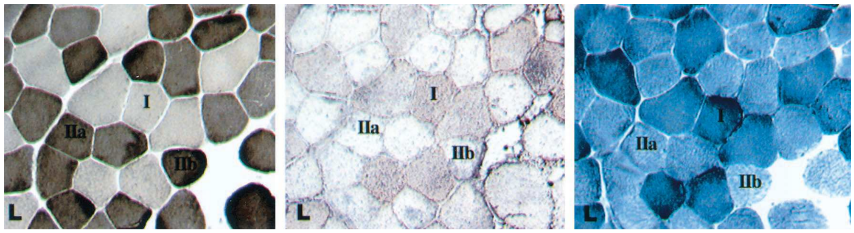


FIGURE 5. Photographs of cross-sectionals of the vastus lateralis muscle from lean volunteer, serial sections are stained for fiber type, lipid content and enzyme activity, respectively (modified from He et al. 2001).

Fast-twitch fibres reach peak tension in about 40 ms, when slow-twitch ones reach it in 80-100ms. In addition, type I fibres have longer relaxation time compared to type II fibres. Differences in conduction velocities are due to motor neurons. Fast fibres are innervated by fast conducting neurons, which have large diameter and high activation threshold. Neurons innervating slow fibres are smaller, thus they have also slower conduction speed. Activation threshold is however relatively low. Motor units of human muscle are activated based on the size principle by Henneman et al. (1965). Therefore, the order of recruitment is from type I to type IIa to type IIb. When the intensity of exercise increases, recruitment proceeds from type I to type IIb fibres. (Maughan et al. 2005, 10-13.)

Fast fibres are larger and they are able to release rapidly calcium ions due to extensive sarcoplasmic reticulum. Calcium ions are needed in order to start the muscle action. Fast fibres use glycogen as an energy substrate. Thus, they have larger amounts of glycolytic enzymes, fewer mitochondria and less blood supply compared to slow fibres. (Guyton & Hall 2006, 80-81.) Fast fibres have two primary subdivisions called IIa and IIb fibres. Both types exhibit a high capacity for glycolysis and rapid muscle action. Distinction between the subgroups is due to aerobic capacity (McArdle et al. 2001, 163.) Other subdivisions of type II fibers are also possible. Type IIa and IIb are referred as fast twitch-fatigue resistant and fast twitch-fatiguable fibres, respectively. (Maughan et al. 2005, 10.)

On the contrary, slow fibres are smaller in size and innervated nerve fibres are also smaller. Because slow fibres use energy through oxidative metabolism, they have extensive blood vessel system and increased number of mitochondria. In addition, there is a lot of myoglobin in slow fibres. Myoglobin resembles hemoglobin. They both are proteins, containing iron and acting as storage for the oxygen. Myoglobin enhances the delivery of oxygen to mitochondria and also gives the red colour for the slow fibres. Furthermore, fast fibres lack in myoglobin and are called as white muscle fibres. Slow fibres are adapted for prolonged, endurance-like muscle activity, when fast fibres are adapted for rapid force production, such as jumping. (Guyton & Hall 2006, 80-81.)

Contrary to other species, human skeletal muscles have heterogeneous fibre composition. For example, the m. vastus lateralis consists of type I, IIa and IIb fibres, 40 %, 50 % and 10 % respectively. (He et al. 2001.) The proportions of fibres determine whether the muscle reacts and produces force rapidly or slowly. The amount of slow and fast fibres is largely an inherited property. By training, one can alter distribution only a little, if at all. (Guyton & Hall 2006, 1061.) Most of the training adaptations take place in the metabolic capabilities of the muscle, independently of the fibre types (Maughan et al. 2005, 10).

There are gender-related differences in the whole muscle function. But single muscle fibres are similar in older males and females because single muscle fibre specific force or power does not differ between sexes. Therefore, gender-related differences in whole muscle strength and power are not due to contractile elements. (Krivickas et al. 2006.)

Myosin. Myosin is a contractile protein in muscle fibres. It is related to ATPase activity and shortening velocity, thus being one factor determining contractile properties of muscle. Myosin is formed from two heavy chains (MHC) and two pairs of light chains (MLC). These six subunits have multiple variation possibilities due to different isoforms of MHC and MLC subunits. (Bottinelli et al. 1991.)

From rodents, MHC isoforms 1, 2A, 2B and 2X can be found (Bottinelli et al. 1991). On the contrary, human muscles contain only isoforms 1, 2A and 2X (figure 6) (Linari et al. 2004). MHC type 1 is slow and types 2A, 2B and 2X are fast, fibres containing MHC type 2B being the fastest ones. The maximum velocity of shortening in isoforms 2A and 2X is similar. However, the maximal shortening velocities overlap among fast fibres. Muscle fibre can contain a mixture of different isoforms of MHC. The maximal power output is lower in slow fibres compared to fast ones. Fast fibre types 2B and 2X have highest maximal power output. In addition, slow fibres exhibit less force per unit cross-sectional area than fast fibres. But cross-sectional area is not related to fibre type. (Bottinelli et al. 1991.)

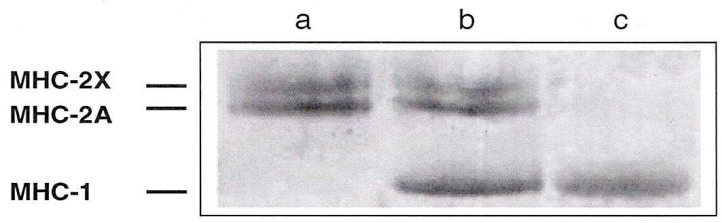


FIGURE 6. MHC isoforms from human muscle biopsy samples (modified from Linari et al. 2004).

During isometric and active shortening actions muscle fibre types produce different amounts of force and stiffness. In a lengthening action, there is no difference in maximum power output and maximal velocity between fibre types. Fast fibres produce 47-76 % higher isometric tension compared to slow fibres. This might be due to a higher number of interacting myosin heads in fast fibres. When maximally activated fibres lengthen, they increase steady force and stiffness. Force in slow fibres increases by 120% and stiffness by 65%. In fast fibres, force and stiffness are increased by 40% and 20%, respectively. (Linari et al. 2004.)

During lengthening both fibre types produce similar amounts of force and stiffness. In addition, stretch increases force twice as much as the stiffness. MHC isoform determines the isometric force produced by activated fibre. Isoform modulates the force per each myosin head and proportion of strong bound force-generating heads. MHC isoform provide also resistance to stretch to the fibre. Bonds are equally strong independently of the myosin head, which are able to interact with actin. (Linari et al. 2004.)

Slow fibres work more economically than fast fibres in isometric and concentric muscle actions. During the isometric tension, both fibre types produce almost similar tension but slow fibres use seven times less ATP than fast ones. In the concentric actions, ATP consumption is increased in direct relation to the velocity of shortening. Slow fibres produce about six times less maximal power output compared to fast fibres. Slow fibres are however more efficient in transforming chemical energy into mechanical power. (Reggiani et al. 1997.)

3.3 Adaptation of muscle fibres

Training. Resistance training increases the amount of contractile proteins of muscle fibres, thus leading to increased muscle size and strength. The mechanisms responsible for increased RNA synthesis and following protein synthesis are not entirely understood. (Evans 1992.) Heavy-load resistance training induces changes in fibre type composition. The number of type IIb fibres is decreased, while the number of IIa fibres is increased. After resistance training, the average size of type II fibers is larger compared with the size of type I fibres when there is no difference in pre-training values. No hypertrophy is usually observed in the type I fibres. (Andersen & Aagaard 2000.)

Training increases contractile activity in the skeletal muscle and induces changes in the relative distribution of MHC isoforms. Heavy-load resistance training results in down-regulation of MHC IIX content and corresponding elevation of MHC IIA content. No

changes are observed on MCH I, however, a period of detraining reverses the effect and causes overshoot of MHC IIX. Thus, after detraining the MHC IIX content can be higher than at the pre-training phase. Also, endurance training seems to decrease the amount of MHC IIX in favour of MHC IIA. (Andersen & Aagaard 2000.)

Obesity. A relationship exists between muscle fibre type and obesity. Compared to their lean counterparts, obese subjects have reduced percentage of type I and elevated percentage of IIb muscle fibres. (Tanner et al. 2002.) Particularly, android obesity is strongly linked with the amount of IIb fibres (Lillioja et al. 1987). It is not known however, whether the lower amount of slow muscle fibres is the cause or the effect of obesity. Altogether, high percentage of type I muscle fibre tends to increase the amount of weight loss during a weight loss intervention. (Tanner et al. 2002.)

Weight loss increases the oxidative capacity of each muscle fibre type. In addition, capillary density in the muscle and capillary/fibre ratio is elevated. Enhancement of oxidative capacity may be explained by relative hypoinsulinemia, hyperglucagonemia and elevated nonesterified fatty acids apparent during a prolonged weight loss period. However, weight loss does not necessarily induce changes in the muscle fibre type distribution. (Kern et al. 1999.)

Obesity and type 2 diabetes mellitus. Obesity and type 2 diabetes seem to have similar effect on the muscle fibre type distribution. Gaster et al. (2001) found obese diabetic and obese non-diabetic subjects to have a lower fraction of oxidative slow-twitch fibres in skeletal muscles compared to lean, young controls. There was no difference between obese and diabetic subjects. In addition, diabetics have reduced glucose transporter protein 4 (GLUT4) density in slow muscle fibres compared to obese and lean counterparts. Decreased expression of GLUT4 could partly explain reduced insulin-stimulated glucose uptake, leading to insulin resistance in diabetes. (Gaster et al. 2001.)

GLUT1 and GLUT4 are the isoforms, which mediate glucose transport in human skeletal muscles (Kern et al. 1990). GLUT4 is suggested to determine the responsiveness and sensitivity of glucose uptake in insulin-sensitivity cell systems. GLUT4 mediated glucose uptake is stimulated either insulin or muscle action in human skeletal muscle. (Gaster et al. 2000.)

Muscle fibre type is dependent on the total amount of GLUT4 glucose transporter protein. Expression of GLUT4 is higher in slow fibres compared to fast ones. Differences in GLUT4 expression could explain, at least partly, the insulin responsiveness variation between fibre types. (Kern et al. 1990.) Expression level is higher in slow fibres compared to fast fibres in subjects with reduced glucose tolerance. Physical inactivity seems to be related to reduced insulin sensitivity and GLUT4 levels, and lower fraction of type II fibres. In addition, GLUT4 expression is reduced in fast fibres with aging. (Gaster et al. 2000.)

Metabolic capacity of fibre types may be affected by obesity and type 2 diabetes. Obese and diabetic subjects have lower oxidative enzyme activity than their lean counterparts. Reduction is apparent in each of the fibre types indicating the basis of decreased oxidative enzyme activity in obesity and diabetes. However, differences in glycolytic enzyme activity and fibre type distribution (figure 7) have not been detected. (He et al. 2001.) On the contrary, Oberbach et al. (2006) found type 2 diabetic patients to have increased glycolytic capacity and decreased oxidative capacity. Moreover, diabetics had reduced fraction of slow fibres and elevated proportion of fast glycolytic fibres (figure 8). In addition, intramyocyte content of triglyceride in muscle fibres' is elevated in obesity and obesity-related diabetes (He et al. 2001).

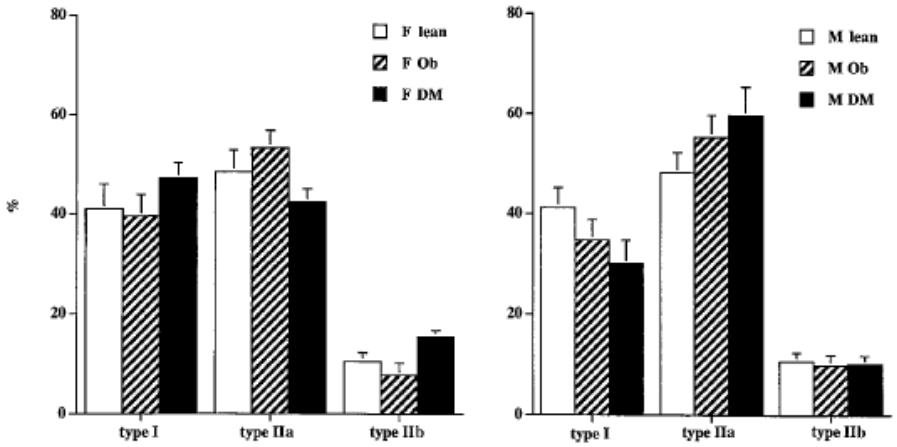


FIGURE 7. Skeletal muscle fiber distribution is not statistically significantly different between type 2 diabetics and healthy controls in females and males (modified from He et al. 2001).

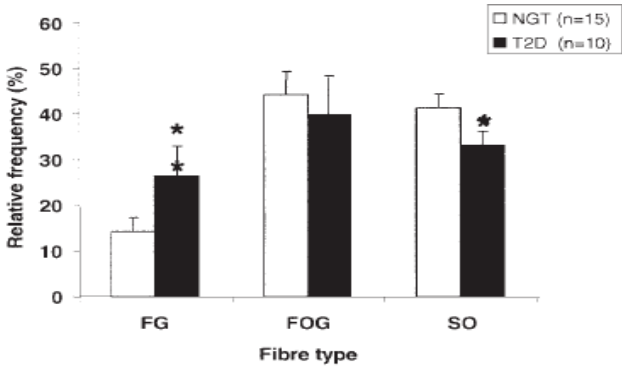


FIGURE 8. Differences in skeletal muscle fiber distribution between healthy volunteers and type 2 diabetics, groups include females and males. * p< 0,05 (modified from Oberbach et al. 2006).

Muscle fibre characteristics may influence on the development of glucose tolerance. The study of Larsson et al. (1999) indicated that postmenopausal women with impaired glucose tolerance (IGT) had larger type IIa and IIx muscle fibres. In addition, the degree of capillarization of type I fibers affected glucose tolerance. However, muscle fibre composition was not proven to determine development of IGT. Lillioja et al. (1987) found that capillary density of skeletal muscle and fractions of muscle fibre types are related to insulin action, in vivo. This could be explained by biochemical changes in cellular oxidative capacity or limitation of insulin diffusion when capillaries are widely spaced.

Immobilization and aging. Immobilization and aging induce similar changes on skeletal muscles, muscle atrophy. The rate of protein breakdown exceeds the rate of synthesis leading to loss of contractile protein content from the muscle cells. Muscle atrophy is seen in reduced muscle fibre size. The fixed length of muscle influences on the rate of atrophy, which is slower in muscles that are fixed at greater than resting length compared to muscle in shortened position. Due to reduced protein content, cross-sectional area of fibres decreases causing strength loss in absolute and relative values (figure 9). In addition to strength loss, functional capacity, insulin sensitivity, bone density and energy requirements are age-relatively decreased. Skeletal muscle maintains however the ability to respond to strengthening exercise with increased strength and muscle size. (Evans 1992.)

Both quantitative properties, such as loss of muscle mass, and qualitative mechanism of muscle, i.e. capacity of muscle fibre to produce force, are influenced by aging. Loss of force of single muscle fibre is higher than decline of cross-sectional area in elderly compared to young people. Lowered force production might be due to decreased number of strongly bound acto-myosin interactions during maximal activation or such an interaction produces less force. The maximal shortening velocity of type I and type IIa fibres is lower in elderly subjects compared to younger ones. Same kind of trend is also observed with type IIab, containing MHC isoforms 2A and 2X, and IIb fibres that contain only MHC 2X. Thus, aging might influence on both fast and slow fibres. Especially, immobilisation enhances the effect of aging. (D'Antona et al. 2003.)

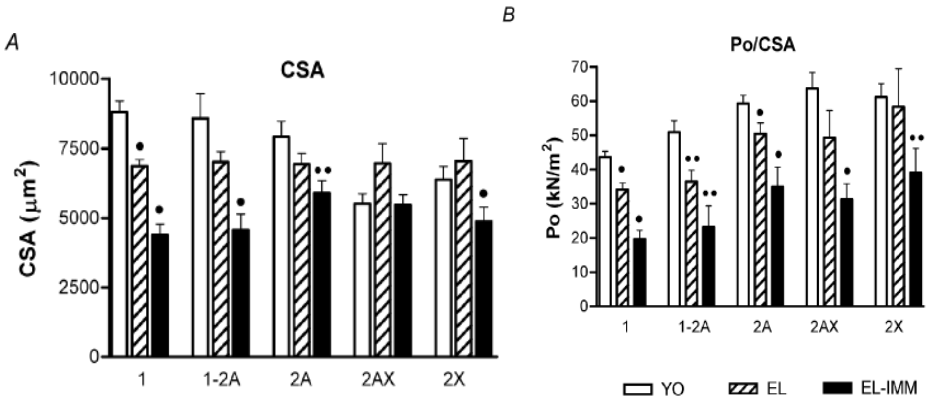


FIGURE 9. (A) Cross-sectional area, and (B) specific force of the single muscle fiber from young (YO), elderly (EL) and immobilized elderly (EL-IMM) (modified from D'Antona et al. 2003).

Muscle characteristics are altered due to aging. The cross-sectional area of fast type II fibers decreases progressively, but the area of type I fibres remains almost the same. Alterations may start already at 30 years of age. Although the relative areas of type I and IIb changed, the percentage of different fibres has no age-related alterations. (Korhonen et al. 2006.)

The synthesis rate of skeletal muscle myosin heavy chain (MHC) declines in aging but sarcoplasmic proteins are observed to maintain their synthesis rate (Balagopal et al. 1997). Composition of slow MHC is elevated, and proportion of fast MHC IIx and shortening velocity of muscle cells are decreased (Korhonen et al. 2006). The rate of MHC synthesis is related to muscle mass and to muscle strength per unit muscle mass. In addition, age has effect on muscle strength independently of muscle mass. On the contrary, synthesis rates of mixed muscle protein and sarcoplasmic protein demonstrates to have no correlations with the muscle strength. Levels of IGF-I, dehydroepianrosterone (DHEAS) and testosterone (in males) in circulation have anabolic effect on muscle and also they affect MHC synthesis. (Balagopal et al. 1997.) Age-related loss of absolute maximal force is largely due to loss of contractile tissue (Korhonen et al. 2006).

Reduced MHC synthesis declines ability of skeletal muscle to remodel and maintain the quality and quantity of MHC. Decline in the quality is observed as reduced muscle strength per unit muscle mass. Probably, MHC synthesis has direct effect on the muscle mass. Thus, it could be a part of sarcopenia mechanisms of aging. Other factors leading to sarcopenia are vascular and neuronal factors, and reduced synthesis of other proteins. (Balagopal et al. 1997.)

There might be gender-related differences in the parameters regulating sarcopenia. In females, the relation between muscle strength and synthesis rate of MHC is stronger than with males. On the contrary, in males the hormonal levels and MHC had stronger relation. (Balagopal et al. 1997.)

Transcript levels of MHC decrease in aging. Especially, transcript levels of MHCIIa and MHCIIx are reduced significantly in messenger RNA. This could explain the lower number of fast-twitch muscle fibres in elderly. These results are contrary to a hypothesis that suggests MHC synthesis rate to be diminished in posttranscriptional phase. (Balagopal et al 2001.) In a rat study, reduced protein synthesis was mainly due to decreased protein translation (Haddad & Adams 2006).

Age-related decline in protein synthesis might be caused by a DNA damage or defects at the transcriptional level. In the study of Balagopal et al. (2001), resistance training program increased muscle strength and MHC synthesis rate. The changes occurred however at transcript level of MHCI in middle-aged and older subjects, thus partly explaining the relatively high number of type I fibres in elderly subjects.

There is a linear relationship between loss of specific force and myosin concentration. Young people have higher amounts of myosin in a single muscle fibre compared to elderly. Immobilisation is observed to increase the concentration of very fast myosin isoform 2X. During immobilisation in elderly, atrophy influences mostly on the slow mus-

cle fibres. In aging, variables such as immobilisation, partial denervation, exercise habits, physiological and pathological conditions effect on muscle phenotypes. (D'Antona et al. 2003.)

4 ENERGY EXPENDITURE

Carbohydrates, fats and proteins provide substrates for chemical reactions catalyzed by enzymes and cofactors. Substrates can be obtained from diet or from endogenous stores in the body. Substrates are converted to adenosine triphosphate (ATP), chemical energy that body can use, through different pathways in order to maintain cells ATP concentration. (Coyle 2000.) Energy is freed when phosphate bonds in the ATP are broken (figure 10), and inorganic phosphate (P_i) is liberated. From each degraded mole of ATP, muscles can perform up to 24 kJ of work. (Maughan et al. 2005, 56-58.)

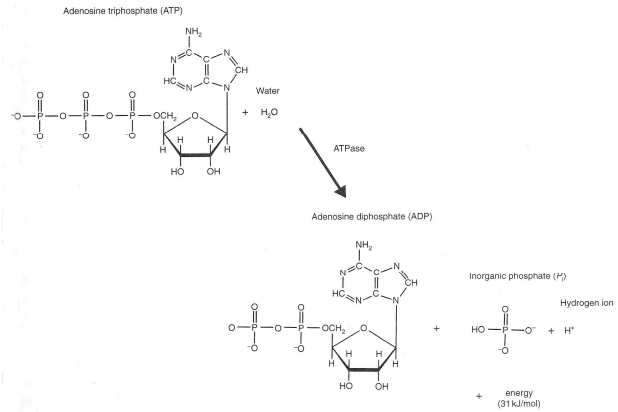


FIGURE 10. Hydrolysis of ATP (modified from Maughan et al. 2005, 57.)

The utilization of energy substrates is integrated and affected by substrate availability, exercise intensity and time. Decrease in the utilization of one energy substrate leads to compensatory increase in the use of another energy supply. Energy can be formed from

four different pathways. Two fastest ways to generate energy are the enzymatic breakdown of ATP to adenosine diphosphate (ADP) and P_i, and the enzymatic breakdown of phosphocreatine (PCr) to creatine and phosphate. They are also referred as the phosphagen (alactic) system. (Maughan et al. 2005, 54-58.)

Anaerobic synthesis of ATP is called glycolysis, also known as the glycolytic system. Reaction series begin from dissolving either glucose or glycogen in the cell cytoplasm. Aerobic pathway takes place in the cell mitochondria in the tricarboxylic acid (TCA) cycle. Metabolic rate is influenced by exercise intensity which leads to training adaptations of the cell. Alterations on the balance between protein synthesis and degradation result in adaptations. (Coyle 2000.)

4.1 Factors affecting substrate utilization

4.1.1 Exercise mode, intensity and duration

Skeletal muscle acts in isometric, eccentric and concentric ways. Metabolic cost of isometric work is lower compared with dynamic muscle actions. Muscle is continually active during isometric action, and it needs about half of the energy required in shortening actions. On the contrary in movements, such as sprinting, muscle is active only a short time period. In dynamic muscle actions, there is also a possibility for some recovery during the movement. (Newham et al. 1995.)

In animal studies, differential coactivation or recruitment in voluntary actions does not affect significantly the energy cost of movement involving different muscle action types. Intrinsic property of the muscle determines the energy cost in action modes. During lengthening actions the energy cost per unit force-time integral is the lowest. In addition, the metabolic cost of lengthening actions in maximally stimulated (rat) muscles does not significantly differ from isometric and shortening ones. This might be due to

relatively low velocity of the muscle actions and large variation of high-energy phosphate consumption values. Decline in PCr/Cr ratio is significantly smaller after lengthening actions compared to after shortening ones. (Beltman et al. 2004b.) In human studies however, oxidative metabolic response to metabolic strain is similar in concentric and eccentric muscle actions in skeletal muscle. Therefore, the ATP synthesis rate is determined by metabolic strain, not muscle action. (Combs et al. 1999.)

Altogether, dynamic muscle actions induce more fatigue than isometric ones. Fatigue is observed as a loss of force and higher energy requirements. If isometric actions are intermittent imitating dynamic actions, the metabolic cost and pattern of fatigue are similar with dynamic movements. This is explained by a combination of two factors. Prolonged muscle activation and repeated external work increase the metabolic cost. (Newham et al. 1995.)

An uptake and oxidation of long-chain fatty acids in skeletal muscle are increased at the onset of exercise. Prolonged exercise for several hours enhances lipid utilization for energy. Exercise intensity plays a major role in determining the energy fuel selection. With increasing intensity, also use of carbohydrates for fuel is increased at the expense of fat oxidation. Endurance training results in enhanced lipid utilization. Although the uptake and oxidation of fatty acids are well described in several studies, the regulatory mechanisms behind phenomena are not fully known. (Kiens 2006.)

Traditionally, endurance training (ET) is emphasized and recommended for treating the metabolic syndrome or type 2 diabetes. Strength training (ST) is proven to be as good, or even better, exercise mode compared with ET. ST improves significantly long-term glycemic control, seen as decreased glycosylated hemoglobin, and insulin resistance on type 2 diabetics. In addition, strength training changes body composition positively: the amount of muscle mass increases and fat mass decreases. Thus, muscle tissue plays a major role in insulin resistance and type 2 diabetes. Changes in body composition are

strongly associated with alterations in blood lipid profile. After a ST intervention, total cholesterol, low-density lipoprotein cholesterol and serum triglycerides are reduced and high-density lipoprotein cholesterol is increased. Effects of ET on the metabolic parameters, such as glycosylated hemoglobin and insulin resistance, and lipid profile are only moderate. (Cauza et al. 2005.)

Eccentric exercise. Kinetics of VO_2 differs between exercise modes due to variations in muscle recruitment patterns. For example, running and cycling have different proportions of eccentric and concentric muscle activation. Eccentric action has lower physiological cost compared to concentric muscle action. In addition, fewer muscle fibres are recruited in eccentric cycling compared to concentric exercise performed at the same work rate. (Perrey et al. 2001.)

Eccentric exercise alters signaling-transporter mechanisms in the muscle and reduces insulin-mediated whole body glucose disposal. In older subjects, reduced CHO oxidation and increased lipid oxidation have been observed during hyperglycemia. Substrate metabolism after eccentric exercise varies in relation with age. In normal compensation after exercise-induced muscle damage, CHO oxidation is maintained and lipid oxidation diminished during hyperglycemia. However, in older subjects oxidation of glucose is depressed and lipid oxidation elevated (figure 11) after eccentric exercise. (Krishnan et al. 2003.)

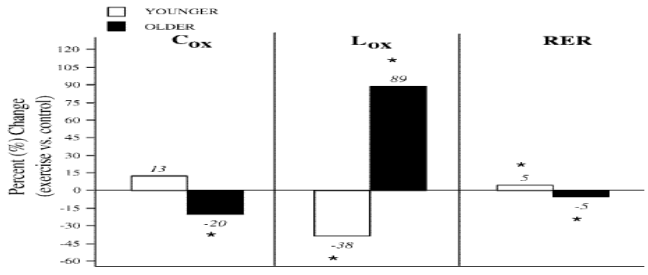


FIGURE 11. Age-related differences in substrate oxidation rates (C_{ox} carbohydrate oxidation; L_{ox} lipid oxidation) after eccentric exercise, * exercise significantly different from control, p< 0,05 (modified from Krishnan et al. 2003).

Muscle fibre characteristics influence on lipid oxidation. Individuals with high number of type I fibres have more active lipid oxidation at rest and during submaximal exercise. Especially at basal state, the difference in lipid oxidation is significant between subjects with high type I contribution compared with subjects with low number of type I fibres. Tendency decreases at submaximal exercise but it is still apparent. (Turpeinen et al. 2006.)

Eccentric exercise increases glycogenolysis leading to reduced mean resting glycogen content, especially in the type II muscle fibres. Also, maximal concentric exercise capacity is decreased. Resting glycogen level seems to be affected more type I fibres after concentric exercise. After prior eccentric exercise, muscle works at higher relative workload during concentric exercise. Therefore, the utilization of muscle glycogen increases and endurance performance is decreased. (Asp et al. 1998.)

After eccentric exercise to unaccustomed muscle, whole-body and muscle glucose uptake are impaired during maximum insulin stimulation for two days. However, no effect has been seen at submaximal insulin concentrations. This might be due to activation of glycogen synthase, which is impaired only at the submaximal insulin concentration after eccentric exercise. In addition, GLUT4 protein and glycogen contents are decreased. GLUT4 protein content is not the only factor determining muscle glucose uptake at maximal insulin stimulation. (Asp et al. 1996.)

In a fast-twitch muscle, insulin action on skeletal muscle glucose transport is impaired prior to eccentric actions. In a mice study, decrement of insulin action was most pronounced in the white quadriceps muscle. Suppression of insulin action was less in the red quadriceps muscle but in the soleus muscle no suppression was detected. Whole body insulin resistance seems to be caused by local muscle effects, at least partly. Muscle glucose uptake, induced by insulin, is dependent of the GLUT-4 protein content of

the muscle. After eccentric exercise, the muscle content of GLUT-4 and insulin-mediated glucose transport is decreased, and glycogen degradation is increased. (Asp & Richter 1996.)

Decrement of the GLUT-4 protein content is found locally, in the eccentrically exercised muscle, and it coincides with low muscle glycogen concentrations. In the study of Asp et al. (1995), low muscle glycogen concentrations during few subsequent days after eccentric exercise could not be explained by increased number of inflammatory cells. It is possible, that the concentration of GLUT-4 influences on the capacity to synthesize glycogen. Plasma membrane damage caused by eccentric exercise may also be involved in the development of insulin resistance after eccentric exercise. Insertion of GLUT-4 into the plasma membrane is more difficult due to membrane damages leading to faster degradation or defective translocation of GLUT-4. The GLUT-4 concentration and muscle glycogen stores are restored within four days after eccentric exercise.

4.1.2 Hormones and exercise

Energy substrate contribution is affected by hormones during exercise. Interaction between insulin, catecholamines and glucagon has the greatest effect on the substrate availability and usage. Increased blood glucose concentration stimulates the insulin secretion, when exercise usually reduces it. Insulin promotes synthesis of lipid, glycogen and protein by inhibiting lipolysis and stimulating the uptake of glucose from the blood by tissues and cellular uptake of amino acids. It also inhibits release of glucose from the liver and release of FFA from the adipose tissue. Glucagon is a counter actor for insulin by increasing the rate of glycogen breakdown and gluconeogenesis in the liver. Glucagon secretion is stimulated by a drop in blood glucose concentration. (Maughan et al. 2005, 136.)

Catecholamines adrenaline and noradrenaline affect the heart rate and contractility, and alter blood vessel diameters. Adrenaline impacts more to the substrate availability than noradrenaline by promoting lipolysis in the adipose tissue, and glycogenolysis in the muscle and liver. Adrenaline elevates FFA availability in the plasma and inhibits insulin secretion. Hypotension, hypoglycaemia and exercise, when the intensity is over 50% VO_{2max}, activate secretion of catecholamines to the blood plasma. (Maughan et al. 2005, 137.)

In addition, cortisol and growth hormone have minor impact on substrate availability. Growth hormone stimulates the mobilization of FFA from the adipose tissue. Exercise intensity influences on the growth hormone secretion. Cortisol promotes protein degradation, amino acid release from the muscle, gluconeogenesis in the liver, and the effect of the catecholamines. Prolonged strenuous exercise elevates the release of cortisol. Cortisol release is stimulated by the secretion of adrenocorticotrophic hormone. Cytokines are protein messenger molecules acting hormone-like manner. During prolonged exercise cytokines, such as interleukin-6 (IL-6), are released by active muscles when muscle glycogen stores start to diminish. IL-6 stimulates liver glycogen breakdown and lipolysis in the adipose tissue. (Maughan et al. 2005, 137-138.)

4.1.3 Nutrition

Intensity and duration of the training session can be influenced by a diet. High-intensity training requires adequate dietary CHO in order to maintain muscle glycogen stores. Glycogen availability is not often a limiting factor in middle distance events, but it is important during intensive training phases. Adequate CHO intake, immediately after exercise, ensures fulfilled muscle and liver glycogen stores before next training session. Before extreme prolonged exercise, the glycogen stores of body should be filled by reducing training volume and increasing CHO intake. When exercise lasts over 1h, endurance capacity can be improved by carbohydrate ingestion during exercise. This delays

the fatigue development by slowing the rate of liver glycogen depletion and maintaining the blood glucose concentration. (Maughan et al. 2005, 110; 140-143.)

Consuming fat does not influence on the substrate availability significantly. Concentration of long-chain fatty acids (LCFA) in plasma is high, when pre-exercise diet has high fat content and time from last meal is long. Arterial LCFA concentration is initially decreased during exercise, which is followed by a slow increase. Depressed concentration may be a result of slow mobilization of fatty acids from adipose tissue and rapidly increased utilization of LCFA in skeletal muscles. (Kiens 2006.) Direct infusion of triaglycerol into circulation increases fat oxidation when plasma fatty acid concentration is low. However, ingestion of medium-chain or long-chain triacylglyserols during exercise has only limited effect on substrate metabolism. (Horowitz & Klein 2000.)

4.1.4 Obesity and type 2 diabetes mellitus

Obese, type 2 diabetics have about 7% higher 24-h energy expenditure (EE) compared to obese, nondiabetic individuals, when it is adjusted to fat free mass, fat mass, spontaneous physical activity, sex and age. Also during the development of type 2 diabetes, rest EE may be increased. This could partly be due to elevated blood glucose by hepatic glucose production. Increased lipid oxidation may provide energy for the glucose production. This leads to elevated levels of plasma FFAs which is associated with mechanisms increasing rest EE. (Bitz et al. 2004.)

These mechanisms could be impaired insulin-mediated suppression of hepatic glucose production and glucose uptake by muscles. Synthesis of VLDL may also be increased and mitochondrial uncoupling proteins stimulated. Although diabetic individuals have higher EE, they are more resistant to lose weight on weight management programs. This suggests that appetite regulation is effected more by other pathophysiological abnor-

malities. In addition, hormones and sympathetic nervous system (SNS) activity modulate the EE. (Bitz et al. 2004.)

Amount of muscle lipid may have some regulatory processes with enzymatic capacity for substrate utilization. In lean subjects, ratio of muscle triglycerides to oxidative enzyme activity seems to be consistent in all fibre types. Differences on oxidative enzyme activity and lipid content are apparent in each fibre type when parameters are considered independently (figure 12). In obesity and diabetes, interaction between muscle lipid and enzymatic capacity appears to be unbalanced. Muscle lipid stores are much greater in relation to oxidative capacity. Imbalanced interaction is unrelated to muscle fibre type. The exact mechanism for the disturbed balance is unknown. (He et al. 2001.)

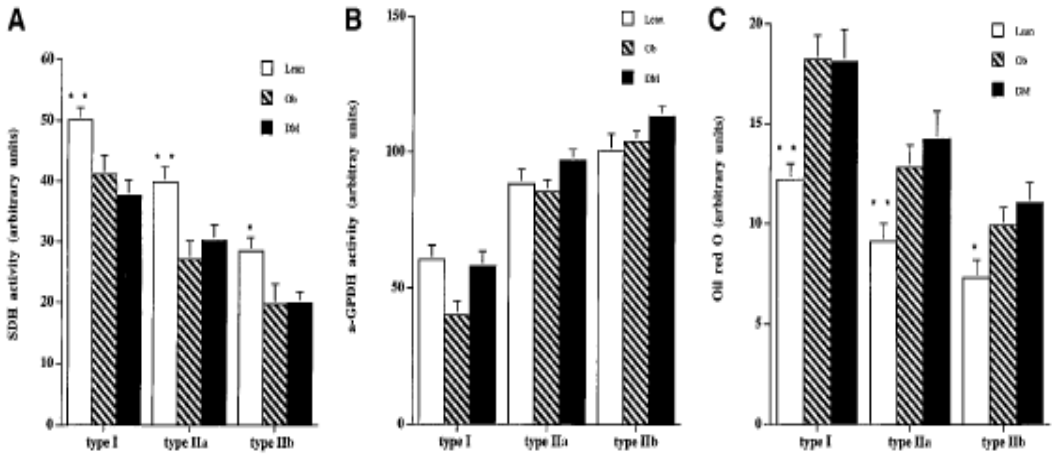


FIGURE 12. Differences on the skeletal muscle (A) oxidative and (B) glycolytic enzyme activity, and (C) skeletal muscle lipid content in three fibre types between lean, obese and diabetic subjects, ** p< 0,01, * p< 0,05 (modified from He et al. 2001).

Impaired lipid utilization in obesity or type 2 diabetes may be explained by several mechanisms. Different muscle characteristics may enhance fat storage at the expense of fat oxidation in obese, insulin-resistant or type 2 diabetic muscle. Characteristics, affecting the fat utilization alone or combined with another factor, include fatty acid transport capacity, potential for β-oxidation, oxidation capacity, fibre type pattern, degree of capillarization and tissue blood flow. Reduced fatty acid uptake and oxidation could be

partly due to diminished catecholamine-mediated lipolysis. Thus, the efficiency of plasma fatty acid uptake is depressed during post-absorptive conditions and β-adrenergic stimulation in abdominally obese and obese diabetic subjects. (Blaak 2005.)

Reduced lipid oxidation is most prominent in very obese subjects (BMI> 35 kg/m^2), especially with abdominal fat distribution. Diminished fatty acid utilization is also observed in obese, type 2 diabetics. Abnormal fat metabolism promotes fat storage in muscle and liver, thus enhancing development of insulin resistance and type 2 diabetes. Reduced fat oxidative capacity is suggested to be an early factor for the development of insulin resistance and type 2 diabetes. (Blaak 2005.)

In the development of insulin resistance, the accumulation of lipid in skeletal muscle and also in tissues has a major role. Bruce et al. (2003) found a strong relation between insulin sensitivity and oxidative enzyme capacity. Association was independent of age and training status. In addition, whole-body maximal aerobic power may predict insulin sensitivity. Elevated intramuscular triglyceride content is related with insulin resistance in sedentary individuals (Goodpaster & Wolf 2004), which may lead to obesity related type 2 diabetes. However, lipid pool in muscles is reduced after diet-induced weight loss. Amount of decreased muscle triglycerides is systematically related with the magnitude of reduced fat mass. (Goodpaster et al. 2000.)

Type 2 diabetes mellitus. Type 2 diabetics are insulin resistant and majority of them are also obese. In insulin resistance, tissues are unable to increase glucose uptake in response to insulin. In normal conditions, most of the insulin-mediated glucose disposal takes place in skeletal muscles. Healthy muscles utilize lipid or carbohydrate as fuels, and transit effectively between two substrates according to exercise stimulus and energy demands. It is possible that insulin resistance alters also muscles ability to transit between different energy pathways. (Goodpaster & Wolf 2004.)

Permeability-surface area product for glucose and insulin in muscle describes their capacity to reach interstitial fluid. It is depended of molecular size of insulin and glucose, and capillary surface area, and it is determined by capillary recruitment. In diabetics, the permeability-surface is subnormal during steady-state insulin clamp conditions, thus affecting the glucose and insulin metabolism. During insulin infusion, reduced muscle capillary recruitment and low permeability-surface for glucose are apparent in type 2 diabetics. (Gudbjörnsdóttir et al. 2005.)

Skeletal muscles of diabetics have elevated glycolytic and reduced oxidative capacity. Alterations are due to changes in fibre composition and fibre-specific metabolism. In addition, contractility of skeletal muscles is diminished by chronic hyperglycemia and insulin resistance. Oxidative and glycolytic enzyme capacities are increased parallel in muscle fibres in diabetes. (Oberbach et al. 2006.)

Contradictory results have also been obtained. In the study by He et al. (2001) diabetics had decreased oxidative enzyme capacity and glycolytic enzyme capacity was unchanged. Differences in fibre classification and characteristics of diabetics, such as age, antidiabetic treatment, and duration of diabetes, could explain controversial results. Reduced oxidative enzyme activity in type 2 diabetes might be explained by imbalance between muscle lipid content and enzymatic capacity for substrate oxidation. Increments in enzyme activity may be a compensatory mechanism to insulin resistance or unbalanced glucose metabolism. (Oberbach et al. 2006.) Scheuermann-Freestone et al. (2003) observed type 2 diabetic subjects to have impaired high-energy phosphate metabolism in cardiac and skeletal muscle. However, diabetics seemed to have normal cardiac morphology and function. In addition, diabetics had faster loss of PCr in skeletal muscle, pH decline and deoxygenation.

Obesity. Carbohydrate and lipid metabolism are affected by the skeletal muscle mass during rest and exercise state. Disturbance in muscle metabolism may enhance the de-

velopment of obesity, or vice versa. If the oxidative metabolism of muscle is impaired, body's energy stores start to accumulate leading to obesity. Sedentary lifestyle is strongly linked with obesity. Low physical activity diminishes energy expenditure and changes muscle metabolism by reducing muscle mass, muscle capillary density, substrate delivery and muscle oxidative capacity. (Blaak 2005.)

Abdominal adiposity is associated with changes in substrate metabolism. Increased fat store in abdominal area elevates lipid availability and oxidation, thus suppressing utilization of carbohydrates. (Krishnan et al. 2003.) Therefore, concentrations of total and subfractions of long-chain fatty acyl-CoAs are higher in obese and overweight subjects compared to normal-weight counterparts. Hulver et al. (2003) also observed reduced fatty acid oxidation in extremely obese subjects. Moderately obese subjects' fatty acid oxidation did not differ from lean controls. Impaired fatty acid oxidation in extremely obese individuals may result from up-regulated triacylglycerol (TG) synthesis and down-regulated TG hydrolysis.

Prior significant obesity seems to have effect on the substrate utilization even after a massive weight loss. After the weight loss previously morbidly obese women still use more carbohydrates and less fat as energy fuel compared to their weight-matched controls during submaximal exercise. This occurs even at very low exercise intensities. On the other hand, it also might be a part of the reason exposing individuals for significant obesity. (Guesbeck et al. 2001.)

Insulin resistance reduces the proportion of exercise energy expenditure derived from nonplasma glucose, such as muscle glycogen. Braun et al. (2004) observed that obese or overweight, insulin-resistant subjects utilize less nonplasma glucose compared to their weight-matched, insulin-sensitive counterparts. However, insulin resistance does not influence on blood glucose uptake during exercise. Location of fat store impacts on the metabolism. Visceral adiposity is linked with insulin resistance, thus affecting substrate

utilization. Whether the effect is direct (regulation of glycogenolytic and lipolytic pathways) or indirect (altered nutrient storage), it is not known.

Relationship between obesity and insulin resistance is strong, although the exact etiology is unknown. Constant hyperinsulinemia leads to insulin resistance which may down-regulate insulin-stimulated pathways in tissues. (Blaak 2005.) Lifestyle plays an important role in glucose tolerance. Inactivity and obesity increase insulin resistance. Weight reduction improves insulin sensitivity substantially in individuals with impaired glucose tolerance. Degree of weight loss determines the degree of improvement in insulin sensitivity. In addition, weight reduction may slightly influence on insulin secretion. Thus, moderate weight loss and healthy lifestyles result in improved insulin sensitivity, and postponed or inhibited development of diabetes mellitus. (Uusitalo et al. 2003.)

4.1.5 Other factors

During isometric maximal voluntary muscle action, higher mean and peak glycolytic rates have been observed in males compared to females. Higher glycolytic rates also result in lower pH-values in males. (Russ et al. 2005.) In the course of aging, basal metabolic rate reduces and amount of body fat increases. The increment of body fat is normal consequence of the absence of strenuous physical activity in daily life. (Krishnan et al. 2003.) Aging is often associated with a lack of physical activity or even immobilization, but their effects on the energy production are also independent. Immobilization decreases capacity for fatty acid, glucose and pyruvate utilization. In addition, glucose tolerance may rapidly be decreased. (Evans 1992.)

Age-related reduction in the basal metabolic rate is due to decline in lipid oxidation. Carbohydrate metabolism remains normal in elderly. (Krishnan et al. 2003.) Also contradictory results have been obtained. Hunter et al. (2002) found oxidative and glycolytic capacity to decrease in aging. But by exercise training, the metabolic capacity of

the skeletal muscles can be improved, or maintained at levels allowing high quality of life. It seems that aging might not influence uniformly on the mitochondrial capacity of the skeletal muscles. The study of Houmard et al. (1998) showed that in the gastrocnemius citrate synthase activity, influencing on the TCA cycle, decreases with aging, but corresponding alterations were not monitored in the vastus lateralis muscle.

4.2 Methods for measuring energy expenditure and substrate utilization

Daily energy expenditure (EE) consists of basal metabolic rate (BMR), energy consumed in physical activity and thermogenesis induced by nutrition, drugs and stress factors. BMR is the minimum amount of energy that is used when individual is awake. (Klausen et al. 1997.) BMR takes about 60% of the energy expenditure, and maintains for example cardiopulmonary activity. Feeding increases EE due to digestion, transport and deposition of nutrients. (Leibel et al. 1995.) Body composition and age affect the energy expenditure. Age-related decline in EE can be sum of factors such as more sedentary lifestyle, changes in FFM composition and in thermogenic hormones, or down-regulation of metabolism in mitochondria. If energy expenditure is adjusted to body composition, gender related differences are not always detected. (Klausen et al. 1997.)

Energy expenditure can be measured by direct and indirect calorimetry. Direct calorimetry is based on the heat production of the body at rest and during exercise detected by an insulated calorimeter. The technique has only limited practical applications due to its high requirements on time, expense and engineering expertise. Direct calorimetry has been used for the validation of indirect methods. (McArdle et al. 2001, 175-176.)

Indirect calorimetry. Indirect calorimetry is based on the measurement of whole body oxygen (VO_2) consumption and production of carbon dioxide (VCO_2). At the tissue level, respiratory quotient (RQ) refers to the quantity of CO_2 production in relation to O_2

consumption. In the lungs, this gas exchange is an approximate measure called respiratory exchange ratio (RER). Therefore, RER is indirect measurement of RQ. Due to differences in the chemical compositions, CHOs, fats and proteins differ in the amounts of O_2 needed and produced CO_2 when oxidized. Thus, RER values allow measuring the mixture of fuels being oxidized. (Jeukendrup & Wallis 2005.)

Calculations of carbohydrate and fat oxidation are based on stoichiometric equations. Often the equations assume glucose to be major energy source from CHO during exercise, although glycogen is in many conditions the predominant source. In addition, indirect calorimetry cannot distinguish different sources of fat used as energy. Amount of oxidized protein can be estimated from the excreted amount of nitrogen in urine and it varies according to the protein. But many studies use classical value in which 1g of nitrogen equates to the oxidation of 6.25g of protein. Stoichiometry for CHO and fat differ due to various lengths of FA or type of CHO. These differences explain partly several versions of the equations for calculating substrate utilization. (Jeukendrup & Wallis 2005.)

Table 1 shows the equations for substrate utilization that have been used in the previous studies. Proposed equations for CHO oxidation take into account that during exercise glycogen is predominant energy source, not glucose. Thus, relative contribution of various CHO sources is taken into consideration. For low intensity exercise, there is a different equation which assumes that 50% of CHO oxidation comes from glucose and 50% from glycogen. (Jeukendrup & Wallis 2005.)

TABLE 1. Equations for substrate utilization used in different studies (modified from Jeukendrup & Wallis 2005).

Author	Equation	Calculated oxidation rates * (g/min)
Carbohydrate oxidation		
Lusk [48]	derived from tables	2.18
Brouwer [15]	$4.170 \cdot \dot{V}CO_2 - 2.965 \cdot \dot{V}O_2 - 0.390 \cdot p$	1.97
Frayn [24]	$4.55 \cdot \dot{V}CO_2 - 3.21 \cdot \dot{V}O_2 - 2.87 \cdot n$	2.21
Ferrannini [22] (glc)	$4.55 \cdot \dot{V}CO_2 - 3.21 \cdot \dot{V}O_2 - 2.87 \cdot n$	2.21
Ferrannini [22] (gly)	$4.09 \cdot \dot{V}CO_2 - 2.88 \cdot \dot{V}O_2 - 2.59 \cdot n$	2.00
Péronnet and Massicotte [52]	$4.585 \cdot \dot{V}CO_2 - 3.226 \cdot \dot{V}O_2$	2.25
Proposed equation for low intensity exercise (40 – 50 % $\dot{V}O_{2max}$)	$4.344 \cdot \dot{V}CO_2 - 3.061 \cdot \dot{V}O_2 - 2.37 \cdot n$	2.12
Proposed equation for moderate to high intensity exercise (50 – 75 % $\dot{V}O_{2max}$)	$4.210 \cdot \dot{V}CO_2 - 2.962 \cdot \dot{V}O_2 - 2.37 \cdot n$	2.07
Fat oxidation		
Lusk [48]	derived from tables	0.44
Brouwer [15]	$1.718 \cdot \dot{V}O_2 - 1.718 \cdot \dot{V}CO_2 - 0.315 \cdot p$	0.43
Frayn [24]	$1.67 \cdot \dot{V}O_2 - 1.67 \cdot \dot{V}CO_2 - 1.92 \cdot n$	0.42
Ferranninni [22]	$1.67 \cdot \dot{V}O_2 - 1.67 \cdot \dot{V}CO_2 - 1.92 \cdot n$	0.42
Péronnet and Massicotte [52]	$1.695 \cdot \dot{V}O_2 - 1.701 \cdot \dot{V}CO_2$	0.41
Proposed equation (all exercise intensities)	$1.695 \cdot \dot{V}O_2 - 1.701 \cdot \dot{V}CO_2 - 1.77 \cdot n$	0.41

n = urinary nitrogen excretion, p = protein oxidation, gly = glycogen, glc is glucose. * calculations of carbohydrate and fat oxidation are based on a $\dot{V}O_2$ of 2.500 L/min and $\dot{V}CO_2$ of 2.250 L/min (RER = 0.90) and negligible protein oxidation (p = 0, n = 0). New proposed equations are based on 50% of carbohydrate oxidation derived from plasma glucose and 50% from muscle glycogen at low exercise intensities (40 – 50% $\dot{V}O_{2max}$) and 20% from plasma glucose and 80% from muscle glycogen at the moderate to high intensities (50 – 75% $\dot{V}O_{2max}$)

Indirect calorimetry assumes that RER reflects RQ adequately. However, VO_2 from the lungs reflects only reliable the O_2 uptake of the tissue. Similarly, VCO_2 is only an estimate of CO_2 production. At low to moderate intensities (50-75% VO_{2max}), equations are highly reliable because hydrogen ions do not accumulate, and the production and clearance of lactate are in balance. At high intensities (>75% VO_{2max}) VCO_2 is increased in order to excrete excess hydrogen ions. This leads to overestimation of the utilization of CHO at the expense of fat oxidation. Therefore, the technique is accurate for measuring substrate utilization when exercise intensity is $\leq$ 75% VO_{2max}. (Jeukendrup & Wallis 2005.)

In addition, indirect calorimetry is based on an assumption that other metabolic processes do not affect the substrate utilization, although processes, such as ketogenesis, gluconeogenesis and lipogenesis, could influence on RER. Particularly gluconeogenesis from alanine requires energy and may influence on the RQ. But contribution to energy production from alanine is fairly small during exercise. Therefore, its effect on the substrate oxidation is negligible. Lipogenesis occurs during relative carbohydrate overfeed-

ing, thus it is not important during exercise. Ketogenesis provides energy during prolonged exercise. However, the effect on the substrate utilization, and thus on the equation is minimal. (Jeukendrup & Wallis 2005.)

Metabolic chamber. Energy expenditure is measured by indirect whole-body calorimetry. BMR, EE during sleep and 24-h are standardized measurements done in the chamber. In addition, body composition and spontaneous physical activity are measured. Spontaneous physical activity is monitored by microwave motion detectors. Measurements have high accuracy and precision. (Klausen et al. 1997.)

The chamber is "environmental walk-in room" with walls of aluminum. Air conditioning, heating and cooling system conjugate in order to keep the room temperature at fixed state. The chamber is furnished which allows all the necessary daily activities. The VO_2 and VCO_2 are continuously measured because the chamber itself is an open-circuit, indirect calorimeter. Fresh, atmospheric air is drawn through the camber, and the mixed air leaves the chamber at three levels. Ventilation measurement module monitors the flow rate at the outlet, air temperature and the barometric. Dew point hygrometer determines water vapor pressure from the outflowing air. (Ravussian et al. 1986.)

Oxygen and carbon dioxide are analyzed from the outflowing air after flow, temperature, barometric pressure and humidity are determined. Then, oxygen and carbon dioxide concentrations are compared to their concentrations in the fresh air. Oxygen consumption is the sum of the decrease in oxygen in the chamber and the net amount of oxygen added to the chamber. Carbon dioxide production is similarly the sum of carbon dioxide buildup in the chamber and the net amount of carbon dioxide extracted from the chamber. (Ravussian et al. 1986.)

Doubly labeled water (DLW). Doubly labeled water is a well established and validated indirect calorimetry method for assessing total daily EE in free living humans. Method

is noninvasive. When DLW is compared to metabolic chamber measurements, it has accuracy of 1-2% and precision of 5-7%. DLW method assumes that energy intake and energy expenditure are in balance. (de Jonge et al. 2007.)

Subject drinks solutions with two isotopes ($H_2{}^{18}O$ and ^2H_2O). Urine samples are collected at fixed time points. From the urine samples, elimination rates of the isotopes are calculated. Rate of CO_2 production is calculated from the equation of Schoeller's (1988). Energy expenditure is calculated by multiplying rate of CO_2 production by energy equivalent of CO_2 at measured respiratory quotient (RQ). (de Jonge et al. 2007.) DLW provides more clinically relevant data compared to measurements in the respiratory chamber because participants can be physically active as normally (Klausen et al. 1997). DLW is used in the validation of other methods. But its use in practical applications is limited due to its relatively high expense. (McArdle et al. 2001, 185.)

5 THE PURPOSE OF THE STUDY

The purpose of the present study is to examine the energy expenditure during eccentric exercise. This study focuses on the effects of diabetes and obesity on energy expenditure and use of different energy substrates. In addition, associations with muscle fibre type, maximal isometric force and energy expenditure are under examination. The present study is based on following study problems:

1. Does total energy expenditure differ between diabetic and obese subjects during eccentric exercise?
2. Does the use of substrates (lipids vs. CHO) differ between diabetic and obese subjects during eccentric exercise?
3. What are the relationships between muscle fibre type distribution, maximal isometric force and energy expenditure during eccentric exercise in diabetic and obese subjects?

Hypotheses for the study are:

1. Diabetic subjects have higher total energy expenditure during eccentric exercise.
2. Diabetic subjects use more CHO as energy substrate compared to obese and normal weight controls in eccentric exercise.
3. Diabetic subjects have more fast-twitch fibres compared to obese subjects. Furthermore, energy expenditure in eccentric exercise is related to muscle fibre distribution and maximal isometric force.

6 METHODS

6.1 Subjects

Subjects (males, 37-61 years) were divided into three different study groups according to their health status and weight. Group DM consisted of subjects with type 2 diabetes mellitus (n= 14). In addition, diabetic subjects had other diseases related to diabetes mellitus, such as high blood cholesterol, and elevated or high blood pressure. Most of the diabetic subjects were also obese. In the group OND were non-diabetic and obese subjects (n= 12). Some of the obese subjects had metabolic syndrome, because they had elevated blood glucose level and other symptoms related to the disease. For the groups DM and OND, a criterion for obesity was a body mass index (BMI) over 30 kg/m^2.

Normalweight, non-diabetic subjects were in group LND (n= 3). Subjects were non-diabetic and normal weight (BMI $\leq$ 25 kg/m^2) or lean. If fat percent was in normal range (10-20%), subject was considered as lean. Due to difficulties in recruitment of lean non-diabetics subjects, the number of subjects of group LND was small. Therefore, for most of the statistical analyses, groups OND and LND were combined to group healthy controls (HC). Forming the group HC was possible because the data from groups OND and LND were often similar when anthropometrical results were excluded. In the results, the main emphases are in the differences between diabetic and obese non-diabetic subjects, and differences between diabetics and healthy controls.

Subjects were recruited by public advertisements. Before attending to measurements, the subjects underwent a physical examination performed by a physician. If the subject had difficult injuries in spine or joints that could inhibit exercise, or high risk of heart attack, he was excluded from the study. Subjects were volunteers and they were in-

formed about the study and risks involved with the measurements. Written informed consent (appendix 1) and health questionnaire (appendix 2) were obtained from all volunteers before starting any measurements. Study plan was approved in the ethical committee at the University of Jyväskylä.

6.2 Study schedule

The study schedule is summarized in Table 2. During weeks 1 and 2, the subjects underwent a physical examination, anthropometrical measurements and maximal oxygen consumption test. During the adaptation period with eccentric bicycle ergometer (weeks 3-6), also the maximal isometric force was measured and fasting blood sample was collected. In week 7, energy expenditure was measured with the eccentric cycling ergometer. Finally, muscle biopsy was collected in week 8.

TABLE 2. The study schedule.

Week	Measurements
1-2:	Physical examination Anthropometrical measurements and maximal oxygen consumption test
3-6:	Blood sample Adaptation trainings with eccentric bicycle ergometer Maximal isometric force test (knee extension)
7:	Energy expenditure test with eccentric ergometer
8:	Muscle biopsy

6.3 Anthropometrical measurements

Body mass index (BMI) and waist circumference. Body weight and height of the subjects were measured while they wore only light clothing. BMI value was calculated as the body mass (in kilograms) divided by the height (in metres) squared (unit kg/m^2). Waist circumference was measured with a soft measure tape at the midpoint between

lowest rib and the iliac crest when subject was standing normally. Waist circumference is related to amount of visceral fat, which is adipose tissue surrounding organs in the abdominal cavity. High amount of visceral fat is a risk factor for diabetes mellitus.

Body composition. Body composition was measured by InBody 3.0 (Biospace Co., Korea; figure 13). During the measurement, subject stood straight on the feet electrodes and held electrode handles of the InBody 3.0. Hands were straighten and slightly away from the trunk. Low electric currency was conducted through the electrodes to hands and legs at four frequencies (5, 50, 250 & 500 kHz). The InBody 3.0 displays automatically the measurements of total body water, fat percentage, and the amounts of fat-free mass (FFM), fat mass (FM) and visceral fat. The test-retest reliability of the device is considered as high (0.995). In the percentage of total body fat no significant differences are found between InBody 3.0 and the dual-energy x-ray absorptiometry (DXA), or between InBody 3.0 and hydrostatic weighting (HW). (Demura et al. 2004.)

The device estimated the percentage of body fat (fat percentage, %) from the estimations of intracellular water and total body water, and summing the segmental impedance values from the right arm, left arm, right leg, left leg and trunk. The InBody 3.0 calculated the amount of FFM by summing the predicted muscle mass and bone mass. The estimation of the amount of muscle mass from the total water was based on an assumption that the hydration of fat-free mass is 73,2%. FM was calculated by subtracting FFM from the body mass. (Demura et al. 2004.) The cross-sectional area of visceral fat is estimated by the CT method. From this estimation and the subject’s impedance value, a regression equation is formed and the amount of visceral fat is calculated.

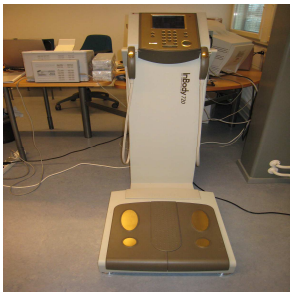


FIGURE 13. Bioelectrical impedance (InBody 3.0) for measuring the body composition.

6.4 Isometric force measurement

Isometric force production of the right knee extensor muscles was measured on a knee extension dynamometer (David Sports ltd, Finland). As a warm-up, subjects cycled with a bicycle ergometer for 5 minutes and performed isometric contractions on the dynamometer. Warm-up contractions were done at a submaximal force level, and their aim was to familiarize subject to the performance.

The subjects sat on the knee extension dynamometer and their knee joints were fixed at 107 degrees. During the force measurement, the subjects did isometric muscle action against ankle bar as fast and maximally as possible. Each subject had 3-5 attempts to reach the maximal force level. Maximal isometric force is calculated as an average from three best attempts. In addition to maximal isometric force, the rate of force development (RFD) was measured from 5ms. The rest period between the attempts was 1-2 minutes. This ensured that the subjects were mentally ready for maximal performance and that their fast energy stores in the muscles (ATP, PCr) were replenished.

6.5 Maximal oxygen consumption

Maximal oxygen consumption (VO_{2max}) was measured in a graded bicycle test. Warm-up was done on a bicycle ergometer (Ergoselect 200P Ergoline, Germany) for ten minutes at a low intensity (70-80 W). The workload for the first grade was determined from

the warm-up and it was from 75 to 100 watts. In the test, load increased by 25 watts in every three minutes. Subject maintained a cycling pace between 60 to 80 revolutions per minute (rpm). Heart rate (Polar, Finland) and blood pressure (Omron, Finland) were monitored during the test. Respiratory gases O_2 and CO_2 were measured with open-circuit spirometry (Oxycon Pro Jæger, Germany) in breath-by-breath mode. The subjects evaluated the exercise subjectively by the scale of Borg (RPE scale from 6 to 20).

The test continued until the subject wanted to stop it, or heart rate, blood pressure or oxygen consumption started to decrease. The maximal oxygen consumption was considered as the highest 30s average of oxygen consumption (VO_2) in relative value (ml/kg/min). After the test, the subjects continued cycling for ten minutes with the load of 75 W for cooling down and recovery purposes. The heart rate was monitored during cooling down. Stretching was advised and guided after the test, in order to diminish the possible muscle soreness during the following days.

6.6 Eccentric bicycle ergometer and adaptation protocol for eccentric exercise

Subjects sat on the eccentric bicycle ergometer (Metitur, Finland) and their feet were in the pedals in front of the body (figure 14). Bicycle was a motor driven ergometer, which rotated the pedals backwards. The subjects had to resist the movement of the pedals with constant and steady force only when pedal was moving towards the subjects' torso. When the pedal was moving away from the torso, subject relaxed the leg. Resisting was done with both legs, one after another. During the resisting movement, at least a part of the m. quadriceps femoris was lengthening while contracting.

FIGURE 14. Eccentric bicycle ergometer.

In order to learn to use the eccentric ergometer, each subject performed eight training sessions twice a week. Thus, the adaptation period lasted for four weeks. Exercise sessions were light but they changed gradually into more demanding ones. Different velocities, loads and intensity level durations were introduced to the subjects. Main focus was to familiarize the subjects to the performance, in order to enable the energy expenditure test with the eccentric bicycle. The subjects evaluated exercise with the Borg's scale and evaluations were used when designing the velocities and loads for the energy expenditure test.

6.7 Energy expenditure test on eccentric bicycle

Subjects' respiratory gases were measured at rest before the maximal oxygen consumption and energy expenditure tests. Before any physically demanding measurements were done, subject sat on a chair and respiratory gases were collected for 5 minutes. These control measurements were taken in order to improve the reliability of the study. In many studies, the day-to-day intra-individual coefficient of variation (CV) of BMR has been 3-5% (Shetty 2005).

The energy expenditure test started with a 10 minute warm-up on a treadmill. Warm-up was done by walking and the aim was to reach 60% of subject's HR_{max} measured during the VO_{2max} test. Initial velocity was 4 km/h, and it was increased gradually up to 6 km/h.

If the target heart rate was not reached, the grade of the treadmill was increased. Both heart rate and respiratory gases were monitored throughout the warm-up.

In the energy expenditure test, the eccentric exercise was divided to two (2) intensity levels, and each of these lasted seven minutes. The velocities for the intensity levels were determined according to the subject's capabilities to perform at different velocities during the adaptation training sessions. The velocities were 40-50 rpm in the first level and 50-60 rpm during the second one. For the most of the subjects, the pedaling rates during the first and second intensity level were 50 and 60 rpm, respectively. Heart rate and respiratory gases were measured during the test as in the maximal oxygen consumption test. The subject evaluated the exercise with Borg's scale. After the test, the subject pedaled on the eccentric bicycle without resisting for 5 min while cooling down.

Energy expenditure and substrate utilization. The total energy expenditure and the utilization of carbohydrates and fatty acids as energy substrates were calculated from the respiratory gas measurements according to Jeukendrup & Wallis (2005). Equations 1 & 2 for substrate utilization were based on oxygen consumption (VO_2) and carbon dioxide production (VCO_2). During the eccentric exercise, the metabolic cost and the exercise intensity were fairly low. Because the exercise intensities were below 75% VO_{2max}, equations for the substrate utilization were accurate.

The respiratory gas values were measured as an average for 30s. VO_2 and VCO_2 values in the equations were the averages of two last minutes of each intensity level because the subjects reached the steady-state during both levels. Excretion of urinary nitrogen (n) was not collected in this study. Due to the duration and the nature of the exercise, it was fair to assume that the effect of protein oxidation was negligible. Thus, from the equations for the substrate utilization the protein oxidation was left out.

Equation 1. CHO oxidation= $4.210*VCO_2-2.962*VO_2-2.37*n$
Equation 2. Lipid oxidation= $1.695* VO_2-1.701*VCO_2-1.77*n$

6.8 Blood sample

Blood sample (10-12 ml) was collected from the brachial arterial after an overnight fast (minimum of 8 h). From the sample, total cholesterol (TC), high-density lipoprotein (HDL) and low-density lipoprotein cholesterol (LDL), blood triglycerides (TG) and glucose were analyzed. All blood variables were measured in plasma by standard enzymatic methods using Roche Diagnostic's reagents with an automated analyzer (Roche Modular P800, Roche Diagnostics GmbH, Germany).

The reagents for the TC, HDL and LDL were Cholesterol Chod PAP, HDL-C plus 3rd generation and LDL-C plus 2nd generation, respectively. The LDL cholesterol values can be calculated according to the formula of Friedewald (equation 3; Friedewald et al. 1972). From some of the samples, LDL was calculated with modified equation of Friedewald (equation 4). The reagents for the determining of the TG and glucose were Triglycerides GPO-PAP and Gluco-quant Glucose/HK, respectively. All the analyses were performed in the Central Hospital of Central Finland, in Jyväskylä.

Equation 3. $C_{LDL} = C_{plasma} – C_{HDL} – TG/5$
Equation 4. $LDL = TC – HDL – (TG/2.2)$

6.9 Muscle biopsy

Prior to collecting the muscle biopsy from the m. vastus lateralis, subjects retained from physical exercise for 48 hours. The skin area, where the biopsy would be taken, was shaved and cooled with ice for about ten minutes before local anaesthetic (Lidocain 20

mg/ml c. adrenalin) was injected s.c. The muscle biopsy was taken with the Bergström biopsy needle from the vastus lateralis muscle approximately 15 cm above the patella tendon and 2 cm away from the fascia. The samples were covered with Tissue-Tek and frozen immediately in isopentane cooled with liquid nitrogen. The samples were stored at -80ºC until further analyses. From the muscle biopsies, 10 μm sections were cut in a cryostat at -20ºC (Leica CM 3000, Germany) for the muscle fibre typing and myosin heavy chain analysis. Data from the muscle biopsy samples in the group LND was supplemented (sLND) with the data from study by Mero & Hulmi in order to have enough samples (n= 10) for statistical analyses.

Fiber typing. ATPase histochemistry analysis for the fibre typing was performed according Brook & Kaiser (1970). Biopsy sections were stained in four different pH-solutions (4.37, 4.55, 4.60, and 10.3). Sections stained in different pH-levels were studied with microscope (Olympus system microscope BX50, Japan) and micrographs were taken with Sanyo Color CCD camera (Sanyo electric Co. Ltd., Japan). The micrographs were analysed with Tema image-analysis software (Scanbeam, Denmark). Fibre types I, IIa, IIab and IIb were identified. Relative proportions of various fibre types were used in the statistical analysis. Minimum of 200 muscle cells were examined for the fibre typing.

Myosin heavy-chain (MHC) composition. Myosin heavy-chain analyses were performed from 30-50 muscle biopsy sections using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to Andersen & Aagaard (2000). The gels were stained with Coomassie Blue and MCH isoform contents were determined with a densitometer (Bio-Rad Molecular Imaginer® ChemiDoc™ XRS System, USA) and Quantity One®–software (Biorad, USA). MHC isoforms I, IIA and IIX were identified as relative proportions.

6.10 Statistical analyses

Statistical analyses were done with SPSS 15.0 for Windows. Because measured parameters were not normally distributed, and sample sizes were relatively small, statistical analyses were performed with the non-parametric tests. Differences between study groups were defined with the Mann Whitney test. Control measurements at rest were compared with the Wilcoxon signed ranks test. Correlations were calculated with the Spearman's correlation coefficients. Level of significance was set at $p < 0.05$.

7 RESULTS

7.1 Anthropometric measures

Age and anthropometrical values are summarized in Table 3. All the groups were similar according to age. There were no statistical differences in anthropometrical measures between DM and OND, and between DM and HC.

TABLE 3. Anthropometrical measures from whole group and subgroups.

ALL SUBJECTS (n= 28)	Mean ± SD	OND (n= 11) Mean ± SD	HC (n= 14) Mean ± SD	DM (n= 14) Mean ± SD
Age (yrs)	52 ± 7	50 ± 10	51 ± 9	53 ± 6
Body mass (kg)	101,0 ± 18,9	99,6 ± 14,2	95,3 ± 15,4	106,7 ± 20,8
Height (m)	1,77 ± 0,07	1,76 ± 0,07	1,76 ± 0,07	1,79 ± 0,07
Waist circ.(1	115,3 ± 17,4	118,5 ± 22,6	113,9 ± 21,9	116,7 ± 12,1
BMI(2	32,0 ± 4,2	32,0 ± 2,8	30,8 ± 3,5	33,2 ± 4,7
FFM(3	71,7 ± 10,2	69,3 ± 9,7	68,5 ± 9,2	74,9 ± 10,4
SMM(4	41,0 ± 5,9	40,2 ± 5,8	39,5 ± 5,6	42,5 ± 6,1
FM(5	29,0 ± 10,9	30,4 ± 8,9	26,9 ± 10,4	31,2 ± 11,4
Fat percent (%)	28,6 ± 6,6	30,2 ± 6,0	27,6 ± 7,4	29,6 ± 5,8
Visceral fat (cm^2)	163,1 ± 36,9	164,5 ± 26,4	153,3 ± 32,4	172,9 ± 39,6

(1 Waist circumference (cm), measured at the midpoint between lowest rib and iliac crest
(2 BMI= body mass index (weight divided by height squared, kg/m^2)
(3 FFM= fat-free mass (kg)
(4 SMM= skeletal muscle mass (kg)
(5 FM= fat mass (kg)

7.2 Blood glucose and cholesterol

Table 4 shows that DM had significantly higher blood glucose compared to the OND ($p=0,001$) and HC ($p<0,001$). There were no statistical differences in total cholesterol and HDL cholesterol levels between the groups. Lower LDL cholesterol in DM com-

pared to the OND approached statistical difference (p=0,092). In addition, triglycerides did not differ between the groups.

TABLE 4. Variables from the blood samples (comparisons to OND: ‡ p< 0.01, † p< 0.05; to HC: ** p< 0.01, * p< 0.05).

ALL SUBJECTS			OND (n= 11)	HC (n= 14)	DM	
	Mean ± SD	n	Mean ± SD	Mean ± SD	Mean ± SD	n
Total Chol (mmol/l)	4,7 ± 1,3	28	4,8 ± 1,1	4,8 ± 1,2	4,5 ± 1,5	14
HDL[1 Chol	1,4 ± 0,8	28	1,6 ± 1,2	1,6 ± 1,1	1,2 ± 0,3	14
LDL[2 Chol	2,6 ± 0,9	27	2,9 ± 1,0	2,8 ± 1,1	2,3 ± 0,7	13
Triglycerides (mmol/l)	2,1 ± 1,6	28	1,7 ± 1,0	1,5 ± 0,9	2,6 ± 2,0	14
Blood glucose (mmol/l)	6,4 ± 2,9	28	5,1 ± 1,4	5,1 ± 1,2	7,8 ± 3,5‡**	14

[1 HDL Chol= high density lipo-protein (mmol/l)
[2 LDL Chol= low density lipo-protein (mmol/l)

In the DM group, seven subjects used medication for cholesterol (e.g. Lipitor). In addition, 10 DM subjects had medication for high blood pressure (e.g. Norvasc, Cardace, Emconcor). On the contrary, only one subject from OND used cholesterol medication, and three subjects had blood pressure medication. When subjects with medical treatment for cholesterol were excluded, there were no statistical differences between diabetics and obese non-diabetics, as well as between diabetics and healthy controls.

7.3 Muscle fibre distribution and myosin heavy chain variation

Four muscle fibre types (I, IIa, IIab and IIb) were separated from the muscle biopsy samples. From myosin heavy chain (MHC), three isoforms were found from the whole subject group. Both MHC and muscle fibre type values are presented in Table 5. There were no statistical differences in MHC contents between the groups. On the contrary, the muscle fibre distribution differed in the groups. DM had lower percentage of type I muscle cells compared to group OND (p=0,048) and group HC (p=0,020). Percentages of fast-twitch muscle cells seem to be homogenous in the subject groups.

TABLE 5. Percentage (%) values of MHC isoforms and muscle fiber distribution (comparisons to OND: ‡ p< 0.01, † p< 0.05; to HC: ** p< 0.01, * p< 0.05).

ALL SUBJECTS			OND		HC		DM	
	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	n
MHC[1 IIX	28,2 ± 9,2	12	28,1 ± 12,0	6	28,1 ±12,0	6	28,2 ± 6,5	6
MHC IIA	40,9 ± 18,9	21	34,6 ± 18,0	8	36,8 ±17,5	10	44,7 ± 20,1	11
MHC I	43,0 ±8,4	21	44,3 ± 6,9	8	46,4 ± 9,4	10	40,0 ±6,4	11
Fibre type I	46,2 ± 11,4	21	49,1 ± 10,1	8	50,8 ±11,5	10	42,0 ± 10,0†*	11
Fibre type IIa	27,1 ± 8,3	21	26,4 ± 8,7	8	27,0 ± 9,1	10	27,3 ± 8,0	11
Fibre type IIab	22,5 ± 9,0	21	21,2 ± 7,3	8	19,0 ± 8,4	10	25,7 ± 8,6	11
Fibre type IIb	4,2 ± 6,5	21	3,2 ± 3,1	8	3,2 ± 2,9	10	5,1 ± 8,7	11

[1 MHC= myosin heavy chain isoform

Supplemented LND (sLND) group had statistically significantly higher proportion of type IIab fibers (p=0,011) compared to diabetics. In addition, the data displayed a trend (p=0,078) of lower proportion of type IIa fibers in sLND compared to DM. When sLND was compared to OND, the data displayed trends of higher proportion of type IIa fibres (p=0,051) and lower fraction of type IIab fibres (p=0,076) in the sLND.

7.4 Maximal force and force production

Maximal isometric force showed no statistical differences or trends between the groups. Also, the rate of force development (RFD) was at similar levels in all the groups (table 6). Maximal isometric force did not correlate with muscle fibre distribution in the whole group nor in any of the subgroups. In addition, there were no correlations between the maximal force and myosin isoform content. Similarly, RFD did not correlate with muscle fibre types, or with MCH isoforms.

TABLE 6. Isometric maximal force and rate of force development (RFD).

ALL SUBJECTS (n= 28)		OND (n= 11)	HC (n= 14)	DM (n= 14)
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
F_{max} (N)	830 ± 233	843 ± 342	826 ± 305	834 ± 141
RFD (N/s)	12434 ± 6752	14375 ± 9292	13653 ± 8390	11214 ± 4592

7.5 Maximal performance capacity

Diabetics had lower relative VO_{2max} (table 7) compared to the OND (p=0,035) and HC (p=0,008). Diabetics reached significantly lower maximal heart rate (HR_{max}) during the maximal test compared to the obese non-diabetics (p=0,020) and healthy controls (p=0,020).

TABLE 7. Results from the maximal oxygen consumption test (comparisons to OND: ‡ p< 0.01, † p< 0.05; to HC: ** p< 0.01, * p< 0.05).

ALL SUBJECTS (n= 28)	Mean ± SD	OND (n= 11) Mean ± SD	HC (n= 14) Mean ± SD	DM (n= 14) Mean ± SD
VO_{2max} (1 (L/min)	2,6 ± 0,6	2,8 ± 0,7	2,8 ± 0,7	2,4 ± 0,4
VO_{2max} (ml/kg/min)	26,0 ± 6,5	27,8 ± 6,0	29,2 ± 6,1	22,9 ± 5,5†**
W_{max} (2	206 ±53	219 ± 67	224 ± 63	188 ± 32
HR_{max} (3	160 ± 18	168 ± 18	167 ± 17	154 ± 16†*

(1 VO_{2max}= maximal oxygen consumption
(2 W_{max}= maximal workload (watts)
(3 HR_{max}= maximal heart rate (bpm)

7.6 Respiratory gases during eccentric exercise

The control respiratory gases measured before VO_{2max} and energy expenditure test did not differ in the whole subject group, or in the subgroups. Therefore, the respiratory gas measurements are comparable. The two intensity levels differed significantly with each other in terms of respiratory gases. Oxygen consumption during the higher intensity level (also referred as: second level) was significantly higher compared to the lower intensity level (first level) both in absolute and relative values. Differences were found in the whole group (p<0,001), and among the diabetics (p=0,001), obese non-diabetics (p=0,005), and healthy controls (p=0,001).

Respiratory gases during the eccentric test did not differ statistically between the diabetics and obese non-diabetics. On the contrary, statistical differences were found between the diabetics and healthy controls (table 8) from the second level, when the diabetics had higher absolute oxygen consumption (p=0,033) and production of carbon dioxide (p=0,033). In addition, the data displayed a trend of higher absolute oxygen consumption (p=0,069) in the DM during the first level compared to the HC.

TABLE 8. Respiratory gases during the energy expenditure test on the eccentric bicycle (comparisons to HC: ** p< 0.01, * p< 0.05).

ALL SUBJECTS (n= 27)	Mean ± SD	OND (n= 10) Mean ± SD	HC (n= 13) Mean ± SD	DM (n= 14) Mean ± SD
1st level				
VO_2(1 (L/min)	0,84 ± 0,24	0,83 ± 0,28	0,79 ± 0,25	0,89 ± 0,23
VO_2 (ml/kg/min)	8,3 ± 1,8	8,3 ± 2,3	8,3 ± 2,0	8,3 ± 1,6
VCO_2(2	0,76 ± 0,23	0,76 ± 0,25	0,71 ± 0,24	0,80 ± 0,23
RER(3	0,90 ± 0,06	0,91 ± 0,04	0,90 ± 0,05	0,90 ± 0,08
2nd level				
VO_2 (L/min)	1,18 ± 0,31	1,15 ± 0,36	1,09 ± 0,33	1,25 ± 0,29*
VO_2 (ml/kg/min)	11,7 ± 2,3	11,6 ± 3,1	11,5 ± 2,8	11,8 ± 2,0
VCO_2	1,06 ± 0,31	1,03 ± 0,33	0,97 ± 0,31	1,15 ± 0,29*
RER	0,90 ± 0,05	0,90 ± 0,05	0,88 ± 0,05	0,91 ± 0,05

(1 VO_2= oxygen consumption
(2 VCO_2= production of carbon dioxide (L/min)
(3 RER= respiratory exchange ratio

Oxygen consumptions during both intensity levels were compared to the maximal oxygen consumption value. This was done in order to reveal possible differences in the proportion of oxygen consumption used in the test between the groups (table 9). There were no statistical differences between the intensity levels but between the groups the proportions varied significantly.

Diabetics used higher proportions of their oxygen consumption capacity compared to healthy controls during both intensity levels. Differences were found in absolute (1st level: p=0,033; 2nd level: p=0,026) and relative values (p=0,023). Between the DM and

OND no statistical differences were detected. However, during the higher intensity level a trend of higher proportion of absolute oxygen consumption (p=0,089) was found in the diabetics (38,0%) compared to the obese non-diabetics (30,8%).

TABLE 9. Relative proportions (%) of oxygen consumption during energy expenditure test on the eccentric bicycle (comparisons to HC: ** p< 0.01, * p< 0.05)

ALL SUBJECTS (n= 27)	Mean ± SD	OND (n= 10) Mean ± SD	HC (n= 13) Mean ± SD	DM (n= 14) Mean ± SD
1st level				
Absolute VO_2/VO_{2max} (%)	33,8 ± 10,3	31,1 ± 10,1	29,3 ± 9,5	37,9 ± 9,6*
Relative VO_2/VO_{2max} (%)	47,4 ± 14,0	44,2 ± 16,5	41,4 ± 15,3	53,0 ± 10,2*
2nd level				
Absolute VO_2/VO_{2max} (%)	33,8 ± 10,2	30,8 ± 9,7	29,2 ± 9,1	38,0 ± 9,5*
Relative VO_2/VO_{2max} (%)	47,5 ± 13,8	43,8 ± 16,0	41,2 ± 14,7	53,2 ± 10,4*

7.7 Energy expenditure and utilization of the substrates during eccentric exercise

Mean energy expenditure and substrate utilization were calculated from the average values of oxygen consumption and carbon dioxide. Between the diabetics and obese non-diabetics, there were no differences in the oxidation of lipid or carbohydrates for energy during the eccentric test (table 10). Total energy expenditure had no statistically significant difference during the first and second intensity levels of the test.

Healthy controls had lower energy expenditure during both intensity levels compared to the diabetics. During the first level, the EE approached statistical significance (p=0,065), but during the second level the energy expenditure was statistically significantly different (p=0,037). In addition, higher oxidation of carbohydrates in the diabetics during the second level compared to the healthy controls approached statistical significance (p=0,081).

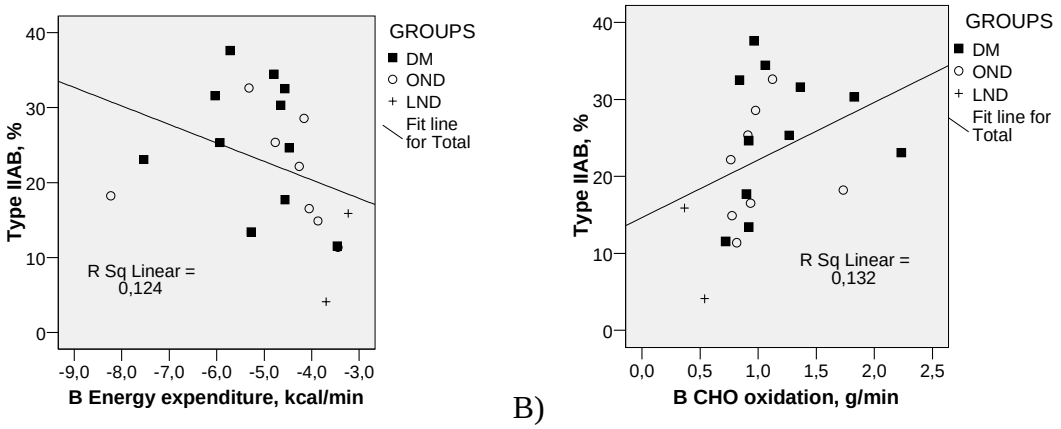
TABLE 10. The energy expenditure and the utilization of substrates during the energy expenditure test on the eccentric ergometer (comparisons to HC: ** p< 0.01, * p< 0.05).

ALL SUBJECTS (n= 27)	Mean ± SD	OND (n= 10) Mean ± SD	HC (n= 13) Mean ± SD	DM (n= 14) Mean ± SD
1st level				
CHO oxidation (g/min)	0,7 ± 0,3	0,7 ± 0,3¤	0,7 ± 0,3	0,8 ± 0,4
FAT oxidation (g/min)	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,1	0,1 ± 0,1
EE (kcal/min)	-3,3 ± 1,0	-3,3 ± 1,1	-3,1 ± 1,0	-3,5 ± 0,9
2nd level				
CHO oxidation (g/min)	1,0 ± 0,4	1,0 ± 0,4¤	0,9 ± 0,4	1,1 ± 0,4
FAT oxidation (g/min)	0,2 ± 0,1	0,2 ± 0,1	0,2 ± 0,1	0,2 ± 0,1
EE (kcal/min)	-4,7 ± 1,2	-4,6 ± 1,4	-4,4 ± 1,3	-5,0 ± 1,1*

(1 EE= energy expenditure

The maximal isometric force did not correlate with the energy expenditure in the whole group nor in the subgroups. In the obese non-diabetic group however, the data displayed a positive trend (r=0,552; p=0,098) between the isometric force and the energy expenditure during the second level.

Muscle fibre distribution showed a few correlations with the energy expenditure and the utilization of substrates. In the whole group, the proportion of type IIab muscle cells correlated negatively with the energy expenditure (r=-0,599; p=0,004) and positively with the oxidation of carbohydrates (r=0,588; p=0,005) during the second intensity level (figure 15). In addition, the proportion of type I muscle cells correlated negatively with the oxidation of CHO (r=-0,452; p=0,040) during the second level.



A) B)

FIGURE 15. Correlations during the second level between the proportion of type IIab fibres and the energy expenditure (A), and between the proportion of type IIab fibres and the oxidation of CHO (B).

In the diabetics, the proportion of type IIb cells correlated negatively with the oxidation of CHO (r=-0.661; p=0,027) during the second intensity level. No other significant correlations were found in the diabetics, but the data showed positive trends between the percentage of type IIb cells and the energy expenditure during the first level (r=0,541; p=0,085), and the proportion of type IIa cells and the oxidation of CHO during the second level (r=0,564; p=0,071).

From the obese non-diabetic group could be found only a positive trend between the percentage of type IIb muscle cells and the lipid oxidation during the first level (r=0,667; p=0,071), and a negative trend between the percentage of type IIab cells and the energy expenditure (r=-0,690; p=0,058) during the second level. From the healthy controls, positive correlations were obtained during the first level between the proportion of type IIab and the oxidation of CHO (r=0,661; p=0,038), and between the percentage of type IIb and the lipid oxidation (r=0,648; p=0,043). In addition during the second level, the percentage of type IIab and the energy expenditure correlated negatively (r=-0,758; p=0,011), and the percentage of type IIab and the CHO oxidation displayed a positive trend (r=0,624; p=0,054).

8 DISCUSSION

The aim of the study was to examine the effects of type 2 diabetes mellitus and obesity on the energy expenditure and substrate utilization during eccentric exercise. In addition, the study focused on the muscle fibre distribution and its relations to energy expenditure and maximal isometric force. The main findings of this study were:

1) Energy expenditure differed between the diabetics and healthy controls. However, no statistical differences were found in the energy expenditure between diabetic and obese non-diabetic subjects.

2) Utilization of substrates did not differ between the diabetics and the obese non-diabetics. The oxidation of carbohydrates was higher in the diabetics compared to the healthy controls.

3) Diabetics had lower percentage of slow-twitch muscle fibres compared to obese non-diabetics and healthy controls. Muscle fibre distribution was not related to maximal isometric force or to energy expenditure during eccentric exercise.

8.1 Effect of obesity on energy expenditure

Bitz et al. (2004) have reported that type 2 diabetics have about 7% higher 24-h energy expenditure compared to obese non-diabetics, when EE is adjusted to FFM, FM, spontaneous physical activity, sex and age. In the present study however, differences in the energy expenditure between the diabetics and the obese non-diabetics were not detected during submaximal exercise. In addition, the energy expenditure was not related to the maximal isometric force at the whole group level or any of the subgroups. Thus, it is

possible that the difference in the energy expenditure may exist only at basal state, not during exercise, although the intensity of the eccentric exercise was from light to moderate.

The regulatory processes of energy metabolism could hide possible differences between the diabetics and the obese subjects. Furthermore, the difference between the groups was so small during exercise, that it is not statistically seen. The type of exercise could also influence on the energy expenditure differences between the groups. Kyröläinen et al. (1990) found that energy expenditure is lower in eccentric exercise compared to concentric mode, thus leading to higher mechanical efficiency. In addition, the mechanical efficiency increased when the mechanical work increased. Because almost every subject reported that the second intensity level was easier to perform, it is possible that the mechanical efficiency was better during the second level. There are also contradictory results about the metabolic strain in various muscle action modes. Combs et al. (1999) found that concentric and eccentric exercise cause similar metabolic strain. On the contrary, Perrey et al. (2001) detected that during eccentric action, physiological cost is lower compared to concentric one. Altogether, the group differences in the present study could become apparent at higher intensities and by altering the muscle action modes.

Differences in the energy expenditure were obtained between the diabetics and the healthy controls, which was greatly due to the lean non-diabetic subjects in the HC group. It seems that diabetes alone does not affect the total energy expenditure but the obesity plays a greater role. According to Klausen et al. (1997), body composition and age affect the energy expenditure. Because age did not differ in the groups, the body composition was primary variable causing differences in the energy expenditure between the diabetic and healthy subjects.

In general, the effect of age is related to sedentary lifestyle, changes in fat-free mass composition, as well as hormone and metabolism regulation. However, determining the

cause and the effect is not straightforward. Sedentary lifestyle accelerates the changes in the body composition and increases the likelihood for obesity, thus leading to other related diseases such as diabetes mellitus. The age-related changes in metabolism also induce the alteration of body composition, which can increase the possibility for physical inactivity. (Blaak 2005; Krishnan et al. 2003.)

8.2 Metabolic characteristics of muscle fibre distribution

Muscle fibre distribution. Muscle fibre distribution is closely linked to the substrate utilization due to the metabolic characteristics of the various muscle fibre types. Slow-twitch (type I) fibres are more insulin-sensitive and they have high oxidative enzyme capacity compared to fast-twitch fibres with higher glycolytic enzyme capacities (He et al. 2001). However, studies have shown controversial results on the effect of diabetes or obesity on the muscle fibre distribution (He et al. 2001; Gaster et al. 2001; Oberbach et al. 2006). This is partly due to the procedure which is vulnerable to variations. Muscle biopsy sample is very small, and thus it is possible that it does not reflect the whole muscle status. The classification of fibre types depend on the typing procedure and it varies between studies. In addition, the vastus lateralis muscle has often quite heterogeneous muscle fibre distribution (He et al. 2001).

Gaster et al. (2001) did not find differences in the fibre distribution between age-matched diabetics and obese non-diabetics. Only differences were lower fractions of type I fibres in young lean subjects compared to both obese subjects and diabetic subjects. In the present study, age was not significant factor because groups were age-matched. The diabetics had significantly lower proportion of type I fibres compared to the obese non-diabetics and the healthy controls as according to Oberbach et al. (2006). But these results are contrary to the results of Gaster et al. (2001). Difference could be partly due to different classification of muscle fibres. In the present study fibres were classified as type I, IIa, IIab or IIb fibres while Gaster et al. (2001) classified fibres only

as type I or type II fibres. The study of Tanner et al. (2002) found that obese subjects had elevated percentage of type IIb fibres and reduced percentage of type I fibres compared to their lean counterparts. In the present study, differences between lean and obese non-diabetics could not be confidently compared due to small number of lean non-diabetics.

High portion of type II fibres in the diabetics was expected in the hypotheses. The diabetics had about 42% of slow fibres, when obese non-diabetics had over 49%. Because slow fibres are more sensitive to insulin, higher proportion of type I fibres could resist or slow down the development of type 2 diabetes mellitus. In addition, a high proportion of type II fibres could accelerate, or be a risk factor to, the development of diabetes mellitus. Because the muscle fibre distribution is largely an inherited property (Guyton & Hall 2006, 1061), its effect on the development of diabetes through muscle metabolism is great. However, lifestyle patterns are as important as the inherited properties. It seems that genetic factors define individuals 'sensitivity' to the development of diabetes but lifestyle is the final trigger for the disease. To more 'diabetes-sensitive' individuals, smaller trigger is needed for the development of diabetes, and vice versa.

It is not known, whether muscle fibre distribution is the cause or the effect of obesity. But correlation has been found particularly between higher proportion of type IIb fibres and abdominal obesity (Lillioja et al. 1987). In the present study, each diabetic and obese non-diabetic subject had waist circumference over 100cm; the range was up to 180cm. Thus, they had abdominal obesity which was also seen in the amount of visceral fat. But the proportion of type IIb fibres was very small in the subject groups. Depending on the group, it varied from about 5% (DM) to about 3% (LND). Lillioja et al. (1987) did not classify hybrid fibres (type IIab) as in the present study, which could explain the small proportion of type IIb fibres in the subject groups in the present study.

Between the subject groups, the muscle fibre distribution varied only at type I fibres which produce less force compared to type II fibres. Because also the MHC isoforms, which are related to the contractile properties of the muscle (Bottinelli et al. 1991), did not differ between the subject groups, the muscle fibre distribution did not correlate with maximal isometric force. Likely, for the same reason the rate of force production did not differ between the groups.

Substrate utilization. Diabetes mellitus is assumed to influence on the substrate utilization at basal state and during exercise. However, regulation of the oxidation of substrates is not fully known. According to Oberbach et al. (2006), diabetics have elevated glycolytic and depressed oxidative capacity which could be due to imbalance between muscle lipid content and enzymatic capacity. Partly contradictory results suggest that the oxidative enzyme capacity is decreased but the glycolytic one is maintained (He et al. 2001). In the present study, the diabetics had higher CHO oxidation compared to the healthy controls, which is in agreement with Oberbach et al. (2006). But once again, differences were not apparent between the diabetics and the obese non-diabetics. It is possible that the differences between the diabetics and the healthy controls are caused mainly by the lean non-diabetic subjects. Like in the energy expenditure, obesity seems to influence more on the substrate utilization than type 2 diabetes mellitus alone.

Lipid oxidation in obesity and type 2 diabetes can be affected by fatty acid transport capacity, oxidation capacity, and fibre type pattern, degree of capillarization and tissue blood flow. Reduced lipid oxidation is most prominent in very obese subjects (BMI >35) and obese diabetics, particularly with abdominal fat distribution. (Blaak 2005.) In the present study, the lipid oxidation did not differ between the study groups. From the OND and DM subjects only four had BMI around or over 35, so it is possible that subjects were not enough obese to have depressed lipid oxidation. Or, perhaps with greater number of lean controls, the difference would have been apparent.

Amount of skeletal muscle mass affects the CHO and lipid metabolism. Muscle metabolism may enhance the development of obesity, or vice versa. The effect of sedentary lifestyle is strongly linked on obesity by reducing muscle mass, muscle capillary density, substrate delivery and muscle oxidative capacity. (Blaak 2005.) Therefore, could the effect of obesity and type 2 diabetes be compensated, at least partly, by training even without significant weight loss? Could good physical fitness overcome the effects of obesity and T2DM?

Substrate utilization is affected by the substrate availability, exercise intensity and duration (Maughan et al. 2005, 54). In the present study, the exercise intensity was moderate, thus emphasizing oxidation of lipids. Short duration of the test (14min) might promote the oxidation of carbohydrates. In addition to the test, the subjects walked 10min warm-up and they reached 60% of their maximal heart rate during the last minutes of the warm-up. Thus, energy production from fatty acids might be promoted due to the warm-up. It is possible that the various walking styles of the subjects have effect on the oxygen consumption and substrate utilization during the warm up. Substrate availability is assumed to be normal because subjects did not do any demanding exercises few days prior to the eccentric bicycle test. They consumed 'normal' diet without any restrictions. If the subjects had followed a predetermined strict diet, the differences on the substrate utilization may have been seen. Specially, if the protocol would have been prolonged or otherwise highly demanding.

During the energy expenditure test, the percentage of oxygen consumption (VO_2/VO_{2max}) used during the eccentric exercise did not change between the intensity levels within the group. VO_2 kinetics was according to Bigland & Lippold (1954) who found that during lengthening muscle actions oxygen consumption does not change, when tension is constant and velocity is increased. But the differences in the oxygen consumption between the subject groups were apparent in the present study. Diabetics had significantly higher values during both intensity levels in absolute (l/min) and rela-

tive (ml/kg/min) values compared to the HC. The difference between the diabetics and obese diabetics approached statistical significance. With bigger subject groups, the difference might have been seen between DM and OND. The diabetics used higher percentage of their oxygen consumption capacity for both of the intensity levels. This might be partly due to lower proportion of type I fibres in DM. In addition, the lowered oxidative enzyme activity in diabetics, found by both Oberbach et al. (2006) and He et al. (2001), could explain the phenomenon.

Muscle fibre distribution showed few trends and relations with energy expenditure and substrate oxidation. High number of type I fibres is related to more active lipid oxidation particularly at basal state but also at submaximal exercise (Turpeinen et al. 2006). In the present study, however, such a relation was not seen. In all subjects, the proportion of type IIab fibres correlated negatively with the energy expenditure and positively with the CHO oxidation during the second intensity level of the test. Type I fibres correlated negatively with the oxidation of CHO at the second level.

The correlations with the CHO oxidation are logical with the metabolic properties of muscle fibres. Type IIab fibres use more glucose for energy, and type I fibres use more lipids. The second intensity level was more demanding than the first one, but still the intensity was moderate. Perhaps, it was enough demanding for the energy expenditure to really start to utilize substrates for energy. It is possible that IIab fibres produced force for eccentric exercise and used less energy than would have been needed by type I fibres in the same exercise. Thus, a high amount of type IIab fibres could diminish energy expenditure. Explanation could be the difference in the electrical activity of motor units during lengthening muscle actions. In eccentric actions, less electrical activity is required compared to the concentric contractions. In addition, the motor activity is short burst action potentials launched at equal time intervals. Because fast-twitch fibres use more CHO as energy source, could eccentric training be more beneficial with diabetic subjects, especially, when diabetics usually have more fast fibres than slow ones.

In the OND group, the data displayed a trend of negative correlation between type IIab fibres and energy expenditure during the second level, similarly as the whole group. In addition, type IIb fibres showed positive trend with lipid oxidation during the first intensity level. Because type IIb fibres are recruited when intensity increases (Maughan et al. 2005, 10-13) and fibres have high glycolytic enzyme activity (Guyton & Hall 2006, 80-81), the positive trend was unexpected. In the HC group these trends were changed into statistical differences. Type IIab correlated positively with the CHO oxidation during the first stage.

The diabetics showed a positive trend between type IIb cells and energy expenditure during the first intensity level. Because no relations were seen during the second level, it can be suggested that during very light exercise the regulation of energy expenditure is not so well controlled. Type IIb fibres use glucose for energy, so maybe the excess amount of available glucose effects on the energy expenditure although the physiological cost is not very high. Because eccentric exercise is not so physiologically demanding, it is possible that the relations would be stronger in concentric exercise or at higher intensity level. In addition, the CHO oxidation during the second level was negatively correlated with the type IIb fibres, and positively correlated with the type IIa fibres.

8.3 Evaluation of the data and methods

Subject groups. The effect of aging is excluded from the results because all the groups had no statistical difference according to the age. The groups DM and OND were matched for anthropometrical variables. At rest state, only difference was higher blood glucose in the diabetics, as required. Therefore, comparing the effect of diabetes in the exercise was easier. The LND group differed naturally in the anthropometrical variables. Unfortunately, the small size of the sample inhibited the comparisons with the DM and OND. The group HC presented quite good venue for comparing healthy and

diabetic subjects. Often the results between the HC and the DM showed stronger differences compared to the difference between the OND and the DM, thus emphasizing the effect of overweight on the physiological variables.

Blood sample. The medications for different diseases influenced on the parameters measured from the blood samples. Blood pressure and cholesterol could not be compared between the groups at 'natural state'. Diabetics had higher blood glucose, although some diabetics used their medication for diabetes before fasting sample for safety reasons. Because no glucose tolerance test was performed, it is possible that few subjects from the OND have impaired glucose tolerance (IGT) or even diabetes mellitus, which would cause interference to the results.

Energy expenditure and substrate utilization. Calculating variables always includes a risk for underestimation or overestimation. Because the eccentric exercise was performed at low to moderate intensities, the accuracy of the equations is much better than at high intensity exercises. Exclusion of the urinary nitrogen excretion from the measurements was justified with the low intensity requirements and short duration of the exercise.

Eccentric exercise in the energy expenditure test seemed quite easy to perform. Oxygen consumption reached from 30% to 50% percent levels in all groups. Still, the RPE values varied quite much, ranging from 7 to 18. The average values in all groups were around 10 and 13 during first and second level, respectively. During the eccentric test the difference between fit subjects and less fit subjects was apparent. The energy expenditure test lasted only 14min and the intensity of the exercise levels were not highly demanding. Moderate intensity was justified because usually obese individuals do not necessary exercise much so they are not able to sustain high intensity exercise. Also, the high intensity exercise includes always a health risk, particularly to individuals with many risk factors such as obesity, high cholesterol, high blood pressure and diabetes.

Therefore, exercise at the submaximal level is not only a safety issue but also the results can be better applied to practise. In addition, the eccentric ergometer used in the study has its limits on increasing the intensity.

Muscle biopsy. There is always a debate about the reliability of the muscle biopsy. Sample size is small compared to whole muscle mass of the m. vastus lateralis. But the biopsy procedure is widely used and contains a lot valuable information of the body's functions. Muscle biopsy was collected by an experienced physician, which enhanced the reliability of the samples in the groups. Fibre typing was performed by one researcher, so the criteria for different fibre types were similar. For the typing, at least 200 cells were counted which should give a reliable result.

8.4 Conclusions

1) Energy expenditure during eccentric exercise is similar in obese and diabetic subjects; although at rest state Bitz et al. (2004) have reported higher EE in diabetics. Obesity influences more on the energy expenditure than diabetes mellitus probably by the regulatory mechanisms of energy metabolism.

2) Lower percentage of type I fibres in the diabetics is reflected as the higher oxidation of CHO for energy compared to the healthy controls. In addition, the diabetics used higher proportion of their oxygen consumption capacity which could be a sign for increased glycolytic enzyme activity. These variables could partly explain the subjects' sensitivity to develop type 2 diabetes mellitus.

3) Eccentric exercise could provide a safe exercise mode for subjects with low exercise tolerability due to its low impact on ventilation and heart rate. Motor activity pattern during eccentric exercise could enhance the oxidation of CHO, which is important especially for the diabetic patients.

The field of research related to type 2 diabetes mellitus is wide. Further studies could concentrate on the lean and the obese diabetics. How would the body composition influence on the energy metabolism when diabetes already occurs? The role of muscle fibres is also interesting. Is there a difference in the muscle fibre distribution between the lean and the obese diabetics and how is it shown in the substrate utilization? The prevention of diabetes is one of the biggest challenges in world wide. Effect of physical activity is known to be crucial in the prevention of diabetes. Therefore, more research on the resistance and endurance training and its influences is required. The role of every day activity compared to training intervention should also be addressed.

9 REFERENCES

Abbruzzese, G., Morena, M., Spadavecchia, L. & Schieppati, M. 1994. Response of arm flexor muscles to magnetic and electrical brain stimulation during shortening and lengthening tasks in man. J Physiol 481: 499-507.

Adams, G.R., Cheng, D.C., Haddad, F. & Baldwin, K.M. 2004. Skeletal muscle hypertrophy in response to isometric, lengthening and shortening bouts of equivalent duration. J Appl Physiol 96: 1613-1618.

Andersen, J.L. & Aagaard, P. 2000. Myosin heavy chain IIX overshoot in human skeletal muscle. Muscle Nerve 23: 1095-1104.

Asp, S., Daugaard, J.R., Kristiansen, S., Kiens, B. & Richter, E.A. 1996. Eccentric exercise decreases maximal insulin action in humans: muscle and systemic effects. J Physiol 494: 891-898.

Asp, S., Daugaard, J.R., Kristiansen, S., Kiens, B. & Richter, E.A. 1998. Exercise metabolism in human skeletal muscle exposed to prior eccentric exercise. J Physiol 509: 305-313.

Asp, S., Daugaard, J.R. & Richter, E.A. 1995. Eccentric exercise decreases glucose transporter GLUT4 protein in human skeletal muscle. J Physiol 482: 705-712.

Asp, S. & Richter, E.A. 1996. Decreased insulin action on muscle glucose transport after eccentric contraction in rats. J Appl Physiol 81 (5): 1924-1928.

Balagopal, P., Rooyackers, O.E., Adey. D.B., Ades, P.A. & Sreekumaran Nair, K. 1997. Effects of aging on in vivo synthesis of skeletal muscle myosin heavy-chain and sarcoplasmic protein in humans. Am J Physiol Endocrinol Metab 273: E790-E800.

Balagopal, P., Coenen Schimke, J., Ades, P., Adey. D. & Sreekumaran Nair, K. 2001. Age effect on transcript levels and synthesis rate of muscle MHC and response to resistance exercise. Am J Physiol Endocrinol Metab 280: E203-E208.

Beltman, J.G.M., Sargeant, A.J., van Mechelen, W. & de Haan, A. 2004a. Voluntary activation level and muscle fiber recruitment of human quadriceps during lengthening contractions. J Appl Physiol 97: 619-626.

Beltman, J.G.M., van der Vliet, M. R., Sargeant, A.J. & de Haan, A. 2004b. Metabolic cost of lengthening, isometric and shortening contractions in maximally stimulated rat skeletal muscle. Acta Physiol Scand 182: 179-187.

Bigland, B. & Lippold, O.C.J. 1954. The relation between force, velocity and integrated electrical activity in human muscles. J Physiol 123: 214-224.

Bitz, C., Toubro, S., Larsen, T.M., Harder, H., Rennie, K.L., Jebb, S.A. & Astrup, A. 2004. Increased 24-h energy expenditure in type 2 diabetes. Diabetes Care 27: 2416-2421.

Blaak, E.E. 2005. Metabolic fluxes in skeletal muscle in relation to obesity and insulin resistance. Best Pract Research Clin Endocrin Metab 19 (3): 391-403.

Bottinelli, R., Schiaffino, S. & Reggiani, C. 1991. Force-velocity relations and myosin heavy chain isoform compositions of skinned fibres from rat skeletal muscle. J Physiol 437: 655-672.

Braun, B., Sharoff, C., Chipkin, S.R. & Beaudoin, F. 2004. Effects of insulin resistance on substrate utilization during exercise in overweight women. J Appl Physiol 97: 991-997.

Brook, M.H. & Kaiser, K.K. 1970. Three “myosin adenosine triphosphatase” systems: the nature of their pH lability and sulfhydryl dependence. J Histochem Cytochem 18: 670-672.

Bruce, C.R., Anderson, M.J., Carey, A.L., Newman, D.G., Bonen, A., Kriketos, A.D., Cooney, G.J. & Hawley, J.A. 2003. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. J Clin Endocrinol Metab 88: 5444-5451.

Cauza, E., Hanusch-Enserer, U., Strasser, B., Ludvik, B., Metz-Schimmerl, S., Pacini, G., Wagner, O., Georg, P., Prager, R., Kostner, K., Dunky, A. & Haber, P. 2005. The relative benefits of endurance and strength training on the metabolic factors and muscle function of people with the type 2 diabetes mellitus. Arch Phys Med Rehab 86: 1527-1533.

Chen, Y-W., Hubal, M.J., Hoffman, E.P., Thompson, P.D. & Clarkson, P.M. 2003. Molecular responses of human muscle to eccentric exercise. J Appl Physiolo 95: 2485-2494.

Combs, C.A., Aletras, A.H. & Balaban, R.S. 1999. Effect of muscle action and metabolic strain on oxidative metabolic responses in human skeletal muscle. J Appl Physiol 87 (5): 1768-1775.

Coyle, E.F. 2000. Physical activity as a metabolic stressor. Am J Clin Nutr 72 (suppl): 512S-520S.

D'Antona, G., Pellegrino, M.A., Adami, R., Rossi, R., Carlizzi, C.N., Canepari, M., Saltin, B. & Bottinelli, R. 2003. The effect of ageing and immobilization on structure and function of human skeletal muscle fibres. J Physiol 552 (2): 499-511.

Demura, S., Sato, S. & Kitabayashi, T. 2004. Percentage of total body fat as estimated by three automatic bioelectrical impedance analyzers. J Physiol Anthropol Appl Human Sci 23 (3): 93-99.

Enoka, R.M. 1996. Eccentric contractions require unique activation strategies by the nervous system. J Appl Physiol 81 (6): 2339-2346.

Enoka, R.M. 2002. Neuromechanics of human movement. 3rd edition. Human Kinetics, USA.

Evans, W.J. 1992. Exercise, Nutrition and Aging. J Nutr 122: 796-801.

Fleck, S.J. & Kraemer, W.J. 2004. Designing resistance training programs. 3rd Edition. Human Kinetics, Canada.

Friedewald, W.T., Levy, R.I. & Fredrickson, D.S. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18 (6): 499-502.

Gaster, M., Poulsen, P., Handberg, A., Schrøder, H.D. & Beck-Nielsen, H. 2000. Direct evidence of fiber type-dependent GLUT-4 expression in human skeletal muscle. Am J Physiol Endocrinol Metab 278: E910-E916.

Gaster, M., Staehr, P., Beck-Nielsen, H., Schrøder, H.D. & Handberg, A. 2001. GLUT4 is reduced in Slow Muscle Fibers of Type 2 Diabetic Patients Diabetes 50: 1324-1329.

Goodpaster, B.H. & Wolf, D. 2004. Skeletal muscle lipid accumulation in obesity, insulin resistance, and type 2 diabetes. Ped Diab 5: 219-226.

Goodpaster, B.H., Theriault, R., Watkins, S.C. & Kelley, D.E. 2000. Intramuscular lipid content is increased in obesity and decreased by weight loss. Metab 49 (4): 467-472.

Gudbjörnsdóttir, S., Sjöstrand, M., Strindberg, L. & Lönnroth, P. 2005. Decreased muscle capillary permeability surface area in type 2 diabetic subjects. J Clin Endocrinol Metab 90:1078-1082.

Guesbeck, N.R., Hickey, M.S., MacDonald, K.G., Pories, W.J., Harper, I., Ravussin, E., Dohm, G.L. & Houmard, J.A. 2001. Substrate utilization during exercise in formerly morbidly obese women. J Appl Physiol 90: 1007-1012.

Guyton, A.C. & Hall, J.E. 2006. The textbook of medical physiology. 11th edition, Elsevier Saunders. China.

Haddad, F. & Adams, G.R. 2006. Aging-sensitive cellular and molecular mechanisms associated with skeletal muscle hypertrophy. J Appl Physiol 100: 1188-1203.

He, J., Watkins, S. & Kelley, D.E. 2001. Skeletal muscle lipid content and oxidative enzyme activity in relation to muscle fiber type in type 2 diabetes and obesity. Diabetes 50: 817-823.

Henneman, E., Somjen, G. & Carpenter, D.O. 1965. Functional significance of cell size in spinal motoneurons. J Neurophysiol 28 (3): 560-580.

Horowitz, J.F. & Klein, S. 2000. Lipid metabolism during endurance exercise. Am J Clin Nutr 71 (S1): 558S-563S.

Hortobágyi, T, Barrier, J., Beard, D., Braspennincx, J., Koens, P., Devita, P., Dempsey, L. & Lambert, J. 1996. Greater initial adaptations to submaximal lengthening than maximal shortening. J Appl Physiol 81 (4): 1677-1682.

Houmard, J.A., Weidner, M.L., Gavigan, K.E., Tyndall, G.L., Hickey, M.S. & Alshami, A. 1998. Fiber type and citrate synthase activity in the human gastrocnemius and vastus lateralis with aging. J Appl Physiol 85 (4): 1337-1341.

Hulver, M.W., Berggren, J.R., Cortright, R.N., Dudek, R.W., Thompson, R.P., Pories, W.J., MacDonald, K.G., Cline, G.W., Shulman, G.I., Dohm, G.L. & Houmard, J.A. 2003. Skeletal muscle lipid metabolism with obesity. Am J Physiol Endocrinol Met 284: E741-E747.

Hunter, G.R., Newcomer, B.R., Weinsier, R.L., Karapondo, D.L., Larson-Meyer, D.E., Joanisse, D.R. & Bamman, M.M. 2002. Age is independently related to muscle metabolic capacity in premenopausal women. J Appl Physiol 93: 70-76.

Ingalls, C.P., Warren, G.L., Williams, J.H., Ward, C.W. & Armstrong, R.B. 1998. E-C coupling failure in mouse EDL muscle after in vivo eccentric contractions. J Appl Physiol 85 (1): 58-67.

Jeukendrup, A.E. & Wallis, G.A. 2005. Measurement of substrate oxidation during exercise by means of gas exchange measurements. Int J Sports Med 26 (Suppl 1): S28-S37.

de Jonge, L., DeLany, J.P., Nguyen, T., Howard, J., Hadley, E.C., Redman, L.M. & Ravussin, E. 2007. Validation study of energy expenditure and intake during calorie restriction using doubly labeled water and changes in body composition. Am J Clin Nutr 85: 73-79.

Kern, M., Wells, J.A., Stephens, J.M., Elton, C.W., Friedman, J.E., Tapscott, E.B., Pekala, P.H. & Dohm, G.L. 1990. Insulin responsiveness in skeletal muscle is determined by glucose transporter (Glut4) protein level. Biochem J 270: 397-400.

Kern, P.A., Simsolo, R.B. & Fournier, M. 1999. Effect of weight loss on muscle fiber type, fiber size, capillarity, and succinate dehydrogenase activity in humans. J Clin Endocrinol Metab 84: 4185-4190.

Kiens, B. 2006. Skeletal muscle lipid metabolism in exercise and insulin resistance. Physiol Rev 86: 205-243.

Klausen, B., Toubro, S. & Astrup, A. 1997. Age and sex effects on energy expenditure. Am J Clin Nutr 65: 895-907.

Korhonen, M.T., Cristea, A., Alén, M., Häkkinen, K., Sipilä, S., Mero, A., Viitasalo, J.T., Larsson, L. & Suominen, H. 2006. Aging, muscle fiber type, and contractile function in sprint-trained athletes. J Appl Physiol 101: 906-917.

Krishnan, R.K., Evans, W.J. & Kirwan, J.P. 2003. Impaired substrate oxidation in healthy elderly men after eccentric exercise. J Appl Physio 94: 716-723.

Krivickas, L.S., Fielding, R.A., Murray, A., Callahan, D., Johansson, A., Dorer, D.J. & Frontera, W.R. 2006. Sex differences in single muscle fiber power in older adults. Med Sci Sports Exerc 38 (1): 57-63.

Kyröläinen, H., Komi, P.V., Oksanen, P., Häkkinen, K., Cheng, S. & Kim, D.H. 1990. The mechanical efficiency of locomotion in females during different kinds of muscle action. J Appl Physiol 61: 446-452.

LaMonte, M.J., Blair, S.N. & Church, T.S. 2005. Physical activity and diabetes prevention. J Appl Physiol 99: 1205-1213.

Larsson, H., Daugaard, J.R., Kiens, B., Richter, E.A. & Ahrén, B. 1999. Muscle fiber characteristics in postmenopausal women with normal or impaired glucose tolerance. Diabetes Care 22: 1330-1338.

Leibel, R.L., Rosenbaum, M. & Hirsch, J. 1995. Changes in energy expenditure resulting from altered body weight. N Eng J Med 332: 621-628.

Lillioja S., Young, A.A., Culter, C.L., Ivy, J.I., Abbott, W.G.H., Zawadzki, J.K., Yki-Järvinen, H., Christin, L., Secomb, T.W. & Bogardus, C. 1987. Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. J Clin Invest 80: 415-424.

Linari, M., Bottinelli, R., Pellegrino, M.A., Reconditi, M., Reggiani, C. & Lombardi, V. 2004. The mechanism of the force response to stretch in human skinned muscle fibres with different myosin isoforms. J Physiol 554 (2): 335-352.

Lynn, R., Talbot, J.A. & Morgan, D.L. 1998. Differences in rat skeletal muscles after incline and decline running. J Appl Physiol 85 (1): 98-104.

Malm, C., Sjödin, B., Sjöberg, B., Lenkei, R., Renström, P., Lundberg, I.E. & Ekblom, B. 2004. Leukocytes, cytokines, growth factors and hormones in human skeletal muscle and blood after uphill or downhill running. J Physiol 556 (3):983-1000.

Maughan, R., Gleeson, M. & Greenhaff, P.L. 2005. Biochemistry of exercise and training. Oxford University Press, Great Britain.

McArdle, W.D., Katch, F.I. & Katch, V.L. 2001. Exercise physiology. Energy, nutrition, and human performance. 5th edition. Lippincott Williams & Wilkins, USA.

Morgan, D.L. 1990. New insights into the behaviour of muscle during active lengthening. Biophys J 57: 209-221.

Morgan, D.L. & Allen, D.G. 1999. Early events in stretch-induced muscle damage. J Appl Physiol 87 (6): 2007-2015.

Nardone, A. & Schieppati, M. 1988. Shift of activity from slow to fast muscle during voluntary lengthening contractions of the triceps surae muscles in humans. J Physiol 395: 363-381.

Nardone, A., Romanò, C. & Schieppati, M. 1989. Selective recruitment of high-threshold human motor units during voluntary isotonic lengthening of active muscle. J Physiol 409: 451-471.

Newham, D.J., Jones, D.A., Turner, D.L. & McIntyre, D. 1995. The metabolic costs of different types of contractile activity of the human adductor pollicis muscle. J Physiol 488 (3):815-819.

Oberbach, A., Bossenz, Y., Lehmann, S., Niebauer, J., Adams, V., Paschke, R., Schön, M.R., Blüher, M. & Punkt, K. 2006. Altered fiber distribution and fiber-specific glycolytic and oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes. Diabetes Care 29: 895-900.

Ochala, J., Dorer, D.J., Frontera, W.R. & Krivickas, L.S. 2006. Single skeletal muscle fiber behaviour after a quick stretch in young and older men: a possible explanation of the relative preservation of eccentric force in old age. Eur J Physiol 452: 464-470.

Perrey, S., Betik, A., Candau, R., Rouillon, J.D. & Hughson, R.L. 2001. Comparison of oxygen uptake kinetics during concentric and eccentric cycle exercise. J Appl Physiol 91: 2135-2142.

Proske, U. & Morgan, D.L. 2001. Muscle damage from eccentric exercise: mechanism, mechanical signs, adaptation and clinical applications. J Physiol 537 (2): 333-345.

Ravussin, E., Lillioja, S., Anderson, T.E., Christin, L. & Borardus, C. 1986. Determinants of 24-hour energy expenditure in man: methods and results using a respiratory chamber. J Clin Invest 78: 1568-1578.

Reggiani, C., Potma, E.J., Bottinelli, R., Canepari, M., Pellegrino, M.A. & Stienen, G.J.M. 1997. Chemo-mechanical energy transduction in relation to myosin isoform composition in skeletal muscle fibres of a rat. J Physiol 502 (2): 449-460.

Russ, D.W., Lanza, I.R., Rothman, D., Kent-Braun, J.A. 2005. Sex differences in glycolysis during brief, intense isometric contractions. Muscle Nerve 32: 647-655.

Scheuermann-Freestone, M., Madsen, P.L., Manners, D., Blamire, A.M., Buckingham, R.E., Styles, P., Radda, G.K., Neubauer, S. & Clarke, K. 2003. Abnormal cardiac and

skeletal muscle energy metabolism in patients with type 2 diabetes. Circulation 107: 3040-3046.

Shetty, P. 2005. Energy requirements of adults. Publ Health Nutr 8 (7A): 994-1009.

Schoeller, D.A. 1988. Measurement of energy expenditure in free-living humans bu using doubly labeled water. J Nutr 118: 1278-1289.

Stupka, N., Tarnopolsky, M.A., Yardley, N.J. & Phillips, S.M. 2001. Cellular adaptation to repeated eccentric exercise-induced muscle damage. J Appl Physiol 91: 1669-1678.

Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. & Kasuga, N. 2001. Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. J Physiol 533: 571-583.

Tanner, C.J., Barakat, H.A., Dohm, G.L., Pories, W.J., MacDonald, K.G., Cunningham, P.R.G., Swanson, M.S. & Houmard, J.A. 2002. Muscle fiber type is associated with obesity and weight loss. Am J Physiol Endocrinol Metab 282: E1191-E1196.

Turpeinen, J-P., Leppävuori, J., Heinonen, O.J., Kaila, K., Salo, J., Lilja, M. & Kesäniemi, Y.A. 2006. Muscle fiber type I influences lipid oxidation during low-intensity exercise in moderately active middle-aged men. Scand J Med Sci Sports 16: 134-140.

Uusitalo, M., Lindi, V., Louheranta, A., Salopuro, T., Lindström, J. & Tuomilehto, J. 2003. Long-term improvement in insulin sensitivity by changing lifestyles of people with impaired glucose tolerance. Diabetes 52: 2532-2538.

Vallejo, A.F., Schroeder, E.T., Zheng, L., Jensky, N.E. & Sattler, F.R. 2006. Cardio-pulmonary responses to eccentric and concentric resistance exercise in older adults. Age and Ageing 35: 291-297.

van Loon, L.J.C., Koopman, R., Manders, R., van der Weegen, W., van Kranenburg, G.P. & Keizer, H.A. 2004. Intramyocellular lipid content in type 2 diabetes patients compared with overweight sedentary men and highly trained endurance athletes. Am J Physiol Endocrinol Metab 287: E558-565.

Vijayan, K., Thompson, J.L., Norenberg, K.M., Fitts, R.H. & Riley, D.A. 2001. Fiber-type susceptibility to eccentric contraction-induced damage of hindlimb-unloaded rat AL muscles. J Appl Physiol 90: 770-776.

Warren, G.L., Hayes, D.A., Lowe, D.A. & Armstrong, R.B. 1993. Mechanical factors in the initiation of eccentric contraction-induced injury in rat soleus muscle. J Physiol 464: 457-475.

Whitehead, N.P., Allen, T.J., Morgan, D.L. & Proske, U. 1998. Damage to human muscle from eccentric exercise after training with concentric exercise. J Physiol 512: 615-620.

10 APPENDICES

Appendix 1: Written consent

SUOSTUMUSLOMAKE

Ylipainoisuuden/2 tyypin diabeteksen vaikutukset lihassolujakaumaan sekä energianlähteiden käyttöön levossa ja rasituksessa

Tutkimuksen toteuttaja: LIKES-tutkimuskeskus ja Jyväskylän yliopisto, liikuntabiologian laitos
Vastaava tutkija: Heikki Kainulainen, FT, professori, Jyväskylän yliopisto, liikuntabiologian laitos
Lisätiedot tutkimuksen osalta: Riikka Kivistö, liik. yo (040-5872015) rimakivi@jyu.fi

Laki lääketieteellisestä tutkimuksesta (488/1999) ja sitä täsmentävä asetus (986/1999) edellyttävät, että tutkimuksiin osallistuvat koehenkilöt ovat tietoisia tutkimuksen kulusta ja eri mittauksista, sekä niihin liittyvistä hyödyistä ja mahdollisista riskeistä. **Koehenkilöiden tulee olla vapaaehtoisia ja heillä on oikeus keskeyttää tutkimus niin halutessaan.**

Tämän tutkimuksen pääpiirteet käyvät ilmi liitteenä olevasta koehenkilötiedotteesta, jonka lukemisen vahvistatte allekirjoituksella. (Keski-Suomen sairaanhoitopiirin eettinen toimikunta on antanut tämän tutkimuksen suorittamisesta puoltavan lausunnon 30.05.2007.)

Halutessanne voitte keskeyttää mittaukset tai jättää jonkin osan väliin ilman erityistä syytä. Panoksenne tutkimuksellenne on kuitenkin erittäin tärkeä ja toivomme, että olisitte valmis kaikkiin tutkimuksen mittauksiin.

Näitä samansisältöisiä asiakirjoja on kaksi kappaletta, joista toinen jää koehenkilölle ja toinen tutkijalle.

Olen selvillä tutkimuksen mittauksista ja niihin liittyvistä riskeistä. Minulla on ollut mahdollisuus esittää mieltäni askarruttavia kysymyksiä ja olen saanut niihin minua tyydyttävät vastaukset. Osallistun mittauksiin vapaaehtoisesti. Tutkimustuloksiani voidaan käyttää tutkimusraporteissa osana keskiarvoa tai muita tunnuslukuja, tai tunnistamattomina esimerkkeinä yksittäisistä havainnoista.

Jyväskylässä ____/____ 200__ Syntymäaika _____

Allekirjoitus _____ Osoite _____

Nimenselvennys _____ _____

Puhelinnumero _____

Yhteyshenkilö tutkimuksen aikana Riikka Kivistö

Puhelinnumero 040-5872015

Vastaanottajan allekirjoitus _____

Nimenselvennys _____

Appendix 2. Health questionnaire

LIKES

Lihasharjoituslaite 2004/
TERVEYSKYSELYLOMAKE

Testauksen turvallisuuden kannalta on tärkeää, että tiedämme mahdollisista sairauksistasi, oireistasi, elintavoistasi ja liikuntatottumuksistasi ennen kuin testaamme Sinut.

Nimi:_____ **Synt.aika:**_____

Osoite:_____

Ole hyvä ja vastaa seuraaviin kysymyksiin huolellisesti!

Liikunnan harrastus:

Laji	Ei lainkaan	Joskus	1-2 krt/vko	3-4 krt/ vko	yli 4 krt/vko	Keskimääräinen kesto (min)
Kävely						
Juoksu						
Pyöräily						
Uinti						
Palloilu						
Kuntosali						
Aerobic/jumppa						
Tanssi						
Talon työt						

Asumismuoto
Omakotitalo ____
Rivitalo ____
Kerrostalo ____ kerros Kuljen ___ portaita ___ hissillä

Oma kuntoarvio: ☐ heikko ☐ välttävä ☐ keskitasoinen ☐ hyvä ☐ erinomainen

Oireet viimeisen 6 kk aikana:	**kyllä**	**ei**	**en osaa sanoa**
1. Onko Sinulla ollut rintakipuja?	____	____	____
2. Onko Sinulla ollut rasitukseen liittyvä poikkeavaa hengenahdistusta?	____	____	____
3. Onko Sinulla ollut huimausoireita?	____	____	____
4. Onko Sinulla ollut rytmihäiriötuntemuksia?	____	____	____
5. Onko Sinulla toistuvia, liikkumista haittaavia selkäkipuja?	____	____	____

LIKES

6. Onko Sinulla toistuvia niska-hartiaseudun kipuja? ____ ____ ____

7. Onko Sinulla toistuvia liikkumista haittaavia nivelkipuja? ____ ____ ____

   Missä nivelissä?_____

9. Oletko tuntenut poikkeavan voimakasta uupumusta

   liikkuessasi? (esim. jalat ovat valahtaneet voimattomiksi.) ____ ____ ____

10. Aiheuttaako fyysinen rasitus Sinulle usein päänsärkyä? ____ ____ ____

11. Onko lähisuvussasi veritulpan saaneita? ____ ____ ____

    Kuka ja missä iässä ensimmäinen kohtaus on ilmennyt?_____

Lääkärin toteamat sairaudet: Onko Sinulla tai onko Sinulla ollut jokin/joitakin seuraavista? Rastita!

☐ sepelvaltimotauti	☐ sydäninfarkti	☐ kohonnut verenpaine	☐ sydänläppävika
☐ aivohalvaus	☐ aivoverenkierron häiriöitä	☐ sydämen rytmihäiriö	☐ sydämen tahdistin
☐ katkokävely	☐ sydänlihassairaus	☐ syvä laskimotukos	☐ muu verisuonisairaus
☐ kr. keuhkoputkentulehdus	☐ keuhkolaajentuma	☐ astma	☐ muu keuhkosairaus
☐ allergia	☐ kilpirauhasen toim.häiriö	☐ diabetes	☐ anemia
☐ korkea veren kolesteroli	☐ korkea veren sokeri	☐ nivelreuma	☐ nivelrikko, -kuluma
☐ krooninen selkäsairaus	☐ mahahaava	☐ pallea-, nivus-, tai napatyrä	☐ ruokatorven tulehdus
☐ mielenterveyden ongelma	☐ kasvain tai syöpä	☐ leikkaus äskettäin	☐ tapaturma äskettäin

☐ matala veren kalium- tai magnesiumpitoisuus ☐ kohonnut silmänpaine ☐ näön tai kuulon heikkous

☐ muita sairauksia tai oireita, mitä?_____

Lisätietoja:_____

Säännöllisesti käyttämäni lääkkeet ja annostus:_____

_____

_____

Tupakointi: ☐ Ei ☐ Kyllä ☐ Satunnaisesti, määrä:_____

Alkoholin käyttö viimeisen 48 tunnin aikana: ☐ Ei ☐ Kyllä

Kuumetta, flunssaista oloa tai muuten poikkeavaa väsymystä viimeisen viikon aikana ennen testiä: ☐ Ei ☐ Kyllä

Olen ymmärtänyt testaukseen tarkoituksen ja siihen liittyvät riskit. Lisäksi olen saanut riittävää ennakkoinformaatiota testeistä ja suostun suorittamaan testit omalla vastuullani. Allekirjoituksellani suostun myös siihen, että henkilötietoni sekä testitulokseni saa tallentaa LIKESin tietojärjestelmään.

Käsittelemme antamasi tiedot luottamuksellisesti.

Jyväskylässä ___ /___ 200___

_____