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The Impact of Low Frequency Neuromuscular Electrical Stimulation on Glucose Regulation in Individuals with Type 2 Diabetes

(Spine Title: Low Frequency NMES and Glucose Regulation in Diabetes)

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by

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of the requirements for the degree of

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Abstract

The purpose of this study was to examine the effect of low-frequency neuromuscular electrical stimulation (NMES) on glucose regulation in individuals with type 2 diabetes. Eight individuals with type 2 diabetes between 41 to 65 years of age volunteered for this study. Participants completed two experimental sessions in a randomized order, a control session and an NMES session. During both sessions, an initial blood sample was collected at rest, a beverage containing 75 g of glucose was then consumed, and further blood samples were drawn at 60 and 120 minutes. On the NMES day, participants completed one hour of low frequency (8 Hz) NMES immediately following the glucose ingestion. Participants regulated the intensity of the NMES to their maximum tolerable level. A significant increase ($p < 0.01$) from 7.59 ± 2.06 to16.11 ± 3.73 and from 7.75 ± 2.29 to 15.9 ± 3.01 mmol/l in blood glucose was observed after 60 minutes of the NMES and the control sessions, respectively. This was followed by a significant ($p < 0.01$) decrease from 16.11 ± 3.73 to 13.3 ± 3.16 and from 15.9 ± 3.01 to 14.5 ± 3.1 mmol/l after 120 minutes of the NMES and the control sessions, respectively. The changes in blood glucose levels were not different between the NMES and control conditions. A positive correlation was found between the delta difference in blood glucose at time 60 and the intensity of the low-frequency NMES ($r^2 = 0.89$, $P < 0.01$). The positive correlation between glucose concentration and the intensity of the low frequency NMES may indicate a dose response to NMES in individuals with type 2 diabetes. Overall, low frequency NMES had no impact upon plasma glucose concentration in individuals with type 2 diabetes following acute glucose ingestion.

Keywords: electrical stimulation, diabetes, glucose regulation

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Introduction

Diabetes is the most prevalent metabolic disorder in the world and it is reaching epidemic dimensions. The global impact of diabetes in 2000, estimated by the World Health Organization (WHO), was over 171 million people. This number is expected to increase to 366 million in 2030 (Wild, Roglic, Green, Sicree, and King, 2004). Nationally, the number of Canadians who are currently diagnosed with diabetes is 1.7 million and its prevalence continues to increase at an alarming rate (Health Canada, 2005). The highest prevalence recorded in Canada was observed in a First Nations population in 1997, reporting 26 % (Harris et al., 1997). The economic burden of diabetes and its complications on the Canadian Health Care System is estimated to be 13.2 billion dollars every year, and is expected to increase to 15.1 billion in 2010 and 19.2 billion in 2020 (Hogan, Dall, and Nikolov, 2003).

The three major types of diabetes are type 1, type 2 and gestational diabetes. Exercise is an essential component of management and treatment of all forms of diabetes. Glucose uptake can be increased by two separate mechanisms; one insulin-dependent pathway and one insulin-independent or contraction/exercise induced pathway. In type 1 diabetes or insulin-deficient individuals, exercise improves insulin sensitivity, (Koivisto et al., 1986; Wallberg-Henriksson et al., 1982) but not glucose control (Wallberg-Henriksson et al., 1982; Zinman et al., 1984). Individuals with type 1 diabetes sometimes have blunted or impaired ability to increase glucose uptake via the contraction/exercise pathway. Nonetheless, exercise training, in individuals with type 1 diabetes, can improve cardiovascular fitness, blood lipid profiles and reduce insulin requirements.

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Type 2 diabetes is the most prevalent and accounts for 90-95 % of all diabetes cases in the world. Individuals with type 2 diabetes individuals are known to have defects in the insulin-signaling cascade that include decreases in insulin receptor substrate 1 (IRS-1), tyrosine phosphorylation, IRS-1 association with PI 3-kinase, and PI 3-kinase activity (Patti et al., 1999). This results in a greater difficulty absorbing plasma glucose into their skeletal muscles. Glucose uptake in skeletal muscles may occur by insulin-dependent or insulin-independent (contraction-induced) mechanism. Exercise increases insulin-stimulated glucose disposal in individuals with type 2 diabetes (Trovati et al., 1984). Exercise in type 2 diabetes can not only increase insulin sensitivity but also increase rates of whole-body glucose uptake (Devlin JT, 1987; Zierath JR., 1995).

Both endurance training and strength training are beneficial in the treatment of type 2 diabetes. The Canadian Diabetes Association (CDA) recommends that persons with type 2 diabetes exercise for at least 150 minutes per week, at a moderate intensity. In addition, these recommendations also include three sessions of resistance training per week. Recently, a study comparing strength training (ST) to aerobic endurance training (ET) in individuals with type 2 diabetes found that blood glucose and insulin resistance improved more in the ST then the ED group. ST was found to be more effective then ET in improving glycemic control (Cauza et al., 2005). Resistance training has also been found to improve glycemic control and insulin sensitivity independently of an increase in muscle mass (Yaspelkis et al., 2006). This improvement in insulin action could be due to an increase in GLUT-4 content in skeletal muscles, and to insulin signaling protein expression or biochemical adaptations to resistance training (Holten et al., 2004). Both acute (Fenicchia et al., 2004) and chronic (Castaneda et al., 2002) resistance exercise are

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helpful in glucose control. Two prolonged session of resistance training per week, at intensities of 50-80%, can increase muscle strength, decrease abdominal fat, and improve insulin sensitivity. Even if exercise has been postulated to be beneficial and essential in the treatment of type 2 diabetes, most persons with type 2 diabetes do not follow the recommended guidelines for exercise. In a study of 1480 individuals with type 2 diabetes, about one third of these individuals were found to be completely sedentary and another third reported less than recommended levels of physical activity (Nelson, Reiber, and Boyko, 2002). Several factors can explain the lack of exercise by this population, including age, technology, obesity, high blood pressure and hypertension. In addition, chronic complications like retinopathy, neuropathy, and heart problems can limit their ability to perform some types of exercise.

Novel approaches that take advantage of the contraction-induced response might benefit individuals with type 2 diabetes. Chronic electrical muscle stimulation (EMS) has been used in sedentary individuals to improve physical fitness (Banerjee et al., 2005). To date, electrical stimulation (ES) has few studies which have examined glucose regulation and ES in type 2 diabetes. In the past decade, most of the research on glucose regulation and electrical stimulation has been done on individuals with spinal cord injuries (SCI). Previous research on glucose regulation and functional electrical stimulation (FES) has shown favorable results in individuals with SCI (Mohr et al., 2001).

When individuals with SCI are compared to able-bodied, they are more likely to have oral carbohydrate intolerance, insulin resistance, elevated low-density lipoprotein cholesterol, reduced high-density lipoprotein cholesterol (Bauman, and Spungen, 2001), and they have higher risk of developing type 2 diabetes (Jeon et al., 2003). These

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individuals also have a higher incidence of cardiovascular diseases, impaired insulin action and are limited or restricted from voluntary exercise. Functional electrical stimulation is the application of low-voltage currents to enhance function of paralyzed muscles. This modality to elicit involuntary muscle contraction was found to improve glucose tolerance and insulin sensitivity chronically in diabetic individuals with SCI (Mohr et al., 2001). Another method that can assist involuntary muscle contraction is neuromuscular electrical stimulation (NMES). During NMES an electrical impulse is passed from a device to electrodes placed on the skin over a targeted muscle or muscle group. The stimulation causes the muscles to contract. Clinically, NMES could be a useful modality to elicit involuntary muscle contraction and increase glucose uptake in skeletal muscles of diabetic individuals who have difficulties performing physical activity.

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Research Question

The potential role of low-frequency NMES in non spinal injured diabetics has yet to be examined. The purpose of this study is to determine the acute perturbation of low-frequency NMES on glucose metabolism in patients with type 2 diabetes. The plasma glucose response to the oral glucose tolerance test is well documented, and it is simple to administer; therefore, it was the choice for this study.

Hypothesis

Glucose uptake in skeletal muscles can be increased despite decreased insulin secretion, since exercise causes the translocation of the GLUT-4 from a different pool than insulin. A single bout of exercise can not only markedly increase rates of whole-body glucose uptake, but also increase the sensitivity of skeletal muscle glucose uptake by insulin. The most important tissue for glucose uptake following a glucose tolerance test is skeletal muscles, which account for 70-90 % of the observed uptake (DeFronzo et al., 1981). It is therefore hypothesized that blood glucose concentration following a glucose tolerance test will decrease during and following the NMES protocol compared to a control day.

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Literature review

Glucose Metabolism during Exercise

The four main substrates during prolonged exercise in active people can come from any of the following sources: muscle triglycerides, blood glucose, muscle glycogen, and plasma free fatty acids (FFA). At low-intensities comparable to walking (25% maximum oxygen uptake), almost all of the energy is derived from plasma fatty acids and a small contribution from blood glucose when performed in the fasted state. As exercise intensity increases from 25% to 65%, comparable to running, the total fat oxidation increases. Plasma fatty acid and muscles triglycerides account equally to total fat oxidation; however, carbohydrate oxidation (muscles glycogen and blood glucose) provides one-half of total energy, since the rate of fat oxidation is limited (Martin et al., 1993). At higher intensities (85% VO_2 max), carbohydrate oxidation provides more than two-thirds of the total energy and the remaining contribution comes from plasma free fatty acids and muscles triglycerides. Despite the large amount of potential energy by the plasma free fatty acids and muscles triglycerides, their rate of oxidation is limited; therefore, the glycogen stored in the muscles and liver and blood glucose is needed to provide the additional substrates for oxidation as the intensity of exercise is increased.

Glucose transport across the cell surface is a key regulatory step for glucose metabolism in skeletal muscle (Goodyear, and Kahn, 1998a). Glucose transport in the skeletal muscle occurs primarily by facilitated diffusion and utilizes a family of transport carriers. In human skeletal muscles, the primary glucose transporter is called GLUT-4 (Klip, and Paquet, 1990). The major mechanism by which exercise increases glucose uptake in skeletal muscles is through the translocation of GLUT-4 from an intracellular

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location to the plasma membrane in skeletal muscles. Two separate pools of GLUT-4 have been identified, one that is insulin dependent and the other which is exercise-induced (Goodyear, and Kahn, 1998a). In the insulin-dependent mechanism, insulin first binds with the extracellular α-subunit of the insulin receptor, then there is autophosphorylation of tyrosine residues in the receptor β-subunit, tyrosine phosphorylation of the insulin receptor 1 (IRS-1) and insulin receptor 2 (IRS-2), and activation of phosphatidylinositol 3-kinase (PI-3 kinase) (Clarke, Young, Yonezawa, Kasuga, and Holman, 1994). In the exercise induced or insulin-independent mechanism, the signaling steps are not the same since contraction does not stimulate autophosphorylation of isolated insulin receptors (Treadway, James, Burcel, and Ruderman, 1989), receptors of IRS tyrosine phosphorylation (Goodyear, Giorgino, Balon, Condorelli, and Smith, 1995; Wojtaszewski, Hansen, Urso, and Richter, 1996), or PI 3- kinase activity (Goodyear, Giorgino, Balon, Condorelli, and Smith, 1995; Wojtaszewski, Hansen, Urso, and Richter, 1996). These two mechanisms have also been found to be additive when combined (Wallberg-Henriksson, Constable, Young, and Holloszy, 1988).

Glucose is the only type of carbohydrate that skeletal muscles can metabolize for energy and that can be stored as glycogen. During exercise, glucose uptake is known to increase in working skeletal muscles. A number of factors impact upon glucose metabolism during exercise. The entry of glucose in skeletal muscles has been found to be relative to the intensity of exercise (Wilkinson, and Liebman, 1998; Hargreaves, 1995). At rest, skeletal muscles only account for 15-20% of total glucose uptake in the body. A study using a cycling bout of 55-60% VO_2max, demonstrated that skeletal

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muscle glucose uptake was responsible for as much as 80-85% of glucose utilized in the body (Kjaer, Kiens, Hargreaves, and Richter, 1991). A match between glucose uptake and hepatic production (gluconeogenesis, glycogenolysis) usually maintains euglycemia homeostais in non-diabetic individuals exercising. The amount of gluconeogenesis that occurs during exercise depends on the glycogen reserve prior to exercise, and the intensity and duration of the exercise. In the first 30 minutes of moderate to intense exercise, most of the glucose output from the liver is derived from glycogen stores (glycogenolysis) and not gluconeogenesis (Wahren, Felig, Ahlborg, and Jorfeldt, 1971). After one hour of moderate exercise, the proportion of gluconeogenesis used is less than 15 % of total glucose output (Kjaer, 1995).

Nutritional status is another important factor that effect glucose metabolism during exercise. In a significant fasted state (a day or two) during an exercise bout, a large proportion of glucose released from the liver is from gluconeogenesis (Bjorkman, and Eriksson, 1983). In a fed state, glucose production and release from the liver is not necessary. Previous research has found lower plasma glucose levels after exercise in the fed state compared to the fasted state. This may be explained by the fact that plasma insulin levels were higher in the fed state, and this probably blunted hepatic glucose production, which resulted in higher glucose utilization than production (Poirier et al., 2001).

Glucose metabolism during exercise is also influenced hormonally. A number of hormonal changes occur during exercise that signal the body to breakdown stored glycogen for fuel, and then can be used by the skeletal muscles for energy. Insulin, which is secreted by the β-cells of the islet of Langerhans in the pancreas, is usually at a low

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level during exercise or is maintained at a low concentration, while glucagon levels increase. Glucagon, which is secreted by the α-cells of the pancreas, responds to low blood glucose by activating cyclic AMP in the liver, stimulating both gluconeogenesis and glycogenolysis. Within the first few seconds of exercise, levels of epinephrine and norepinephrine rise dramatically. These hormones stimulate the breakdown of stored fat in both skeletal muscles and adipose tissues, and the breakdown of glycogen in the liver and in skeletal muscles. Glucose production by the liver is either increased by decreased insulin and unchanged or increased by sensitizing it to glucagons. (Wasserman, Williams, Lacy, Goldstein, and Cherrington, 1989; Wasserman et al., 1989).

Skeletal muscle uptake can however be increased despite decreased insulin secretion, because exercise causes translocation of the GLUT-4 from a different pool than insulin (Douen et al., 1989; Coderre, Kandror, Vallega, and Pilch, 1995). A single bout of exercise can not only markedly increase rates of whole-body glucose uptake, but also increase the sensitivity of skeletal muscle glucose uptake by insulin (Devlin JT, 1987; Zierath JR., 1995). These effects can persist for several hours after the exercise ends. Exercise training can also increase peripheral insulin action on diabetic individuals (Hughes et al., 1993). Insulin action improvement can be explained by an increase in GLUT-4 levels, oxidative enzymes activity, type I skeletal muscles fibres, and capillary density (Ivy, 1997). The contraction-induced mechanism and the enhanced insulin sensitivity mechanism explain the utility of exercise in the management of type 2 diabetes.

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Glucose metabolism and diabetes

Type 2 diabetes is characterized by a defect in the insulin-dependent signaling pathway that contributes to insulin resistance. Insulin resistance can be defined as the inability of the body to respond appropriately to insulin. Therefore, to maintain euglycemia, the pancreas compensates by secreting an increase amount of insulin. The following period of compensation is called impaired glucose tolerance, which results despite an elevated insulin concentration as insulin resistance increases. Then, the failure of the pancreatic β-cells to respond adequately results in a decrease insulin secretion. Clinical diabetes can be diagnosed when impaired β-cell function and insulin resistance occurs simultaneously (DeFronzo, 2004). Impaired insulin action influences the amount of glucose uptake by the skeletal muscles of diabetic individuals.

When whole body glucose regulation is altered, individuals with type 2 diabetes have difficulties absorbing plasma glucose, which results in excess glucose circulating in the bloodstream. Individuals with type 2 diabetes have altered muscle fiber composition compared to normal healthy subjects (Hickey et al., 1995; Marin et al., 1994). Sensitivity of whole-body glucose disposal to insulin, in human skeletal muscles is positively correlated with the percentage of type I fibers and negatively correlated with the percentage of type IIB fibers in the vastus lateralis muscle (Lillioja et al., 1987). Type II B fibers include a reduced oxidative enzyme activity and an increased glycolytic enzyme activity in comparison with type I fibers. Individuals with type 2 diabetes have been found to have a low percentage of type I fibers, elevated type IIB fibers, and a low capillary density (Marin P., et 1994). Reduced oxidative enzyme activity in skeletal

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muscle of individuals with type 2 diabetes is most likely due to a reduction in the proportion of type 1 fibers (Oberbach et al., 2006).

However, it is believed that the GLUT-4 translocation is functioning properly in diabetic individuals and exercise training can positively affect the GLUT-4 protein in skeletal muscles (Kennedy et al., 1999). GLUT-4 content was measured in individuals with type 2 diabetes compared to asymptomatic individuals after an acute bout of exercise. The investigators demonstrated that the total muscle content of GLUT-4 after acute exercise was not different between non-diabetic individuals and individuals with type 2 diabetes. Two muscle biopsies were taken from five individuals with type 2 diabetes and five normal control individuals. The first biopsies were collected on one leg at rest and the second on the opposite leg 3-6 weeks after 45-60 minutes of cycle exercise at 60-70 % VO_2 max. Plasma membrane GLUT-4 increased in both groups after acute exercise (Kennedy et al., 1999).

Henriksen et al. (1990) demonstrated the correlation between GLUT-4 content, different muscle fiber types and glucose transport in an animal study with male Wistar rats. They found that glucose transport was highest in the soleus muscles (80% type 1 fiber) and had a higher GLUT-4 content than in the epitrochlearis (65% type IIB fibers) muscles which had a lower GLUT-4 content (Henriksen et al., 1990). Increasing GLUT-4 levels can benefit glucose transport in diabetic individuals since oxidative type I and type IIA muscles fibers are more insulin sensitive. These oxidative muscles fibers have a higher GLUT-4 content than the glycolytic muscles fibers (MacLean, Zheng, and Dohm, 2000).

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A single bout of exercise increase rates of whole-body glucose uptake, and the sensitivity of skeletal muscle glucose uptake by insulin (Devlin JT, 1987; Zierath JR., 1995). Exercise training improves insulin action in skeletal muscles tissue (Dela et al., 1992; Holten et al., 2004) in both healthy and individuals with type 2 diabetes. A change in protein expression in the insulin signaling cascade as well as proteins involved in glucose uptake and storage in skeletal muscles is responsible for this improvement (Dela et al., 1992; Holten et al., 2004). Chronic resistance training in rats, has been found to increase the activation of PI 3-kinase, a PKC, and Akt, and increasing total GLUT-4 protein concentration (Yaspelkis et al., 2006). In individuals with type 2 diabetes, resistance training has been found to change protein content in PBK, GLUT-4 and GS (Holten et al., 2004).

Electrical Stimulation

During NMES, contractions are triggered by eliciting action potentials of the motor nerves. Motor units with smaller motor neurons are recruited first and then motor units with larger motor neurons are recruited as the intensity increases (Henneman, and Olson, 1965). However, the reverse recruitment procedure with electrical stimulation is controversial, and determined partly by the stimulation parameters (Lertmanorat et al., 2006). A narrow pulse width (50 micros) has been shown to reverse the recruitment procedure of peripheral nerve stimulation by recruiting the small axons before the large axons (Lertmanorat et al., 2006). Voluntary exercise may have more difficulties activating faster contracting motor units than NMES, because these smaller motor units may only be activated at higher intensities. Research regarding the effect of electrical stimulation on skeletal muscles found that NMES in complement with voluntary exercise

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may provide a more successful training modality for high threshold motor units (Trimble, and Enoka, 1991). High-frequency and low-frequency ES are two commonly used types of electrical stimulation. NMES can induce significant levels of both high frequency (HFF) and low frequency fatigue (LFF). HFF is characterized by an extreme loss of force at high frequencies of stimulation and LFF is characterized by a less significant loss of force at low frequencies of stimulation (Jones, 1996).

In the early 1990's the majority of research on glucose regulation and electrical stimulation was performed on rodents. Glucose transport in skeletal muscles of rodents was examined following electrical stimulation of the sciatic nerve (Etgen, Farrar, and Ivy, 1993). This study indicated an increase in the number of the glucose transporters GLUT-4 translocated to the plasma membrane. Studies on glucose uptake and the intensity of electrical stimulation in rat muscles typically found that glucose uptake increases with increasing stimulus intensities (Johannsson, Jensen, Gunderson, Dahl, and Bonen, 1996).

In humans, glucose uptake and electrical stimulation was examined on individuals with spinal cord injuries. Individuals with type 2 diabetes and spinal cord injuries were treated three times per week with electrical stimulation and improved glucose regulation (Mohr et al., 2001). The frequency used was 30 Hz with a preset maximal intensity of 120 mA. This study examined individuals with SCI and FES for one year, in order to examine insulin sensitivity, glucose tolerance, and glucose transporter (GLUT-4) content. Individuals with SCI performed 30 minutes of computer-controlled FES exercise ergometer, three times per week for a year. The training was then reduced to 30 minutes once a week for the following 6 months. This study demonstrated that one year of FES enhanced whole-body insulin sensitivity and GLUT-4 content in subjects with SCI.

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Glucose transport was measured by comparing the GLUT-4 content before and after the FES protocol, which increased by 105 % after 1 years of FES. (Mohr et al., 2001)

Similarly, a study using healthy adults demonstrated a positive relationship regarding glucose regulation through electrical stimulation (ES) (Hamada, Sasaki, Hayashi, Moritani, and Nakao, 2003). Hamada et al. (2003) examined whether involuntary contraction induced by low-frequency electrical stimulation could enhance glucose uptake in healthy adults. A stimulation pattern with 0.2-ms biphasic square pulses at 20 Hz and a 1-s on-off duty cycle was used. Whole-body glucose uptake was determined by the glucose disposal rate measured using a euglycemic clamp. Glycemic clamp was acutely increased by 2.5 $mg \cdot kg^{-1} \cdot min^{-1}$ in response to electrical stimulation, and remained elevated by 3-4 $mg \cdot kg^{-1} \cdot min^{-1}$ for at least 90 minutes after cessation of stimulation on skeletal muscles of healthy subjects. (Hamada et al., 2003)

Only one study has examined the effect of high-frequency electrical muscle stimulation (EMS) on glucose uptake in neurologically intact individuals with type 2 diabetes (Poole, Harrold, Burridge, Byrne, Holt et al., 2004). Their high-frequency EMS protocol consisted of a 30 minutes control period; then a current of 30 mA was used for 30 minutes and then increased to 40mA for a further 30 minutes. Three out of the five subjects increased their glucose uptake; however, no statistical significance was found, and an allergic response to the EMS was found in 25 % of participants. These participants had to stop the EMS protocol and could no longer participate in the study.

Low-frequency electrical stimulation with a frequency of 8 Hz is tolerated comfortably and safely by individuals. A low-frequency electrical stimulation protocol might benefit participants by increasing their ability to tolerate longer periods of time and

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by decreasing the drop-out rate due to allergic responses. Nonetheless, individuals with type 2 diabetes might be impacted by hypoglycemia if they are taking sulfonylureas and performing exercise. Previously, post absorptive individuals with type 2 diabetes patients taking sulfonylureas were found to have an enhanced plasma glucose- lowering effect because of the interaction of the medication and exercise. However, none of these participants developed hypoglycemia during exercise after taking the sulfonylureas. Both sulfonylureas and exercise are known to decrease plasma glucose; the risk of hypoglycemia is therefore increased when they are combined. When extensive exercise is performed in the fasting state, individuals with type 2 diabetes treated with sulfonylureas need to be cautious (Larsen, Dela, Madsbads, Vibe-Petersen, and Galbo, 1999). The risk of developing hypoglycemia is associated with the energy expenditure in response to exercise (Larsen, Dela, Kjaer, and Galbo, 1997). However, an hour of low-frequency electrical stimulation with a frequency of 8 Hz should not cause extensive energy expenditure.

Regulation of glucose uptake, in skeletal muscles during and after electrical stimulation or exercise, can be explained by two main phases. The first phase, an insulin-independent effect of muscle contraction is apparent during and for a short period after the electrical stimulation. Phase two, the insulin-dependent mechanism, occurs mostly during the latter part of the post-stimulation period (Hamada et al., 2003). These two distinct mechanisms of glucose uptake by electrical stimulation, insulin-dependent and independent, highlight the possibility of a novel modality to treat individuals with type 2 diabetes.

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Methods

Participants

Eight participants with type 2 diabetes volunteered for this study. Five men and three women were included, and the average age was 52 ± 13 years. These participants were identified by a physician as having met the current diagnosis of type 2 diabetes (Canadian Diabetes Association 2003 Clinical Practice Guidelines for the Prevention and Management of Diabetes in Canada) with mean diabetes duration of 6.25 ± 8.75 years. Exclusion criteria were: insulin use, anginal symptoms, hepatic or renal insufficiency, progressive ventricular dysrhythmia, chronic lung disease, intermittent claudication, symptomatic lower limb osteoarthritis, surgically implanted electronic devices (i.e. cardiac pacemakers), and lower limb amputation. Five participants reported none or very little exercise on a weekly basis, and three participants reported they walked, biked or played leisure activities two to three times a week. They all continued their hypoglycemic medications as prescribed for all testing and exercise days. Except for one subject treated with diet alone all other subjects (n= 7) were treated with diet plus oral hypoglycemic agents (Glyburide, and/or Metformin, and/or Gluconorm, and/or Avandia and/or Actos). Two subjects were only taking Metformin, one subject was taking Avandia alone, and the other four subjects were taking a combination of these agents; Metformin and Glyburide; Metformin and Gluconorm; Metformin, Glyburide, and Avandia; Metformin, Actos, and Glyburide. This study was approved by the Ethics Review Committee of Lakehead University.

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Participants were asked to complete three sessions over a two-week period. Each session could last up to three hours. The first session was a screening session that gave participants the opportunity to familiarize themselves with the low-frequency NMES protocol which would be used. In the course of the second and third sessions, a glucose tolerance test (GTT) was performed. During one of these two sessions, participants also completed 1-hour protocol of NMES along with the GTT. The participants (N = 8) were randomly assigned to either the NMES session or the control session on two separate days. The NMES was always completed during the first hour of the protocol.

Glucose Tolerance Test

Subjects underwent a 2-h glucose tolerance test. This test is the most used method for accessing whole-body glucose tolerance (Soonthornpun et al, 2003). After an overnight fast (12 hours), the GTTs were performed at 07: 30 AM for both conditions. Fasting blood sample was collected upon arrival (time 0). This fasting blood glucose value provided a baseline for comparing other glucose values. The subjects were then asked to drink a solution containing a known amount of glucose (75g) within 5 minutes. In total, three blood samples were collected, at rest and at 60, and 120 minutes after consumption of glucose load. Blood was drawn from a vein (venipuncture), usually from the inside of the elbow or the back of the hand.

Neuromuscular Electrical Stimulation

The electrical stimulation was delivered to the knee extensor muscles of the right and left legs by a portable battery-powered stimulator (Respond Select, Empi Inc; 300 g approximate weight) and two 7.5-cm diameters round reusable adhesive electrodes (Pals Plus, Empi). The current used was a balanced symmetrical biphasic, with a frequency of

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8 Hz for a period of one hour, the pulse duration was set at 200 μs, and within the first two minutes, participants regulated the intensity of the contraction to the maximum tolerable level (The portable stimulator ranged from 0mA to 120mA, but our participants could tolerate from 30mA to 60mA). The electrodes were placed on the extensor muscles of the legs and produced a rhythmic contraction. This protocol is well tolerated for long periods of time (Theriault et al., 1994b).

Statistical Analysis

Statistical comparisons were made using a 2 (condition: NMES or Control) by 3 (time: 0, 60, 120 minutes) repeated measures ANOVA to determine whether significant changes in absolute glucose levels occur with time or groups. The Student-Newman-Keuls Method test was used to make multiple comparisons when appropriate. Values are presented as means ± standard error. Level of significance was set at $p<0.05$. Relative changes in plasma glucose were determined by calculating the difference between the participants' values at 0 and 60 minutes, as well as between 60 and 120 minutes. A paired t-test was used to statistically assess relative changes.

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Results

A significant increase ($p < 0.01$) from 7.59 ± 2.06 to 16.11 ± 3.73 and from 7.75 ± 2.29 to 15.9 ± 3.01 mmol/l in blood glucose was observed after 60 minutes of the NMES and the control sessions, respectively. This was followed by a significant ($p < 0.01$) decrease from 16.11 ± 3.73 to13.3 ± 3.16 and from 15.9 ± 3.01 to 14.5 ± 3.1 mmol/l after 120 minutes of the NMES and the control sessions, respectively. The results of the 2 (condition: control and NMES) x 3 (time: 0, 60, and 120 minutes) repeated measures ANOVA indicated that there was a significant main effect for time, F (2) = 52.339, $p <$ 0.01. The main effect for condition was not significant, F (1), = 0.441, $p > 0.05$. The condition by time interaction was also not significant, F (2) = 1.56, $p > 0.05$.

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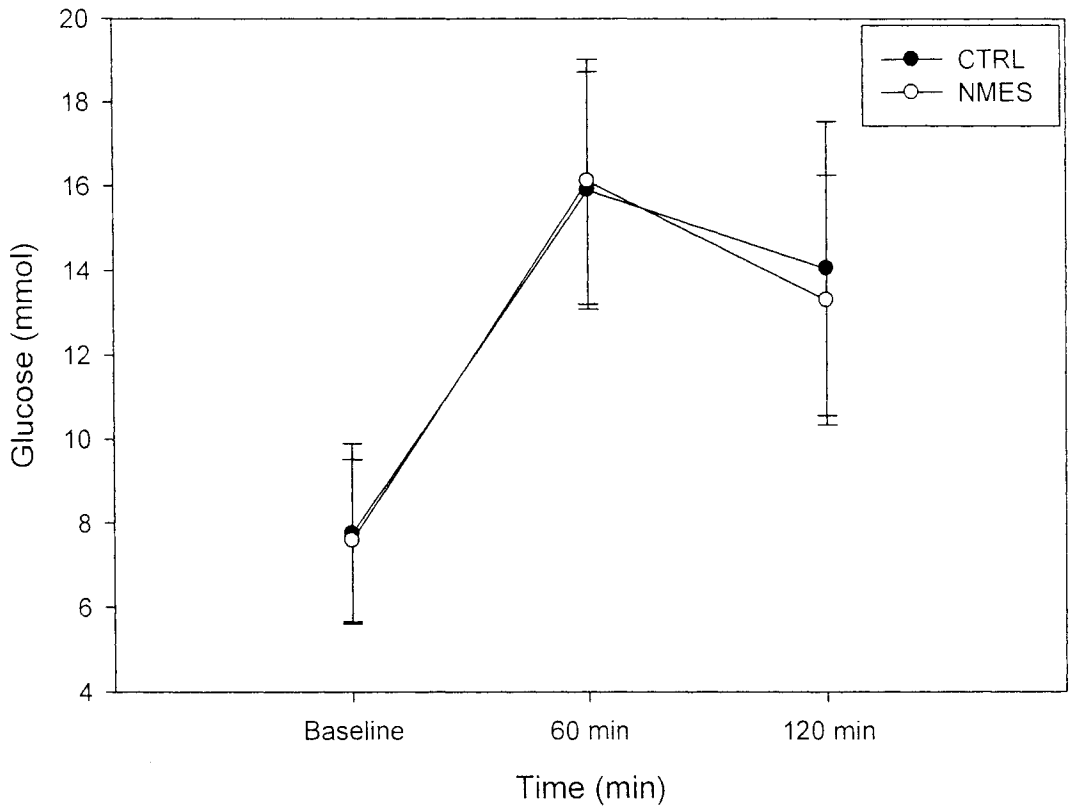

Figure 1. Absolute plasma glucose concentrations at times 0, 60, and 120 minutes during NMES and control conditions. Values are means ± SE.

No difference was observed in the relative glucose change from 0 to 60 minutes between the control (8.15 ± 3.05 mmol/l) and NMES (8.53 ± 2.95 mmol/l) conditions. A positive correlation was found between the relative difference in blood glucose at time 60 and the intensity of NMES ($r^2 = 0.89$, $P < 0.01$; all participants) (Fig 2). No difference was observed in the relative glucose change from 60 to 120 minutes between the control (-1.85 ± 1.78 mmol/l) and NMES (-2.81 ± 1.57 mmol/l) conditions ($p = 0.145$).

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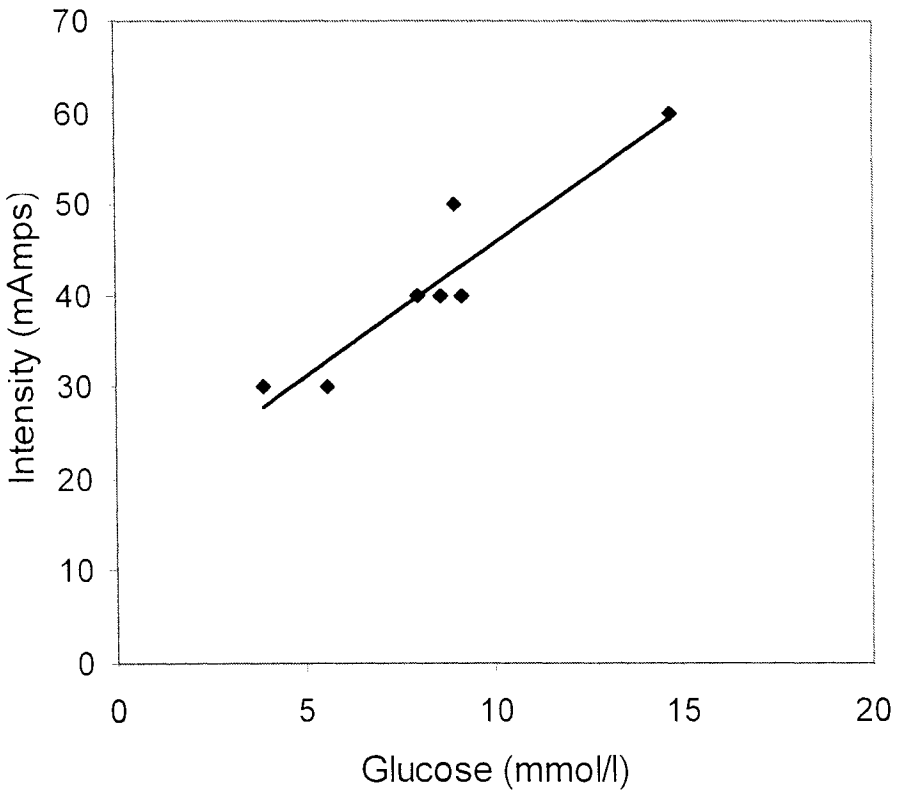

Figure 2. Relationship Between Glucose Delta Difference and NMES Intensity Level at 60 minutes in Individuals with type 2 diabetes. ($y = 2.9319x + 16.256$)

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Discussion

To our knowledge, this is the first study to examine low-frequency NMES and glucose metabolism in individuals with type 2 diabetes without spinal cord injuries. The present study did not find any significant impact on plasma glucose concentration in response to the NMES protocol. However, we did observe a significant positive correlation between the increase in blood glucose concentration and the intensity of the NMES. Information regarding the impact of NMES on glucose regulation in diabetics is sparse. Only one other study has examined the effect of acute electrical muscles stimulation (EMS) in neurologically intact individuals with type 2 diabetes (Poole et al., 2004). Their high-frequency EMS protocol consisted of a 30 minutes control period; then a current of 30 mA was used for 30 minutes and then increased to 40mA for a further 30 minutes. The participants were all individuals with type 2 diabetes, and were in a post-absorptive state. Acute glucose uptake was evaluated using a 5-h hyperinsulinaemic, euglycaemic clamp during ES. The uptake increased markedly in three of the five volunteers, but did not reach statistical significance (p=0.089). Chronically, no significant differences were found in glucose concentration during the OGTT. The stimulation frequency was higher (50Hz vs. 8 Hz) than the one chosen in our study, and they utilized a stimulation current of 30mA to 40mA. Our protocol encouraged the subjects to find the highest current level that could be tolerated comfortably for an hour, which ranged from 30mA to 60mA.

Along with Poole et al., our study supports the overall finding that acute electrical stimulation is insufficient to modulate glucose metabolism in individuals with type 2 diabetes. This may be explained by the fitness level of the participants. Poole et al.

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(2004) noted that the individuals with a low response to NMES were thinner, had lower fasting insulin, fasting glucose, and HbA1C levels than the three individuals who responded better to EMS (Poole et al., 2004). This may indicate that healthier individuals with type 2 diabetes may benefit less from NMES compared to less fit individuals. It is therefore possible that overall finding of our study is due to the fact that the participants were fitter participants not in a severe diabetic condition. Although we did not assess fitness levels directly, our exclusion criteria, length of disease, and resting glucose levels suggests a fitter group of participants. Nonetheless, both studies identified responders and non-responders to NMES. A positive correlation between glucose concentration and the intensity of the low-frequency NMES in our study indicated a dose response to NMES. A number of factors might explain this finding: i) stimulation intensity, ii) counter-regulatory hormone response, and iii) timing of glucose ingestion and NMES.

Firstly, although individuals without type 2 diabetes tolerate low-frequency NMES comfortably for up to eight hours (Theriault et al., 1994), one hour of NMES for some individuals with type 2 diabetes might represent an intense muscular activity. During intense exercise, glucose production increases more then glucose utilization (Marliss and Vranic, 2002). ES is a unique type of muscular contraction, very different from voluntary exercise (VE). One potential difference between both modalities is the reverse size principle. In ES, the fast twitch muscles are usually activated along with the slow twitch. Also, glycogen depletion has been found to be more pronounced in electrical stimulation than voluntary contractions at identical low intensities (30 W), and both types I and type II fibers are recruited (Kim, Bangsbo, Strange, Karpakka, and& Saltin, 1995). Moreover, carbohydrate utilization has been compared between ES and VE. When

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individuals performed at identical intensities and duration exercises, a single bout of ES to lower limb muscles stimulated greater carbohydrate utilization than VE. These differences could be due to larger activation of type II fibers in ES compared with VE at the same intensity level. As for fiber type differences in ES, rates of glycogenolysis have been found to be higher in type II fibers, or fast twitch muscles, than in type I fibers or slow twitch muscles (Greemhaff, Soderlund, Ren, and Hultman, 1993). Also, studies in animals have shown that glucose transport is higher in type II fibers than in type I fibers when electrical stimulation is used (Johannsson, Jensen, Gundersen, Dahl., and Bonen, 1996; Roy, Johannsson, Bonen, and Marrette, 1997). A combination of the unique characteristics of ES and altered metabolic profile of skeletal muscles in individuals of type 2 diabetes (Simoneau, and Kelley, 1997) might result in a particularly intense activity and could explain the increased blood glucose concentration noted during the NMES protocol. Studies evaluating muscles strength and fatigue in individuals with type 2 diabetes are very limited. Recent data suggest that individuals with type 2 diabetes have similar contractile function compared with age, gender, and activity matched control (Singh-Peters et al., 2006). This study examined the M-wave, isometric contractile properties and central activation of the tibialis anterior in individuals with non-neuropathic type 2 diabetes. However, another study found a relationship between raised glucose levels, and weaker grip muscles strength in individuals with type 2 diabetes (Sayer- Aihie et al., 2004). To our knowledge, there are no studies quantifying muscle strength and fatigue in the extensor muscles of the legs in individuals with type 2 diabetes. Secondly, the increase in blood glucose concentration might also have been due to an altered counter-regulatory hormonal response. Plasma epinephrine and glucagon

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responses to exercise are higher in individuals with type 2 diabetes than in the control subjects (Kjaer et al., 1990), and longer hyperglycemia periods may occur because of glucose uptake increases less than glucose production. Thirdly, timing between the GTT, NMES protocol, and measurement of glucose tolerance may also have affected our results. We evaluated our subjects immediately after the one-hour NMES protocol. Blood glucose concentration may have drop during the NMES protocol. One study found that blood glucose concentration decreased rapidly fifteen minutes after the beginning of exercise, and then as exercise continued, blood glucose concentration increased (Tokmakidis et al., 1999). Therefore, it is possible that blood glucose concentration may have drop during the initial phase of the NMES protocol, and then increased when we measured blood glucose concentration at time 60 and 120. Also, it is possible that the second phase of glucose regulation, the insulin dependent effect, peaked after we measured the last blood sampling at 120 minutes. At 60 minutes, three individuals showed higher delta difference on the control day compared to the NMES day. After 120 minutes, we observed that six individuals had higher delta differences on the control day. This might indicate that the NMES had more of a post-exercise effect and lowered blood glucose concentration in subjects with type 2 diabetes. In addition, the subjects in our study arrived in a fasted state, and consumed a glucose load of 75g glucose. This method was chosen because it would be similar to evaluating electrical stimulation as a mean of regulating acute meal impact on blood glucose. Poirier et al. (2000) compared diabetic individuals in the fasted and fed state to evaluate the lowering affect of exercise and the timing of meals. After exercise, plasma glucose levels were much lower in the fed state compared to the fasted state. This may be explained by the fact that plasma insulin levels

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were higher in the fed state, and this probably blunted hepatic glucose production, which resulted in higher glucose utilization than production. Therefore, a greater decrease in plasma glucose levels was seen in individuals in the fed state compared with the fasted state (Poirier et al., 2000; Poirier et al., 2001). Exercise of moderate intensity is usually associated with a decreased in plasma insulin (Wasserman, Williams, Lacy, Goldstein, and Cherrington, 1989), an increase in glucagon (Bottger, Schlein, Faloona, Knochel, and Unger, 1972) and in plasma catecholamines (Christensen, and Galbo, 1983). However, in our study, insulin levels might have increased following the glucose load and it has been shown that a decrease in insulin concentration may not be essential for the increase in glyconeogenesis during exercise (Zinman, Vranic, Albisser, Leibel, and Marliss, 1979). It is possible that the increased demand for energy was met by glyconeogenesis by the liver; therefore, increasing blood glucose concentration at time 60. It is also feasible that an hour of low-frequency NMES managed to activate and recruit both type I and type II fibers, and significantly diminish muscles glycogen. NMES is typically associated with an increased fatigue rate in comparison to volitional exercise due to motor unit recruitment. The possible reduction in muscles glycogen and hyperglycemic response might have favored an optimal setting for restoration of muscles glycogen. We might have observed decreased blood glucose concentration after the NMES protocol if the post-exercise GTT would have been performed at a later time.

Although we did not investigate chronic impact, the GTT might also have been used to study the delayed impact of ES on glucose metabolism. Fenicchia et al. (2004) performed their OGTT 12 to 24 hours after the first exercise session, in women with type 2 diabetes in a fasting state. Their exercise protocol consisted of 50 minutes of resistance

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training, 3 nonconsecutive days per week, for a period of 6 weeks. They found that glucose concentration improved 12-24 hours after the first resistance training exercise session.

Delimitations

This study was delimited to the use of low-frequency electrical stimulation on glucose regulation in type 2 diabetes. Individuals with type 2 diabetes were chosen because of the well documented role of contraction induced glucose uptake. Low-frequency electrical stimulation is well tolerated and easily administered by a portable device. Since we wanted to complete the study in a safe and supervised environment, the portability of the unit enabled us to collect data locally in a clinical facility.

Limitations

Although we received excellent support from the local family physician, total recruitment over a six-month period was limited to eight participants. Due to this small sampling size, we were unable to create subgroups to investigate the role of metabolic and lifestyle issues involved in glucose regulation. These issues include physical fitness levels, dietary habits, length of disease, and drug regimens. Due to funding limitations for this initial study, a muscle glucose uptake level was not directly measured and a glucose tolerance test was used to quantify glucose metabolism. The euglycemic clamp method, which would have directly measured peripheral glucose utilization and not hepatic glucose production, would have been favorable.

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Summary

To date, few studies have examined NMES in individuals with type 2 diabetes. These studies have found that some individuals respond to electrical stimulation, while other do not, or have a less pronounced response. Based on studies on rodents, it is believed that a positive correlation between glucose uptake and intensity levels of electrical stimulation is to be expected. However, we found a trend toward increased glucose concentration during NMES, in five participants, which correlated positively with increasing intensity levels of NMES. The positive correlation found in this study between blood glucose concentration and intensity levels of NMES are in disagreement with previous studies performed on rodents. These studies typically found that glucose uptake increases with increasing stimulus intensities (Johannsson, Jensen, Gunderson, Dahl, and Bonen, 1996; Lund, Holman, Schmitz, and Pedersen, 1995; Nesher, Karl, and Kipnis, 1985). Electrical stimulation and glucose regulation has been studied mostly on SCI individuals. These individuals have sensory impairment which allows them to tolerate much higher current than in the present study (120mA vs. 30-60 mA). The positive correlation between glucose concentration and the intensity of low-frequency NMES found in our study might be explained by fatigue mechanism, hormonal response, and/or methodological procedures. In the present study it could not be ascertained whether glucose uptake or hepatic glucose release was changed, resulting in increased glucose concentration in some participants at time 60. It is possible that overall the individuals with type 2 diabetes in our study were too healthy to benefit from NMES. Individuals with more severe diabetes condition or a more controlled stimulation paradigm for a longer duration would be necessary to induce an effect. A continuous

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glucose monitoring system before, during and after exercise might be useful for recording glucose for a longer period. In addition, evaluating lactate concentrations and the percentage of maximal contraction voluntary (MCV) during NMES might help understand the unique fatigue in NMES in individuals with type 2 diabetes.

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