RELATION OF SUBMAXIMAL CONCENTRIC EXERCISE TO MUSCLE FIBER COMPOSITION AND SUBSTRATE UTILIZATION IN TYPE 2 DIABETIC, OBESE AND HEALTHY MEN

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ABSTRACT


Muscle fiber type distribution, substrate utilization and force production are altered by the development of type 2 diabetes mellitus (T2DM) and obesity. Exercise that can ameliorate above three factors plays a great role in the treatment of these diseases. However, it is not well understood how muscle fiber type distribution is reflected in the substrate utilization in obesity and T2DM.

In order to address the relationship between force production, muscle fibre type distribution and substrate utilization at rest and during exercise, 28 subjects (n_{type II diabetic}=14, n_{obese}=11 and n_{healthy}=3) were recruited to this study. The following steps were performed: physical examination, body composition analysis, isometric force measurement, bicycle ergometer exercise, muscle biopsy, fiber typing and myosin heavy chain (MHC) analysis. They cycled 21 minutes on a bicycle ergometer at concentric exercise intensity of 30%, 50% and 70% of maximal power (W_{max}) that was obtained from (maximal oxygen consumption) VO_{2max} test.

Carbohydrate (CHO) oxidation was similar at rest in type 2 diabetic and obese groups, but higher during exercise in obese than in diabetic group. The difference was increased with an increase in exercise intensity. Fat oxidation increased first at lower exercise intensity, and then started to decrease with the increase of exercise intensity and duration. The obese group with lower proportion of fast twitch fibers had higher force production than the T2DM group. Force production was lower in the T2DM group than in the obese group. The proportions of type IIA and MHCIIx muscle fibres were significantly higher, and type IIA was lower significantly in diabetic and obese subjects compared with control subjects. The proportions of type I fibers and type II fibers were lower and higher, respectively, in T2DM than in obesity. CHO oxidations at rest and during exercise indicated direct correlation with type II fibers. Fat oxidation at moderate intensity of exercise correlated with MHCII. Type IIAB, MHCIII and force production bore a close relationship to the incidence of T2DM/obesity.

Key words: Submaximal concentric exercise, type 2 diabetes mellitus (T2DM), obesity, healthy, muscle fiber type distribution, substrate utilization, force production
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1 INTRODUCTION

Nowadays, the prevalence of both diabetes and obesity is sharply rising in the world. The complications, such as hyperlipidemia, heart disease and hypertension etc. (ACSM 2006; NDIC 2005), are becoming overabundant. Muscle fiber type distribution, substrate utilization and force production are associated with the development of obesity and type 2 diabetes mellitus (T2DM), the most common form of diabetes. Therefore, studies on T2DM/obesity become extremely important to know more about these worldwide health problems.

Skeletal muscles consist of type I (slow-twitch) and type II (fast-twitch) muscle fibres with different metabolism and functions. Type I muscle fibres (range 13–96% of skeletal muscle, mean value 50-60% of skeletal muscle) that are mainly genetically determined, and have a high capacity for oxidative energy metabolism, whereas type II fibres have a high capacity for glycolytic energy production. (Hernelahti et al. 2005; Karjalainen et al. 2006.)

Skeletal muscle takes a great role in carbohydrate and lipid metabolism at rest due to 30 / 40% of body weight composed of skeletal muscle in a reference women/men. During exercise, skeletal muscle has primary dominance over whole body energy flux due to the increase of metabolic flux in skeletal muscle. Once muscle oxidative metabolism is impaired, the bodily energy stores will be saturated, which will cause the development of obesity. (Blaak 2005.)

Generally, obese patients can obtain proportionally higher values of isotonic strength and a greater average force during exercise due to a greater amount of fat-free mass (FFM). It should be noticed that there is a large imbalance between the mass of the contractile element and inert fat mass in obesity, which compromises the motor performance (Lafortuna et al. 2005). Nevertheless, motor performance is related to force
production. Diabetics also have a reduction in muscle mass because of insulin resistance, which further impairs the force.

Adjustments of oxygen uptake ($\text{VO}_2$) to increased work load are related to muscle fibre types. Changes in $\text{VO}_2$ and muscle phosphocreatine are faster in muscles consisting predominantly of slow-twitch fibres. Changes in $\text{VO}_2$ are similar at different exercise intensities, and in incremental and constant power output exercises. (Barstow et al. 2000.) The peak of fatty acid oxidation is reached at intensities between 44-65% $\text{VO}_{2\text{max}}$. At higher intensities, intramuscular factors limit fatty acid oxidation and transport. Fatty acid oxidation is related with proportion of type 1 muscle fibres. (Sahlin et al. 2007.)

Obese subjects express a decreased number of type I muscle fibres. An increased number of IIX and IIA muscle fibres leads to reduced oxidative capacity and increased risk for T2DM. However, subjects with impaired glucose tolerance have been reported no different muscle fibre compositions compared to healthy subjects. (Larsson et al. 1999.)

In T2DM, utilization of plasma free fatty acid (FFA) is impaired in skeletal muscle without influencing whole-body lipid oxidation at rest, which further alters the pattern of substrate oxidation during exercise (Borghouts et al. 2002). In both obesity and T2DM, insulin resistance of muscles is characterized by a decreased ability to oxidize fatty acids during conditions with a relatively high lipolytic rate (like exercise). In addition, triacylglycerol storages in muscles are increased due to inability to oxidize fatty acids. (Blaak 2004.) Compared to a healthy control group, substrate utilization matches for body composition at rest and during moderate intensity exercise in non-obese Type II diabetic patients. (Borghouts et al. 2002.)

Interestingly, plasma glucose concentrations generally decline during exercise for both obese and non-obese type 2 diabetics. This results from either the impairment of splanchnic glucose output or the increase of plasma glucose uptake by exercising
Nevertheless, it has been reported that the development of skeletal muscle insulin resistance is related to the fuels (carbohydrate and lipid) and substrate utilization by muscle, which is the potential factor to impinge on T2DM/obesity. (De Beaudrap et al. 2006.)

Exercise is considered as a basic strategy for the treatment of diabetes, because it can improve insulin resistance. Furthermore, insufficient exercise is a major risk factor for obesity and T2DM. It has also been reported that physical fitness can significantly prevent diabetes. (Ahn et al. 2004.)

However, the relation between force production, muscle fibre type distribution and substrate utilization has yet to be examined in depth. The present study focused on the influence of T2DM and obesity on skeletal muscle fibre types, substrate utilization and force production. The purpose of the present study was to examine substrate utilization between T2DM, obese and control subjects at rest and during exercise, the difference of force production and muscle fibre type distribution in skeletal muscle between those three groups and the relationship between muscle fibre type distribution, substrate utilization and force production.
2 REVIEW OF THE LITERATURE

2.1 Categorization of diabetes mellitus

Diabetes mellitus illustrates a rapidly growing threat to the health of world populations, and is regarded as one of the most widespread epidemics. There are two general types of diabetes: type 1 and type 2 diabetes mellitus. Diabetes mellitus is caused by hyperglycemia due to the reduced quantities of a hormone called insulin, which moves the glucose from the bloodstream into the individual cells, and is a syndrome of impaired carbohydrate and lipid metabolism (Guyton & Hall 2000, 894). Also, the uncontrolled or improperly controlled diabetes can cause complications, such as heart disease, eye disease, kidney disease, and degeneration of nerve fibers. (Laaksonen 2002.)

Type 1 diabetes mellitus (T1DM) is characterized by deficient or absent insulin production by beta cells of the pancreas and usually occurs before middle age. Type 2 diabetes mellitus (T2DM) is the most common form of diabetes, which is caused by decreased sensitivity of target tissue to the metabolic effects of insulin. (Guyton & Hall 2000, 895.) The prevalence of type 2 diabetes is on the rise, which is reported largely due to the rise in obesity. The mechanisms that link obesity with insulin resistance and the pathogenesis of type 2 diabetes are still poorly explained. Currently, the WHO uses a fasting plasma glucose level of \( \geq 7.0 \) or a two-hour post-load level of 11.1 mmol/l in a 75-g oral glucose tolerance test as cutoffs for type 2 diabetes. These criteria are similar to the American Diabetes Association criteria. In contrast, the American criteria recommend an oral glucose tolerance test only when the fasting glucose level is below 7.0 mmol/l with high suspicion of diabetes. (Laaksonen 2002.)
2.2 Muscle fiber type composition

Bundles of single muscle fibers compose the skeletal muscle. Each muscle fiber contains many myofibrils, which are strands of proteins (actin and myosin) that can shorten the muscle and cause muscle contraction with their interplay. (Quinn 2007.) Muscle fiber types can be divided into three different types. Myosin ATPase content (i.e., I, IIa, IIb) or myosin heavy chain (MHC) composition (i.e., I, IIa, IIx) is the common basic categorization technique used for these fibers (Parcell et al. 2003). There may be more than one MHC isoform (i.e., I/IIa, IIa/IIx, I/IIa/IIx) in each single muscle fiber (Parcell et al. 2003). Slow twitch (Type I) muscle fibers, also called slow-twitch-oxidative fibers (Rauramaa 1981) have a small motor neuron and fiber diameter, a high mitochondrial and capillary density, as well as a high myoglobin content. Type I fibers generate adenosine triphosphate (ATP) by the oxidative metabolism of fatty acids, glucose and glycogen; have a slow contraction time and a high resistance to fatigue. Type I fibers are recruited during prolonged, low to moderate intensity activity (Nehasil 2001), such as most activities of daily living: walking and maintaining posture (Karp 2008).

Fast twitch (Type II) muscle fibers generate the energy source adenosine triphosphate (ATP) rapidly, mainly by glycogenolysis (the breakdown of their glycogen stores), with a quick contraction time and a low resistance to fatigue. Additionally, type II fibers produce lactic acid, or more correctly, lactate and hydrogen ions, which induce fatigue. Type II fibers can be further classified into fast-twitch A (FT-A or type IIA) and fast-twitch B (FT-B or type IIB) fibers. FT-A fibers, also called fast-twitch-oxidative-glycolytic fibers (Rauramaa 1981), have a large motor neuron and fiber diameter, a high mitochondrial density, a medium capillary density, and a medium myoglobin content. These fibers have high creatine phosphate (CP) and glycogen content a medium content of triglyceride stores, a high glycolytic and oxidative enzyme
activity, and a moderate resistance to fatigue. Type IIB fibers create energy almost equally by utilizing both aerobic and anaerobic metabolism, which promotes their recruitment during prolonged anaerobic activities with a relatively high force output, such as racing 400 meter sprint. (Karp 2008.)

Fast-twitch B fibers or fast-twitch-glycolytic fibers (Rauramaa 1981) have a large motor neuron and fiber diameter, but a low mitochondrial, capillary density and myoglobin content. They have a much faster rate of fatigue development due to many glycolytic and few oxidative enzymes. In this way, they are recruited during short anaerobic, high force production activities, such as sprinting and jumping. Fast-twitch B fibers are high in creatine phosphate and glycogen, but low in triglycerides with more power output compared to ST fibers. Table 1 generalizes some major features of the three fiber types. (Karp 2008.)

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Slow Twitch (ST)</th>
<th>Fast Twitch A (FT-A)</th>
<th>Fast Twitch B (FT-B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contraction time</td>
<td>Slow</td>
<td>Fast</td>
<td>Very fast</td>
</tr>
<tr>
<td>Size of motor neuron</td>
<td>Small</td>
<td>Large</td>
<td>Very large</td>
</tr>
<tr>
<td>Resistance to fatigue</td>
<td>High</td>
<td>Intermediate</td>
<td>Low</td>
</tr>
<tr>
<td>Activity used for</td>
<td>Aerobic</td>
<td>Long term anaerobic</td>
<td>Short term anaerobic</td>
</tr>
<tr>
<td>Force production</td>
<td>Low</td>
<td>High</td>
<td>Very high</td>
</tr>
<tr>
<td>Mitochondrial density</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Capillary density</td>
<td>High</td>
<td>Intermediate</td>
<td>Low</td>
</tr>
<tr>
<td>Oxidative capacity</td>
<td>High</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Glycolytic capacity</td>
<td>Low</td>
<td>High</td>
<td>High</td>
</tr>
<tr>
<td>Major storage fuel</td>
<td>Triglycerides</td>
<td>CP, Glycogen</td>
<td>CP, Glycogen</td>
</tr>
</tbody>
</table>

When exercise intensity is increased, type I fibres are recruited first, followed by type II
fibres. ATP generation in type II fibers depends on anaerobic metabolism, which makes muscle glycogen become depleted more rapidly in type II than type I fibers. Also, the speed of muscle glycogen resynthesis in type I fibers after prolonged exercise is significantly lower than that in type II fibers after repeated bouts of high intensity exercise. It is well known that each fiber type is unique in its ability to contract in a certain way with genetic factors. On average, most human skeletal muscles have about 50 percent slow twitch and 50 percent fast twitch fibers. For example, in arm and leg muscles of children and adults, there are 45-55% ST fibers. (Nehasil 2001.)

2.2.1 Muscle fiber type proportion in obese and patient with type 2 diabetes

Generally, muscle fiber type proportion in humans is influenced by gender, age, obesity, and the waist-to-hip ratio (Marin et al. 1994). In obese and T2DM individuals, there is a reduced oxidative enzyme activity, increased glycolytic activity, and lipid content in skeletal muscle, which is related to deficient insulin sensitivity of skeletal muscle and muscle fiber distribution (He et al. 2001). It has been reported that obese and T2DM subjects have abnormal muscle morphology, such as a higher proportion of type II (especially type IIB) fibers and decreased proportion of type I fibers (Marin et al. 1994, Tanner et al. 2002; Oberbach et al. 2006). The reason for abnormal muscle morphology is due to insulin sensitivity that is inversely related to the proportion of the glycolytic or low oxidative type IIB muscle fibers and directly related to the proportion of type I (red, oxidative) muscle fibers (Kern et al. 1999).

2.2.2 Muscle fiber type composition and force production

Given a fixed velocity of movement, fiber type determines the amount of force output. When muscles contract, the muscle fibers are either shortening or lengthening. Fast-twitch (FT) fibers produce more force than slow-twitch (ST) fibers (Fitts & Widrick 1996). During isometric contractions, FT fibers produce exactly the same
amount of force as ST fibers. The difference in force occurs when muscles are doing
dynamic contractions. Therefore, at any given velocity or force output, the higher the
percentage of FT fibers, the higher is the muscle force output or velocity. (Karp 2008.)

Colliander et al. (1988) reported that skeletal muscle force production decreases more in
the subjects with a high percentage of type II fibers than those with high percentage of
type I fibers during a single bout of maximal voluntary concentric contractions. Also,
force output recovers less in subjects with a low percentage of type I fibers than those
with high percentage of type I fiber distribution after exercise. During repeated bouts of
exercise, energy imbalance is greater in the subjects with high percentage of type II
fibers than those with low percentage of type II fibers. After a single episode of
supramaximal cycle ergometer exercise, marked shifts of the energy state of skeletal
muscle are indirectly indicated in subjects with high percentage of type II fibers. During
repeated bouts of maximal voluntary concentric exercise, maintenance of force is lower
in the subjects with low percentage of type I fibers than those with low percentage of
type II fibers. Between the repeated bouts of maximal voluntary concentric exercise, the
recovery of force is also lesser in type I fibers than type II fibers. (Colliander et al.
1988.)

2.2.3 Exercise and muscle fiber distribution in type 2 diabetes and obesity

Normally, regular exercise is beneficial to increase HDL cholesterol and the HDL/total
cholesterol ratio. It has been reported that LDL cholesterol and triglyceride levels can be
decreased by endurance training (Laaksonen 2002). Ingjer (1979) presented that
endurance training significantly increases the number of type IIA fibres and decreases
the number of type IIB fibres. In the study by Gaster et al. (2001), it was shown that the
slow-fiber proportion is reduced to 86% in the obese subjects and to 75% in the diabetic
subjects compared with the control group. Some studies also have reported that
high-intensity intermittent training significantly increases the proportion of type I and
decreases the proportion of type IIB fibers, while the proportion of type IIA remains unchanged (Simoneau et al. 1985). Training increases the cross-sectional area of type I and IIB fibers as well (Simoneau et al. 1985). Generally, fiber type composition in human muscles may be changed by exercise training among the population of fast-twitch fibers (i.e., type IIA to IIB) and in lesser extent from fast- to slow-twitch fibers. (Ingalls 2004.)

2.3 Substrate utilization and exercise

Exercise training is a recommended and powerful treatment for T2DM as well as a beneficial pathway to cure and prevent most metabolic dysfunctions (insulin resistance and lipid disorders etc.) (Ghanassia et al. 2006). Exercise is also a good preventive method against the current obesity epidemic due to its beneficial effect on weight loss (Stiegler et al. 2008). During exercise, carbohydrate and fat are the two predominant substrates used as fuels for muscular contraction, whereas protein is utilized as auxiliary fuel during muscular work (Nehasil 2001). Moreover, it has been presented that training improves lipids oxidation and reduces fat mass in both adult and adolescent subjects, which improves metabolic defects, including an increase in insulin sensitivity (Ghanassia et al. 2006). However, the mechanism of metabolic adaptation to exercise in these diseases is still unclear.

2.3.1 Substrate depots of the body

A normal man expends approximately 5kJ/min in his resting state. During the exercise bout, energy expenditure rises 10-20 times of that value at rest. The blood glucose pool alone cannot support prolonged work, thus glycogen that is stored in the liver and skeletal muscle starts to release glucose for maintaining the exercise. Triglycerides in adipose tissue are the largest energy reserve. Table 2 shows all the substrate depots in a healthy man. (Wahren 1982.)
Muscle glycogen depots are affected by the initial glycogen content of the muscle and an individual’s fitness. Fat is the principal form of stored energy, which serves as fuel for exercising human skeletal muscle. The adipose tissue triglycerides release FFA and glycerol to the blood stream by lipolysis, which plays an important role in the transport of fuel from the adipose tissue to other tissues (i.e. muscle). (Wahren 1982.)

### 2.3.2 Effects of exercise intensity

Exercise intensity (expressed as %VO$_{2\text{max}}$) is a decisive factor in determining which substrate is used by muscle contraction. During rest and low intensity exercise (less than 40 % VO$_{2\text{max}}$), fat is the predominant fuel. At moderate exercise intensities (ranging from 40-60% VO$_{2\text{max}}$), muscle contraction is fueled by fat and carbohydrate equally. During the exercise with high intensity (over 70% VO$_{2\text{max}}$), carbohydrate becomes the preferred and then the exclusive fuel for working muscles. (Kolkhorst 2007.)

There are four major fuels for exercise: muscle glycogen, plasma glucose, muscle triglyceride, and plasma fatty acids. Figure 1 shows the usages of these fuels change with the increase of energy intensity. These data have been derived from the study of
Romijn et al. (1993). As the exercise intensity increases from 25% to 65%, then to 85% of VO₂max, muscle glycogen and plasma glucose use increases, and inversely, plasma fatty acid use decreases. Utilization of muscle triglyceride increases at 65% of VO₂max and decreases at 85% of VO₂max. Total utilization of fat and carbohydrate are highest at 65% and 85% of VO₂max, respectively. (Romijn et al. 1993.)

FIGURE 1. Contribution of the above four substrates to energy expenditure after 30 min of exercise at 25%, 65% and 85% of VO₂max in fasted subjects (Kolkhorst 2007). Total amount of calories (cal) available from plasma does not change with the exercise intensity (Romijn et al. 1993). There is no muscle glycogen utilized at the 25% intensity. Muscle glycogen becomes the primary substrate fueling as exercise intensity increases (Kolkhorst 2007).

2.3.3 Effects of exercise duration

As exercise continues, the fuels for exercise are changing. Fat utilization increases and carbohydrate utilization decreases, which is due to the depletion of muscle glycogen content that declines within 2-4 hours of continuous exercise according to different intensity. Nevertheless, blood glucose generally remains almost constant, which is due to more liver glycogen broken down by the liver and the subsequent release of glucose into the blood. As time goes on, the onset of fatigue and reduced blood glucose occurs
due to the depletion of liver glycogen. In figure 2, it is shown clearly how the substrate utilization affected by the exercise duration. (Kolkhorst 2007.)

![Figure 2: Percentage of energy derived from the four major substrates during prolonged exercise at 65-75% of maximal oxygen uptake. Initially, approximately one-half of the energy is derived each from carbohydrate and fat. As muscle glycogen concentration declines, blood glucose becomes an increasingly predominant source of carbohydrate energy for muscle. After 2 h exercise, carbohydrate ingestion is needed to maintain blood glucose concentration and carbohydrate oxidation. FFA (Free fatty acids). (Kolkhorst 2007.)](image)

**2.3.4 Exercise duration and intensity**

During aerobic exercise, fats provide 50-60% of the body’s energy. During the first several minutes (less than 20 minutes) of aerobic exercise, carbohydrates are the primary fuel. Following 20-30 minutes of aerobic exercise, fat and carbohydrate are almost equal fuel sources (50%). After one hour of aerobic exercise, fat utilization greatly increases, approximately seven times. (Kolkhorst 2007.)

Specifically, during the low intensity exercise (<30% VO$_2$ max) of long duration, fat is the predominant fuel, which is almost the same as when at rest. Carbohydrate, in contrast, is the preferred fuel used for high intensity exercise (>70% VO$_2$ max). In other words, during lower intensity submaximal exercise, there is higher proportion of fat and
a lower proportion of carbohydrate utilization. Also, there is a gradual shift from carbohydrate to fat metabolism in prolonged low intensity exercise (i.e. more than 30 minutes). Proteins are used in prolonged exercise as a fuel supply more after the first hour of exercise (less than 2% of total energy substrate utilization). (Kolkhorst 2007.) It has been reported that protein utilization can reach 5-15% of the energy source in prolonged exercise lasting 3 to 5 hours (Berg et al. 1980; Cerretelli et al. 1977; Hood & Terjung 1990; Lemon & Mullin 1980; Lemon et al. 1980). When glycogen stores and energy intake are insufficient for prolonged and intense exercise, proteins can provide around 10% of total energy substrate utilization (Brooks 1987). During high intensity and short duration aerobic exercise, more carbohydrates or glycogen is utilized for muscle contraction. However, muscle glycogen is depleted by intense or prolonged exercise. When lactate output is greater, fat metabolism is inhibited. (Turcotte et al. 1995.), however, during continuous and prolonged intense exercise, fat is utilized more (Mulla et al. 2000; Phelain et al. 1997).

2.3.5 Mechanisms for substrate utilization at exercise

In the light of the previous literature, several mechanisms are proposed for substrate utilization during exercise. For instance, fatoxidation is enhanced by the increase in availability of fatty-acids (FA), which causes increased concentrations of acetyl-CoA and citrate. Phosphofructokinase (PFK) and pyruvate dehydrogenase (PDH) are down-regulated by this fat-induced increase in acetyl-CoA and citrate, which further results in decreased carbohydrate oxidation. Glucose-6-phosphate accumulates by the decreased activation of PFK, which further causes decreased activation of hexokinase. Therefore, a reduction in glucose uptake occurs. This theory was the originally accepted metabolic process and is called the classical glucose-fatty acid cycle. (Jeukendrup 2002.) Another metabolic pathway has been proposed by Coyle et al. (1997). Fat oxidation can be directly regulated by carbohydrate availability during exercise. Glycolytic flux is increased during exercise, which directly inhibits long-chain fatty acid oxidation by
muscle mitochondria and further regulates substrate utilization. (Coyle et al. 1997.) The decreased activation of muscle malonyl CoA mediates uptake of FA by the mitochondria. Malonyl CoA inhibits the activation of carnitine palmityl transferase 1 (CPT 1), which further inhibits β-oxidation that is a cyclic pathway to break FA down to acetyl CoA. (Diwan 2007; Winder 1998.) Vavvas et al. (1997) suggested dual regulation of glycolysis and fatty acid oxidation (See figure 3). In this model, 5’-AMP-activated kinase (AMPK) activity in muscle cells is increased by exercise, which further phosphorylates and inactivates acetyl-CoA carboxylase, an enzyme for converting acetyl-CoA to malonyl-CoA. Consequently, concentration of malonyl-CoA declines accompanied by increased activation of CPT 1, which results in increased FA oxidation. Once the rate of glycolysis is increased, FA oxidation will be reduced by enhanced malonyl-CoA concentrations, which is followed by inhibited CPT 1. (Vavvas et al. 1997.)

FIGURE 3. Proposed dual mechanism of regulation of glycolysis and fatty acid oxidation. In this model, acetyl-CoA carboxylase-β (ACC-β) and PFK are acutely activated and inhibited by increased glucose availability, respectively, which is due to the increase in cytosolic levels of citrate. However, in increased energy expenditure (during exercise), ACC-β and PFK are inhibited and activated individually due to the increased concentration of free 5’-AMP. When AMPK is activated, ACC-β will be affected by 5’-AMP. In this model, the resultant change in the concentration of malonyl-CoA and glycolysis changed with the activity of PFK will at least partly restrain or enhance FA oxidation. In the intense contraction, the 5’-AMP-mediated
effects show the priority in regulating fatty acid oxidation with the increase of both cytosolic citrate and 5’-AMP.(Vavvas et al. 1997.)

As mentioned above, in the main metabolic pathway, increased FA availability leads to the increase in fat oxidation and decrease in CHO oxidation, while the increase in availability of CHO causes decreased fat utilization and increased CHO utilization during submaximal exercise. (Holloszy et al. 1998.)

2.3.6 Substrate metabolism in type 2 diabetes and obesity

Carbohydrate can produce ATP rapidly and is the only energy substrate to produce ATP via anaerobic metabolism, which is used for fueling moderate to high intensity exercise. However, fats/ lipids can produce ATP slowly via only aerobic metabolism, which fuels exercise with low intensity and short to long duration. (Ghanassia 2006.)

There have been few studies to illustrate substrate metabolism in T2DM and obesity. In the study of Ghanassia et al. (2006), it is shown that lipid oxidation is lower at exercise and there is an earlier shift towards a predominance of carbohydrate oxidation with increased exercise intensities in type 2 diabetic group compared to control group. There is increased proportion of triglyceride use within muscle fibers in fatty acid metabolism by skeletal muscle in individuals with obesity and T2DM (Kelley & Mandarino 2000). As demonstrated in figure 4, substrate utilization changes with metabolic deficiency in T2DM and obesity. During fasting, there is a predominant use of lipids as an energy source in the lean group but in the obese and diabetic groups, there is a reliance on carbohydrates as an energy source. During insulin infusion, there is a reliance on carbohydrates in all groups. Furthermore, in the obese and diabetic groups, the fuel choices remain almost unchanged. In total, carbohydrate oxidation is increased during basal measurements and decreased under insulin-stimulated conditions in T2DM and obesity, which increases the production of triglyceride within muscle fibers and further
decreases insulin sensitivity. (Kelley & Mandarino 2000.) Noticeably, a metabolism for a substrate is also determined by muscle fiber distribution regardless of exercise intensity. It has been found that the rate of FA oxidation is higher in type I muscle fibers than in type II fibers (Sahlin & Harris 2008).

FIGURE 4. Illustration of metabolic inflexibility of oxidative fuel selection between carbohydrate and lipid in skeletal muscle of insulin-resistant obese and type 2 diabetic subjects (Kelley & Mandarino 2000).

2.4 Overweight and an abdominal fat distribution

Body mass index (BMI: kg/m²) is most widely used for the evaluation of adiposity, which is unrelated to height, and it is a good index of overall adiposity at the population level (Laaksonen 2002). There are three other indicators of obesity, namely, excess fat mass, waist circumference and abdominal visceral fat (Bouchard 2007). Figure 5 shows the correlations between these four indicators (Bouchard 2007). Other measures, such as skinfold measures and bioelectrical impedance, are more accurate calculations of percent body fat, but require sex and age-dependent norms that may alter from population to population (Laaksonen 2002). In adults, BMI from $\geq 25$ kg/m² to $< 30$ kg/m² is regarded as overweight while a value $\geq 30$ kg/m² is defined as obesity (Cole et al. 2000; ACSM 2006, 216).
FIGURE 5. Correlations between BMI, FM (excess fat mass), WAIST (waist circumference) and CT-assessed AVF (abdominal visceral fat). The bidirectional arrows show that there are specific interplays among these indicators. Weighted mean of six correlations is illustrated by each coefficient computed in samples of black men and women and white men and women from the Heritage Family Study, and of Caucasian men and women from the Quebec Family study. (Bouchard 2007.)

It is well known that abdominal fat distribution can be measured by waist circumference and the waist-hip ratio, which is deleterious. Waist circumference is reported to be a preferred measure in comparison to waist-hip ratio. Noticeably, abdominal obesity generally increases with the rise in the degree of obesity. It has been suggested that cutoffs for waist circumference between 94 cm and 102 cm should be the interventional action levels for men. (Laaksonen 2002.)

Visceral fat is intra adipose tissue around the abdomen region. High amount of visceral fat is a risk factor for T2DM. (Ahn et al. 2006.) It has been reported that visceral abdominal fat is related to insulin resistance in obesity and T2DM (Gastaledlli et al. 2007), independent of total body fat or subcutaneous abdominal fat. According to many other studies, however, subcutaneous abdominal adipose tissue has been found to have a strong or stronger correlation to insulin resistance. The relationship between the independent contribution of waist circumference or waist-hip ratio over BMI and the development of diabetes is still unknown. (Laaksonen 2002.)
2.5 Cardiorespiratory responses to physical activity

It is commonly known that aerobic physical exercise decreases weight, visceral fat accumulation, triglyceride levels and blood pressure, improves insulin sensitivity, increases HDL cholesterol and enhances cardiopulmonary fitness. It is still only partly understood, what the mechanisms are by which exercise increases insulin sensitivity regardless of weight loss. (Rauramaa 1981.)

2.5.1 Maximal oxygen consumption

Maximal oxygen consumption (physical performance capacity, VO$_{2\text{max}}$), also called aerobic power, is a function of cardiac output and oxygen extraction from blood (Misquita et al. 2001), which is utilized to predict individual ability to perform muscular activity. The most valid measurement of physical performance capacity is direct measurement of oxygen consumption during exercise. Generally, VO$_{2\text{max}}$ is measured during a continuous, multistage exercise test of increasing intensity (Misquita et al. 2001).

Normally, muscle respiratory capacity is an important determinant of maximal oxygen consumption for a healthy man. Physical training can increase maximal oxygen uptake, which is reliant on initial aerobic power, as well as on the frequency and intensity of exercise, and on the duration of training program. (Rauramaa 1981.) Ara et al. (2004) pointed out that physical fitness is increased by regular physical activity (at least 3 hours/week).

It has been reported that cardiorespiratory fitness is decreased in T2DM (LaMonte et al. 2005). However, there are only few studies where maximal oxygen consumption and the effects of regular physical training on cardiovascular fitness in diabetic subjects have been measured. There are no great differences in the physical activity capacity
between diabetic and healthy subjects. Previously, it was reported that there is no
correlation between physical working capacity and duration of diabetic disease. As
mentioned by Rauramaa (1981), there were similar increases in physical performance
capacity after ten weeks’ running training in 12 insulin-dependent diabetic men
compared to 13 non-diabetic men, and maximal oxygen consumption was increased
after bicycle training for half a year in six non-insulin dependent diabetic patients. Also,
it has been reported that local fatigue in working muscles is the reason why many
diabetic patients are unable to achieve the estimated age-specific maximal heart rate
without sufficient training. (Rauramaa 1981.)

In general, obese individuals have low physical fitness and aerobic exercise capacity
(Hulens et al. 2001). Mustelin et al. (2008) presented a significantly decreased VO_{2\text{max}} in
an obese co-twin when comparing to a non-obese co-twin. Whereas, Lazzer et al.
discovered that there is no significant difference in VO_{2\text{max}} between obese and nonobese
adolescents (Lazzer et al. 2007). The study of Brien et al. (2007) demonstrated that the
lower probability of obesity in 2002-04 is correlated with the higher VO_{2\text{max}} in 1981-88.
Seng et al. (2003) also found that mean aerobic capacity is increased by 11% in
overweight and obese sedentary men after 6 weeks of moderate aerobic exercise. There
is still a scarcity of studies on the effects of physical activity on VO_{2\text{max}} in obese and
diabetic populations.

### 2.5.2 Heart rate

Usually, the resting heart rate of healthy subjects is lower than that of diabetic ones.
During submaximal exercise loads in diabetic subjects, the heart rate is higher and
increases more quickly with the decrease in VO_{2\text{max}}. Except for one study, by Larsson et
al. (1964) a lower maximal heart rate is attained at a lower level of external work in
diabetic than healthy subjects. (Rauramaa 1981.) There are multiple factors that affect
the differences in heart rate responses to physical exercise between diabetic and healthy
subjects. The lower physical performance capacity may be a result of decreased physical activity.

2.6 Blood pressure

Diabetic patients have various metabolic dysfunctions that can cause hypertension; moreover, hypertension is an independent risk factor for type 2 diabetes. Hypertension is one of the most prevalent forms of cardiovascular disease (ACSM 2006, 213), which has a well established association with obesity and abdominal fat distribution. The risk of hypertension increases significantly with the increase in age and BMI (Rankinen et al. 2007). In one cohort study, hyperinsulinemia shows a close correlation with the morbidity of hypertension and dyslipidemia (Laaksonen 2002), but the cause-effect relationship between diabetes and hypertension still needs to be proven.

2.7 T2DM and obesity

In a normal man, overweight causes health problems due to the association of overweight with several comorbidities, such as T2DM, cardiovascular diseases and hypertension. Figure 6 shows the presence of insulin resistance / hyperinsulinemia that is the most common abnormality shown in obesity and has close relationship with the metabolic and cardiovascular complications of obesity (Weiss et al. 2005). It has been illustrated that the risk of diabetes increases between 4.5-9% with every kilogramme of weight gain. (Golay et al. 2005.) The development of T2DM is triggered by the vertiginous rise in obesity (Golay et al. 2005.), which is due to the incidence of T2DM significantly correlated with the increase in body fatness (Kumanyika et al. 2002). Obesity is identified by the deposition of excess body fat (Stieglar et al. 2008; Guyton & Hall 2000). Usually, obese subjects are insulin resistant, which results in T2DM among susceptible subjects (Kern et al. 1999). Therefore, the term ‘diabesity’ has been coined to demonstrate the interdependence between diabetes and weight (Golay et al.
It describes that excessive weight leads to diabetes and those who are diabetics have excessive weight as well (Crabs 2009).

It has been reported that 60–90% of all T2DM patients are or have been obese. Generally, obesity is regarded as a strong risk factor for the later development of T2DM (Guo & Zhou 2004). Increasing insulin resistance and defective insulin secretion cause obesity to develop into diabetes. Therefore, the question arises whether or not obesity is only a risk factor, but also a cause of T2DM. The mechanism behind obesity-related insulin resistance is unclear (Kern et al. 1999), and the mechanisms of the pathway from obesity to T2DM need to be further researched (Golay et al. 2005).
2.8 Exercise, T2DM and obesity

It has been proven that regular physical activity can help manage T2DM and obesity (Perez-Martin et al. 2001). In a study by Myers et al. (2003), it is stated that the risk for mortality is twice as high in physically inactive diabetic men as in physically active men. The development of T2DM can be prevented by moderate-to-vigorous leisure-time
physical activity (Laaksonen et al. 2002). Obviously, exercise is important for diabetics, because it helps insulin work better to lower blood sugar, helps to keep weight down, reduces the risks of complications and gives more energy. In obesity, regular exercise along with proper nutritional diet is beneficial to reduce body fat (Tecco 2001; Perez-Martin et al. 2001), which improves insulin sensitivity (Kern et al. 1999; Perez-Martin et al. 2001). Sixteen weeks aerobic or resistance training programme combined with diet elucidates similar weight reduction but higher improvement in insulin sensitivity compared to diet alone (Rice et al. 1999).

As a whole, physical activity can be used as a tool to prevent excessive body fat accumulation (Goris & Westerterp 2008), which further changes body composition and improves insulin sensitivity (Perez-Martin et al. 2001). Exercise can increase lipid oxidation and muscle mitochondrial oxidative capacity, which can further establish the molecular mechanisms for lipid-induced insulin resistance and exercise efficacy in this situation (Kiens 2006).
3 RESEARCH QUESTIONS AND HYPOTHESES OF THE STUDY

The present study was to address the relationship between force production, muscle fibre type distribution and substrate utilization in T2DM and obesity. The results were expected to develop a simple method to describe the individual characteristics of muscles. By knowing the muscle characteristics, individual exercise prescriptions can be made more effective.

The main research questions of the study:
(1) How does substrate utilization function between T2DM, obese and control subjects at rest and during exercise?
(2) What are the differences of force production and muscle fibre type distribution in skeletal muscle between T2DM, obese and control subjects?
(3) What kind of relationship is there between muscle fibre type distribution, substrate utilization and force production in T2DM/obesity?

The hypotheses are as follows:
1) Obese and diabetic subjects use less oxidation of fatty acids during exercise compared with control subjects;
2) Force production of muscle is unchanged in obese subjects and impaired in T2DM when compared to control group;
3) In obese and diabetic subjects, the proportion of type 2 muscle fibres is higher in comparison with control subjects; impaired force production relates to muscle fibre type distribution and substrate utilization in the development of obesity and T2DM.
4 METHODS

All subjects were volunteers and they could remove themselves from the study at any point. Subjects were fully informed about the study and its measurements. Written approval of volunteering and fully understanding the study was obtained from the subjects before all the measurements started. The study protocols were approved by the ethical committee at the University of Jyväskylä.

4.1 Subjects

Subjects, aged 35-60 years old, were recruited not only from LIKES-weight management groups and Diabetes Association of Jyväskylä but also by the advertisement in local newspapers. They were obese, and healthy or diabetic men ($n_{type II\text{ diabetic}}=14$, $n_{obese}=11$ and $n_{healthy}=3$; $n_{total}=28$). Lean and healthy subjects were used as a control group. Results were compared between obese healthy and obese diabetic groups. A criterion for obesity was a BMI value >30 kg/m$^2$ and control group’s BMI was below 25 kg/m$^2$. Before performance measurements were done, subjects underwent an examination performed by a physician.

4.2 Anthropometric measurements

Body composition was measured by Inbody 720 analyzer (Biospace, Seoul, Korea) and the results were expressed as the percentage of fat mass. BMI was calculated as the body mass (in kilograms) divided by the height (in metres) squared. Body weight and height were measured in the morning with subjects wearing light clothing. Waist circumference was measured with a soft tape, midway between the lowest rib and the iliac crest while subjects were standing.
4.3 Blood sample, blood pressure and heart rate

Blood samples were collected by a physician after overnight fasting. The following biochemical parameters were analyzed from the blood samples: plasma glucose, total cholesterol, triglycerides, high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol.

Blood pressure was taken at the second minute of each load when the subjects performing the VO$_{2\text{max}}$ test. During the submaximal concentric test, blood pressure was taken at the second last minute of each grade. It was measured by the automatic blood pressure monitor (OMRON 705CP, Hamburg, Germany) from the right arm, which was recorded as the systolic and diastolic blood pressure.

Heart rate was recorded at the last minute of each load in the VO$_{2\text{max}}$ test. While during the submaximal concentric test, heart rate was recorded at the third, fifth and seventh minute of each grade, which was judged with heart rate monitoring model (Polar Electro Oy, Finland). Rating of Perceived Exertion (RPE) was asked from the subjects directly from the Borg scale as the same time as heart rate was recorded during both tests.

4.4 Exercise testing

*Maximal oxygen consumption.* Maximal oxygen consumption (VO$_{2\text{max}}$) and maximal workload were measured to determine workloads for the energy expenditure measurement using a bicycle ergometer for concentric exercise. VO$_{2\text{max}}$ was measured (Oxycon Pro Jaeger, Germany) during a graded maximal bicycle ergometer test with the subjects cycling at a self-determined rate above 50 repetitions per minute (Hulens et al. 2001; Borghouts et al. 2002). Before the test, subjects warmed up on the bicycle ergometer at a low intensity (50-70 W) for ten minutes. Initial workload (around 60-70 W) was determined from the warm-up. Each grade of the test lasted three minutes and
workload was increased by 25 watts for each grade. Heart rate, blood pressure, respiratory gases and RPE were measured during the test. The test was ended with either the decrease of HR, BP or VO$_2$, the subject’s inability to maintain desired pedalling rate, or subject’s request to stop the test. A cool-down was performed on the bicycle ergometer for ten minutes after the test with HR monitoring for the duration of cool-down. After cooling down, a guided stretching session was completed to diminish the possible muscle soreness during the following days.

**Force measurement.** Isometric force was measured on a knee extension bench (David 200, David Sports ltd, Finland) with subjects’ right leg. Subjects warmed up on a bicycle ergometer for 5 minutes and rehearsed the isometric contraction on the knee extension bench with submaximal levels of force. During the test, subjects were instructed to perform maximal voluntary isometric contractions as fast and hard as possible by sitting on the adjustable bench with a knees joint angle of 107 degrees for 3-5 times. Rest between the maximal contractions was 1-2 minutes. The average value of two or three highest attempts was taken for the analysis. Maximal force and rate of force development (RFD) were measured. (Kyröläinen et al. 1990.)

**Measurement of respiratory gases and substrate utilization.** Subjects performed warm-up (walking) on a treadmill at a self-selected pace for ten minutes. The aim was to reach 60% of HR$_{max}$ measured during the VO$_{2max}$ test. Heart rate level was reached by altering treadmills velocity from 4-6 km/h and grade, if necessary. The test was performed on a bicycle ergometer at 50-60 rpm. In the test, there were three grades at the intensity of 30%, 50% and 70% of Wmax (watts), respectively. Each grade lasted seven minutes. HR, BP, RPE and respiratory gases were monitored. A cool-down was done on the bicycle ergometer for 5 minutes and followed by stretching. Energy expenditure was calculated for each grade from the respiratory gases. The calculation was according to Peronnet and Massicote’s equations (Ghanassia et al. 2006):

- Glucox (mg/min)=4.585*$V_{CO2}$–3.2255*$V_{O2}$
- Lipox (mg/min)=1.6946*$V_{O2}$–1.7012*$V_{CO2}$. 
During the maximal oxygen consumption test and measurement of respiratory gases, volumes of oxygen and carbon dioxide were measured and adjusted to standard temperature. Metabolic rate and rate of substrate utilization was calculated from respiratory gas exchange. (Sale et al. 2006.) Before any of these tests, a control measurement of respiratory gases was performed for 5 minutes to ensure reliability of the measurements.

### 4.5 Muscle biopsy

In order to prevent the acute effects of exercise on muscle triglycerides, subjects were asked not to perform physical exercise for 48 hours prior to the muscle biopsy procedure. Before the biopsy, local anesthesia was applied 5–10 minutes with adrenaline. Muscle biopsy specimens were obtained from the vastus lateralis (15 cm above the patella) (Goodpaster et al. 2000; Oberbach et al. 2006) and approximately 2 cm away from the fascia using a Bergström needle (4.5–5.0 mm external diameter). Two muscle samples were taken. One sample was examined to determine fibre orientation by using a magnifying glass and then mounted on a cork with embedding medium (OTC-compound, Tissue-Tek) and frozen in isopentane, cooled to its freezing point with liquid nitrogen (N₂). The other was directly wrapped in the aluminum foil and frozen immediately in liquid N₂. They were stored at -80°C so that they could be used for histochemical analysis (Gravholt et al. 2001) and electrophoretic separation later.

### 4.6 Fibre typing

In the identification of muscle fiber types, myofibrillar ATPase staining was utilized together with preincubations of pH 10.3, 4.55–4.6, and 4.37. A Track Eye Motion Analysis (TEMA) image analysis system was used to analyze the computer image. The
classifications of fibers of the vastus lateralis specimen was type I, type IIa, and type IIb. When the fibers classified, the distinct fibers should be counted as many as possible (>200).

4.7 Statistical analysis

All the statistical analyses were performed using SPSS 13.0 and 16.0. P< 0.05 was considered as significant difference. Variables were expressed as means ±SD. The data were analyzed with the nonparametric Mann-Whitney rank-sum tests due to their nonnormal distribution. Differences in variables were examined in type II diabetic and obese groups. Kruskal-Wallis H tests were only used to compare differences in muscle fiber type distributions between diabetic, obese and healthy groups. The relation between selected variables was assessed by using correlation, partial correlation and Pearson’s correlation analysis. Logistic regression was used to estimate the association of force production, substrate utilization and muscle fiber distribution with the incidence of obesity/T2DM.
5 RESULTS

The anthropometric characteristics of the subjects were summarized in table 3. Due to insufficient number of healthy subjects (n=3), the following statistical differences were analyzed between the diabetic and obese groups with the exception of the differences in muscle fiber type distributions.

**TABLE 3.** Characteristics of anthropometric and biochemical data in three groups. *HRmax P=0.02 (correlated with diabetic and obese groups: r = 0.403, P= 0.046) and *blood glucose P=0.001 (correlated with diabetic and obese groups: r = -0.441, P= 0.027) were significant difference between diabetic and obese groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type II diabetes (n=14)</th>
<th>Obesity (n=11)</th>
<th>Control (n=3)</th>
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<tbody>
<tr>
<td>Age (yrs)</td>
<td>53±6</td>
<td>50±10</td>
<td>54±8</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>106.7±20.8</td>
<td>99.6±14.2</td>
<td>79.6±7.1</td>
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<tr>
<td>Height (cm)</td>
<td>178.9±7.0</td>
<td>176.1±7.3</td>
<td>173.9±4.2</td>
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<td>WC (cm)</td>
<td>116.7±12.1</td>
<td>118.5±22.6</td>
<td>96.7±1.8</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>33.2±4.7</td>
<td>32.0±2.7</td>
<td>26.2±1.0</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>74.9±10.4</td>
<td>69.3±9.7</td>
<td>65.3±7.8</td>
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<td>SMM (kg)</td>
<td>42.5±6.0</td>
<td>40.1±5.8</td>
<td>37.0±5.0</td>
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<tr>
<td>FM (kg)</td>
<td>31.2±11.4</td>
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<td>14.3±1.5</td>
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<tr>
<td>Fat (%)</td>
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<td>Visceral Fat (cm²)</td>
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<td>164.5±26.4</td>
<td>112.0±9.5</td>
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<td>Isometric Fmax (N)</td>
<td>834±141</td>
<td>843±342</td>
<td>761±114</td>
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<td>RFD (N/s)</td>
<td>11214±4592</td>
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<td>11009±3526</td>
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<td>VO₂max (L/min)</td>
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<td>2.9±0.5</td>
</tr>
<tr>
<td>Wmax (W)</td>
<td>187.5±32.2</td>
<td>218.6±67.2</td>
<td>241.7±52.0</td>
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<td>HRmax (bpm)</td>
<td>154±16*</td>
<td>168±18*</td>
<td>164±10</td>
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<td>Total Cholesterol (mmol/l)</td>
<td>4.5±1.5</td>
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<td>4.8±1.8</td>
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<td>HDL-Cholesterol (mmol/l)</td>
<td>1.2±0.3</td>
<td>1.6±1.2</td>
<td>1.6±0.3</td>
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<td>LDL-Cholesterol (mmol/l)</td>
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<td>Triglycerides (mmol/l)</td>
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<td>Blood glucose (mmol/l)</td>
<td>7.7±3.5*</td>
<td>5.1±1.4*</td>
<td>5.0±0.2</td>
</tr>
</tbody>
</table>

Note: WC- Waist circumference; BMI- Body mass index; FFM- Fat-free mass; SMM- Skeletal muscle mass; FM- Fat mass; RFD- Rate of force development; VO₂max- Maximal oxygen consumption; Wmax- Maximal isometric force; HRmax- Maximal heart rate.

Maximal oxygen consumption and isometric force. The mean VO₂max and Fmax in
diabetic and obese groups were similar (figure 7). According to rate of force development, force production in obese group was higher than the other groups though not statistically significant.

FIGURE 7. Mean levels of VO2max and maximal isometric force in three groups. Error bars represent SD. The three groups were not statistically significant.
Substrate utilization at rest and during concentric exercise. Fat and carbohydrate oxidation at rest and during exercise were summarized in table 4 and figure 8.

Table 4 illustrates the relative amounts of substrate oxidized. As seen from this table, fat oxidation accounted for 1.4 % higher (No Significant) energy expenditure at rest in diabetes than in obesity, whereas CHO oxidation was 1.4% lower (No Significant) at rest in diabetics group than in the obese group. Fat/CHO oxidation showed no significant difference at exercise intensities of 30%, 50% and 70% in type II diabetic patients or the obese group. Figure 8 shows the contribution of fat and CHO substrates to energy provision at rest and during exercise. As shown in the figure, total fat oxidation and total CHO oxidation were not significantly different between diabetic and obese groups. When exercise intensity increased, CHO oxidation became a more dominant part of energy consumption. At the exercise intensity of 70% \( W_\text{max} \), fat oxidation turned out an almost negative value, which was not theoretically reasonable. However, it seems to have shown that CHO provided almost all the energy consumed when exercise intensity and duration are increasing.
a. The absolute amounts substrate oxidized

b. The relative contribution to energy expenditure

FIGURE 8. Substrate utilization at rest and during exercise in diabetics, obese and healthy groups.
**Muscle fiber type distribution.** The characteristics of muscle fiber types and myosin heavy chain were presented in table 5. Figure 9 shows their muscle fiber distributions.

Due to low number of healthy subjects recruited to the study, some data about muscle fiber types of healthy subjects from another study (table 5. a) were added into this study in order to increase the reliability. In this case, there was the lowest proportion of MHCIIx in healthy group, which was statistically significant (P<0.05). Comparing with healthy group, type IIA decreased 32% and 35% (P<0.05) in T2DM and obesity, respectively. Type IIAB increased 78% and 48% (P<0.05) in T2DM and obesity. If the comparison was just between T2DM and obesity, then type I fiber would decrease 7.1% significantly in T2DM than in obesity (P=0.048<0.05).

**TABLE 5. Characteristics of muscle types.** *MHCIIx, Type IIA and IIAB fibers were significant difference among three groups (1: P =0.036; 2: P =0.016; 3: P =0.034; 4: P =0.020; 5: P=0.043) (a. Healthy group: Age(yrs): 53±6; Weight(kg): 73.4±11.0; Height(cm): 173.4±8.9; FFM(kg): 57.1±7.6; FM(kg): 16.4±4.8; Fat(%): 22.0±3.9)

<table>
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<th>Characteristics</th>
<th>Type II diabetes (n=11)</th>
<th>Obesity (n=8)</th>
<th>Control (n=8)</th>
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<tr>
<td>MHCIIx/5ul (%)</td>
<td>28.2±6.5*</td>
<td>28.1±12.0*</td>
<td>6.3±8.9*</td>
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<tr>
<td>MHCIIa/5ul (%)</td>
<td>44.6±20.1</td>
<td>34.6±18.0</td>
<td>49.0±9.4</td>
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<td>MHCI/5ul (%)</td>
<td>40.0±6.4</td>
<td>44.3±6.9</td>
<td>46.8±14.9</td>
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<td>Number of cells (n)</td>
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<tr>
<td>TYPE I (n)</td>
<td>91±21.0</td>
<td>105.8±19.6</td>
<td>95.9±37.5</td>
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<tr>
<td>TYPE IIA (n)²</td>
<td>59.5±19.6*</td>
<td>57.4±19.4*</td>
<td>88.0±23.8*</td>
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<tr>
<td>TYPE IIAB (n)³</td>
<td>55.9±19.5*</td>
<td>46.5±18.4*</td>
<td>30.6±19.2*</td>
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<tr>
<td>TYPE IIB (n)</td>
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<td>6.9±6.6</td>
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<td>TYPE I (%)</td>
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<td>TYPE IIA (%)⁴</td>
<td>27.3±8.0*</td>
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<td>TYPE IIAB (%)⁵</td>
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<td>TYPE IIB (%)</td>
<td>5.1±8.7</td>
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</tr>
</tbody>
</table>
a. Muscle fiber type distributions

b. Myosin heavy chain distributions

FIGURE 9. Muscle fiber type and myosin heavy chain distributions in three groups.
Correlations of physical fitness, substrates utilization, muscle fiber types and anthropometric, biochemical parameters. VO$_{2\text{max}}$ correlated with W$_{\text{max}}$ ($r=0.767$, $P<0.001$) and HR$_{\text{max}}$ ($r=0.641$, $P=0.001$, $n=25$). BMI and Waist circumference (WC) correlated with percent body fat ($r=0.784$, $P<0.001$ and $r=0.556$, $P=0.004$, respectively, $n=25$) and fat mass ($r=0.905$, $P<0.001$ and $r=0.576$, $P=0.003$, respectively, $n=25$). HDL-cholesterol negatively related with F$_{\text{max}}$ ($r=-0.543$, $P=0.005$, $n=25$). Type I correlates with W$_{\text{max}}$ ($r=0.525$, $P=0.021$, $n=25$). Type IIAB correlated with MHCIIx ($r=0.644$, $P=0.024$, $n=25$). Carbohydrate (CHO) oxidation at rest was correlated with type IIB ($r=-0.548$, $P=0.018$, $n=25$; figure 10). CHO oxidation on concentric exercise at the intensity of 30% W$_{\text{max}}$ was correlated with W$_{\text{max}}$ ($r=0.463$, $P=0.020$, $n=25$) and MHCIIa ($r=-0.519$, $P=0.023$, $n=25$; figure 10). CHO oxidation at the exercise intensity of 50% W$_{\text{max}}$ correlated with type IIB ($r=-0.470$, $P=0.042$, $n=25$; figure 10) and W$_{\text{max}}$ ($r=0.699$, $P<0.001$, $n=25$). CHO oxidation at the exercise intensity of 70% W$_{\text{max}}$ correlated with type I ($r=0.560$, $P=0.013$, $n=25$; figure 10) and W$_{\text{max}}$ ($r=0.849$, $P<0.001$, $n=25$). Fat oxidation at the exercise intensity of 30% W$_{\text{max}}$ correlated with W$_{\text{max}}$ ($r=0.459$, $P=0.021$, $n=25$). Fat oxidation during 50% W$_{\text{max}}$ intensity of exercise correlated with MHCII ($r=0.489$, $P=0.033$, $n=25$; figure 11).
FIGURE 10. Scatterplot of CHO oxidation at rest and during exercise related with muscle fiber type in the diabetic and obese men. CHO oxidation at rest had stronger correlation with type IIB in obese men (R²=0.547). CHO oxidation during 30%Wmax concentric exercise correlated more strongly with MHCIIa in obese ones (R²=0.476). CHO oxidation at 50% Wmax intensity of exercise showed stronger association with type IIB in obesity (R²=0.36). CHO oxidation at 70% Wmax intensity of exercise presented stronger relation with type I in obese group (R²=0.542).
FIGURE 11. Scatterplot of fat oxidation at 50% Wmax intensity of exercise correlated with muscle fiber type in the diabetic and obese men. It showed stronger association with MHCI in obesity ($R^2=0.336$).

In terms of the data in force production, muscle fiber distribution and substrate utilization, logistic regression was used to examine the association of these parameters with incidence of obesity and T2DM in the study. It showed that high/low force production and low/high proportion of type II AB fibers bore a close relationship to the incidence of obesity/T2DM, respectively.
6 DISCUSSION

CHO oxidation was similar at rest in type II diabetic and obese subjects, but higher during exercise in obese than in diabetic subjects. The difference was enhanced with an increase in exercise intensity. Fat oxidation increased first at lower exercise intensity, and then decreased with increasing exercise intensity and duration. The obese group with lower proportion of fast twitch fibers had higher force production than the T2DM group. Force production was higher in the obese group than in the T2DM group. The proportions of type IIAB and MHCIIx muscle fibres were significantly higher, and type IIA was significantly lower in diabetic and obese groups than those in the healthy group. The T2DM group had lower proportion of type I fibers and higher proportion of type II fibers in comparison to the obese group. There was linear correlation between CHO oxidations at rest and during exercise and the proportion of type II fibers. Fat oxidation at moderate intensity of exercise correlated with MHC1. Type IIAB, MHCIIx and force production were closely related to the incidence of these diseases.

6.1 Substrate utilization

In the present study, 50% $W_{\text{max}}$ concentric exercise fat oxidation correlated with MHC1 ($P=0.033$), which was in agreement with research by Sahlin et al. (2007). Due to the T2DM subjects being almost obese diabetics, there was not a significant difference in substrate utilization between the two groups. It has been mentioned that type II fibres have a high capacity for glycolytic energy production (Hernelahti et al. 2005; Karjalainen et al. 2006) and anaerobic energy expenditure becomes predominant with the increase of exercise intensity. In this study, CHO oxidations at rest and during exercise showed linear correlation with type II fibers. As can be seen from figure 8, CHO oxidation gradually increased with the increase of exercise intensity, which accored with the theory and previous studies that higher percentage of fat and less
percentage of carbohydrate utilizations at lower intense submaximal exercise, a gradual shift from carbohydrate to fat metabolism in the low intense and prolonged exercise and higher carbohydrate utilization at higher intensity (Ghanassia et al. 2006; Mulla et al. 2000; Phelain et al. 1997). At rest, fat oxidation was higher in healthy group than in the other two groups. Whereas there was no significant difference regarding CHO oxidation between three groups during exercise, independent of exercise intensity. These observations were also matched with previous studies in which fat was a predominant fuel at rest in healthy subjects and CHO was a predominant fuel at exercise in all subjects (Kelley & Mandarino 2000). CHO oxidation at 70% \( W_{\text{max}} \) exercise was the dominant fuel for working muscle, an observation that is in agreement with Kolkhorst (2007) in which carbohydrate became the preferred and then the exclusive fuel to maintain the high intensity (over 70% \( VO_{2\text{max}} \)) of exercise. According to previous studies, the rate of fat oxidation showed direct relationship with high proportion of type I fibers (Sahlin & Harris 2008), in contrast, fat oxidations at rest and during exercise presented association with type II fibers in this study. Only at the moderate intensity of exercise, fat oxidation showed direct correlation with type I fibers. Nevertheless, the reason for this is still unclear and more research is needed.

The development of type 2 diabetes can be controlled by exercise with moderate intensity and duration that is considered as part of the treatment programme in T2DM patients (Laaksonen et al. 2005; Lehmann et al. 1995; Rauramaa 1981). Also, the abdominal fat that is a major problem for obese patients can be decreased by weight reduction that can be controlled by regular physical activity combined with diet (Rauramaa 1981). It has been presented that exercise improves insulin sensitivity and induces weight loss. The reason is that reductions in visceral and abdominal subcutaneous visceral adipose tissue are closely related to changes in glucose and insulin. (Rice et al. 1999.) The benefits from concentric exercise for the T2DM and obesity patients are still unspecified. More research is required to help T2DM and obesity patients to know the benefits of individual concentric exercise.
6.2 Muscle fiber type distribution

Regarding the muscle fiber type distribution, type I and type II fibers were lower and higher in T2DM than in obesity, respectively, which has been reported in previous studies (Larsson et al. 1999; Marin et al. 1994; Tanner et al. 2002; Oberbach et al. 2006). The reason for this difference might be the result of higher insulin resistance related with higher type IIB muscle fibers and lower type I muscle fibers (Kern et al. 1999). As shown in the study of Karjalainen et al. (2006), type I fibers have a strong correlation with obesity. On the other hand, MHC isoform expression could be changed by exercise training with a transformation from type IIB to IIX and IIA and seldom to type I muscle fibers (Röckl et al. 2007). Type IIB fibers might be renamed type IIX because recently it has discovered the close correspondence to the type IIX fiber in the rat (Gravholt et al. 2001). As mentioned in the result, type IIA, type IIAB and MHCIIx presented statistically significant among diabetics, obese and healthy groups after more control data about muscle fibers was added. The proportion of type II AB closely related to the incidence of T2DM/obesity. There was direct association between type II AB and MHCIIx. In other words, MHCIIx was somehow related to the incidence of these diseases and muscle fiber type distribution was closely correlated to MHC. Although muscle fiber type distribution is different based on gender, age, obesity, and the waist-to-hip ratio (Marin et al. 1994), research on MHC in T2DM/obesity is still lacking. Noticeably, the proportion of type II muscle fibres in obese and diabetic subjects was higher compared with control subjects, though not statically significant, which was in accordance with the theoretical prediction.

6.3 Aerobic fitness and force production

Fat and CHO oxidations during the exercise with 30% $W_{\text{max}}$ intensity both related with $W_{\text{max}}$. When exercise intensity increased, maximal power output only correlated with CHO oxidation. Therefore, it was understandable that $W_{\text{max}}$ only correlated with type I
fibers, which explained that slow-twitch fibers could maintain the exercise with higher intensity and longer duration. The study showed that \( \text{VO}_2\text{max} \) had positive association with \( W_{\text{max}} \) and \( \text{HR}_{\text{max}} \). Specifically, diabetic and obese individuals had lower \( \text{VO}_2\text{max} \) due to their low physical fitness and aerobic exercise capacity, which has been mentioned earlier (Hulens et al. 2001).

Force production in the obese group was the highest among all groups, which might correlate with muscle fiber type distributions. As mentioned in the literature, force output is related to muscle fiber types. For example, the higher percentage of FT fibers, the higher muscles force output at the given velocity (Karp 2008). According to our theoretical hypothesis, compared with the healthy subjects, the unchanged and impaired force production should be observed in obese and type II diabetic subjects, respectively. In the present study, force production was lower in the T2DM group than in the obesity group, which also supports our hypotheses. The obese group had a lower distribution of FT fibers, but had higher force production which did not correspond to the hypothesis. Further studies are needed in muscle fiber distribution and force production in T2DM and obesity subjects. Nevertheless, in this study, the healthy subjects showed lower force production, which was not representative of the whole population and might be due to the subjects’ own lower muscle mass. Still, little data is available on force production in T2DM/obesity.

### 6.4 Anthropometric characteristics

There were strong positive correlations among BMI, WC, fat mass and visceral fat, which was similar to data presented in Bouchard’s study (2007). However, the associations of these anthropometric parameters with the development of T2DM/obesity are still unclear. Interestingly, although there were larger isometric force and higher HDL-cholesterol level in the obese group than those in the T2DM group, HDL-cholesterol showed inverse correlation with isometric force. The reason for above
case has yet to be tested. Normally, the higher HDL-cholesterol level is; the healthier person is due to the lower risk of coronary artery diseases (MedicineNet 2002). In this study, decreased force production showed a close correlation with the incidence of T2DM, which agrees with part of the hypothesis that impaired force production relates to the development of T2DM.

### 6.5 Study design and methods

In this cross-sectional study, there was almost no statistical difference between T2DM and obesity groups because the control sample size was not large enough for statistical calculations. Additionally, some subjects had some physical problems, such as knee joint and back pain. When they did the exercise, they could not perform with maximal effort. Thus, the effect of VO\(_{2\text{max}}\) test on physical fitness would influence the exercise intensity that was used in concentric exercise.

Before the tests started, health questionnaire was filled out in order to keep the exercise safe and more accommodating for subjects. Also, food diary records were kept for one week before exercise started and guidance that they were asked to consume the same food 24 hours before the tests was given to the subjects in order to get the similar energy fuel during VO\(_{2\text{max}}\) test and concentric exercise. The focus of this study was on fat and carbohydrate utilization at rest and during exercise. As mentioned above, due to the small sample size of healthy group, there were no comparisons of fatty acid oxidation during exercise and muscle force production in obese and diabetic subjects with those in healthy ones.

Respiratory gas exchange analysis was directly used to measure VO\(_{2\text{max}}\) during an incremental cycle ergometer exercise test, which could measure cardiorespiratory fitness precisely and with high reproducibly (Rauramaa 1981). BMI and body fat distribution etc. were measured by bioimpedance (Inbody 720 body composition...
analyzer) with providing a normal rage for individuals. Feedback was given to the subjects after the entire test, which focused on fitness index, exercise and diet recommendations according to their exercise testing and health questionnaires.
7 SUMMARY AND CONCLUSIONS

Muscle fiber composition and substrate utilization were studied in obese and diabetic subjects. Type 2 diabetic patients (n=14), obese subjects (n=11) and healthy subjects (n=3) were recruited to participate in this study. A bicycle ergometer test was used for determination of VO$_{2\text{max}}$ and concentric exercise tests, where heart rate, blood pressure and RPE were recorded. Biopsies from vastus lateralis muscle were obtained to histochemically examine muscle fiber distribution (diabetes=11, obesity=8 and health=2+6 table 5. a).

According to the results obtained from this study, the conclusions could be drawn as follows:
(1) When exercise intensity increased, CHO oxidation during the exercise was higher in the obese group than in the diabetic group. While at rest both groups were almost the same. Compared to the control subjects, CHO oxidation was decreased at rest and increased during exercise in both diabetic and obese subjects. CHO oxidation at 70% W$_{\text{max}}$ of concentric exercise was the dominant fuel for maintaining the exercise intensity. Fat oxidation increased first at lower exercise intensity, and then started to decrease with the increased exercise intensity and duration.

(2) The obese group with lower FT fiber had higher force production than the T2DM group. Force production in the obese group was the highest among all three studied groups. Force production decreased more in the T2DM group than in the obese group. In the obese and diabetic groups, the proportion of type II muscle fibres was higher compared with the control group. Specifically, the proportions of type IIA and type IIAB/MHCIIx were significantly lower and higher in the obese and diabetic groups than in the healthy group. The proportions of type I and type II fibers were lower and higher in T2DM than in obesity.
(3) Muscle fibre type distribution was related with substrate utilization and force production. CHO oxidation at rest and during exercise showed a direct correlation with type II fibers. Fat oxidation at 50% $W_{\text{max}}$ intensity of concentric exercise correlated with MHCI. In this study, the incidence of obesity/T2DM was closely related with high/low force production and low/high proportion of type II AB fibers, respectively. The incidence of these diseases might bear a close relationship to the amount of MHCIIx due to direct association of type II AB fibers with MHCIIx.

(4) Additionally, obese individuals showed low physical fitness and aerobic exercise capacity. VO$_{2\text{max}}$ had positive association with $W_{\text{max}}$ and HR$_{\text{max}}$. $W_{\text{max}}$ was related to CHO oxidations during the exercise with all the intensities, furthermore, $W_{\text{max}}$ only correlated with fat oxidation at low intensity of the exercise.
8 FUTURE DIRECTIONS

Individuals with obesity and/or T2DM can easily develop complications, such as hyperlipidemia and hypertension etc. Physical exercise with moderate intensity and duration are beneficial for the treatment of both T2DM and obesity. However, more knowledge on the mechanism of force production in relation to muscle fiber distribution in T2DM/obesity patients is still scarce. The mechanism of substrate utilization at rest and during exercise correlated with fiber type distribution in T2DM and obesity is still unclear. Further study of impaired force production associated with the development of T2DM/obesity is needed.

Good physical fitness implies that people have a strong physique and can avoid illness or diseases better. Therefore, further research is needed to clarify the role of physical exercise in relation to body composition and VO$_{2\text{max}}$, indexes of physical fitness, and to investigate the effect of concentric exercise on T2DM and obesity. Also, the long-term influence of physical activity on the treatment of these diseases should be thoroughly understood by the doctor and patient. A little alteration of lifestyle could keep the incidence of T2DM and obesity far away.
9 REFERENCES

ACSM’s guidelines for exercise testing and prescription. 2006. American College of Sports Medicine, the seventh edition.


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