University of Alberta

Effects of Functional Electrical Stimulation-Assisted Cycling Exercise on Glucose Tolerance and Insulin Sensitivity in People with Spinal Cord Injury

by

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"The fear of the Lord is the beginning of knowledge, but fools despise wisdom and discipline"

Proverbs 1:7

For the message of the cross is foolishness to those who are perishing, but to us who are being saved it is the power of God. For the foolishness of God is wiser than man's wisdom, and the weakness of God is stronger than man's strength.

1 Corinthians, 1: 18, 25

ABSTRACT

The purpose of the study was to determine the effect of functional electrical stimulation (FES) - assisted cycling (ERGYS II, Therapeutic Alliance) on glucose tolerance and insulin sensitivity in people with SCI. Seven subjects with SCI (upper motor neuron lesion, duration of injury from three to 40 years, aged 30 to 53 years) participated in the study. Two-hour oral glucose tolerance tests (OGTT) and hyperglycemic clamp tests were performed before and after eight weeks of training with ERGYS II. OGTTs were performed on seven subjects and hyperglycemic clamp tests were performed on three out of seven subjects before and after eight weeks of training. The results indicated that subjects' glucose tolerance improved significantly after eight weeks of training with ERGYS II (139.9±16 vs 122.4±10, P=0.014). According to two-hour hyperglycemic clamp tests, all three subjects improved on either glucose utilization or insulin sensitivity. Two subjects' insulin sensitivity improved by 30.9 and 35.9 percent and two subjects' glucose utilization was increased by 23.9 and 65 percent. These results suggested that eight weeks of training with ERGYS II improved glucose tolerance and may improve insulin sensitivity in people with SCI.

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ACRONYMS

AUC:	Area Under the Curve
DM:	Diabetes Mellitus
ERGYS II:	Functional Electrical Stimulation Assisted Cycle Ergometer
IGT:	Impaired Glucose Tolerance
FES:	Function Electrical Stimulation
FFA:	Free Fatty Acid
FPG:	Fasting Plasma Glucose
FPI:	Fasting Plasma Insulin
GLUT-4:	Glucose Transporter - 4
HR:	Heart Rate
PO:	Power Output
RER:	Respiratory Exchange Ration
SCI:	Spinal Cord Injury
SV:	Stroke Volume
TA:	Tibialis Anterior
VCO_2:	Carbon Dioxide Production
VE:	Minute Ventilation
VO_{2max} :	Maximal Oxygen Consumption
VO_2:	Oxygen Consumption

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CHAPTER I

INTRODUCTION

Diabetes Mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (The Expert Committee on the Diagnosis and Classification of DM, 1997). The human pancreas secretes a hormone called insulin, which facilitates the entry of the glucose into all tissues of the body. In a person with DM, the entry of glucose into the cell is impaired, resulting from either a deficiency of insulin produced or impaired insulin action (American Diabetes Association, 1996). There are two major types of DM: insulin dependent DM (Type 1) DM and non-insulin dependent DM (Type 2 DM) (The Expert Committee on the Diagnosis and Classification of DM, 1997). In Type 1 DM, the pancreas produces very little insulin or none at all. In Type 2 DM, the pancreas often makes sufficient or excessive amount of insulin, but the tissue cells are resistant to it and cannot use glucose effectively as energy.

DM is one of the major health problems throughout the world (American Diabetes Association, 1996). It affects the health of about 1.5 million Canadians and about 16 million people in the United States (Tan & MacLean, 1995; National Centre for Health Statistics, 1994). In the United States, more than 385,000 people with DM die each year, and nearly half of these deaths are directly related to having DM or its complications (National Centre for Health Statistics, 1994). Also, the economic costs of DM and its complications are significant, requiring approximately 90 billion dollars in the United States alone (American Diabetes Association, 1996).

Individuals with spinal cord injury (SCI) have increased risk of developing Type 2 DM. Bauman et al. (1994) demonstrated that 22% of 100 subjects with SCI had DM as compared to only 6% in an able-bodied control group. Also, 62% of those with quadriplegia and 50% of those with paraplegia had abnormal glucose tolerance, compared to only 18% of the able-bodied control. This high prevalence of abnormal glucose tolerance which includes DM and impaired glucose tolerance

(IGT) is related to physical inactivity, changes in body composition and a large mass of paralysed muscle. The treatment and prevention of DM should therefore be a major area of research for people with SCI.

Aerobic exercise has proven to be beneficial for treatment and prevention of DM in an able-bodied population, yet there is no research on the benefits of exercise for treatment and prevention of DM in people with SCI. Consequently, research on exercise-associated insulin or glucose metabolism changes is essential for the development of quality exercise programs for people with SCI. Since the 1980s, exercise modalities such as functional electrical stimulation (FES)-leg cycle ergometer (ERGYS II) have become more popular among people with SCI and research has clearly demonstrated physiological benefits of exercise with ERGYS in terms of cardiovascular, muscular, pulmonary and hormonal adaptations (Glaser et al., 1989; Hooker et al., 1992; Nash et al., 1991). However, effects of exercise with ERGYS II on glucose metabolisms has not yet been investigated.

Statement of the problem

Although people with SCI have a high risk of developing DM, there is limited research examining the effects of physical activity on glucose tolerance and insulin sensitivity in people with SCI. Therefore, it is important to investigate how training with ERGYS II may affect glucose metabolism in people with SCI. The purpose of the study was to determine the effects of exercise training utilizing ERGYS II on glucose tolerance and insulin sensitivity in people with spinal cord injury.

Research Hypotheses:

Hypothesis 1:

Insulin sensitivity measured by hyperglycemic clamp test improves after eight weeks of training

with ERGYS II.

Hypothesis 2:

Glucose tolerance measured by two hour oral glucose tolerance test improves after eight weeks of training with ERGYS II.

Limitations:

1) Small number of subjects for the hyperglycemic clamp test
2) No control on the subject's diet during the study
3) No control on the subject's daily activity
4) Study started in spring time and completed in early summer. No control for changes in glucose tolerance and insulin sensitivity as a result of seasonal differences.

Delimitation

1) The study was confined to FES-assisted cycling (ERGYS II) not to other types of exercise.
2) Subjects were spinal cord injured with upper motor neuron injury
3) No previous history of DM or cardiovascular disease.
4) The result of hyperglycemic clamp test could not be generalized due to small number of subject and individual case data analysis.

CHAPTER II

REVIEW OF THE LITERATURE

High prevalence of DM in people with SCI

Individuals with spinal cord injury (SCI) currently have a longer life span because of recent improvements in medical care (Hartkopp, Bronnum-Hansen, Seidenschnur and Biering Sorensen, 1997; Whiteneck et al., 1992). As they live longer, the main causes of death in this population have been altered (DeVivo, Black & Stover , 1993). Cardiovascular disease or diabetes mellitus (DM) is now equally or more important than renal failure as primary causes of death (Brenes, Dearwater, Shapera, LaPorte & Collis, 1986). Duckworth et al., (1980) reported that among 45 subjects with SCI, 23 of the subjects were diabetic and four had impaired glucose tolerance (IGT). Duckworth, Solomon & Pallepall (1983) also reported that among 20 patients with SCI and without known acute complications, glucose intolerance or fasting hyperglycemia, 70% of the subjects had some evidence of glucose intolerance. Also, Bauman et al. (1994) demonstrated that among 100 subjects with paraplegia or quadriplegia, 22% of the subjects had DM as compared to only 6% in an able-bodied control group. Sixty-two percent of those with quadriplegia and 50% of those with paraplegia had abnormal glucose metabolism, compared with only 18% in able-bodied controls. In addition, Zhong et al. (1995) investigated 103 people with paraplegia and 94 people with quadriplegia during their annual physical examination. All subjects had normal liver and renal function and none had a prior history of DM. Zhong et al. (1995) reported that among 197 subjects with SCI, 58 (29.4%) had IGT and 36 (18.3%) had DM according to two-hour oral glucose tolerance test (OGTT). The researchers have concluded that people with SCI seem to be more susceptible to impaired glucose tolerance and DM due to insulin resistance than the able-bodied population. However, the etiology of the high incidence of impaired glucose tolerance and DM in people with SCI is not clear.

Etiology of insulin resistance in people with SCI

Body composition change after SCI. Body composition changes after SCI (Dearwater et al., 1986). Increases in fat mass and decreases in lean body mass due to inactivity, immobilization of limbs or muscle denervation have been reported (Olle, Pivarnik, Klish & Morrow, 1993). Lean body mass, which is the major tissue responsible for the uptake of glucose, decreases by approximately one third in those with SCI compared to able-bodied control matched in gender, age, height and weight (Bauman et al., 1994). Therefore, decrease in lean body mass in people with SCI could be a risk factor for the insulin resistance.

Many SCI individuals suffer from obesity due to decreased physical activity resulting from their injury (Dearwater et al., 1986). Increased fat mass is one of the risk factors for insulin resistance (Bjorntorp, 1988; Despres, Sital, Lupien, Andre & Claude, 1993). Abdominal adipose tissues have an exceedingly sensitive system for the mobilization of free fatty acids (FFA) due to a preponderance of beta-adrenergic receptors and alpha-adrenergic inhibition (Kissebah & Peiris, 1989). This increases the concentration of FFA in the circulation. Increased FFA concentration in circulation inhibits insulin binding action, decreases the number of insulin receptors and decreases clearance by the liver. (Bjorntorp, 1990). Therefore, high FFA concentration increases insulin resistance which favours IGT and DM (Despres et al., 1993). Javinen & Koivisto (1983) studied the effects of body composition on glucose tolerance in males of normal weight who were either weight lifters, long distance runners or untrained controls. They found a strong positive correlation between percent body fat and insulin resistance. Therefore, a strong determinant of a predicted rate of glucose metabolism was body composition, and more specifically body fat. One might speculate that this finding can be generalized to people with SCI. Therefore, body composition changes (increased fat mass, decreased lean body mass) in people with SCI could be one of the major factors of insulin resistance in this population.

Muscular changes following SCI. Following SCI, skeletal muscles below the level of an upper motor neuron lesion undergo marked changes in morphological, metabolic and contractile properties (Burnham et al., 1997; Andersen, Mohr, Biering-Sorensen, Galbo & Kjaer, 1996). One significant skeletal muscle change in people with SCI is a pronounced reduction, or complete disappearance of Type I fibres and Type IIa fibres and an increment in Type IIb fibres. Burnham et al. (1997) obtained a total of 19 vastus lateralis muscle biopsies from 12 subjects with SCI representing time points of 0.5-219 months post SCI. They found that following prolonged upper motor neuron paralysis secondary to SCI, muscle fibres that previously had Type I properties altered their phenotypic expression to become Type II. Also, Andersen, Mohr, Biering-Sorensen Garbo & Kjear (1995) reported that after an average of 11.2 years post injury, paralyzed muscle contained 37.2% of Type IIb, 40.7% of combined IIb and IIa, 21% of Type IIa and 0.5% of Type I, as compared to about 4%, 12%, 25%, and 50% respectively in the able-bodied control respectively. Therefore, it is clear that paralyzed muscles undergo a transformation to become predominantly Type II fibres after SCI.

Furthermore, capillary density and blood flow in paralyzed muscle decreases after SCI. Jozsa et al. (1980) demonstrated that capillary density in paralyzed muscle decreases after denervation in rat muscles. After immobilizing 12 rats with plaster casts, capillary density decreased 23%, 33%, and 28% on days 7, 14, and 21, respectively. In addition, glucose transporter (GLUT-4) content which is known to be responsible for glucose uptake in tissue, decreases with denervation (Etgen, Farrar & Ivy, 1993; Henriksen, Rodnick & Mondon, 1991; Sowell et al., 1989). In one study, Henriksen, Rodnick & Mondon (1991) removed a three millimetre section of rat sciatic nerve and observed the level and activity of GLUT-4 after denervation. The authors reported that after one day of denervation, the level of GLUT-4 was reduced by 18% and after three days of denervation, GLUT-4 was reduced by 52%, relative to controls. Other studies reported that reducing muscle activity by denervation results in a rapid loss of skeletal muscle GLUT-4 content (Etgen et al., 1993).

Interestingly, muscle fibre Type proportion, capillarization, and GLUT-4 content appear to be correlated with insulin resistance. This was demonstrated by Lillioja et al. (1987) who compared the capillary density and muscle fibre type in the vastus lateralis with in vivo insulin sensitivity determined by the euglycemic clamp. They found that insulin sensitivity was positively correlated with percent Type I fibres ($r=0.29$) as well as capillary density ($r=0.63$) and negatively correlated with percent Type II b fibres ($r=-0.38$). Also, the maximal capacity for insulin to stimulate glucose transport has been positively correlated with the total GLUT-4 content of muscle (Henriksen et al., 1991). Therefore, it is suggested that muscle characteristic changes due to paralysis are major risk factors for insulin resistance in people with SCI.

Physical inactivity and immobilization. A third possible contributor to insulin resistance in people with SCI is a lack of physical activity following injury. People with SCI experience several weeks of immobilization after their traumatic injury. After this acute phase of injury, it is very difficult to regain the premorbid level of physical activity. During immobilization, the paralyzed muscle tissue is rapidly catabolized and its mass decreases. Within one to two weeks of bed rest, cardiac output, muscle capillary density, blood flow and maximal oxygen uptake are markedly decreased (Saltin & Rowell, 1980). Also, muscle fibre size is decreased and the activity of mitochondrial oxidative enzymes of muscle cells are decreased leading to a reduced rate of glucose utilization and insulin resistance (Saltin & Rowell, 1980; Lipman, Raskin, Love & Lecocq, 1970). Myllynen, Koivisto & Nikkila (1987) measured oral glucose tolerance and insulin response to glucose in 18 patients during six weeks of bed rest with an acute spine fracture. After one week of immobilization, insulin resistance was seven times higher in immobilized subjects than in able-bodied controls. This elevated insulin resistance reduced with time but still remained elevated 2.3 times higher than in the able-bodied controls. Furthermore, Bauman et al. (1993, 1994) demonstrated that lower

cardiopulmonary fitness in this population due to physical inactivity is one of the major risk factors for insulin resistance and that there is a linear negative correlation between insulin resistance and cardiopulmonary fitness (r=-.88, P=.0015). Therefore, lack of physical activity in people with SCI results in decreased cardiopulmonary fitness and insulin sensitivity.

Denervation and insulin resistance Skeletal muscle is a critical site for glucose homeostasis. Muscle denervation seems to induce insulin resistance through multiple pathways, including glucose transport, insulin binding and insulin receptor activation (Burant, Lemmon, Treutelaar, & Buse, 1984; Henriksen et al., 1991; Smith & Lawrence, 1984; Turinsky, 1987). This is supported by the findings which show that responses to insulin in unweighted muscle differ from those in denervated muscle (Burant et al., 1984; Henriksen & Tischler, 1988; Tischler et al., 1990). Tischler et al. (1990) investigated insulin effects on amino acid uptake and protein metabolism in soleus muscles subjected to denervation or unweighting. They reported that denervated muscle had insulin resistance of both 2-deoxyglucose and alpha aminoisobutyric acid uptake whereas unweighted muscle showed an increased or normal response, respectively. They also observed greater atrophy in denervated muscle compared to unweighted muscle and concluded that effects of denervation must be independent of leg posture.

Henriksen et al. (1991) tested whether there are differences in glucose transport between denervated muscle and unweighted muscle. They reported that after three days of denervation, GLUT-4 content decreased by 52 percent whereas GLUT-4 content increased by 35 percent on hindlimb-suspended rat muscle. This result indicated that in addition to muscle activity, there may also be a neurogenic factor that contributes to the regulation of GLUT-4 expression. Megeney et al. (1994) determined whether there is a putative neurogenic factor that regulates GLUT-4 expression in muscle. This study compared changes in GLUT-4 content over time in denervated rat muscle with either a long

nerve stump or short nerve stump of the sciatic nerve. Results indicated that GLUT-4 content did not change until 48 hours after the denervation in long stump muscles whereas GLUT-4 decreased 12 hours after the denervation in short stump muscles. These findings suggest that, in addition to body composition, physical activity level and muscle characteristic changes after the injury, there is neurological factor for the high prevalence of DM in people with SCI.

Exercise and insulin sensitivity

Exercise has proven to have beneficial effects on insulin sensitivity (Bogardus et al., 1984; Braun, Zimmerman & Kretchmer, 1995; Perseghin et al., 1996; Rogers et al., 1988; Ruderman et al., 1979; Schneider et al., 1984; Wallberg-Henrikssonl, 1992; Yamanouchi et al., 1995). Perseghin et al. (1996) trained 10 subjects with Type II DM who had significant insulin resistance and eight control subjects with one hour of stair climbing for six weeks. Insulin sensitivity was measured before and after six weeks of physical activity with euglycemic clamp and hyperglycemic clamp test. Results demonstrated that six weeks of physical training improve insulin sensitivity in both the insulin resistance group and the control group by more (43%) than that which had been reported for metformin (16-25%) or troglitazone (about 20%). Also, Braun, Zimmerman Kretchmer (1994) demonstrated that low intensity exercise (50% of VO_{2max}) over a longer duration improved insulin sensitivity to a level similar to high intensity exercise (75 % of VO_{2max}) over a shorter duration. According to the research by Rogers et al. (1988), one week of intense physical training (68% of VO_{2max}) can improve oral glucose tolerance in Type II DM. Therefore, it could be concluded that aerobic exercise with low to high intensity (50-80% of $V0_{2max}$) improves insulin sensitivity.

The relation between cardiopulmonary fitness and insulin sensitivity in people with SCI was also determined by Bauman and colleagues. Bauman et al. (1992, 1994) measured insulin sensitivity and maximal oxygen consumption in people with SCI and demonstrated a linear positive correlation

between insulin sensitivity and cardiopulmonary fitness ($r=.71$, $p<.05$ and $r=.88$, $P=.0015$). These findings suggested that people with SCI with better cardiovascular fitness have a lower risk of developing DM.

The benefits of FES-assisted exercise

FES-assisted exercise as a new exercise modality. Advanced computer technology has made it possible to stimulate multiple paralyzed muscle groups sequentially for long periods of time in order to produce relatively complex motor activities such as pedalling a cycle ergometer (Figoni et al., 1988; Hooker, Scremin, Mutton, Kunkel & Cagle, 1995; Ragnarsson, 1988). ERGYS, one brand of electrical stimulation cycle ergometer, is becoming a more popular form of endurance exercise training to develop and maintain musculoskeletal and cardiovascular fitness in people with SCI.

Until recently, the preferred mode of endurance training for SCI was upper body exercise, particularly the arm crank ergometer. However, upper body endurance exercise has been criticized for several reasons. Due to the relatively small mass of the upper body musculature in people with SCI, arm exercise does not elicit a sufficient magnitude of cardiopulmonary responses to elicit central training responses (Vokac, Bell, Bautz-Holter & Rodahl, 1975). Disturbance in breathing mechanics, higher respiratory frequency, and lower tidal volume may adversely affect the circulation in the smaller arm muscle, leading to the earlier onset of fatigue (Cowell, Squires & Raven, 1986). Furthermore, prolonged and excessive upper body endurance exercise for individuals with SCI can be stressful on upper body joints and muscles, particularly on shoulders and wrists which are also required for self-care and wheelchair mobility. Injury to the upper body is commonly seen in the chronic phase of SCI and may negatively affect well being and quality of life for people with SCI (Ragnarsson, Pollack & Twist, 1991).

Figoni et al. (1988) compared subpeak physiological responses in people with SCI during

exercise with ERGYS to those elicited during voluntary arm crank exercise performed in the upright posture. The authors reported that exercise with ERGYS elicited a significantly higher cardiac output (CO), stroke volume (SV), and mean arterial pressure for a given oxygen consumption (VO_2), as well as significantly lower pulmonary ventilation (VE) and heart rate (HR) as compared to arm crank exercise. Therefore, the researchers suggested that exercise with ERGYS may be more beneficial than upright exercise with arm crank to improve cardiovascular fitness for people with SCI (Figoni et al, 1988, Glaser et al, 1989).

Glaser et al. (1989) determined steady-state physiologic responses of 12 subjects with SCI to exercise with ERGYS and compared these responses to 6 able-bodied individuals performing voluntary leg cycle ergometry at the same power output levels. They reported that exercise with ERGYS elicited significantly higher O_2, VE and HR responses from subjects with SCI compared to the able-bodied subjects exercising at the same power output levels. The main reasons for this inefficiency of exercise with ERGYS may be the nonphysiological activation of the paralyzed muscles, the deteriorated condition of these muscles, and inappropriate biomechanics for the movements (Glaser et al., 1989). Although low efficiency would not be beneficial for reliable performance of skilled activities, it has an advantage for exercise effectiveness because of the relatively high metabolic rate (Glaser et al., 1989; Figoni et al., 1988). Thus, despite the extremely low peak power output attained during exercise with ERGYS, it may be a superior alternative to upright arm crank exercise for central cardiovascular conditioning for people with SCI.

Effects of FES-assisted exercise on body composition. FES-assisted exercise results in significant changes in paralyzed muscle characteristics. First of all, exercise with FES increases muscle mass and strength of paralyzed muscles (Roger et al., 1991; Sloan, Bremner, Byrne, Day & Scull, 1994). Sloan, Bremner, Byrne, Day & Scull (1994) examined the effects of three months of exercise with ERGYS in 12 subjects with SCI. All subjects who completed the program increased their duration of cycling, strength and exercise load, indicative of a local training effect. There were

also significant increases in quadriceps and total muscle cross sectional area. Another study by Rogers et al. (1991) reported that FES induced knee extension exercise also increased leg strength in 12 people with SCI after training three times per week for 12 weeks. Thirty minutes of FES cycling at 50 rpm increases energy expenditure in people with SCI. This increase in energy expenditure may reduce obesity in people with SCI. However, researchers have failed to see significant decreases in fat mass after training with ERGYS or FES knee extension possibly due to large individual variability and relatively short durations of the training (Rogers et al., 1991; Sloan et al., 1994).

Effect of FES- assisted training on muscular characteristics. Exercise with FES and ERGYS seems to reverse morphological and histochemical changes in paralyzed muscle (Andersen et al., 1996; Martin, Stein, Hoeppner & Reid, 1992). First of all, exercise with FES increases the proportion of Type I and Type IIa in paralyzed muscle. Martin, Stein, Hoeppner & Reid (1992) demonstrated that SCI subjects have a relatively smaller proportion of Type I fibres (14%) in paralyzed tibialis anterior (TA) muscle compared to an able-bodied control (68%). They trained paralyzed TA muscles with low frequency (20 Hz) FES for 24 weeks and observed fibre type proportion changes with myofibrillar ATPase under alkaline (pH 10) incubation method. They demonstrated increase in the proportion of Type I fibres (25%) after training (Martin et al., 1992). Andersen et al. (1996) trained five male SCI subjects with FES cycling for 30 minutes at 50 rpm for 12 months. The myosin heavy chain (MHC) composition of single fibres from the vastus lateralis muscle was analyzed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. After 12 months of training, the number of fibres containing MHC IIb reduced to 2.3% (37.2% before training), the number of fibres containing both MHC IIa and IIb decreased to 4.6% (40.7% before training), while the number of fibres containing only MHC IIa increased to 91.2% (21.2% before training). The authors therefore concluded that changes in muscle type-proportion may be reversed by exercise with FES.

FES exercise may also increases the level of GLUT-4 content in paralyzed rat muscles (Etgen et al., 1993). Etgen et al. (1993) examined the effect of chronic low frequency electrical stimulation

on GLUT-4 content. Rats were randomly divided into three groups based on the duration of stimulation: 10-20 (n=3), 30-40 (n=3) and 60-90 days (n=4). Chronic low frequency FES enhanced GLUT-4 content in muscles stimulated in all three groups but no significant differences were found between the groups. Therefore, it can be concluded that exercise with FES enhances GLUT-4 content but GLUT-4 level reached a plateau after 30-40 days of training.

Furthermore, exercise with FES increases fibre size, capillary density and oxidative capacity. Chilibeck, Jeon, Weiss, Burnham & Bell (1998) demonstrated with six subjects that eight weeks of training with ERGYS (3 time/week, 30 min/day) increased capillaries per fiber, capillary contacts per fiber, fiber area and capillary density 38%, 29%, 23% and 10% respectively. Petrosfsky et al. (1992) reported similar increases in muscle fibre size and oxidative capacity (increased by 75% as measured by differential staining of muscle biopsies) after training individuals with FES cycling 15 minutes a day, five times a week for two months. Also, Martin et al. (1992) reported that 24 weeks of chronic electrical stimulation training increased capillary density in paralyzed muscle.

Effect of FES-assisted training on the cardiovascular system. Research has proven that exercise with ERGYS improves cardiovascular fitness including increase in CO, SV and myocardial function (Hooker et al., 1990; Nash et al., 1991). Hooker et al. (1990) examined acute cardiovascular and pulmonary responses to a single 30 minute bout of exercise with ERGYS in seven subjects with SCI. VO_2, carbon dioxide production (VCO_2), respiratory exchange ratio (RER), VE, HR, CO, SV and blood lactic acid levels (BLA) were all significantly elevated above resting values during exercise with ERGYS. On the other hand, plasma volume, bicarbonate levels and blood pH values were significantly reduced. HR, SV, Q and VO_2 levels were increased by 33-60%, 45-69%, 113-142% and 106-132% respectively above baseline levels. In addition, Pollack et al. (1989) conducted three-phase investigations on the effects of ERGYS in cardiovascular fitness in 11 individuals with SCI. Resting cardiopulmonary function factors were unchanged at rest after all three stages. However, increases in VO_2, VE and VCO_2 virtually doubled and ventilation volume changes parallelled these increases. Therefore, both authors concluded that these physiological responses were sufficient to produce a

cardiovascular training effect in people with SCI (Hooker et al., 1990).

Nash et al. (1991) trained eight healthy neurologically-stable quadriplegic males between the aged 22 and 39 with ERGYS for 24 weeks. Echocardiograms were performed before and after exercise training to quantify the interventricular septal and posterior wall thicknesses at end-diastole and the left ventricular internal dimension at end-diastole. Results showed that exercise with ERGYS increased the left ventricular mass by 35% following exercise training. Therefore, the author concluded that myocardial atrophy can be reversed in people with SCI following training with ERGYS and that the changes in cardiac architecture are likely to be the result of both pressure and volume challenge to the heart imposed by exercise (Nash et al., 1991).

Summary

People with SCI have an higher incidence of DM due to insulin resistance. The reasons for the high prevalence of insulin resistance are probably related to body composition changes, changes of paralyzed muscle and decreased physical activity level after SCI. Based on previous findings, exercise with FES may reverse risk factors associated with insulin resistance in people with SCI. First of all, exercise with FES changes body composition by increasing paralyzed muscle mass and decreasing fat mass. Secondly, exercise with FES increases the proportion of Type I and IIa muscle fibre, capillary density and the level of GLUT-4 content in the paralyzed muscle which is positively related with insulin sensitivity. Lastly, FES-assisted exercise increases the level of physical activity and cardiopulmonary fitness in people with SCI, which potentially improves insulin sensitivity.

CHAPTER III

METHODS AND PROCEDURES

Subjects

Five male and two female spinal cord injured subjects ranging in age from 30 to 53 years were recruited from the Edmonton area with support from the Rick Hansen Centre, the Spinal Cord Injury Treatment Centre Society (SCITCS) and the Canadian Paraplegic Association. Informed written consent was obtained from subjects after the protocol was approved by the Ethics Committee from the Faculty of Physical Education and Recreation and the Faculty of Medicine, University of Alberta. Also, full medical examinations were conducted by a physician to ensure that subjects were suitable to exercise with ERGYS II. Individuals with the following characteristics or disorders were excluded from the study: pacemaker implants, uncontrolled arrhythmias, uncontrolled angina, congestive heart failure, current deep venous thrombosis, severe skin reaction to surface electrodes or electrical stimulation, less than 90 degrees of flexion range of motion of hips and knees, severe lower extremity spasticity, severe DM and participation in regular physical activity. Subject characteristics are summarized in Table 1.

Exercise training

ERGYS. The ERGYS I system is a computer controlled FES-leg cycle ergometer developed in 1984 by Therapeutic Technology Incorporated and recently upgraded to the ERGYS II by Therapeutic Alliance. In this study, ERGYS II was used. The ERGYS II system applied electrical stimulation through surface electrodes to the gluteal, hamstring and quadriceps muscle groups in a computer controlled sequence to allow pedalling of the cycle ergometer. In preparation for the use of ERGYS II training, carbon-filled silastic electrodes were placed over the surfaces of the quadriceps, hamstring and gluteal muscles. One active electrode and one reference electrode were placed on each muscle group. The computer adjusted the electrical stimulation applied to the muscles so that the subjects were able to pedal the cycle ergometer at 50 rpm. In ERGYS II, if the subject could

not pedal at 50 rpm with maximum stimulation, the computer decreased the resistance automatically in order for the subjects to maintain a pedalling rate of 50 rpm. Continued muscle fatigue, characterized by a failure to maintain a pedalling rate of 35 rpm, automatically terminated the stimulation and allowed subjects a two minute cool down followed by a five minute resting period. The maximum workload achievable on the cycle was 7/8 kp or approximately 40 watts.

Table 1.

Subjects characteristics

	Sex	Age (yrs)	LOI	DOI (yrs)	Height (cm)	Weight (kg)	BMI (kg/m^2)	HDM	Smoking
Subject 1	M	30	C5/6	8	165	56	20.6	N	N
Subject 2	M	40	C5/6	3	185	113.7	33.2	N	N
Subject 3	M	53	T6/7	40	158	74.2	29.7	N	N
Subject 4	M	50	T4/5	30	157	77.9	31.6	N	N
Subject 5	M	42	C6/7	10	186	115	33.2	N	N
Subject 6	F	49	T10	34	165.1	74.5	27.3	N	N
Subject 7	F	50	T8	18	166	76.1	27.6	N	N

Note:

LOI = Level of Injury, DOI = Duration of Injury, BMI = Body Mass Index, HDM = History of Diabetes Mellitus

Exercise protocol. Subjects were asked to complete 24 bouts of exercise with ERGYS II in eight weeks. Subjects sat on the ERGYS II system chair, which was adjusted to allow adequate leg extension and flexion ranges when pedalling the ergometer. Correct foot and knee positioning was

determined before each treatment session. After a one-minute technician-assisted warm-up, electrical stimulation was progressively increased to allow the subjects to pedal unassisted at 50 rpm. Subjects were required to complete 30 minutes of ERGYS II exercise. When subjects fatigued (unable to pedal at 40 rpm) before a 30 minute bout was completed, assistance was provided until the next time interval divisible by ten. For example, if a subject fatigued at seven minutes, assistance was provided until ten minutes or if a subject fatigued at 13 minutes, assistance was provided until 20 minutes. After completing each exercise interval, a two minute cool-down and five minute monitored rest period was provided. Each exercise session had a maximum of three exercise intervals which allowed 30 minutes of total exercise duration. Individuals began cycling at 0 kp, and the resistance was increased by 1/8 of a kp (6.1W), once 30 minutes of continuous work without reaching maximal stimulation with ERGYS II was completed.

Glucose tolerance and Insulin sensitivity measurement

The hallmark of Type 2 DM is a chronic elevation of the plasma glucose and insulin level. Thus, a single blood glucose measurement is not always sufficient to make a diagnosis (Stolk , Orchard & Grobbee, 1995). Therefore, regardless of inconvenience, variability, and the nonphysiological nature of the oral glucose tolerance test (OGTT), the two-hour OGTT remains the gold standard to diagnose IGT or DM (McCance et al., 1995). However, the two-hour OGTT does not provide adequate information as to whether hyperglycemia is due to increased glucose production or defects in peripheral glucose uptake, or whether hyperinsulinemia is due to increased insulin secretion or decreased insulin clearance.

The euglycemic clamp technique by Andres has been the most reproducible and commonly used technique to evaluate insulin action on carbohydrate metabolism in vivo (Elahi, 1996; Scheen & Lefebvre, 1992). Also, there is another way to measure insulin sensitivity which is a frequently sampled intravenous glucose tolerance test with minimal modeling (FSIGT). This approach to measuring insulin sensitivity uses an intravenous injection of glucose to stimulate endogenous insulin

secretion (Anderson et al., 1995). Even though this technique is not able to fractionate the insulin-independent and insulin dependent mechanisms of glucose clearance to hepatic and peripheral component, the technique is much less invasive, requiring less preparation, less staff than glucose clamp(Anderson et al., 1995; Ng, 1988). However, neither euglycemic clamp technique nor FSIGT assess the function of the β-cell. The hyperglycemic clamp technique is a unique test that can measure both β-cell function and peripheral tissue sensitivity (Elahi, 1996). DeFronzo, Tobin & Andres (1979) reported a high correlation ($r=0.816$; $p<0.001$) between the amount of glucose infused during a euglycemic clamp and the amount of glucose infused during a hyperglycemic clamp divided by the plasma insulin concentration in 11 healthy young volunteers. Also, Mitrakou et al. (1992) performed euglycemic clamp tests and hyperglycemic clamp tests on forty-two individuals to compare insulin sensitivity acquired from each test and again reported a high correlation ($r = 0.84$; $p< 0.001$) between insulin sensitivity from euglycemic clamp technique and hyperglycemic clamp technique. Therefore, it was concluded that the hyperglycemic clamp technique be used to assess both insulin sensitivity and insulin secretion in a single experiment. In this study, two hour OGTT and hyperglycemic clamp technique were used to assess glucose tolerance and insulin sensitivity.

Oral Glucose Tolerance Test. Three days prior to testing, each subject was asked to consume a weight-maintaining diet containing at least 200 grams of carbohydrates per day and to refrain from strenuous physical activity. Testing was performed after a 12-hour overnight fast. One intravenous polyethylene catheter was inserted into the antecubital vein to collect samples. Normal saline was used to keep veins from clotting. One baseline blood sample was drawn from the subjects. After collection of the baseline sample, subjects were asked to drink 75 grams of glucose in an orange flavoured drink over a five minute period. After drinking 75 grams of glucose, blood samples were drawn at 30, 60, 90 and 120 minutes. All samples were collected in red top testing tubes and centrifuged within 30 minutes after the collection of the sample. The serums were transferred to polyethylene containers and kept at -20 °C until analyzed.

Analytic Procedure for OGTTs. The blood samples (three ml each) for insulin and glucose analyses were collected in red top testing tubes and centrifuged (2000 rpm, 15 min) at 5°C to obtain serum. Glucose was determined using a glucose analyser based on an enzymatic method (Glucose Analyser II, Beckman Instruments, Irvine, CA). The serum used for insulin analysis was drawn from the centrifuged samples and kept at -20°C until it was analyzed. Insulin was analysed using radioimmunoassay with Coat-A-Count kits (Diagnostic Products, Los Angeles, CA). Determination for serum glucose was made in triplicate and determination for serum insulin was made in duplicate.

Hyperglycemic Clamp Test to Measure Insulin Sensitivity (Defronzo et al., 1979). Three days prior to the test, each subject was asked to consume a weight-maintaining diet containing at least 200g of carbohydrate per day and to refrain from strenuous physical activity. All tests were performed in the morning after a 12-h overnight fast. The test required the insertion of two intravenous lines. One intravenous polyethylene catheter was inserted in the antecubital vein for the infusion of 20% dextrose in water. A second polyethylene catheter was inserted into a dorsal hand, wrist vein or antecubital vein for obtaining blood samples. The hand or arm was kept warm with an electrical heating pad to allow sufficient arterial-venous shunting to "arterialize" the venous blood because there is a gradient between arterial and venous glucose values during these tests.

Three blood samples were collected at 30, 20 and 10 minutes prior to glucose infusion, to measure basal glucose and insulin levels. When a fasting basal level was established, the clock was set to zero and the glucose infusion commenced to obtain the target glucose level which was calculated (98 mg/dl above basal glucose level) based on the basal glucose level. The goal of the hyperglycemic clamp was to raise the blood glucose concentration acutely to a fixed hyperglycemic plateau and to maintain it at that level for 120 minutes. This was accomplished by an intravenous glucose infusion consisting of two phases: a ten minute "priming dose" needed to raise the glucose level in plasma and extravascular glucose compartments to the desired plateau and a "maintenance dose" that was computed at 5-min intervals throughout the test. The priming dose was calculated based on body

surface area. After the priming dose, the computation for the periodic adjustments in the glucose infusion was made every five minutes and was based on a negative feedback principle. Blood samples were collected at two, four, six, eight, and 10 minutes to measure glucose and insulin levels as describe abolve. After 10 minutes, blood samples were taken every five minutes for glucose measurements and every 10 minutes for insulin measurements for the duration of the study (120 min).

Analytic Procedure for hyperglycemic clamp tests. The blood samples for insulin and glucose analyses were collected in red top testing tubes. Blood samples were obtained and analyzed on bed side to determine plasma glucose by using a glucose analyser based on an enzymatic method (Glucose Analyser II, Beckman Instruments, Irvine, CA). The serum used for insulin analysis was drawn from the centrifuged samples(2000rpm, 15 min) and kept at -20°C until it was analyzed. Insulin was analysed using radioimmunoassay with Coat-A-Count kits (Diagnostic Products, Los Angeles, CA). Determination for serum insulin and plasma glucose were made in duplicate.

Study Design

Due to the cost of measuring insulin sensitivity and the limitation of recruiting subjects with SCI for the control group, within group pre- and post-test design was used for the present study. Seven OGTTs were performed on five male and two female subjects and three hyperglycemic clamps were performed on male subjects pre- and post-exercise training. The pre-training OGTTs and hyperglycemic clamp tests were performed before any exercise training and the post-training OGTTs and hyperglycemic clamp tests were performed after the completion of eight weeks of ERGYS II training. The post-training OGTTs were performed 48 hours after the last exercise bout and hyperglycemic clamp tests were performed 72 hours after the last exercise bout. The independent variable was eight weeks of training with ERGYS II, three times per week and thirty minutes per session and the dependent variables were glucose tolerance and insulin sensitivity measured by OGTT and hyperglycemic clamp test. Glucose level at 120 minute was used for the diagnosis of DM and area

under the insulin and glucose curve was calculated. Insulin sensitivity calculated from 90-120 min (average glucose infusion/average insulin), 0-10 minute insulin incremental and total area under the curve (AUC), 20-120 minute insulin incremental and total AUC were calculated to investigate the difference between pre- and post-training. Glucose utilization during 20 to 120 minutes and 90 to 120min was also compared pre- and post-training.

Calculations and statistical analysis

Glucose Utilization.

Glucose utilization (mg glucose/kg body weight/min)

= Glucose infusion (mg glucose/min) - Space correction

The space correction may be a considerable factor when plasma glucose levels are relatively unstable.

1) Calculation of glucose infusion (ml/kg/min) for each five minutes time interval from glucose infusion rate (ml.min) for each five minutes time interval.

$$\text{Glucose infusion} = \frac{\text{The infusion rate (ml/min) x glucose concentration (20 \% Dextrose)}}{\text{subjects's body weight}}$$

2) Calculation of space correction

$$\text{Space correction} = [(\text{Glucose value}_{x\ \text{min}}) - (\text{glucose value}_{x\ \text{min} - 5\ \text{min}})] \times 0.095$$

For example, glucose value at 110 minutes is 189 mg/dl and glucose value at 115 minutes is 165 mg/dl. The space correction calculation is:

(165 mg/dl - 189 mg/dl) x 0.095

3) Glucose utilization during 20 to 120 minutes and 90 to 120 minutes

The average glucose utilization during 20 to 120 minutes was calculated for total glucose utilization. The average glucose utilization during 90 to 120 minutes was also calculated to measure glucose utilization for insulin sensitivity calculation (Defronzo et al., 1979).

Insulin sensitivity. Insulin sensitivity was assessed as an insulin sensitivity index, calculated by dividing the average glucose utilization during the 90 - 120 minutes of each hyperglycemic clamp (mg /kg/min) by average serum insulin during 90 - 120 min (Defrenzo et al., 1979 and Elahi, 1996). Insulin sensitivity was expressed as mg glucose/kg/min perμU/ml of insulin.

Area Under the Curve. Incremental glucose and insulin area under the curve was calculated for two-hour OGTT. Both incremental and total area under the curve was calculated for hyperglycemic clamp. Trapezoidal model was used to calculated for the incremental area under the glucose and insulin curve (Rogers et al., 1998).

Statistical analysis. Statistical evaluation was made by nonparametric Friedman Test for paired data. All statistical tests were evaluated at $P < 0.05$ (2-tailed testing). The data are presented as Mean ±Standard Error of the Mean (SEM).

CHAPTER IV

RESULTS

Exercise training

The beginning of the program, no subject was able to complete 30 minutes of continuous exercise at 0 power output (PO). After the completion of 24 exercise sessions over eight weeks of training with ERGYS II, four out of seven subjects were exercising at 9.15± 3.52 Watts. Three subjects were not able to complete ten minutes of continuous cycling until the end of the eight weeks of exercise training. The mean PO the subjects exercising after the eight weeks of training is 5.2± 2.0 Watt (Table 2).

Table 2.

Pre-training, post-training exercise PO, FPG and FSI

	PO* (Watt)	FPG (mg/dl)	FSI(μU/ml)
		mean ± SEM	
Pre-training	0	84.6 ± 2.8	17.7 ± 3.9
Post-training	5.2 ± 2.0	82.7 ± 2.3	16.4 ± 3.8

Note:

• - denote significant differences between pre and post-training ($p<0.05$)

PO = Power Output, FPG= fasting plasma glucose, FSI = fasting serum insulin

SEM = Standard Error of Mean

Fasting serum glucose and insulin

There was no statistical significant change in fasting serum glucose and insulin between pre- and post-training value(Table 2).

Response to two-hour oral glucose tolerance test

Post-training two-hour glucose and insulin levels were significantly lower than pre-training two-hour glucose and insulin levels. Two-hour glucose level decreased after the eight weeks of training with ERGYS II in all seven subjects (pre-training= 140±16; post-training = 122.4±10 mg/dl, p=0.014) (figure 1 and 3). Also, two-hour insulin level were decreased after the training (pre-training = 118.4±42.6; post-training = 87.5±10μU/ml) (figure 2 and 4). However, due to large individual variability among subjects, there was no statistically significant difference between pre and post-training two-hour insulin level (figure 3 and 4). Results from incremental area under the glucose curve indicate that post-training area under the curve decreased by 16% but due to the large individual variability there was no statistical significance (pre-training=6962±1111.4; post-training=5845±800 mg/dl·time) (figure 5). Incremental area under the insulin curve indicated that post-training are under the curve increased by 6.7% but also the difference between pre- and post-training incremental area under the insulin curve did not reach statistical significance (pre-training=6541.5±1272; post-training=7014±1803 μ U/ml· time) (figure 6).

According to the pre-training OGTTs, five out of seven subjects (71.4%) with SCI had abnormal glucose tolerance (figure - 7). Out of five subjects with abnormal glucose tolerance, four had IGT (57.1 %) and one (14.3%) had DM according to the criteria by Expert Committee on the diagnosis and classification of Diabetes Mellitus (1997). Results of the post-training OGTTs indicate that there were two people whose two-hour glucose value was in the IGT range (28.6%) and five people whose two-hour glucose value were in the normal range (71.7 %) after the eight weeks of training with ERGYS II (figure 8).

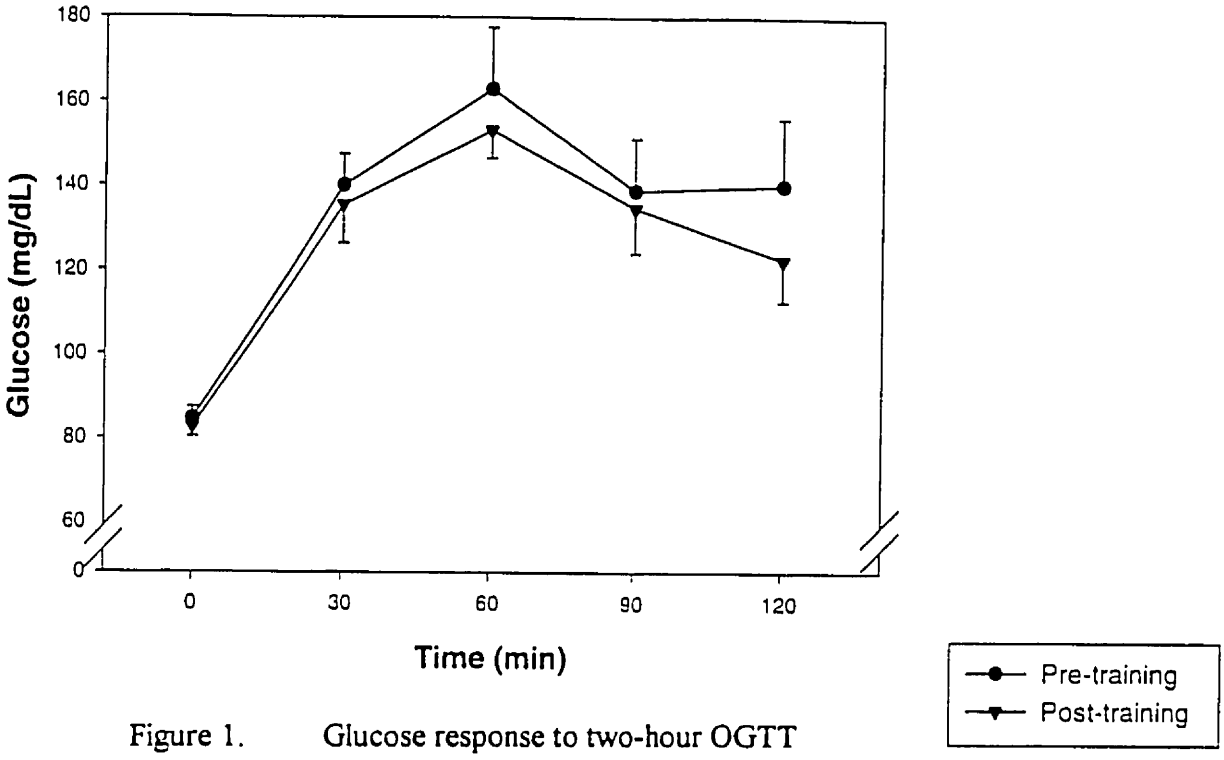

Figure 1. Glucose response to two-hour OGTT

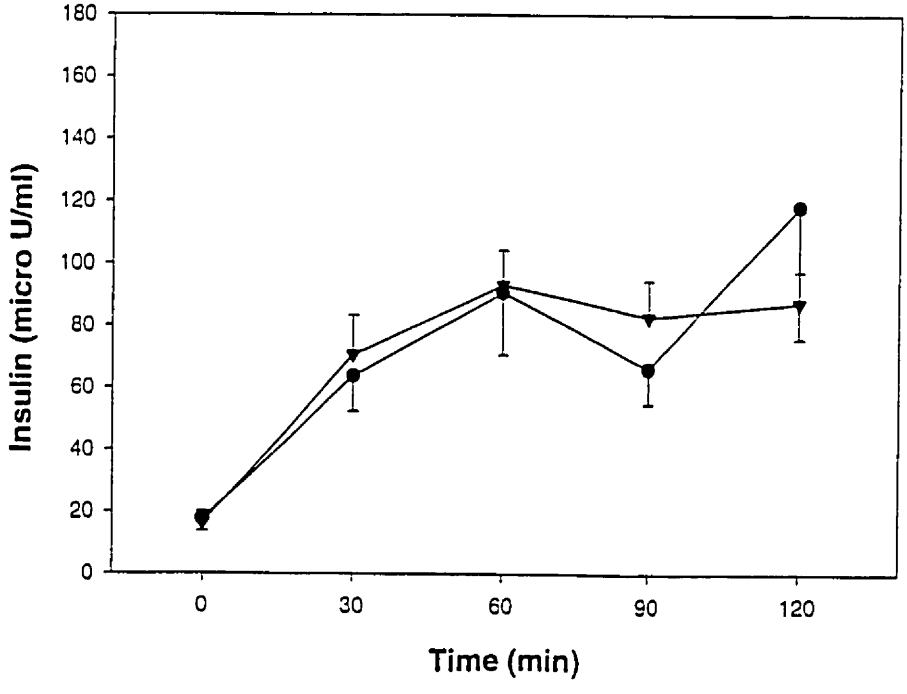

Figure 2. Insulin response to two-hour OGTT

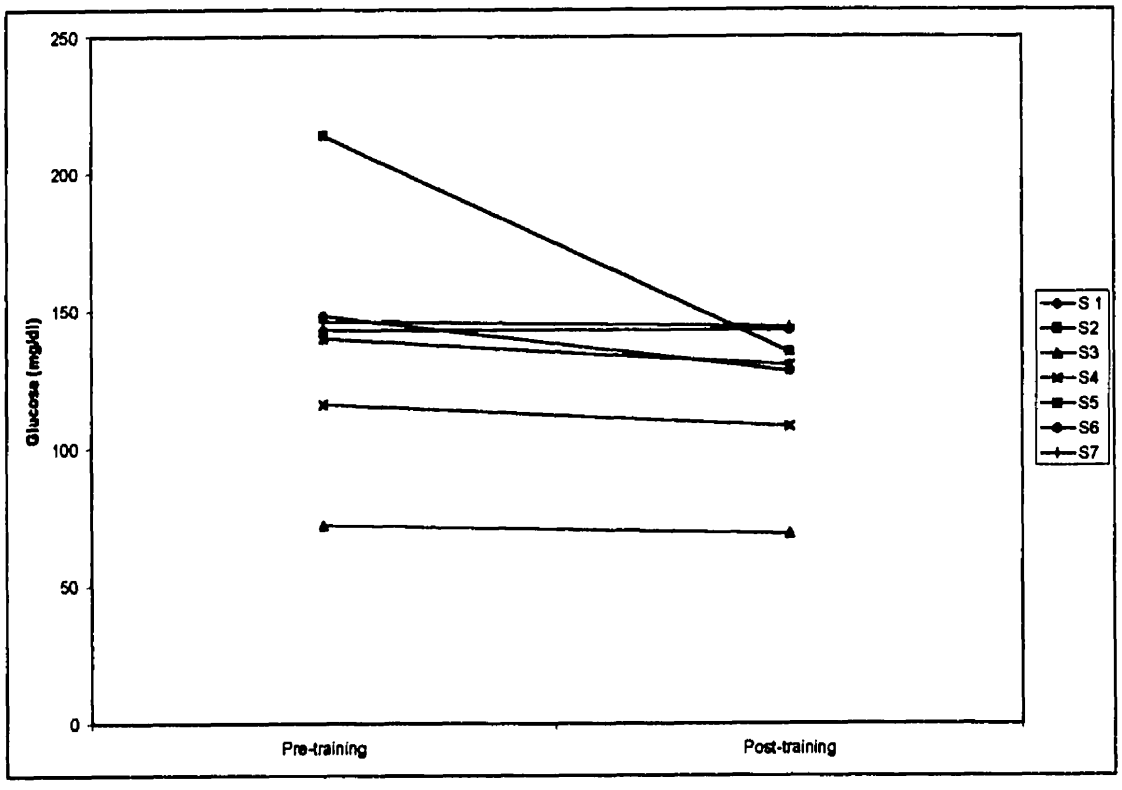

Figure 3. Pre- and post-training 2-hour glucose values during 2-hour OGTT (pre=140 ± 16; post= 122.4 ± 10, p=0.014).

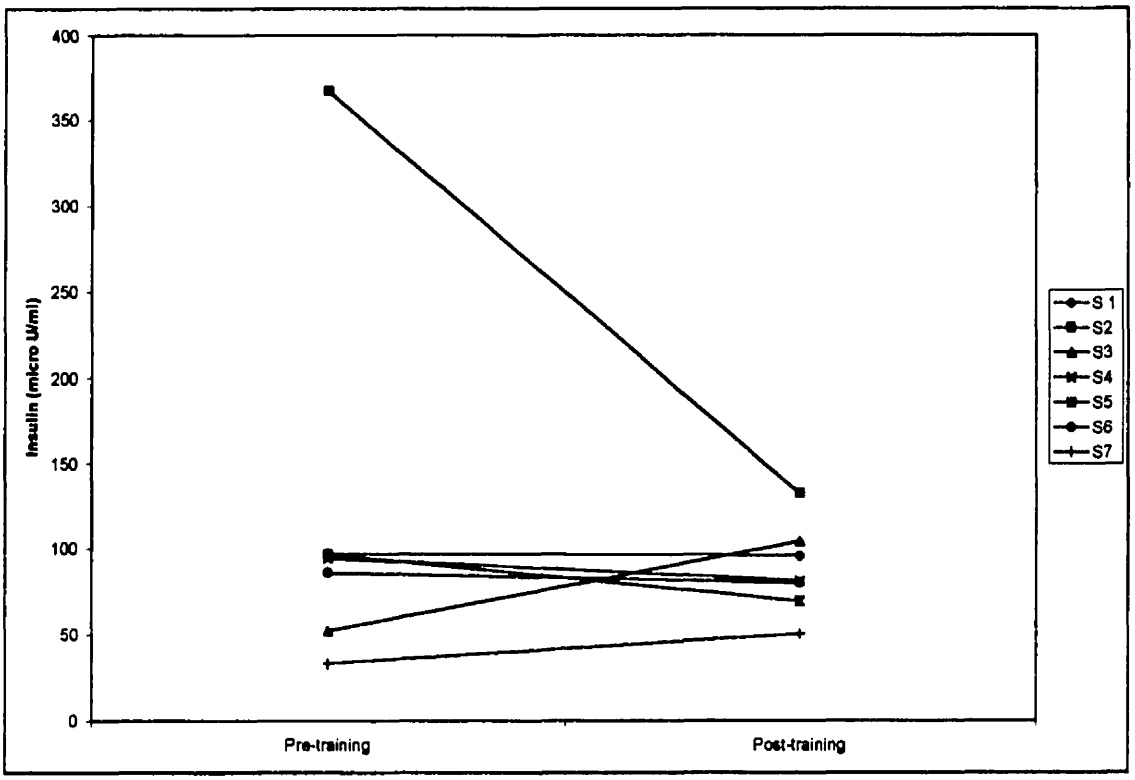

Figure 4. Pre- and post-training 2-hour insulin values during 2-hour OGTT (pre-training = 118.4± 42.6; post-training= 87.5 ± 10 μU/ml)

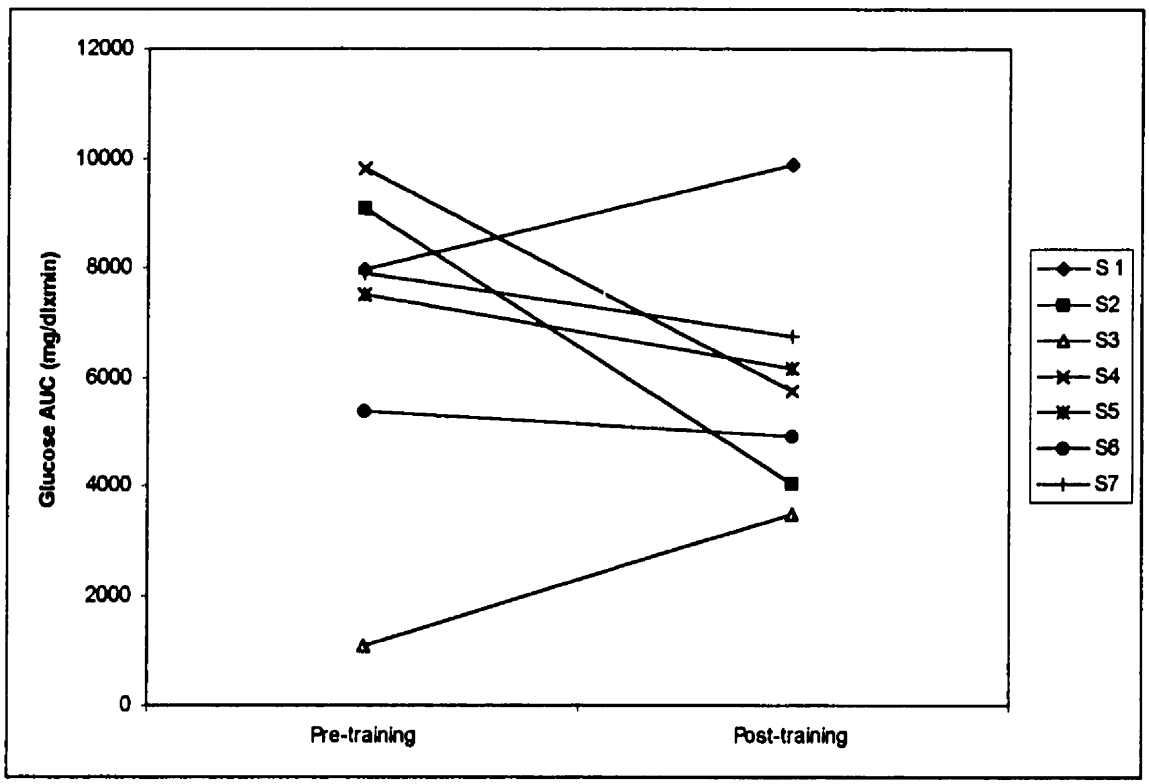

Figure 5. Pre- and post-training incremental area under the glucose curve during 2-hour OGTT (pre-training = 6962 ± 1111.4; 5845 ± 800 mg/dl-time).

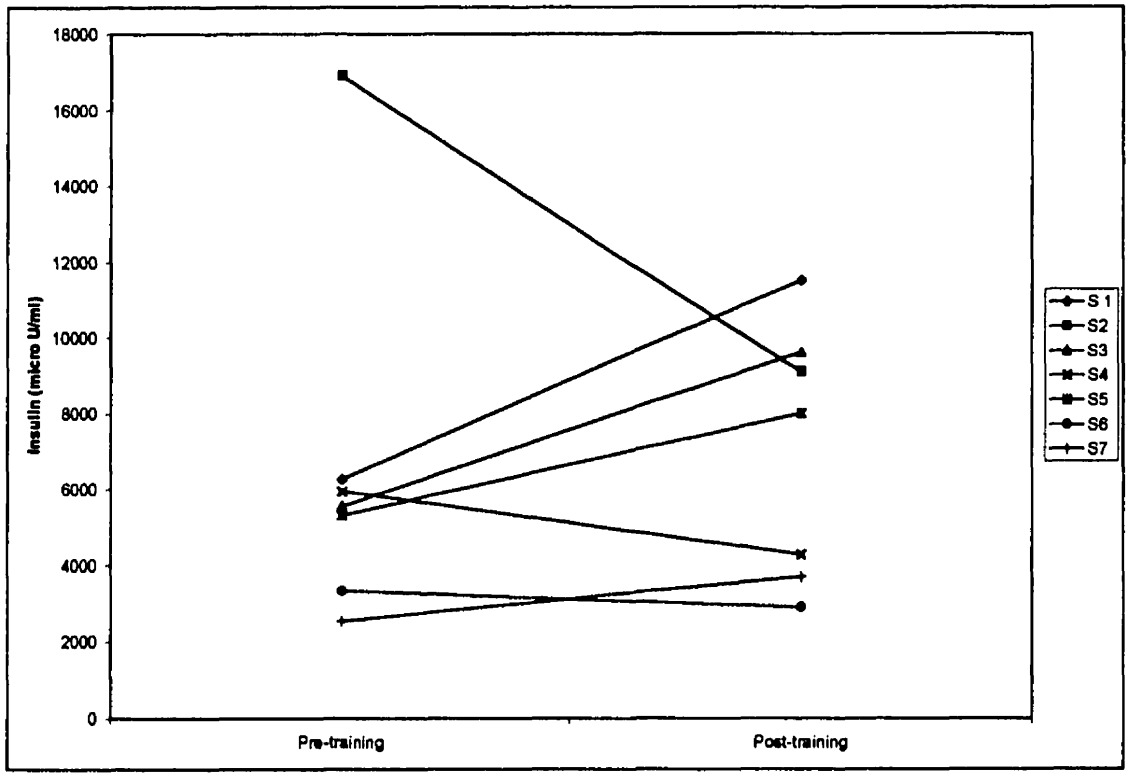

Figure 6. Pre- and post-training incremental area under the insulin curve during 2-hour OGTT. (Pre-training = 6541.5 ± 1272; post-training = 7014 ± 1803 μU/ml)

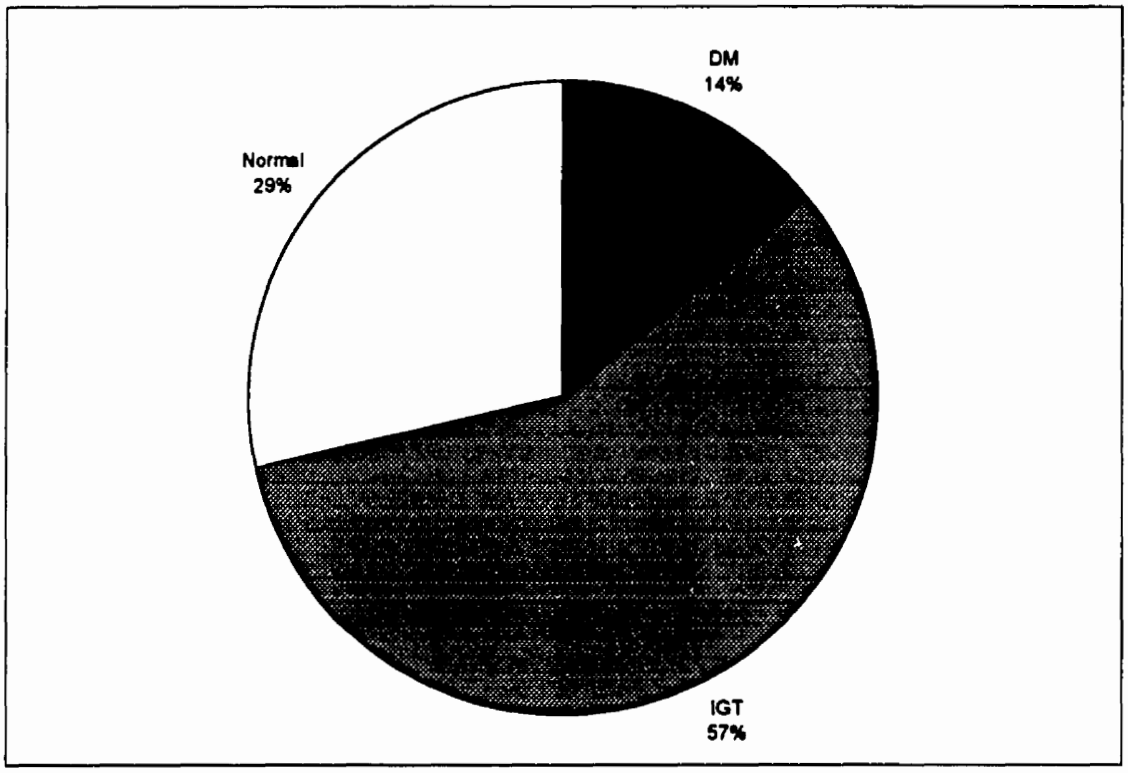

Figure 7. Pre-training Diagnosis of DM and IGT. DM =2 hour glucose value higher than 200 mg/dl, IGT = 2 hour glucose value higher than 140 and lower than 200 mg/dl, Normal = 2 hour glucose value lower than 140 mg/dl

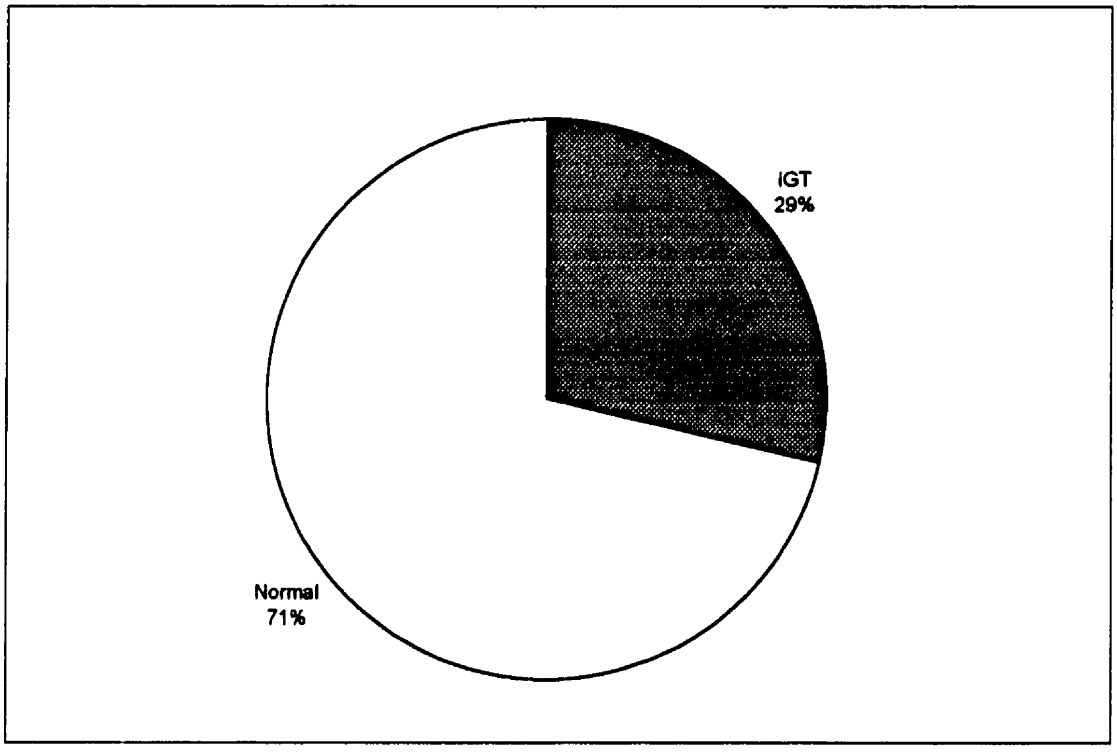

Figure 8. Post-training Diagnosis of DM and IGT. DM = 2 hour glucose value higher than 200 mg/dl, IGT = 2 hour glucose value higher than 140 and lower than 200 mg/dl, Normal = 2 hour glucose value lower than 140 mg/dl

Hyperglycemic clamp test

Hyperglycemic clamp tests were attempted on seven subjects for pre-training insulin sensitivity measurement. Four subjects were excluded from hyperglycemic clamp test due to technical problems. Three pre- and post-training hyperglycemic clamp tests were successfully performed and the results are presented for each individual separately.

Subject 2

Glucose utilization and insulin sensitivity (figure 9 and 10). The glucose utilization during the last 30 minutes of the hyperglycemic clamp test increased from 4.78 $\pm$ .75 to 6.28 $\pm$ 1.04 mg glucose/kg /min after the completion of eight weeks of training with ERGYS II. However, the insulin sensitivity did not change after the completion of training (pre-training: 4.69; post-training: 4.61 mg glucose/kg/min per μU /ml of insulin) (figure 9).

Insulin responses (figure 11, 12, 13 and 14). The early phase (0 to 10 min) serum insulin response to hyperglycemia increased after the training. The peak serum insulin concentration was 83.2 μU/ml for the pre-training and 111.6 μU/ml for the post training. The early phase incremental area under the insulin curve increased from 368.7 to 495 μU/ml·min and total area under the curve increased from 545.3 to 668 μU/ml after the training with ERGYS II. The late phase (20 to 120 min) serum incremental area under the curve increased from 2114.5 to 3500.5 μU/ml - min and the total serum area under the insulin curve increased from 6950 to 10790 μU/ml - min.

Subject 3

Glucose utilization and insulin sensitivity (figure 9 and 10). The glucose utilization during the last 30 minutes of the hyperglycemic clamp test increased from 7.2$\pm$2 to 12.52$\pm$2.47 mg

glucose/kg/min after the completion of eight weeks training with ERGYS II. The insulin sensitivity also improved after the training from 11.45 to 16.58 mg glucose/kg/min per μU /ml of insulin.

Insulin responses (figure 11, 12, 13 and 14). The early phase serum insulin response to hyperglycemia decreased after the training. Pre-training peak serum insulin concentration was 37.4 μ U/ml · min and post-training peak serum insulin concentration was 35.9μU/ml · min. The early phase incremental area under the insulin curve increased from -2.23 to 58.73 μ U/ml · min and total area under the curve decreased from 304.7 to 253.03 μU/ml · min after the training with ERGYS II. The late phase serum incremental area under the curve increased from 119 to 1322 micro U/ml · min and the total serum area under the insulin curve decreased from 3911 to 3866 μU/ml · min.

Subject 4.

Glucose utilization and insulin sensitivity. (figure 9 and 10) The glucose utilization during the last 30 minutes of the hyperglycemic clamp test increased from 5.14 $\pm$ 7.3 to 5.4 $\pm$ 1.04 mg glucose/kg/min after the of completion eight weeks of training with ERGYS II. The insulin sensitivity also improved after the training from 6.77 to 10.43 mg glucose/kg/min per μU /ml of insulin.

Insulin responses. (figure 11, 12, 13 and 14) Post-training serum insulin response during two-hour hyperglycemic clamp decreased. The early phase incremental area under the insulin curve decreased from 130.23 to 75.5 μU/ml and the total area under the curve decreased from 314.53 to 298.7 μU/ml · min after the eight weeks of training with ERGYS II. The late phase serum incremental area under the curve decreased from 1405 to 897 μU/ml · min and the total serum area under the insulin curve decreased from 6205 to 4293 μU/ml · min.

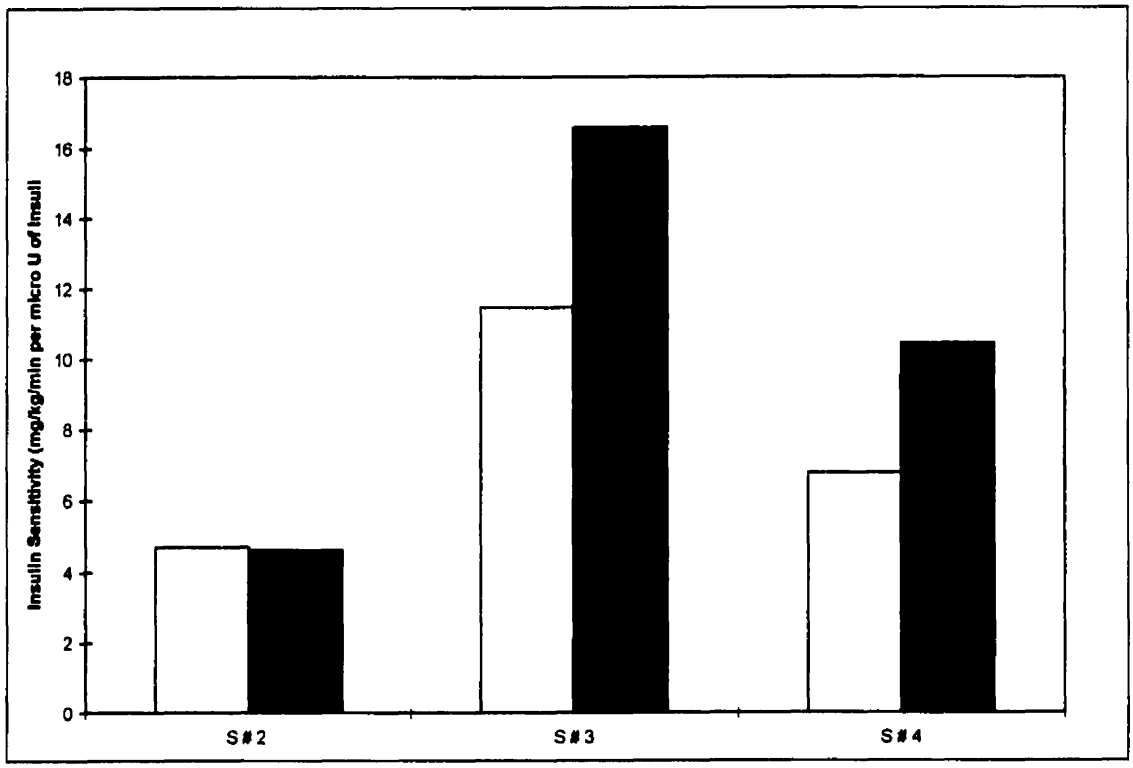

Figure 9. Pre- and post-training insulin sensitivity measure by hyperglycaemic clamp tests on subject # 2, # 3 and # 4 (□=pre-training;■=post-training).

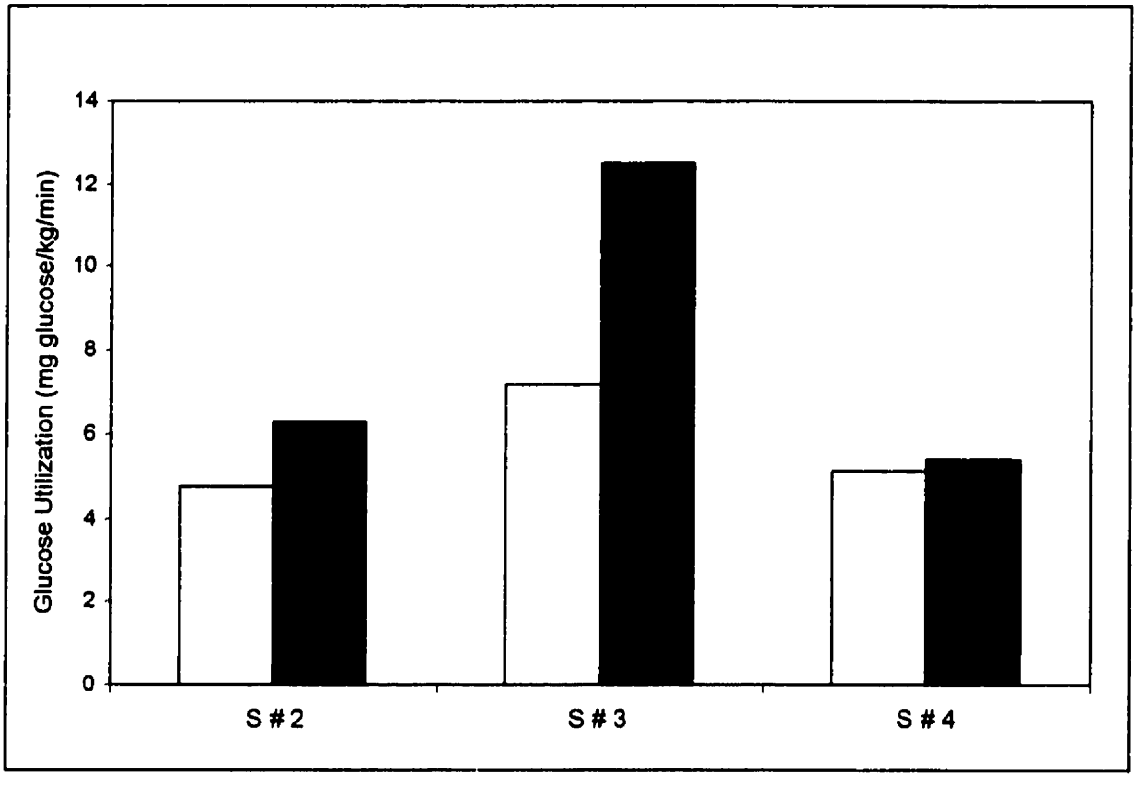

Figure 10. Pre- and post-training 90 - 120 min Glucose utilization during Hyperglycemic clamp test on subject # 2, #3 and # 4. (□=pre-training;■=post-training).

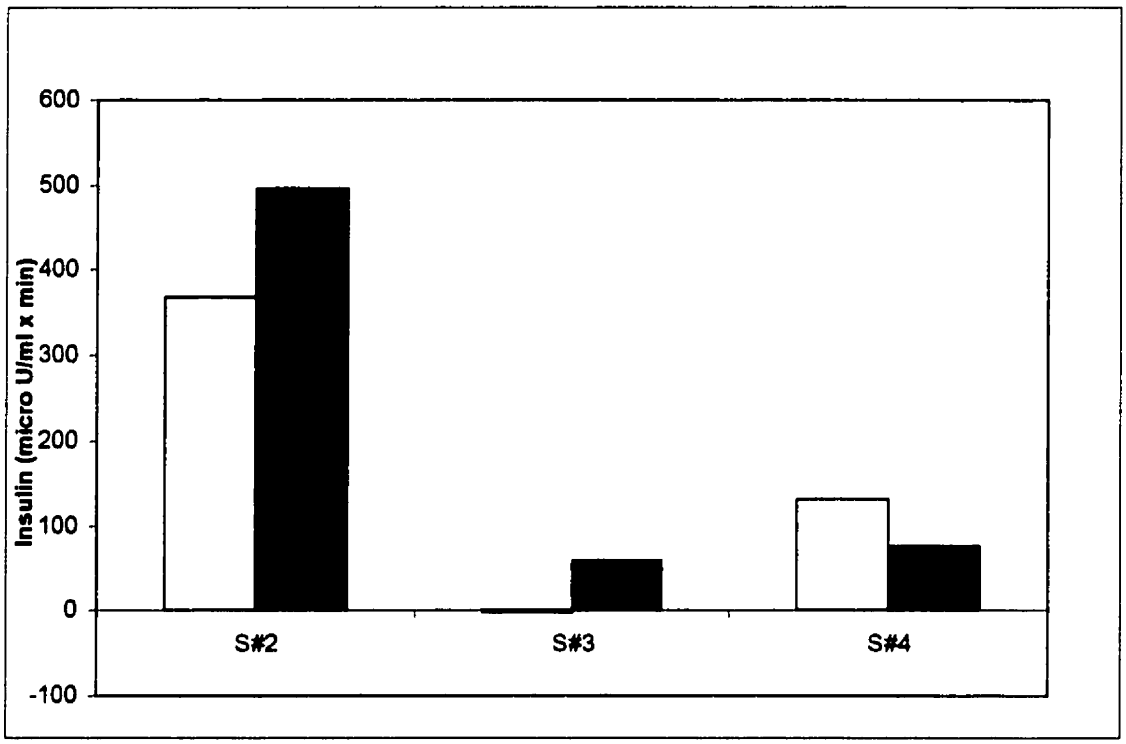

Figure 11. Pre- and post-training early phase (0 - 10 min) incremental area under insulin the curve during Hyperglycemic clamp test on subject # 2, # 3 and # 4 (□=pre-training;■=post-training).

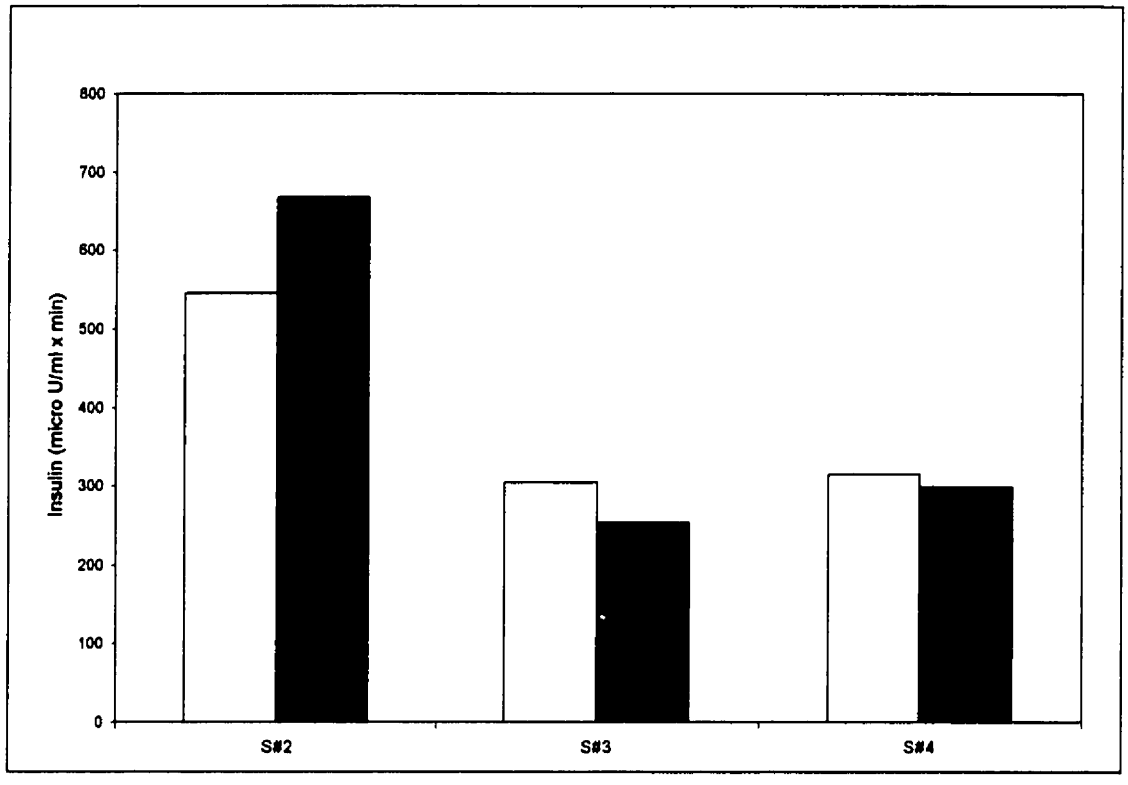

Figure 12. Pre- and post-training early phase (0 - 10 min) total area under the insulin curve during Hyperglycemic clamp test on subject # 2, # 3 and # 4 (□=pre-training;■=post-training).

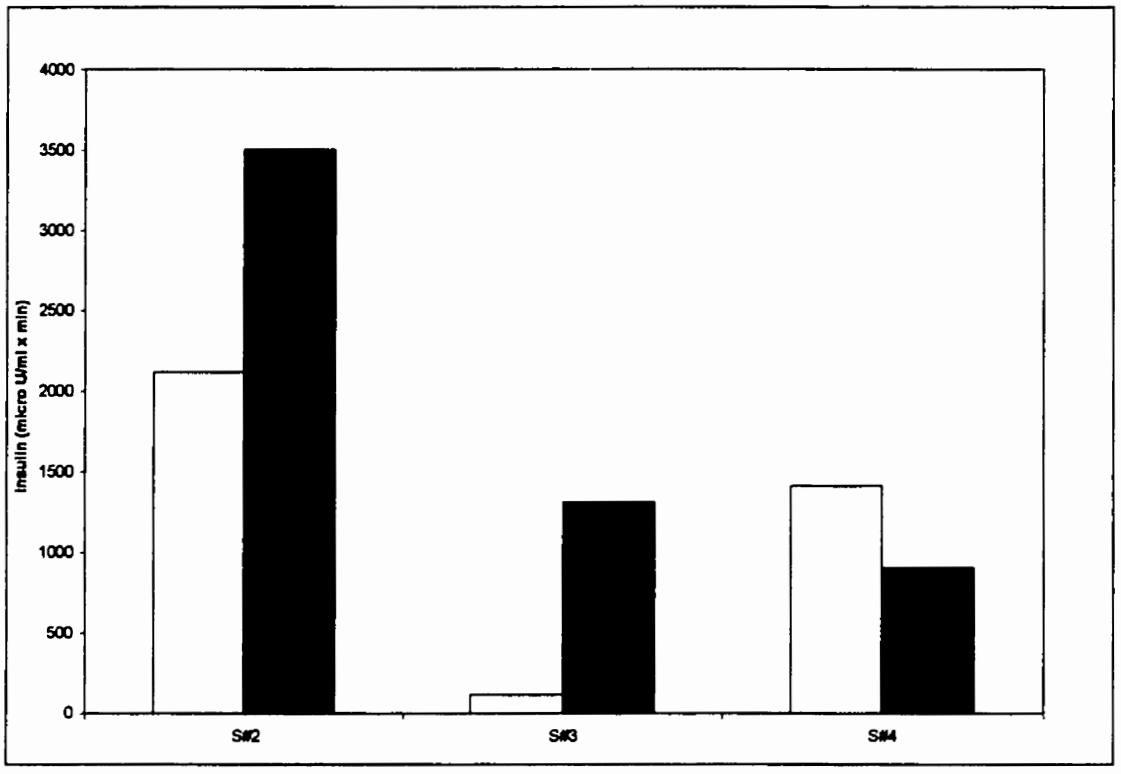

Figure 13. Pre- and post-training late phase (20 - 120 min) incremental area under the insulin curve during Hyperglycemic clamp test on subject # 2, # 3 and # 4 (□=pre-training;■=post-training).

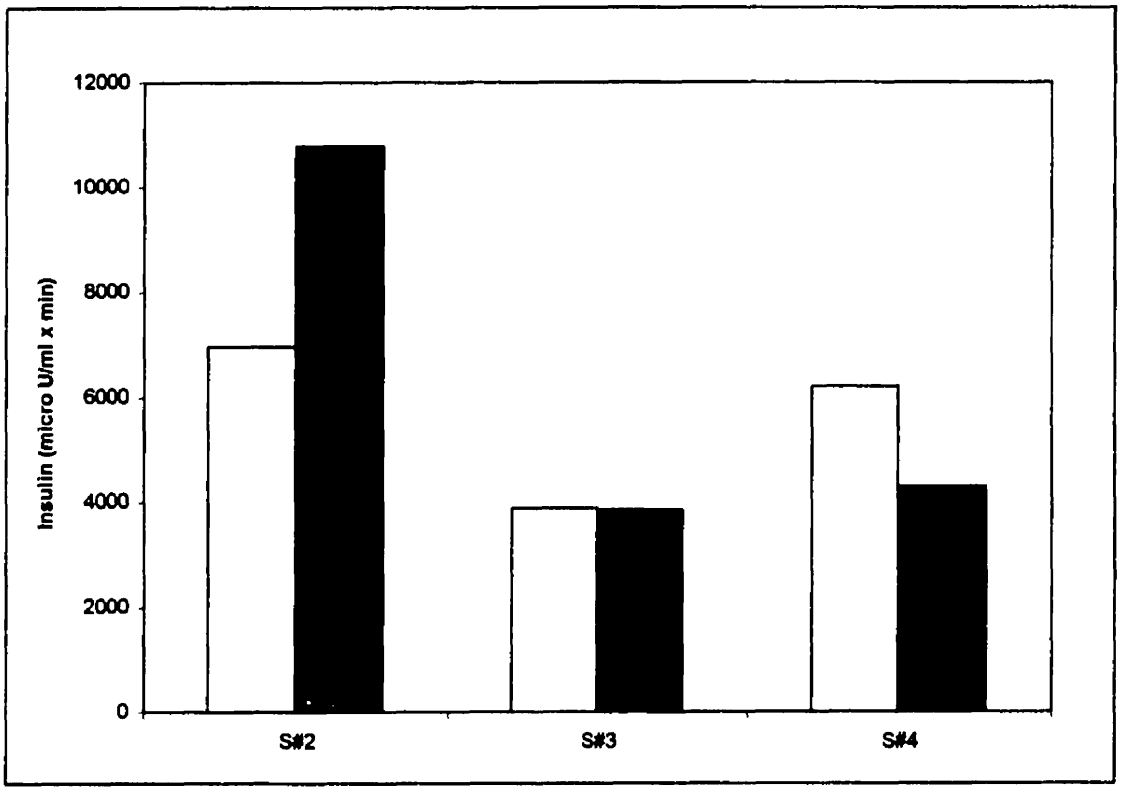

Figure 14. Pre- and post-training late phase (20 - 120 min) total area under the insulin curve during hyperglycemic clamp test on subject # 2, #3 and #4 (□=pre-training;■=post-training).

CHAPTER V

DISCUSSION

The effects of eight weeks of training with ERGYS II on glucose tolerance and insulin sensitivity in people with SCI were investigated in the present study. Results indicated that training with ERGYS II improved glucose tolerance and insulin sensitivity in people with SCI. The results from OGTTs suggested that glucose and insulin levels at the two-hour mark of OGTT decreased by 15.3 and 28.5 percent compared to the pre-training level, respectively, after the completion of eight weeks of training. Since the two hour glucose level of the OGTT is used to diagnose DM or IGT, the diagnosis of DM or IGT among participants changed significantly (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 1997). Five out of seven participants had abnormal glucose tolerance before they started exercise training. After the eight weeks of training with ERGYS II, only two out of five subjects remained diagnosed for IGT and the rest showed normal two-hour glucose levels which supported first hypothesis that training with ERGYS II will improve glucose tolerance.

The incremental area under the glucose curve during the two-hour OGTTs decreased 16 percent (pre-training=6962±1114 mg/dl · min; post-training=5845±800 mg/dl · min) and the incremental area under the insulin curve during the two-hour OGTTs increased by 6.7 percent (pre-training=6541.5±1272; post-training=7014±1803 μU/ml · min) after the eight weeks of training with ERGYS II. However, neither pre- and post-training incremental glucose nor insulin area under the curve reached the statistical significance. Although there were no statistical differences in glucose and insulin area under the curve between pre- and post-training, there was a larger decrease in glucose area under the curve (16%) compared to the increase in insulin area under the curve (6.7%). This result suggested that subjects were using glucose and insulin more effectively after the eight weeks of training with ERGYS II. Because post-training OGTTs were performed 48 hours after the last bout of

exercise with ERGYS II, improvement on post-training glucose profile was assumed to be due to the effects of eight weeks of exercise training rather than from the residual effects of the last exercise bout (Burstein et al., 1985; Schneider, Amorosa, Khachadurina & Ruderman, 1984) .

Results from hyperglycemic clamps tests indicated that either insulin sensitivity or glucose utilization improved in all three subjects after the eight weeks of training (figure 9 and 10). Insulin sensitivity improved on subject number three and four and glucose utilization improved in subjects number two and three. Subject number two had DM and lowest insulin sensitivity (4.69 mg/kg/min per μU of Insulin) among three subjects. The mechanism for improved insulin sensitivity or glucose utilization in the present study was not clear. Subject number two's insulin action was already impaired prior to the training and not able to be restored with eight weeks of training with ERGYS II. In subjects number three and four insulin action was not impaired and eight weeks of exercise training was able to improve insulin sensitivity. Therefore, eight weeks of training with ERGYS II may improve insulin sensitivity among subjects with normal insulin sensitivity or glucose tolerance but my not in subjects with prior impaired insulin action. This result supported the second hypothesis that exercise training with ERGYS II will improve insulin sensitivity in people with SCI.

Improvements in glucose tolerance and insulin sensitivity after regular physical activity were consistent with other research findings in the able bodied. Perseghin et al. (1996) trained ten subjects with Type II DM who had significant insulin resistance and eight normal subjects with forty-five minutes of stair climbing at 65 percent of VO_{2max} for six weeks. Insulin sensitivity and insulin response were measured before and after six weeks of physical activity by euglycemic clamp test and hyperglycemic clamp tests. The training improved insulin sensitivity in both the insulin resistant group and the normal group by more (43%) than that which has been reported for pharmacological treatment. Also, Schneider, Amorosa, Khachadurina and Ruderman . (1984) assessed the effects of physical training on a group of 20 subjects with Type 2 DM and 11 control subjects. The subjects were trained

three times per week (50-75% of the VO_{2max}) for six weeks and oral glucose tolerance test were administered before and 72 hours after the last bout of training. The results indicated that training improved glucose tolerance slightly and decreased glycosylated haemoglobin significantly. Similar intensity (percent VO_{2max}), duration and frequency of the training to the present study were used by Schneider et al. (1984).

In the present study, the maximum resistance achieved by the subjects on the ERGYS II was 2/8 kp which is equivalent to 12.2 watts and the average exercise resistance at which subjects were exercising was 0.86/8 kp. In the eight weeks of training, a large variability in exercise capacity among subjects was observed. Three subjects could not perform pedalling for more than five minutes and were therefore allowed three ten minutes exercise bouts followed by two minutes cool down and five minutes rest for each exercise bout, making the total exercise time of 30 minutes. In contrast, four subjects were able to complete 30 minutes of continuous exercise within the first three weeks of training with ERGYS II. External resistance was loaded on all four subjects who were able to complete 30 minutes of continuous exercise and the average resistance was 1.54/8 ± .59 Kp. This result is consistent with Mohr et al. (1997) who reported that eight out of ten subjects were able to pedal the ERGYS for 30 minutes continuously within the first three weeks of training. One of the reasons for the large variability on the exercise intensity among subjects in the present study was perhaps due to the fact that subjects started ERGYS exercise without a leg-strengthening phase which was recommended by the manufacture and also used by many other investigators (Bloomfield, Jackson & Mysiw, 1994; Mutton et al., 1997; Ragnarsson et al., 1988; Twist, Culpepper-Morgan, Ragnarsson, Petrillo & Kreek, 1992).

The exercise power output of the ERGYS II in the present study was significantly lower than cycle ergometer training used in studies of the able-bodied. However, the physiological responses of people with SCI to ERGYS II training were different from able-bodied people at the same exercise

intensity (Glaser et al., 1989). Kjaer et al. (1996) compared glucose regulation between people with SCI exercising on ERGYS and a normal control group exercising on a cycle ergometer at the same power output and at the same oxygen consumption. They reported that at the same oxygen consumption, the resistance of the cycle ergometer at which the control group exercised was four times higher than the resistance attained on the ERGYS by people with SCI. Also, at the same power output, the metabolic rate was much lower than that measured in people with SCI exercising with ERGYS (Kjaer et al., 1996). Also, Glaser et al. (1989) determined steady-state physiologic responses of 12 subjects with SCI during exercise with ERGYS and compared these to six able-bodied individuals performing voluntary leg cycle ergometry at the same power output. They reported that exercise with ERGYS elicited significantly higher oxygen consumption, ventilation and heart rate responses from subjects with SCI compared to the able-bodied subjects exercising at the same power output levels (Glaser et al., 1989). The authors reported that the main reasons for this inefficiency of exercise with ERGYS was the nonphysiological activation of the paralyzed muscles, the deteriorated condition of these muscles, and inappropriate biomechanics for the movements (Glaser et al., 1989). Although low efficiency would not be beneficial for reliable performance of skilled activities, it has an advantage for training effect because of the relatively high metabolic rate (Glaser et al., 1989; Figoni et al., 1988).

Even if the absolute exercise work output was low in the present study, the relative exercise intensity compared to VO_{2max} achieved by arm crank ergometer was not low according to the oxygen consumption when the subjects were exercising on the ERGYS II. The subject's relative VO_{2max} determined during arm crank exercise was 1.46 ± 1.19 L/min and the peak oxygen consumption determined during ERGYS II exercise was 0.8 ± .34 L/min. Therefore, the oxygen consumption during ERGYS II exercise was about 60% of the VO_{2max} determined during arm crank exercise. Interestingly, Braun et al. (1994) demonstrated that low intensity exercise (50% of VO_{2max}) over a

longer duration improved insulin sensitivity to a level similar to high intensity exercise (75 % of VO_{2max}) over a shorter duration in able bodied subjects. Therefore, the intensity of the exercise in the present study may have reached the required intensity needed to improve glucose tolerance and insulin sensitivity.

According to the pre-training OGTT, 71 percent of the participants were diagnosed as either IGT or DM. However, the mean fasting plasma glucose values among the participants were within the normal range (84.6 ± 2.8 mg/dl) regardless of diagnosis. This result was consistent with other research by Bauman et al. (1994) and Zhong, Levy & Bauman (1995). The reason for normal fasting plasma glucose in people with SCI when their two- hour glucose level show DM or IGT is still not clear. The normal fasting plasma glucose in the population with SCI, regardless of diagnosis, has clinical applications. Orchard (1994) reported from a survey among 76 physicians in Pittsburgh that only one of the physicians used the OGTT as a first test, and more than 70 percent never used it. Fasting plasma glucose (64%), random blood glucose (28%) and urin analysis (13%) were used most frequently. However, due to the normal fasting plasma glucose levels among people with SCI regardless of diagnosis, it is difficult to be able to detect IGT or DM until the DM develops further and becomes symptomatic. It was hypothesized that the mechanism for normal fasting plasma glucose level regardless of elevation post-prandially in people with SCI may due to decrease in muscle mass which is major area for the glucose uptake and down regulation of sympathetic activity which led to decrease in catecholamine level during rest and exercising in people with SCI (Mathias, Frankel, Turner & Christensen, 1979 & 1976; Stjernberg, Blumberg & Wallin, 1986). Therefore, it was recommended to perform the two-hour OGTT in order to diagnose IGT or DM in people with SCI rather than fasting plasma glucose or random blood glucose.

The mechanism for the improved glucose tolerance and insulin sensitivity after eight weeks of training with ERGYS II was not clear from the present data. There are three possible mechanisms

for improved glucose tolerance and insulin sensitivity in the present study; body composition changes, improvement on cardiovascular fitness and muscle characteristic changes after eight weeks of training with ERGYS II. However, since paralyzed skeletal muscle in people with SCI was the one of the major factors for developing DM or IGT, exercise of paralyzed muscle with ERGYS II may be one of the main reasons for the improvement in glucose tolerance and insulin sensitivity in the present study. Andersen et al. (1996) trained five male SCI subjects with ERGYS for 30 minutes at 50 rpm for 12 months. The myosin heavy chain (MHC) composition of single fibres from the vastus lateralis muscle was analyzed by electrophoresis. After 12 months of training, the number of fibres containing MHC IIb were reduced to 2.3% (37.2% before training), the number of fibres containing both MHC IIa and IIb decreased to 4.6% (40.7% before training), while the number of fibres containing only MHC IIa increased to 91.2% (21.2% before training). Also, Mohr et al. (1997) trained ten subjects with SCI with FES cycling, three times per week for one year and measured thigh girth, and also performed muscle biopsy before and after the training for one year. The results from the study indicated that one year training increased thigh girth (1.8 ± 0.3 cm) and muscle mass (12%) significantly. Also, the same study reported increments in enzymatic activity of citrate synthase, an indicator of mitochondrial oxidative capacity after the training. Petrosfsky & Stacy (1992) reported increases in oxidative capacity by 75% after training individuals with FES cycling 15 minutes a day, five times a week for two months. In addition, Etgen et al. (1993) reported that FES exercise increased the level of GLUT-4 content in paralyzed rat muscles. Proportion of Type I and Type IIa muscle fibre, capillary muscle fibre ratio and GLUT-4 content in the muscle are correlated to insulin sensitivity which can be improved by training with ERGYS. Therefore, it was hypothesized that improvement in glucose tolerance and insulin sensitivity in the present study may be due to changes in muscle characteristics.

CHAPTER VI

SUMMARY, CONCLUSION, AND RECOMMENDATION

Summary and Conclusion

The two-hour glucose valuesl during two-hour OGTT indicated that one subject with DM and two subjects with IGT achieved normal range after the completion of eight weeks of training. However, two-hour insulin values, glucose and insulin area under the curve during two-hour OGTT did not reach statistical significance. Even if there was no statistically significant decrease after the training, most of the post-training values were lower than those of pre-training. This supported that glucose tolerance improved after training but some of the variables did not reach statistical significance due to small subject number. According to two-hour hyperglycemic clamp tests, all three subjects improved on either glucose utilization or insulin sensitivity. Subject number three and four's insulin sensitivity improved by 30.9 and 35.9 percent respectively. Subject number two's glucose utilization was increased 23.9 percent but insulin level also increased 25 percent. Thus, subjects number two's insulin sensitivity did not change.

Improvement on glucose tolerance and insulin sensitivity after training in the present investigation supported the initial hypotheses that eight weeks of training with ERGYS II would improve glucose tolerance and insulin sensitivity in people with SCI. Due to the lack of subject number for hyperglycemic clamp tests, the results from hyperglycemic clamp test can not be generalized to whole population with SCI. However, the consistancy with the two-hour glucose tolerance test and data from individual cases suggested that exercise training with ERGYS II may improve insulin sensitivity who do not have severe insulin resistance in people with SCI. The mechanism of improvement on glucose tolerance and insulin sensitivity was still not clear from the present study data. However, it is assumed that improvement on glucose tolerance and insulin sensitivity may be due to increase in muscle mass, changes in muscle characteristics and increased level of physical activity.

Therefore, it is hypothesized that regular exercise with ERGYS II is a useful tool for prevention and treatment of IGT or DM in people with SCI. Also, due to its additional beneficial effects on cardiorespiratory fitness, blood lipid profile, adiposity and blood pressure, exercise training is highly recommended for the prevention and treatment of DM. Thus, regular exercise with ERGYS II is recommended as the way to optimize effects of physical activity and to decrease the risk of developing DM in people with SCI.

Recommendations for the Future Research

- The present study found a statistical significance only on two-hour glucose levels due to small number of subjects, a large individual variability among subjects and low exercise intensity. For future research, it is recommended to have more subjects with less individual variability. Instead of including male, female, subjects with paraplegics and quadriplegics, include only male with paraplegics or female with quadriplegics to decrease individual differences.

- The present study was the first research investigation examining the effect of exercise training on glucose metabolism or insulin sensitivity in people with SCI. FES-assisted exercise device (ERGYS II) was used in the present study. For the future research, training effects of other exercise modalities such as wheelchair exercise, arm crank exercise, swimming and hybrid (arm crank exercise & ERGYS II exercise simultaneously) should be investigated. Also, it was recommended to investigate the mechanism of exercise associated improvement on glucose tolerance or insulin sensitivity such as effects of FES-assisted training on insulin binding or GLUT-4 transporter contents.

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APPENDIX A

INFORMATION TO THE PARTICIPANTS

Effects of electrical stimulation exercise on insulin sensitivity in people with spinal cord injury

Principal investigator: Dr. Robert Burnham, Division of Physical Medicine & Rehabilitation, University of Alberta

Co-investigators: Mr. Justin Jeon, Dr. Robert Steadward, Dr. Garry Wheeler, Rick Hansen Centre, Department of Physical Education, University of Alberta

Information to Participants:

Recent research found that people with spinal cord injury (SCI) tend to use sugars in their body differently than able-bodied population which may put them at higher risk of developing diabetes mellitus (DM). Exercise has proven to be an effective tool to prevent or treat DM in those without SCI. One useful form of exercise for persons with SCI uses electrical stimulation on the paralyzed muscle of the legs enabling them to lift weights or pedal an exercise bike.

You are being asked to participate in a research study to determine the effect of electrical stimulation exercise on the way your body uses its sugar. Prior to the training you will undergo an examination by a medical specialist to ensure you are fit for the training. You will be asked to complete 24 sessions of electrical stimulation assisted bicycle exercise in eight week period . Therefore, you will be asked to make 24 visits for the exercise training. The electrical stimulation training involves applying electrodes on the skin at various sites on your legs through which an electrical current is given under computer control to make the legs pedal a bike. On four occasions during the study (before and after eight weeks of exercise training), blood samples will be taken from your arm to measure insulin sensitivity. On two occasions, 30 small blood samples (3 ml each equivalent to a total of 1 cupful) will be drawn before and during the infusion of glucose via an intravenous needle in a forearm vein. On two occasions, five small sample (3 ml each) will be drawn from you in two hour period (0, 30, 60, 90, 120 minute) after drink a orange juice (75grams of carbohydrate).

There is a very slight chance of side effects occurring with the electrical stimulation exercise training. These side effects may include: skin irritation, muscle or ligament damage, stress fracture, nausea or

faintness, and autonomic dysreflexia (elevated blood pressure, head ache). However, a physician examination conducted prior to the study will identify participants who may be at risk for these side effects, and blood pressure will be monitored during the exercise sessions. The blood tests are invasive (skin is punctured for two intravenous needles) and with any such procedure there is a slight risk of infection or bruising. All lab procedures involving collection and handling of body fluids are conducted under the strict guidelines of Occupational Health and Safety (OHS).

All the information collected will be held in strict confidence and stored on computer disk at the Rick Hansen Centre under the care of Dr. Robert Burnham and Mr. Justin Jeon. Data will be used for abstracts and publications and confidentiality will be maintained via identification number.

You are free to withdraw from the research study at any time, and your continuing medical care will not be affected in any way. If any knowledge gained from this or any other study becomes available which could influence your decision to continue in the study, you will be promptly informed. If you desire to continue with electrical stimulation exercise training at the end of the study, the Rick Hansen Centre will make this program available for you.

If you have further concerns about aspect of this study, you may contact the Patient Concerns Office of the Capital Health Authority, at 474-8892. This office has no affiliation with the study investigator.

Please contact any of the individuals identified below if you have any questions or concerns:

Dr. Robert Burnham at the Edmonton Sport Institute. (403) 451-1234
Dr. Robert Steadward at the Rick Hansen Centre. (403) 492-3182
Mr. Justin Jeon at the Rick Hansen Centre. (403) 492-9389

APPENDIX B

CONSENT FORM

Effect of electrical stimulation exercise on insulin sensitivity in people with spinal cord injury

Principal Investigator: Dr. Robert Burnham (ph:451-1234)

Co-investigator: Mr. Justin Jeon (Ph:492-9389), Dr. Robert Steadward (492-3182)

Dr. Garry Wheeler (492-7158)

Rick Hansen Centre, University of Alberta

CONSENT FORM

	Yes	No
Do you understand that you have been asked to be in a research study ?	☐	☐
Have you read and received a copy of the attached Information Sheet ?	☐	☐
Do you understand the benefits and risks involved in taking part in this research study ?	☐	☐
Have you had an opportunity to ask questions and discuss this study ?	☐	☐
Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your future medical care?	☐	☐
Has the issue of confidentiality been explained to you, and do you understand who will have access to your medical records?	☐	☐
Do you want the investigators to inform your family doctor that you are participating in this research study?	☐	☐

Who explained this study to you? _____

I agree to take part in this study: YES ☐ No ☐

Signature of Research Subject _____

(Printed Name) _____

Date:_____

Signature of Witness _____

Signature of Investigator or Designee _____

APPENDIX C
RICK HANSEN CENTRE FES SAFETY PROTOCOL

The Rick Hansen Centre FES safety protocol is as follows:
The blood pressure will be checked at regular intervals during ERGYS training. Should significant autonomic dysreflexic induced hypertension (defined as .180/120 or .50 % increase of resting blood pressure) occur, the following protocol should be followed:

1. Discontinue the electrical stimulation.
2. Elevate the head of the bed or bring the patient to a sitting position.
3. Loosen any constricting clothing.
4. Check bladder drainage equipment for kink or other cause of obstruction, or drain the bladder if an indwelling catheter is not used.

If blood pressure does not drop below the hypertensive level within five minutes of instituting the above measures:

1. If a physician is available on site, he or she will break open and squeeze the contents of one 10 mg Nifedipine capsule under the tongue. If hypertension is not brought under control, this treatment may be repeated in 30 minutes. If the signs and symptoms are not manageable, the patient will be transferred to emergency.

2. If no physician is available, transfer to Emergency.

APPENDIX D

Glucose values from Two-hour OGTT

subjects	Glucose (mg/dl)				
	0 min	30 min	60 min	90min	120min
Subject 1 Pre-training	81	140	182	156	143
Subject 1 Post-training	79	177	182	175	143
Subject 2 Pre-training	99	166	210	166	214
Subject 2 Post-training	91	118	141	126	135
Subject 3 Pre-training	77	109	94	67	72
Subject 3 Post-training	80	155	125	82	69
Subject 4 Pre-training	87	142	163	153	116
Subject 4 Post-training	76	114	154	135	108
Suject 5 Pre-training	88	153	190	145	140
Subject 5 Post-training	89	144	160	148	130
Subject 6 Pre-training	81	120	133	135	148
Subject 6 Post-training	87	112	154	138	128
Subject 7 Pre-training	79	150	168	149	146
Subject 7 Post-training	77	128	156	138	144

APPENDIX E

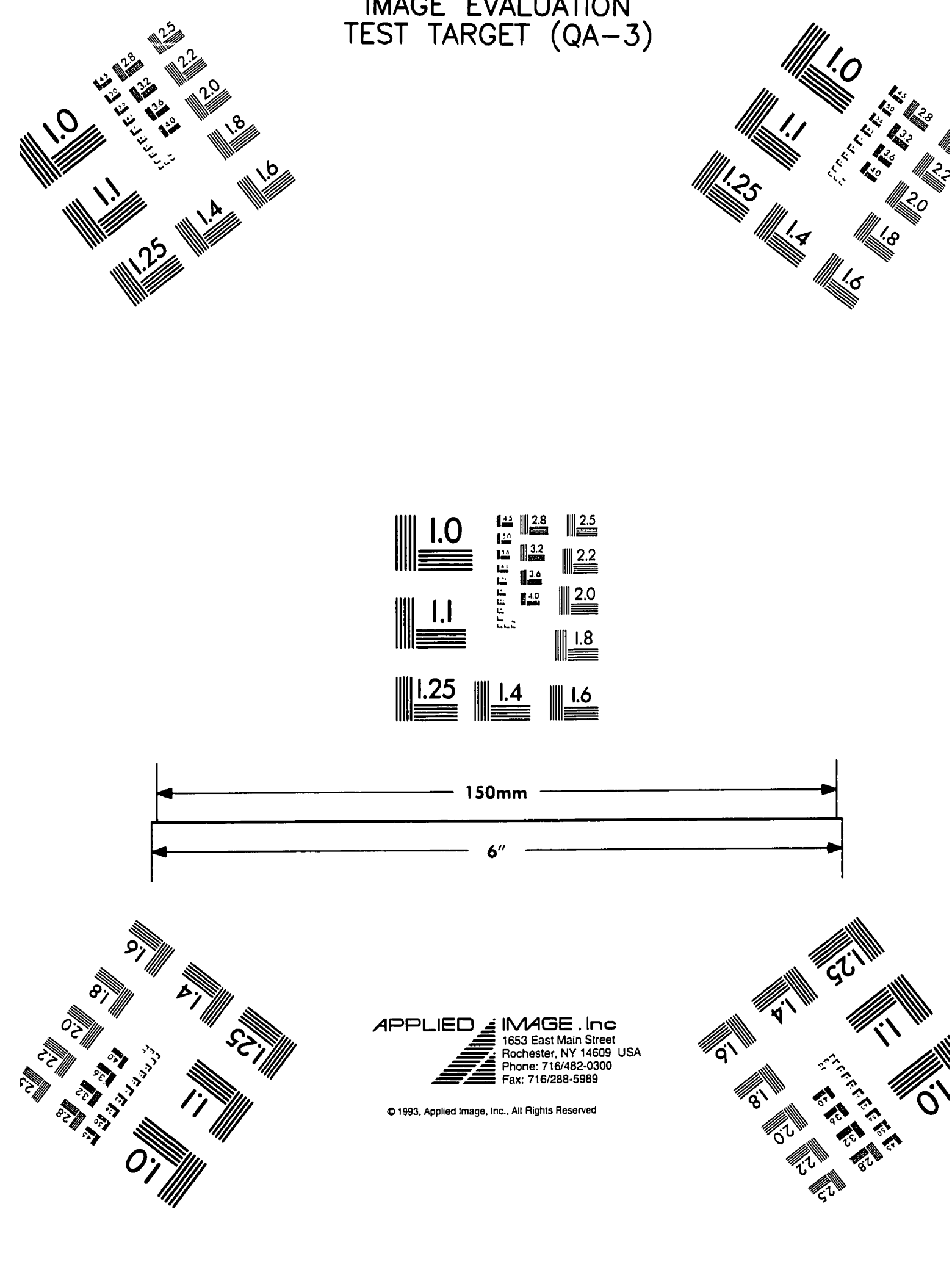
Insulin values during Two-hour OGTT

	Insulin (μU/ml)				
	0 min	30 min	60 min	90min	120min
Subject 1 Pre-training	26.7	59.6	135	59.2	97.2
Post-training	10.4	111.7	142.2	118.6	95.9
Subject 2 Pre-training	13.7	126	189	111.6	367.3
Post-training	20.2	108.3	114.8	84.4	132.2
Subject 3 Pre-training	11.8	61.2	71.6	64.1	52.1
Subject 3 Post-training	7.2	72.1	100.3	118.4	104.3
Subject 4 Pre-training	12.9	42.8	69.9	82.8	94.4
Subject 4 Post-training	12.1	34.7	68.7	40.9	81.1
Suject 5 Pre-training	12.8	64.6	79.1	28.5	98.6
Subject 5 Post-training	14.1	89.5	96.3	95.2	69.2
Subject 6 Pre-training	37	61.4	52.7	83.4	86
Subject 6 Post-training	37	34.7	75.1	76.1	80
Subject 7 Pre-training	9	31	38.1	30.6	32.9
Subject 7 Post-training	13.6	43.6	55.7	46.5	50.1

APPENDIX F

Summary result of hyperglycemic clamp test

	Subject 2	Subject 3	Subject
Pre-training glucose utilization during 20 - 120 min (mg/kg/min)	4.85	6.11	4.84
Pre-training glucose utilization during 90- 120 min(mg/kg/min)	4.78	7.2	5.14
Pre-training average insulin value during 90 - 120 min (μU/ml)	102	62.88	75.88
Pre-training insulin sensitivity (mg/kg/min per μU insulin)	4.69	11.45	6.77
Post-training glucose utilization during 20 - 120 min (mg/kg/min)	6.86	9.4	4.46
Post-training glucose utilization during 90 - 120 min (mg/kg/min)	6.28+1.04	12.52	5.4
Post-training average insulin value during 90- 120 min (μU/ml)	136.13	75.5	51.8
Post-training insulin sensitivity (mg/kg/min per μU insulin)	4.61	16.58	10.43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