From Centre for Family Medicine,
Department of Neurobiology, Care Sciences and Society,
Karolinska Institutet, Stockholm, Sweden

PHYSICAL ACTIVITY IN NORMAL AND IMPAIRED GLUCOSE TOLERANCE AND TYPE 2 DIABETES MELLITUS

Effects of walking and Nordic walking on health-related quality of life, cardiovascular risk factors, and mitochondrial gene expression in overweight individuals

Tomas Fritz

Karolinska Institutet

Stockholm 2012

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Published by Karolinska Institutet. Printed by Universitetsservice US-AB

978-91-7457-839-3

In summary, NIDDM most often develops in subjects at a rather advanced age, wherein the enthusiasm for a life-long physical exercise regime might be fairly limited …

Is the conclusion then that physical exercise as therapy for NIDDM is theoretically interesting, but practically not feasible?

The questions will then arise about the possibility of reaching sufficient intensity in such a group and whether non-intensive exercise (e.g., walking or golf) is effective. Unfortunately there is very little data on these points.

Professor Per Björntorp (1931–2003), in *Diabetes Care* 1992

(NIDDM = Non-insulin-dependent diabetes mellitus,
today known as type 2 diabetes mellitus.)

ABSTRACT

Background and aim Type 2 diabetes mellitus (T2DM) is a disease associated with the risk of severe cardiovascular complications. Genetic predisposition, a sedentary life-style and overweight may increase the risk of developing T2DM. The aim was to study the effects of physical activity on risk factors of cardiovascular disease, health-related quality of life, and on the gene expression of enzymes involved in glucose and lipid metabolism, in overweight people with T2DM, impaired or normal glucose tolerance.
Methods Two different exercise intervention studies were conducted, both for a four-month period. Study 1, presented in paper I, included 52 T2DM patients, 26 controls, 26 in an intervention group. The intervention was to increase physical activity by brisk walking, 45 minutes three times per week. At baseline and after four months we assessed systolic (SBP) and diastolic (DBP) blood pressure, body mass index (BMI), glucose and lipid metabolic parameters, self-reported physical activity and physical fitness. Study 2 (papers II, III and IV) included 212 overweight individuals. The intervention was a weekly physical activity increase by 5 hours of walking with walking poles (Nordic walking). The participants were classified by an oral glucose tolerance test (OGTT) with normal glucose tolerance (NGT), impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM), and randomized into a control group (n=125), or an exercise intervention group (n=87). Health-related quality of life (HRQoL) was recorded by questionnaire (paper II), and anthropometric and clinical data (papers II & III) were assessed at the time of inclusion and after four months. From 79 NGT and 33 T2DM male exercise participants a 20–100 mg biopsy was taken from the quadriceps muscle of the thigh, for the assessment of messenger RNA (mRNA) expression of mitochondrial genes, coding for enzymes involved in glucose and fatty acid metabolism (paper IV).
Results In study 1 there were no significant improvements of anthropometric parameters, physical fitness, blood pressure, glucose or lipid metabolism. The 17 patients in the intervention group who attained ≥80% of the intended increment of physical activity significantly improved SBP, DBP, BMI and total plasma cholesterol, compared with the control group. In study 2 (papers II & III) quality of sleep, body weight, BMI and waist circumference were improved for NGT exercise participants, and in the IGT exercise group exercise capacity improved. Among the exercise participants ≥80% compliant with the scheduled time of Nordic walking, exercise capacity improved significantly in all three (NGT, IGT and T2DM) exercise groups. Blood pressure, glycaemic control and blood lipids were unaffected. Baseline mRNA expression of 3 mitochondrial genes was increased in the T2DM group (paper IV). In the NGT group the expression of the enzyme PDK4 was increased after the exercise period, but not in the T2DM group.
Conclusions The exercise participants ≥80% compliant with the exercise goals in paper I improved SBP, DBP and BMI. The exercise goal of Nordic walking, 5 hours per week, led to improved quality of sleep, body weight, BMI and waist circumference in the NGT group, and exercise capacity improved in the IGT group. The elevated baseline PDK4 expression and the unaltered post-exercise expression in the T2DM cohort may reflect an impaired utilization of glucose and lipid fuels inherent in T2DM, and a dysfunction of the appropriate adaptive responses to exercise in skeletal muscle associated with insulin resistance.

CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following original articles, which will be referred to in the text by their Roman numbers. Paper I is based on study 1, whereas papers II, III and IV are based on results obtained from participants in study 2.

I Tomas Fritz, Per Wändell, Hans Åberg and Peter Engfeldt. Walking for exercise – does three times per week influence risk factors in type 2 diabetes? Diabetes *Research and Clinical Practice* 2006; 71: 21–27.

II Tomas Fritz, Kenneth Caidahl, Megan Osler, Claes-Göran Östenson, Juleen Zierath and Per Wändell. Effects of Nordic walking on health-related quality of life in overweight individuals with type 2 diabetes mellitus, impaired or normal glucose tolerance. *Diabetic Medicine* 2011; 28: 1362–1372.

III Tomas Fritz, Kenneth Caidahl, Anna Krook, Petra Lundström, Fredirick Mashili, Megan Osler, Ferenc Szekeres, Claes-Göran Östenson, Per Wändell and Juleen Zierath. Effects of Nordic walking on cardiovascular risk factors in overweight individuals with type 2 diabetes, impaired or normal glucose tolerance. In manuscript, accepted for publiction in *Diabetes/Metabolism Research and Reviews*.

IV Sameer S. Kulkarni, Firoozeh Salehzadeh, Tomas Fritz, Juleen R. Zierath, Anna Krook and Megan E. Osler. Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus. *Metabolism Clinical and Experimental* 2012; 61: 175–185.

ABBREVIATIONS AND DEFINITIONS

BMI	Body mass index
DBP	Diastolic blood pressure
HbA1c	Glycated haemoglobin
HRQoL	Health-related quality of life
IGT	Impaired glucose tolerance
LADA	Latent autoimmune diabetes in adults
MID	Minimally important differences
mRNA	Messenger RNA
NGT	Normal glucose tolerance
PRO	Patient-reported outcome(s)
SBP	Systolic blood pressure
T2DM	Type 2 diabetes mellitus

INTRODUCTION

Diabetes mellitus is a chronic disease signified by a pathologically increased level of glucose in the blood (hyperglycaemia). This diagnosis comprises several conditions of different pathogenic origins. The underlying cause of hyperglycaemia is either lack of insulin or the impaired effect of insulin in target tissues.

CLASSIFICATION

Diabetes is primarily classified as type 1, type 2, gestational or secondary diabetes. The focus in the studies presented in this thesis is on type 2 diabetes only, but the other main types of diabetes are briefly described below.

Type 1 diabetes and LADA

Type 1 diabetes is an autoimmune disease caused by the formation of antibodies directed against the insulin-producing beta cells of the pancreas. The destruction of beta cells caused by the antibodies leads to the cessation of endogenous insulin production. The treatment of type 1 diabetes is therefore daily insulin injections. The onset is typically in childhood or early adolescence, but it can develop at any age, and hyperglycaemia develops rapidly, and to levels that can be life-threatening if not promptly treated. Latent autoimmune diabetes in adults (LADA) is an autoimmune type of diabetes affecting adults, usually with a more gradual development of insulin deficiency than is the case in "classical" type 1 diabetes. Insulin treatment may therefore not be necessary at the onset of LADA, and oral glucose lowering agents may initially suffice to maintain good glycaemic control. However, insulin deficiency usually occurs in one or a few years' time.

Type 2 diabetes

Type 2 diabetes has a more insidious onset than type 1 diabetes, and blood glucose rarely reaches life-threatening levels. It has by tradition been considered a disease of middle or old age. In recent years, however, type 2 diabetes has become a frequent diagnosis also in young people, particularly in the United States of America [1,2]. Type 2 diabetes is caused by a combination of resistance to the metabolic effects of insulin in various tissues and an insufficient insulin secretion [3]. The result is impaired glucose metabolism and hence a fuel deficit in cells that depend on glucose as a major source of energy. The level of circulating glucose in the blood is elevated but unavailable to the cells of various organs. The diabetic condition has therefore been characterized as "starvation in the midst of plenty".

Insulin resistance seems to appear early in the development of this disease. Skeletal muscle, liver and adipose tissue are the main organs affected by insulin resistance. A pre-diabetic state of impaired glucose tolerance and a low degree of hyperglycaemia (impaired fasting glucose) may be present for a long period of time before the onset of overt diabetes and may cause changes in various tissues, but without clinical symptoms. At the time of inclusion in the United Kingdom Prospective Diabetes Study, 38% of

3041 newly diagnosed type 2 diabetes patients had developed diabetes retinopathy to some degree [4]. A family history of T2DM, old age, a sedentary lifestyle and obesity are all associated with an increased risk of developing T2DM. In addition to obesity and deranged glucose metabolism, T2DM is often associated with elevated blood pressure (hypertension) and a disturbed metabolism of blood lipids (dyslipidaemia). The term “metabolic syndrome” has been used to draw attention to this cluster of cardiovascular risk factors, which is often present in patients with type 2 diabetes. It is not known whether these factors interact or whether the metabolic syndrome per se implies an increased risk of cardiovascular disease and the development of type 2 diabetes [5].

Lifestyle intervention, involving physical activity and reduced caloric intake, has been shown to prevent, or delay, the development of impaired glucose tolerance into overt T2DM [6-8]. Moreover, other studies have shown that people with and without T2DM who are physically active, or who adopt a more physically active lifestyle, have a longer life expectancy[9-12]. In a joint position statement the American College of Sports Medicine and the American Diabetes Association conclude that regular exercise is considered to play “a major role in the prevention and control of insulin resistance, pre-diabetes, gestational diabetes mellitus, type 2 diabetes and diabetes-related complications” [13].

Gestational diabetes

Gestational diabetes develops during pregnancy. It is caused by insulin resistance and not by an autoimmune reaction. It is thus classified as type 2 diabetes and usually subsides after delivery. Women who have had gestational diabetes are at high risk of developing type 2 diabetes later in life [14].

Secondary diabetes

Secondary diabetes can be caused by various factors, other than insulin resistance or the formation of autoantibodies. Diseases of the pancreas, predominantly pancreatitis, may result in extensive destruction of insulin-producing beta cells and the development of insulin-dependent diabetes. Various drugs, particularly corticosteroids, may after long-term treatment cause the development of T2DM as an undesired side effect.

Insulin and its effects

Insulin is synthesized in the beta cells of the islets of Langerhans in the pancreas. It is released from the beta cells into the blood, and the amount of insulin released is regulated by the levels of blood glucose. Insulin is the most important anabolic hormone in the human body. Its effects are of vital importance for the regulation of carbohydrate, lipid and protein metabolism. The main target organs for the effects of insulin are skeletal muscle, the liver and adipose tissue. Insulin mediates the uptake of glucose from the blood into the cells of the various organs, where glucose is utilized as fuel for metabolic processes. Glucose is, in fact, the main substrate for energy production in the human body. When bound to an insulin receptor in the cell membrane of the target organ, insulin mediates several intracellular effects. A glucose transporter protein (GLUT) facilitates the uptake of glucose across the cell membrane, into the cell.

There are 13 different isoforms of glucose transport proteins and GLUT4 is predominant in skeletal muscle cells [15]. Translocation of GLUT4 from the intracellular cytosol to the cell membrane, where it exercises its effect, is an insulin effect but GLUT4 translocation is also an effect of muscle contraction [16]. Furthermore, insulin triggers a cascade of intracellular enzymatic reactions involved in the oxidation of glucose. In adipose tissue the anabolic effect of insulin is mediated by its lipolytic effects and the storing of triglycerides.

Impaired insulin sensitivity, or insulin resistance, is a metabolic disturbance that precedes the development of overt type 2 diabetes [17]. Although the nature of insulin sensitivity is not fully understood it can be measured [18], and it is well established that it can be enhanced by regular physical exercise [19]. Exercise improves the redistribution of GLUT4 to the cell membrane, a factor that seems to contribute to improved insulin sensitivity, whereas this GLUT4 translocation to the cell membrane is impaired in insulin resistance [20]. Furthermore, the dysregulation of certain enzymes involved in the oxidation of carbohydrates and fatty acids in the mitochondria of the skeletal muscle cell may contribute to insulin resistance [21]. Diminished activity of several oxidative enzymes present in the mitochondria, and involved in carbohydrate and lipid oxidation, has been described [21]. This impaired lipid oxidation causes an accumulation of triglycerides in skeletal muscle cells which seems to be correlated with the degree of insulin resistance [22]. Healthy skeletal muscle cells with normal insulin sensitivity have the capacity for a metabolic shift from utilizing glucose as energy substrate in the fed, insulin-stimulated state, to the use of fatty acids as a fuel source in the fasting state. This metabolic flexibility between carbohydrates and lipids as sources of energy is less pronounced in insulin-resistant skeletal muscle, indicating the impairment of both carbohydrate and lipid metabolism in insulin resistance and type 2 diabetes [23]. At the onset of type 2 diabetes insulin sensitivity is impaired, as is the endogenous production of insulin. Initially it is the rapid elevation of plasma insulin levels following the ingestion of food that is impaired. With increasing diabetes duration the beta cell function tends to diminish with a general decline of insulin production. This may develop, over a period of several years, into the cessation of insulin production.

TYPE 2 DIABETES

Diagnosis

Diabetes is diagnosed by the assessment, on two different occasions, of elevated plasma glucose levels, measured in the fasting state. According to the current World Health Organization (WHO) diagnostic criteria, plasma glucose levels between 6.1 and 6.9 mmol/l are classified as impaired fasting glucose, whereas ≥7.0 mmol/l is a diabetic level. Diabetes can also be diagnosed by an oral glucose tolerance test (OGTT), performed after an overnight fast. After assessing fasting plasma glucose a solution of 75 g of glucose is ingested. A second plasma glucose assessment is performed after two hours. A two-hour OGTT value of ≥11.1 mmol/l is diagnostic of diabetes (≥12.2 mmol/l if capillary blood samples are being used). The plasma glucose level at the time of diagnosis does not indicate the type of diabetes that has been diagnosed.

Epidemiology

The prevalence of diabetes shows considerable geographic variation and increases in an ageing population. Since the end of the 20th century type 2 diabetes has been increasing on a global level, a phenomenon that has been named the diabetes pandemic. This rapid development is most pronounced in the Middle East, Asia, Africa and South America and is probably caused by a “westernized lifestyle” comprising less physical activity and excessive dietary intake of fat and carbohydrates. Overweight and/or obesity are known to be strong risk factors for T2DM in both men and women[24,25]. WHO estimates indicate that the prevalence of diabetes in adults will rise on a worldwide basis from 135 million in 1995 to 300 million in 2025 [26]. More recent data indicate that the global diabetes prevalence will continue to increase, even if levels of obesity remain constant, and the number of people with diabetes is estimated to rise to 366 million in 2030 [27].

An increasing number of young persons with pronounced adiposity develop type 2 diabetes, particularly in Japan and the United States [1,2]. This is an alarming development that may become a global reality due to the ongoing increase of mean BMI in the western world as well as in developing countries, a tendency that applies to all age groups.

T2DM is evenly distributed between males/females in most populations. During the first half of the 20th century T2DM was more prevalent among females, but this has shifted towards a more equal sex distribution. In some populations a male preponderance was noted during the latter half of the 20th century, possibly as a consequence of the increasing obesity in men, characterized by abdominal fat distribution [28].

The total prevalence of diabetes in Sweden is approximately 4.5% according to recent surveys, with a slight dominance of men [29,30]. In the age group >75 years the prevalence was 16% in one survey [29]. Type 2 diabetes represents 85–90% of all diabetes in Sweden. It is usually a disease with mild or no symptoms at all, unless blood glucose rises to very high levels or acute cardiovascular complications occur. The patient with type 2 diabetes can therefore be undiagnosed for a variable amount of time, probably for years in some cases. The prevalence of type 2 diabetes in a geographic area in our country most likely depends on whether or not active screening for this disease is performed in the area.

Complications

Type 2 diabetes is a multifaceted disease that entails the risk of impaired function and medical complications in several organs of the human body. The basis of such complications is atherosclerotic changes in blood vessels and subsequent impairment of blood circulation in affected organs. Hyperglycaemia, hypertension and dyslipidaemia often co-exist in type 2 diabetes and constitute the three central risk factors of late diabetes complications, so called since they often occur after years of diabetes duration. Most common in type 2 diabetes are the macro-vascular complications, emanating from the great blood vessels, affecting the heart and central nervous system, causing angina pectoris, acute myocardial infarction or stroke. These complications are often disabling or lethal in their consequences. The micro-vascular complications, emanating from

capillaries, may affect the retina of the eyes, the kidneys and the feet, causing visual impairment, renal failure and ulcerations of the feet.

Management

It has hitherto not been possible to find a curative treatment of type 2 diabetes, and the three main aims of diabetes care are therefore the absence of hyperglycaemic symptoms, the maintenance of good quality of life and the prevention of late diabetes complications. Regulating blood glucose [4], blood pressure [31] and blood lipid levels [32] to as near normal as possible reduces the risk of diabetes complications. Medical monitoring of type 2 diabetes in Sweden is mainly undertaken in primary health care. Primary health care therefore has an important function in educating and supporting patients with type 2 diabetes in disease management, as well as in preventing the development of T2DM in patients at risk.

Management of type 2 diabetes comprises a combination of pharmacological treatment and lifestyle alterations. It is therefore important to assess the possible effects of non-pharmacological aspects of diabetes treatment and to develop methods of conveying to the patient possible means of individually adapted lifestyle alterations.

Changes of dietary habits and increased regular physical exercise are lifestyle changes most commonly called for in type 2 diabetes. It is often difficult, however, to attain satisfactory levels of blood glucose, blood pressure and blood lipids by means of lifestyle alterations only. In the Steno-2 study it was possible to improve the levels of blood glucose, blood pressure and blood lipids, as well as the risk of cardiovascular complications, in patients with type 2 diabetes during 7.8 years of intensive medication [33]. With increasing duration of type 2 diabetes, the difficulty of maintaining satisfactory blood glucose control usually becomes more pronounced.

Pharmacological treatment

Pharmacological treatment of type 2 diabetes involves oral drugs that enhance the endogenous production of insulin or drugs that improve insulin sensitivity, often in combination. Incretins represent a class of anti-diabetic drugs that have been recently introduced for clinical use. They enhance postprandial insulin secretion and inhibit gastric motility. This delays and protracts carbohydrate absorption and contributes to a satiating effect. Incretin drugs can be administered orally (Dipeptidyl peptidase-4 inhibitors) or as subcutaneous injections **(**Glucagon-like peptide-1 analogues) [34]. With decreasing insulin production oral medication becomes less efficient in maintaining adequate blood glucose control. Insulin injections are then the recommended mode of treatment. Patients with type 2 diabetes usually require higher doses of insulin than patients with type 1 diabetes. This is due to the presence of insulin resistance in type 2 diabetes, which does not occur in type 1 diabetes. It has not been possible to prevent, or slow down, the progression of insulin resistance or the diminishing insulin production in type 2 diabetes. Despite high doses of oral anti-diabetic drugs or insulin, given as subcutaneous injections, it may be difficult to attain adequate blood glucose levels with an increasing duration of type 2 diabetes.

Non-pharmacological treatment

Physical exercise

A sedentary lifestyle seems to be a contributory factor in the development of insulin resistance and type 2 diabetes [6,8,35,36]. Skeletal muscle cells at work derive most of their energy from the oxidation of glucose [19]. The amount of physical activity therefore plays an important role in glucose metabolism. At the same time our knowledge is insufficient as to the amount – in terms of time, intensity and mode – of exercise that is required to obtain measurable health effects.

Regular physical exercise is known to have cardiovascular risk-reducing effects in the treatment of type 2 diabetes, hypertension and hyperlipidaemia [37-39]. Studies on exercise in diabetes have, however, often been performed on relatively young persons and involved rather intensive exercise programmes. Many patients with type 2 diabetes are unaccustomed to exercising, middle-aged or elderly and overweight. A 6 month structured exercise and support programme was not effective in increasing physical activity in sedentary patients with type 2 diabetes, aged 60±8 years [40]. In an article reviewing 14 studies of exercise in obese patients with type 2 diabetes, it was concluded that glucose metabolic control did not improve in type 2 diabetes patients aged 57 to 61 years, as it did in type 2 diabetes patients aged 40 to 54 years [41]. Sedentary type 2 diabetes patients thus appear to be less prone to start exercising at age ≥60 than at the age of 40 to 50+. On the other hand, two studies with participants aged 54–58 years have reported good adherence to one 3-year diet-and-exercise intervention and one 12-week walking programme [42,43]. Anthropometric and metabolic parameters were improved in both studies and in the walking study estimated VO_{2max} improved as well.

This large group of patients could probably benefit in several respects from adopting habits of regular physical activities that are adapted to their abilities. Providers of diabetes care may have limited practical knowledge and lack the methodological routines required to help patients to establish feasible exercise habits and to demonstrate the different effects of exercise activities [44]. It is therefore important to obtain practical experience of the effects of low or medium intensive exercise in the treatment of type 2 diabetes.

Dietary recommendations

Current Swedish dietary guidelines for diabetes patients [45] recommend a restriction of saturated fat to 10 E% and carbohydrates to 50–60 E%. The subject of recommended proportions of carbohydrate versus fat intake has caused substantial controversy over the past few years. The scientific evidence has been considered to be insufficient for a definite recommendation of the preferred intake of carbohydrates and fat in the general population as well as in type 2 diabetes.

SIGNIFICANCE TO PATIENTS AND COMMUNITY

Patients

Primary health care has an important task in counselling patients with type 2 diabetes so that they can manage their disease in an optimal way, thereby reducing the risk of late diabetes complications. It is also important to develop cost-effective means for the management of type 2 diabetes. If diabetes complications can be prevented, much human suffering can be avoided, as well as expensive efforts at medical rehabilitation. Furthermore, patients with type 2 diabetes experience lower health-related quality of life (HRQoL) than the general population [46,47]. Low HRQoL in type 2 diabetes is due mainly to the presence of diabetes complications. Medication as well as alterations of lifestyle may, however, also impair HRQoL. It is therefore important to consider aspects of quality of life when making recommendations for diabetes treatment. It is well known that the development of T2DM can be prevented, or delayed in people with impaired glucose tolerance [6,8]. This is a group of patients that can easily be identified in primary health care, and one that can benefit from professional counselling on the diabetes-preventive effects of regular exercise and weight reduction. In one study it was reported that only 25% of patients with type 2 diabetes discussed matters of exercise with health care professionals. Very few patients reported receiving specific guidelines regarding the type, frequency or duration of exercise [44].

Costs of diabetes care

Direct medical costs for the care of patients with type 2 diabetes in Sweden were estimated at approximately 7 billion SEK in 1998 according to the CODE-2 study [48]. CODE-2 compared the costs of medical care of type 2 diabetes in eight European countries. The number of Swedish patients included in the study was 777. The annual per-patient cost was estimated at 25,000 SEK, representing 6% of Swedish health care expenditure in 1998. The largest share (42%) represented costs for hospital care whereas 31 and 27% represented costs for ambulatory care and medication, respectively. Treatment costs were more than three times higher if the patients had micro- and macro-vascular complications.

Lifestyle alterations in patients – a challenge to medical care providers and epigenetic research

The initial cause of my personal interest in type 2 diabetes and physical activity was a frustration with the difficulties of helping my patients to make lifestyle changes. It is a common experience in diabetes care that lifestyle changes concerning physical activity and dietary habits are not easily attained. Alterations of lifestyle cannot be prescribed in the same manner as medication. Motivation is more crucial to the implementation of lifestyle alterations than it is to medication. Motivation to increasing the amount of physical activity ideally ought to involve the demonstration of positive effects following exercise activities. In a public health perspective such effects should be measurable after a reasonably short period of regular exercise that can be performed by people not previously accustomed to exercising.

In a pilot study we demonstrated an acute blood glucose lowering effect of 2.2 mmol/l following brisk walking [49]. This post-walk decrement is short-lived, however, and blood glucose levels rise promptly after the ingestion of food. From a motivational point of view it would be relevant also to demonstrate to patients the possible positive long-term effects of regular walking on the different cardiovascular risk factors known to be connected with type 2 diabetes.

Since type 2 diabetes is mostly an asymptomatic disease, provided that complications have not occurred and blood glucose levels are not excessively elevated, the patient seldom experiences any physical incentives to strict diabetes control. For that reason I also wished to investigate the effects on quality of life in connection with a period of regular physical activity.

Limiting factors to physical exercise, such as pain in joints and musculature [50], high age and being unaccustomed to exercising are common among patients with type 2 diabetes. Medium or low intensity exercise should therefore be preferred when introducing most type 2 diabetes patients to physical activity. In a study of a 1-year diet and exercise intervention in obese people aged ≥65 years, 93 (87%) of 107 participants completed the study [51]. The mode and intensity of exercise were adapted to the individual abilities of the participants. The demonstration of positive health effects following a period of regular exercise could provide pedagogical support in the implementation of practically oriented methods of education for patients with T2DM, or for people at risk of developing this disease. Reliable methods for demonstrating easily measurable effects of low/medium intensity exercise are lacking at present.

The development of genetics in recent years and the technology of assessing the degree to which a gene is being expressed have opened new fields for research. The specific area of genetic research known as epigenetics describes how the expression of genes can be increased or decreased by factors other than changes in the sequence of nucleotides in the DNA molecule. Physical exercise is one factor that can alter the expression of certain genes involved in substrate metabolism for energy production.

Mitochondria are intracellular organelles that play a central role in the continuous generation of energy in the cells. In diabetes research it has been possible to identify genes coding for the formation of mitochondrial enzymes that are involved in glucose and fatty acid oxidation. Future studies of mitochondrial gene expression may contribute to the understanding of the mechanisms involved in the development of insulin resistance and deranged glucose utilization in T2DM.

AIMS

MAIN AIM

The main aim was to study the effects of regular walking (Study 1), and Nordic walking with walking poles (Study 2), on cardiovascular risk factors in overweight people with type 2 diabetes mellitus and in those with impaired and normal glucose tolerance. The parameters studied were glucose metabolic control, insulin resistance, serum lipids and blood pressure, as well as anthropometric measurements (papers I and III).

SECONDARY AIMS

To assess the effects of Nordic walking (Study 2), on

- self-reported health-related quality of life (paper II).
- mitochondrial gene expression in overweight patients with T2DM and in individuals with normal glucose tolerance (paper IV).

None of the studies included any intervention in dietary habits.

MATERIALS AND METHODS

In study 1 patients with type 2 diabetes were enrolled from two different primary care practices in Gustavsberg and Saltsjö-Boo, two suburban communities outside Stockholm, Sweden. Public meetings, advertised in the local press, were arranged in the two communities and attendants were invited to participate. An additional 67 patients with type 2 diabetes were invited to participate when visiting their physician and 100 by a personal letter of invitation. Patients with severe angina pectoris, physical disability or insulin treatment were excluded. We chose to invite the participants to the two groups from different communities in order to diminish the possibility that the patients in the control group would start exercising as they learned about the activities in the intervention group. This was therefore not a randomized study.

Twenty-seven patients consented to participating in the intervention group and 31 in the control group. One patient in each group declined further participation shortly after being included in the study. Four patients in the control group were lost to follow-up, despite repeated reminders. Thus, the intervention group and the control group both consisted of 26 patients.

The patients in the intervention group were instructed to increase their exercise by 45 minutes of brisk walking, three times weekly, for four months. We expected this to be achievable by most patients. Four months was considered to be a sufficient period to allow for changes of the parameters under study. Walking groups were provided four times per week. Walking diaries and a physical activity questionnaire made it possible to identify those individuals who changed their level of exercise. No recommendations were given concerning changes in dietary habits, and medication remained unaltered in both groups. The patients in the control group were informed that their results were to be used as reference values for diabetes patients over a period of four months when no alterations of lifestyle or medication were undertaken. The participants were examined at the time of inclusion and after four months. Resting systolic (SBP) and diastolic (DBP) blood pressures and body mass index (BMI) were assessed. Age-adjusted physical fitness was determined by bicycle ergometry as a submaximal exercise test. Blood samples were analysed for fasting plasma glucose, insulin, glycated haemoglobin (HbA1c) and lipid levels (total cholesterol, HDL and LDL cholesterol and triglycerides). Insulin resistance was obtained from the fasting glucose and fasting insulin levels as HOMA-IR (Homeostasis Model Assessment) [52]. We used the formula HOMA IR=fasting insulin (μU/ml × fasting glucose (mmol/l)/22.5 or the computerized HOMA calculator for this purpose [53].

Blood samples and measurements of other variables in this study were obtained within 24–48 hours after the last exercise walk.

In study 2 we included 213 individuals without severe physical or cardiovascular impairments aged 60±5.3 years, with body mass index (BMI) 30.2±3.8 kg/m^2. Numbers are mean±SD. Papers II, III and IV were based on the results of study 2. Recruitment was achieved by advertisements in the local newspapers and by letters of personal invitation to 447 former participants in the Stockholm Diabetes Prevention Programme [54]. Based on an oral glucose tolerance test (OGTT), the participants were classified with normal glucose tolerance (NGT, n=128), impaired glucose tolerance

(IGT, n= 35) or type 2 diabetes mellitus (T2DM, n= 50). They were randomized to a control group with unaltered physical activity or to an exercise group performing five hours per week of Nordic walking with poles, for a four-month period. For the randomization procedure, blinded labels with the participants' names were drawn from a box and assigned to either the control or intervention group. The study spanned three consecutive summer seasons (2006–2008), between May and September. Each year new entries to the study were randomized into control (unaltered physical activity) or intervention (Nordic walking) group. Individuals randomized to the control group were invited to participate in the intervention group the following year. Those who joined the study in both control (first entry) and intervention (second entry) groups are represented only once in this work, as control subjects, skewing the control/exercise group numbers. The control group consisted of 126/213 individuals. The intervention group consisted of 87/213 individuals. The use of specially designed, rubber-tipped walking poles activates the upper limbs while walking. In three different trials the effect of this additional muscle recruitment was an increase of oxygen consumption, energy expenditure and heart rate, compared with walking without poles. Two trials were conducted on a treadmill [55,56] and one on a level 200-m track [57]. Despite the increased oxygen consumption and energy expenditure, the participants in two of the trials did not report a higher rating of perceived exertion after Nordic walking than after walking without poles.

Anthropometric measurements, blood chemistry, blood pressure, glucose tolerance, glycaemic control, insulin resistance and plasma lipids were assessed, and medication for diabetes, hypertension and dyslipidaemia was recorded at the time of inclusion and after four months.

HEALTH-RELATED QUALITY OF LIFE ASSESSMENT

HRQoL was assessed at the time of inclusion and after four months.

A quality-of-life questionnaire was provided at the time of inclusion and after four months. The Swedish HRQoL questionnaire (SWED-QUAL) [58], adapted from the Medical Outcomes Study (MOS) [59], was used. MOS assessments are generic (as opposed to disease-specific) HRQoL questionnaires that present results as a health profile. The 66 items of SWED-QUAL provide the basis for 13 scales that represent the following aspects of HRQoL: physical functioning, satisfaction with physical health, pain, role limitation due to physical health, role limitation due to emotional health, positive affect, negative affect, cognitive functioning, sleep, general health perception, family functioning, marital functioning and sexual functioning. The multi-item scales are set between 0 and 100 points, where 0 indicates the lowest possible score and 100 the highest. Inter-group differences of SWED-QUAL results are expressed in terms of statistical significance. Effect size (ES) is a complementary method for identifying minimally important differences (MID) of patient-reported outcomes (PRO). The MID is defined as the smallest change in a PRO that is perceived by the patient as beneficial. The effect size is defined by the formula: Effect size = $(M_{treatment} - M_{comparison})/SD_{pooled}$. An effect size of 0.2 is considered small, 0.5 moderate, and 0.8 is a large change [60]. An effect size of >0.4 is considered clinically relevant. SWED-QUAL has been used in previous studies including patients with T2DM [61,62]. A random sample of 2500 Swedish men and women, aged 18 to 85 years, has previously answered the

SWED-QUAL questionnaire [58]. This was done in 1991. Health status and anthropometric data are not known for this Swedish population sample (SPS). The SWED-QUAL results of each study participant were matched, and compared with, the aggregated results of all SPS individuals of the same age and sex. One participant did not complete the SwedQual questionnaire. This explains the difference in the number of participants (212 in paper II and 213 in paper III).

PHYSICAL ACTIVITY AND EXERCISE CAPACITY ASSESSMENT

Physical activity was self-reported. The study participants were asked to indicate, in a questionnaire, an estimate of habitual low, medium and high intensity physical activities. The question was: How often do you perform physical activities more than 30 minutes at a time? Low, medium and high intensity physical activities were specified as low = not strenuous (e.g. bowling golf, slow walk, slow bicycle ride, light gardening), medium = strenuous but still allowing you to continue a conversation (e.g. tennis, dancing, riding, brisk walk, strenuous gardening) and high = strenuous enough to cause sweating, increased heart rate and pronounced shortness of breath (e.g. aerobics, workout, jogging, fast bicycle ride). Alternative answers were: never, irregularly, once a week, twice a week, > 2 times a week. The questionnaire has previously been used in the Stockholm Diabetes Prevention Programme [54]. The participants were also asked to estimate their physical activity level during the six months prior to the study and during study participation using a visual analogue scale (VAS) to rank the frequency of physical activity from 0 mm (none) to 100 mm (intensive daily activity). The duration and date of each Nordic walking bout were recorded in an exercise diary kept by the participants in the exercise group. To compare self-reported physical activity with an objective assessment, 25 consecutive participants (n=11 from the control group and n=14 from the intervention group) agreed to wear an accelerometer (ActiGraph model GT1M; ActiGraph, Pensacola, Florida, USA), in a belt around the waist, for seven days from morning to bedtime, shortly after randomization [63]. Physical activity was recorded as total activity counts per minute, minutes per day of inactivity, low, moderate, or vigorous activity. Peak oxygen uptake (peak VO_2) was assessed at the time of inclusion and after four months. Exercise tests were performed using an ergometer bicycle. The initial work load was set at 50 W and was increased by 10 W/min. An ergospirometer was used to measure gas exchange. Oxygen uptake (VO_2) was calculated breath by breath, and the mean value for the latest 30 seconds was used. The VO_2 at peak exercise (peak VO_2) was determined at the point of subjective exhaustion.

SKELETAL MUSCLE GENE EXPRESSION ASSESSMENT

A total of 112 individuals, with NGT or T2DM, who underwent the exercise intervention, provided biopsies from the vastus lateralis of the Quadriceps muscle of the thigh before and after 4 months of Nordic walking. They were matched for age (61±5 years) and BMI (30±5kg/m^2) with NGT (n=79) or T2DM (n=33). The aim of this study, paper IV, was to compare the expression of messenger RNA (mRNA) of four mitochondrial enzyme proteins involved in the intracellular energy regulation of

glucose and fatty acid metabolism. The balance between glucose and fatty acid metabolism is determined by a number of genes that regulate the synthesis of enzymes that are necessary for the chain reactions leading to the oxidation of glucose and fatty acids. Pyruvate Dehydrogenase Kinases (PDKs) is a group of enzymes that reduce the rate of carbohydrate oxidation. The PDK isoforms primarily expressed in skeletal muscle are PDK2 and PDK4. PDK4 mRNA expression is increased in human skeletal muscle during exercise. Malonyl-CoenzymeA Decarboxylase 1 (MCD1) and Carnitine Palmitoyltransferase 1b (CPT1b) are both involved in the coordination of fuel balance between glucose and fatty acid metabolism during exercise and in metabolic disease. CPT1b has been linked to the regulation of insulin sensitivity and exercise has been shown to increase the expression of MCD1 in human muscle [64,65].

STATISTICS

Stata statistical software (StataCorp., College Station, TX, USA) was used for all statistical analyses in papers I, II and III. In paper IV SPSS version 17.0 (SPSS, Chicago, IL, USA) was used. Comparisons were considered statistically significant at $p<0.05$ in papers I–IV.

Paper I

A power calculation, based on the assumed differences in HbA1c of 0.8% between the control and intervention groups, with SD 1.0, $\alpha=0.05$ and power=0.8, indicated that 25 participants would be required in each group. We aimed at including 30 individuals in each group, to compensate for participants who might leave the study before it was finished. Data are presented as mean±SD or mean (95% CI). Paired t-test was used for the analysis of differences within groups and two-sample t-test was used for differences between groups.

Paper II

A power calculation, based on the assumed differences in SWED-QUAL scales of 0 vs 5 between the control and intervention groups, with $\alpha=0.05$ and power=0.8, indicated that 63 participants would be required in each group. With the skewed randomization, explained above, a power =0.8 would be obtained by 77/54 participants. In the NGT group we included 75/53 individuals (= power 0.7957). The study was underpowered for IGT (20/14) and T2DM (30/20) participants. Differences within and between groups were calculated by non-parametric tests; Wilcoxon's signed-rank test for within-group comparisons and Wilcoxon's rank-sum test for between-group comparisons. Therefore, the score for each quality-of-life scale in the SWED-QUAL questionnaire was calculated as the median (25th;75th percentile) of the items (questions) included in each of the 13 scales. Sex differences between groups were calculated by χ^2 test. Spearman's rho was determined for the assessment of correlation.

Paper III

Data are presented as mean±SD. Within-group differences were analysed by paired t-test and for the analysis of between-group analysis two-sample t-test was used. Differences of sex distribution, medication and physical activity levels were analysed by χ^2 or Fisher's exact test. Analyses in papers II and III were performed as intention-to-treat. Follow-up data were missing for 10 participants who left the study after inclusion. The principle of last-observation-carried-forward was applied to those individuals and baseline data were therefore also included in the statistical analysis as follow-up data.

Paper IV

Data are presented as mean±SEM and a two-sample t-test was used for the analysis of baseline differences in gene expression between the NGT and the T2DM groups. Pearson correlations were calculated for baseline measurements of gene and clinical parameters. Regression models controlled for covariates (age, sex and BMI) were used to examine clinical predictors of gene expression. For the analysis of the fold-change of gene expression following exercise intervention, a paired t-test was used. Natural log transformation was applied when data were not normally distributed.

RESULTS

STUDY 1, PAPER I

Baseline characteristics did not differ between the control and the intervention groups. Although mean SBP at baseline was markedly higher in the control group than in the intervention group, 154.8±18.6 vs 146.9±13.5 mmHg, this difference was not statistically significant by 2-sample t-test (p=0.09). Self-reported exercise at baseline exceeded 3 × 45 minutes of walking per week, by 15 patients in the control group and by 11 in the intervention group. Exercise was increased in the intervention group by 1.2 (0.7 to 1.7) hours per week and in the control group exercise was increased by 0.2 (–0.6 to 1.0) hours per week. Data are mean (95% CI). Nine patients in the intervention group did not alter their exercise habits. Six of them remained on a low physical activity level and three maintained their pre-study level of exercise, which was higher than what was prescribed in the study protocol.

There were no significant changes of parameters reflecting glucose metabolism, i.e. HbA1c, fasting plasma glucose or insulin levels. In the intervention group there was a tendency towards a decrease in SBP and DBP. In the control group the tendency was towards a rise in SBP and DBP. When analysed by paired t-test, HDL and LDL cholesterol levels were significantly (and identically) improved in both groups; 0.14 (0.10 to 0.18) and –0.4 (–0.7 to –0.1) respectively. Data are mean (95% CI).

Seventeen patients in the intervention group attained 80% or more of the increment of physical activity stipulated for the intervention group. In this subgroup SBP, DBP, BMI and total plasma cholesterol were significantly improved.

STUDY 2, PAPER II

Baseline health-related quality of life, reported by the study cohort of 212 overweight or obese individuals with NGT (n=128), IGT (n=34) and T2DM (n=50), was similar to or better than an age-and-sex-matched Swedish population sample, for 12 of 13 quality-of-life scales of the SWED-QUAL questionnaire. The T2DM cohort reported quality-of-life scores at the same level as the NGT cohort, for all 13 SWED-QUAL scales (Wilcoxon's rank-sum test, statistics not shown). Only 5 individuals in the T2DM cohort were newly diagnosed at the time of inclusion in the study. The other 45 had been diagnosed prior to participating in this study. Self-reported physical activity, assessed by visual analogue scale (VAS), increased significantly in the NGT and T2DM intervention groups by 30 (10;40) and 24 (10;40) mm respectively, median (25th;75th percentile). Adherence to the Nordic walking study protocol was assessed from the walking diaries obtained from 91% of the participants in the intervention groups. According to those diaries ≥80% (≥4 h/week) of prescribed Nordic walking was reported by 74% of the participants with NGT, 67% of IGT and by 50% of the T2DM participants. Hours per week of Nordic walking were 4.7 (4.1;5.2), 4.6 (3.7;6.0), and 3.8 (3.1;4.7) for the NGT, IGT, and T2DM groups, respectively. ANOVA did not indicate any significant difference between the three groups (p=0.1468). Comparison by Wilcoxon's Rank-Sum test showed a significant difference between NGT and

T2DM participants (p=0.0218), whereas comparisons between NGT-IGT and IGT-T2DM participants showed no significant differences in hours of Nordic walking per week (p=0.8702 and 0.1557, respectively). Total counts per minute, recorded by accelerometers, were 348 (220;528) for the control group (n=11) and 466 (348;623) for the intervention group (n=14), p< 0.001, Wilcoxon rank-sum test. Data are median (25th;75th percentile).

After four months of Nordic walking, quality of sleep and general health perception improved significantly in the NGT intervention group, compared with the NGT control group; effect size 0.4 and 0.1, respectively. In the T2DM intervention group quality of sleep and satisfaction with physical health improved in the intervention group, compared with the T2DM control group; effect size 0.5 and 0.7 respectively.

Pain tended to be more pronounced in the study group as a whole, compared to the Swedish population sample, although this difference was not statistically significant. After four months pain had not increased in the NGT, IGT or T2DM intervention cohorts and there were no reports of musculoskeletal pain that limited Nordic walking. BMI improved in the NGT intervention group (p<0.01), compared with the NGT control group. Correlation analysis showed no significant relationship between the improved quality of sleep and the lowered BMI (Spearman's rho –0.12).

STUDY 2, PAPER III

At baseline the control (n=126) and intervention groups (n=87) differed in some respects. The most prominent difference was the low degree of self-reported high-intensity physical activity in the NGT intervention group, compared with the NGT control group (p<0.001). Moreover, lipid regulating statin medication was more prevalent in the NGT intervention group (p<0.05) and total plasma cholesterol level was slightly lower (p<0.05) in that group, compared with the NGT control group. In the T2DM intervention group plasma triglyceride levels were higher than in the T2DM control group (p<0.05). Other baseline differences between control and intervention groups were not statistically significant.

The effects of Nordic walking were most pronounced in the NGT intervention group, where body weight (–2.0±3.8 kg), BMI (–0.8±1.4 kg/m^2) and waist circumference (–4.9±4.4 cm), (mean±SD), were significantly improved compared with the NGT control group (p<0.01). Self-reported medium and high-intensity physical activity were also increased in the NGT exercise group (p<0.001). Self-reported physical activity improved less in the IGT and T2DM cohorts, although the increment of high-intensity physical activity reached statistical significance in the T2DM intervention group (p=0.012).

The time spent on Nordic walking, reported as hours/week from the diaries kept by the intervention groups, did not differ significantly between the NGT (4.7±1.5), IGT (4.8±1.8) and T2DM (3.9±1.4) cohorts (p=0.1468 by ANOVA). Among the 25 participants who agreed to wear an accelerometer for one week, the 14 intervention participants were more physically active than the 11 control group participants. Total activity counts per minute, total activity counts per day, minutes of vigorous activity per day and inactive minutes per day all indicated that the intervention participants were more physically active than the control participants (p <0.001, <0.05, <0.005 and <0.05, respectively).

Exercise capacity, assessed by ergometry cycle tests as power output and peak VO_2, were significantly improved in the IGT intervention group, compared with the IGT control group ($p < 0.005$ and < 0.05, respectively).

HbA1c, HOMA insulin resistance, serum lipids, systolic and diastolic blood pressure were unaltered between the NGT, IGT and T2DM exercise and control groups. In the T2DM exercise group, however, the 2h post OGTT plasma glucose (–1.8±2.6 mmol/l), the HbA1c (–0.3±0.6% / –3.3±6.6 mmol/mol), and exercise power output improved (7.4±11.5W) in the intra-group analysis by paired t-test ($p < 0.01$, < 0.05 and < 0.05, respectively).

Adherence to the exercise programme varied within the intervention groups. Therefore we performed a separate analysis for the 55 exercise participants who reported ≥80% of the prescribed time of Nordic walking in their exercise diaries. In this group power output and/or peak VO_2 significantly improved for the NGT, IGT and T2DM participants, compared with their respective control groups. Improvement of body weight, BMI and waist circumference was similar to that of the entire NGT, IGT and T2DM exercise groups.

Ten individuals left the study before follow-up examination. The motives for leaving were personal reasons/lack of time (4), medical reasons (4) and unknown (2). Medication was altered for 4 individuals during the study; 2 were started on anti-hypertensive treatment and 2 had their anti-hypertensive treatment altered.

STUDY 2, PAPER IV

At baseline the mRNA expression of PDK4 was increased in the T2DM patients by approximately 70%, compared with the NGT participants. The mRNA expressions of PDK2 and MCD1 were increased by approximately 50% in the T2DM patients, whereas the mRNA expression of CPT1b was similar between T2DM and NGT participants.

The PDK4 gene expression was significantly associated with BMI, even after adjustment for age, sex and glucose tolerance.

After a four-month exercise intervention, PDK4 gene expression was significantly increased in the NGT group, but not in the T2DM group. PDK4 expression was elevated at baseline in the T2DM cohort, but it did not increase further after the exercise intervention.

DISCUSSION

GENERAL COMMENTS

The strength of our studies is that they were performed in a primary care setting near the homes of the patients, where they also receive their regular diabetes care. Furthermore, no special equipment was utilized. Therefore the intervention could be employed by most patients with type 2 diabetes and our method can be applied to the diabetes care programmes of other primary care centres.

Different levels of physical activity may affect different risk factors for type 2 diabetes assessed in our studies. The results of study 1 would imply that SBP and DBP are improved at a less frequent, or less intensive exercise level, whereas long-term effects on glucose metabolism (HbA1c, insulin sensitivity) may require a higher dose of exercise (frequency or intensity). As a comparison one study has reported significant improvements of HbA1c, SBP, DBP, total cholesterol and triglycerides for type 2 diabetes patients who increased their exercise by ≥30 minutes of daily walking [37]. When motivating the patient with type 2 diabetes to increased physical exercise it may be important to assess an array of known risk factors, since not all of them may be improved. In addition, it is also necessary to know that they will not get worse by exercise. The improvement of one or two risk factors may act as a stimulation to maintain the achieved level of exercise and perhaps to increase that level in order to improve risk factors not yet improved. Our results suggest that 45 minutes of walking 3 times per week represents a frequency at which some well-known risk factors are favourably changed in type 2 diabetes. However, since we could not observe any long-term effects on glucose metabolism, the conclusion should probably be that patients with T2DM ought to aim for more frequent and intensive exercising than that which was applied in study 1.

Already in 1992 doubts were raised [66] about the feasibility of making patients with T2DM adhere to exercise programmes over an extended period of time, pointing out the high drop-out rate from a current exercise study of T2DM patients conducted in the 1980s [67]. Cardiovascular co-morbidity may also prevent many patients with T2DM from participating in more intensive exercise. It has also been demonstrated that sedentary patients with T2DM have a lower physical work capacity (VO_{2max}) than sedentary control subjects [68,69]. Although VO_{2max} improved in the T2DM group after 3 months of regular training it was still lower than that of the untrained sedentary control group. This impaired exercise capacity may suggest that patients with T2DM are less able to benefit from the effects of physical training than healthy individuals. Failing adherence to exercise programmes over time has proved to be an obstacle to obtaining long-term effects of physical activity. A ten-year experience of a comprehensive life-style modification programme, emphasizing physical training, was that after three months only 50% of participants were still enrolled and by the end of one year approximately 90% had dropped out [69]. The mode of exercise was aerobics, and sessions lasted 40–60 minutes 3–4 times per week. After participating for three months, significant improvements were reported in the T2DM cohort for SBP –5 mm, BMI –2.2 kg/m^2, fasting plasma glucose –2.5 mmol/l, HbA1c –1% and triglycerides –42 mg/dl (~0.5 mmol/l).

The effects of brisk walking or Nordic walking in T2DM patients have been described in three recent studies [70-72], two of which continued for four months and one for a year. Low compliance was described as a problem in all three studies with high drop-out rates, mainly due to musculoskeletal symptoms and lack of motivation. HbA1c improved in one study when only participants who attended at least 50% of exercise sessions were included in the analysis. In the other two studies HbA1c did not improve. Systolic and diastolic blood pressure improved in one, but BMI and plasma lipids did not improve in any of the three studies. The overall impression is that brisk walking or Nordic walking does not offer a sufficient amount of exercise to substantially improve glycaemic control, body weight or the cardiovascular risk factors known to be common in T2DM. Moreover, adherence to exercise programmes exceeding three months' duration has been low in most studies, apparently also in studies involving rather low-intensity exercise such as walking.

The parameters recorded in our studies apparently did not show consistent improvement between studies and therefore may not be relied upon as motivational factors to make diabetes patients become more physically active. Immediate decrement of plasma glucose, demonstrated after a bout of exercise, although a short-lived effect, may better motivate diabetes patients to exercise on a daily basis.

Insulin resistance in T2DM causes a shortage of fuel, particularly in skeletal muscle, leading to impaired energy production in muscle cells. A mitochondrial gene expression that favours the use of FFA, as described in our paper IV, may be an indication of this metabolic derangement. It may also imply that patients with T2DM cannot benefit from the health effects of exercise to the same extent as people who do not have T2DM. Since energy metabolism is less deranged in the IGT state it seems probable that the health effects of exercise are more pronounced in this pre-diabetic state than in established T2DM.

Therefore, realistic expectations of exercise effects in T2DM patients should possibly be lower than for people free of T2DM, and individually adjusted. Several studies [73-75] have shown that people who are physically active and physically fit live longer, and this holds true for people with T2DM as well. This ought to be a good incentive for staying physically active, to people with or without T2DM.

HRQoL has seldom been addressed in research concerning physical activity in diabetes care. Aspects related to quality of life may play an important role for the feasibility of implementing regular habits of exercise in diabetes care. The SWED-QUAL results from a Swedish population sample were obtained in 1995. The comparison of HRQoL between the SPS and the participants in our study 2 should therefore be interpreted with some caution. The general perception of quality of life may have changed over time, and this could possibly be one reason for the high level of reported HRQoL in paper III. There were no indications, however, that the modest increase of physical activity implied in our studies impaired the HRQoL of the participating patients. Individuals with known angina pectoris were excluded from participation. This may account for the lack of adverse reactions to exercise in this study. It may be argued that for each individual there is a level of exercise, above which the exercise activity per se may be detrimental to quality of life. Our study indicates that positive effects on risk factors may occur at levels of exercise that do not negatively affect the patients' quality of life. A good clinical strategy may therefore be to encourage stepwise increases of physical exercise to patients with type 2 diabetes, supporting modes and amounts of exercise that are feasible to the individual patient.

Quality of sleep, which improved in study 2, appears to be a sensitive marker of well-being, and insomnia has been identified as one psychological stress factor that contributes to an increased risk of developing pre-diabetes [54], diabetes [76] and hypertension [77]. Participating in physical activity at least once a week, or taking a brisk walk daily, has been reported to reduce the risk of sleep disorders [78]. A meta-analytic review has, however, reported contradictory results concerning the relationship between sleep physiology and physical activity [79].

Limitations to our studies are the small number of patients included and the short period of intervention. Furthermore, the intensity of exercise was not well defined. It may be preferable to specify an exercise intensity that brings about a certain percentage of maximum heart rate. These limitations render our conclusions uncertain. On the other hand, controlling the intensity of exercise would require the supervision of exercise sessions. This would diminish the desired effect of "real life" circumstances to our studies.

Assessing physical activity

To assess the amount of physical activity performed by the individual is not an easy task, and assessments can seldom be completely accurate. The registration of physical activity on a 24-hour basis can be done for a limited period of time using accelero-meters. Bouts of exercise can be registered with respect to duration, work load and heart rate. This method does not account for other modes of physical activity than exercise training, such as transportation or occupational and home activities, and therefore offers a limited picture of the individual's energy expenditure. Self-reported physical activity can be obtained with the use of various questionnaires and may involve other aspects of physical activity than exercise training. The Compendium of Physical Activities [80] is a tool that helps in the classification of self-reported physical activities, where each activity has been assigned a metabolic equivalent intensity level (MET). One MET unit is the resting metabolic rate obtained in a sitting position. By multiplying the MET level and duration of various self-reported modes of physical activity it is possible to estimate individual physical activity in terms of MET hours per week. This method does not require any equipment for registration, but the reliability of the results is limited by recall bias. Unluckily enough, we also found that the results from the physical activity questionnaire, physical activity VAS estimation and walking diaries were not precise enough to be converted to MET hours per week.

Effect of physical activity – hard end-points or surrogate variables?

The effect of physical activity may be assessed in various ways. In our studies we have focused mainly on variables that are commonly measured in clinical practice, generally known as "risk factors" or "surrogate variables". Body weight, BMI, waist circumference, level of physical activity, blood pressure and levels of blood lipids and blood glucose are such risk factors, commonly included when estimating a cardiovascular risk profile in primary health care. It is advantageous to assess clinically adequate risk factors that may improve within a short period of time as a result of intervention. Such improvements are encouraging to the individual.

Regular walking 150 minutes per week prevents, or at least delays, the development of IGT to T2DM [6,8]. Furthermore, walking $\geq$1 mile per day has been shown to

decrease the risk of cardiovascular mortality in male and female patients with T2DM aged 50–90 years [11]. Frequent as well as occasional self-reported physical activity has been shown to correlate to reduced all-cause mortality in male and female individuals aged ≥65, with or without T2DM [81]. The increase of physical activity levels in 50-year-old men over a period of 10 years reduced total mortality, compared with men who maintained a low level of physical activity [9]. To focus on avoiding more definite end-points such as premature death or the development of T2DM or cardiovascular disease may not be as efficient in promoting lifestyle changes as the demonstration of improved clinical risk factors after a limited period of lifestyle intervention.

Better effects on risk factors if they are more pronounced at baseline?

In our studies the effects of exercise intervention on most of the clinical risk factors under study have been mild or non-existent. The exception to this is the improvement of body weight, BMI, waist circumference and exercise capacity in study 2. BMI, SBP and DBP tended to improve in study 1, and this improvement reached statistical significance in the subgroup of seventeen intervention participants who achieved 80% or more of the physical activity intended in study 1. Baseline blood pressure levels in study 1 were higher than in study 2. At baseline SBP in study 1 was 150.8 ±16.6 and in the study 2 cohort 139.7±13.8 ($p<0.001$). DBP was 85.1±7.3 and 84.4±8.2 respectively, $p=0.58$ (mean±SD). The higher baseline SBP in study 1 may explain the more favourable effects on blood pressure in that study.

A meta-analysis of clinical trials has demonstrated that a higher baseline level of HbA1c results in a more pronounced decline following glucose-lowering medication than if the HbA1c was lower at baseline [82]. This supports an intuitive assumption that clinical parameters that are not being well controlled are more likely to improve after appropriate intervention than parameters that are well controlled before intervention.

MUSCLE BIOPSY FINDINGS – A CHALLENGE FOR INTERPRETATION

Previous studies have shown that a reduction of MCD expression enhances glucose and reduces lipid metabolism in human muscle cells [83]. The increased MCD1 expression reported in paper IV may thus play a role in reducing muscle glucose utilization in individuals with impaired glucose tolerance/T2DM.

The elevated PDK4 mRNA expression noted at baseline in the T2DM cohort and following an exercise intervention in the NGT cohort is enigmatic. Elevated PDK4 expression has been associated in other studies with high levels of circulating lipids, in rats, and hyperinsulinaemia has been shown to suppress the PDK4 expression [84,85]. The elevated PDK4 expression at baseline in the T2DM cohort may reflect a state of insulin resistance in that group of participants, providing for the switch to lipid oxidation in order to secure an adequate cellular supply of fuel for energy production. This could be a result of impaired glucose utilization in T2DM. After a period of Nordic walking, gene expression of PDK4 increased in the NGT cohort but not in the T2DM group. This may indicate that individuals with NGT can improve their ability to utilize FFAs for fuel by physical activity, whereas people with T2DM cannot benefit to the same extent from the metabolic effect of physical exercise.

Current recommendations of physical activity

A systematic review and meta-analysis of 47 randomized controlled trials, including 8538 patients has recently presented data from current research on the effects of exercise on glycaemic control in T2DM [86]. The conclusion of this meta-analysis is that structured exercise training that consists of aerobic exercise, resistance training, or a combination of both, is associated with the reduction of HbA1c in patients with T2DM. Furthermore, exercise duration of more than 150 minutes per week was associated with greater HbA1c declines than that of 150 minutes or less per week. The intervention effect was more pronounced among individuals with HbA1c greater than 7% at baseline (NGSP standard, comparable to 53 mmol/mol IFCC standard), than among those with HbA1c levels less than 7%. More intensive exercise was, however, not associated with greater HbA1c declines. The American Diabetes Association supports a recommendation of 150 minutes per week of accumulated moderate physical activity (50–70% of maximum heart rate) for patients with type 2 diabetes. This recommendation is believed to be the most beneficial in the prevention of, and in the early stages of overt type 2 diabetes [87]. Moreover, resistance training 3 times per week, in addition to aerobic exercise, is recommended, in the absence of contraindications. For patients with type 2 diabetes, >65 years of age, the recommendation is to stay as physically active as possible, according to individual abilities [87]. The effect of exercise alone, without dietary restrictions or behaviour modification, on body weight is modest and commonly amounts to a weight loss of ~2kg [88]. Some authors suggest that 45 to 60 minutes of daily moderate-intensity activity is required to prevent the transition to overweight or obesity [89]. The effects of exercise on blood pressure and lipids have also been reported to be relatively modest [88]. For safety reasons it has been recommended that patients with type 2 diabetes undergo a medical examination to evaluate the risk of cardiovascular complications, before increasing the level of physical activity. Such testing is no longer considered necessary for moderate-intensity exercise such as walking. The recommendation to avoid physical activity in case of hyperglycaemia is justified in type 1 diabetes, but in adequately hydrated patients with type 2 diabetes who feel well it is not considered necessary to postpone exercise because of hyperglycaemia alone [88].

CONCLUSIONS

Physical exercise in the form of brisk or Nordic walking is feasible for most people with overweight, normal or impaired glucose tolerance, or type 2 diabetes, if not physically disabled.

Study 1, paper I

After three 45-minute walks per week SBP and DBP tended to improve in the intervention group. For the participants who performed ≥80% of prescribed brisk walking during four months the improvement of blood pressures, BMI, LDL, HDL and total cholesterol reached statistical significance. However, parameters reflecting glucose metabolism did not improve.

Study 2, paper II

Quality of sleep improved in the NGT group following four months of Nordic walking, five hours per week. BMI reduction did not account for this improvement. Nordic walking can be introduced in a primary health care setting as a low-cost mode of exercise that promotes weight loss and improved health satisfaction.

Study 2, paper III

Nordic walking improved anthropometric measurements and exercise capacity. However, unsupervised Nordic walking may not provide sufficient exercise intensity to achieve ultimate health-promoting benefits on the cardiovascular parameters assessed in this study, particularly for those with disturbed glucose regulation.

The intervention effects of Nordic walking were the most pronounced in the NGT group, where body weight, BMI and waist circumference were significantly improved. Self-reported physical activity improved less in the IGT and T2DM cohorts, although the increment of self-reported high-intensity physical activity reached statistical significance in the T2DM intervention group.

Performing ≥80% of the time of Nordic walking prescribed in this study, significantly improved exercise power output and/or peak VO_2 for the NGT, IGT and T2DM participants, assessed by ergometry cycle tests.

Study 2, paper IV

The mRNA expression of PDK4, PDK2 and MCD1 was up-regulated at baseline in skeletal muscle of the T2DM cohort. No further elevation occurred for any of the three enzymes after an exercise intervention. The lack of effects on PDK4 expression following exercise intervention implies a possible resistance to the appropriate adaptive responses to exercise in skeletal muscle associated with insulin resistance. In the NGT cohort, however, the PDK4 expression was significantly elevated following the exercise intervention. This may reflect an increased capacity for lipid oxidation in a

state of increased total energy demand. Skeletal muscle is known to rely on lipid oxidation, particularly when exposed to low-intensity exercise [19,23,90].

FUTURE PERSPECTIVES

According to the official survey of living conditions, conducted on a regular basis by Statistics Sweden [91], the prevalence of overweight (BMI ≥25) and obesity (BMI ≥30) in Sweden has increased over the past 30 years for men and women aged 16–84 years. In 1980–81 overweight was reported by 31% of men and 22% of women, and 5% of men and women were obese. The corresponding figures for 2010–11 were 42/28% for overweight and 12/11% for obesity. It is intriguing to note that for the same period of time self-reported physical activity has increased among Swedish men and women aged 16–84 years [91]. In 1980–81 exercise ≥1 time per week was reported by 47% males and by 43% females. Exercise ≥2 times per week was reported by 30% males and 24% females. Corresponding figures for 2010–11 were 72/76% for exercise ≥1 time per week and 60/66 for exercise ≥2 times per week. These results may indicate that sedentary activities are so predominant in many people's daily lives that negative health effects, such as overweight/obesity, cannot be counterbalanced by bouts of exercise performed on one or more occasions per week. Prolonged sitting has been shown to be a significant risk factor for all-cause mortality independent of age, sex, BMI, physical activity levels, and the presence or absence of cardiovascular disease or diabetes [92]. The accumulated daily amount of physical activity, measured by an accelerometer, has been reported to be a major determinant of insulin sensitivity in healthy, normal-weight men and women. The effect was independent of time spent sedentary, in light-intensity activity or in bouts of moderate or vigorous activity [93]. According to another study, the number of daily interruptions of sedentary time correlate to beneficial effects on waist circumference, BMI, triglycerides and 2-h plasma glucose, in overweight healthy men and women. The beneficial effects were independent of total sedentary time or time spent in moderate-to-vigorous physical activity [94].

It is a challenge to providers of primary health care to develop methods and strategies that help patients with type 2 diabetes, and persons at risk of developing that disease, to incorporate regular physical activities in daily life. Such strategies ought to involve not only "exercise" activities but also aspects of habitual physical activities in everyday life. Transportation to and from work, household activities, shopping and gardening are activities that can be performed in more or less physically demanding ways. Health care providers ought to provide not only objective information on the health benefits of regular physical activities but should also be able to refer the patients to suitable exercise activities offered in the community. The organizing of walking groups could even be included in the services offered by a primary care centre. It is not time-consuming and need not cause any extra expenses for the patients or the primary care provider. Exercise on prescription (EoP) has been used in primary health care in several European countries and in the USA with the aim of providing support to patients for increased physical activity. The aim of EoP is usually to promote activities that are more physically demanding than walking. Two systematic reviews report that EoP appears to have small effects on increasing physical activity in sedentary people [95] but increases physical activity levels in older adults who are already slightly active [96]. In one review, physical activity was increased in half of the studies and EoP

schemes were found to be an acceptable tool for motivating general practitioners to promote physical activity [97]. One Swedish study has reported increased physical exercise and improved quality of life following a period of EoP [98].

The beneficial effects of regular physical exercise in the prevention and treatment of type 2 diabetes and the detrimental effects of a sedentary lifestyle are indisputable. It is therefore important to convey results from research on everyday exercise activities to the public as well as to primary health care providers. Such basic knowledge is crucial to the feasibility of implementing lifestyle alterations in patients with IGT and T2DM. This may be one prerequisite for avoiding future cardiovascular complications that are a threat in T2DM.

However, further studies are needed to establish how different modes, intensities and frequencies of exercise affect cardiovascular risk factors and HRQoL in type 2 diabetes. It is also important to widen our understanding of the physiological mechanisms involved in glucose metabolism and insulin resistance with relation to physical exercise. Although the connection between a sedentary way of life and the development of insulin resistance is well established, the biochemical mechanisms involved are not well understood. We do not know whether different modes of exercise are more or less efficient in enhancing insulin sensitivity in skeletal muscle. Furthermore, it is not clear whether or not the responsiveness to exercise differs between individuals. Studies on the intracellular proteins in skeletal muscle, involved in insulin signalling and glucose metabolism as well as FFA metabolism, may add to our understanding of the role of regular physical exercise in the prevention and treatment of type 2 diabetes.

ACKNOWLEDGEMENTS

A group of exceptionally helpful, creative and dedicated co-workers, representing four different departments of Karolinska Institutet, have made it possible for me to finally complete this PhD thesis, and I wish to express my deepest gratitude to all of you.

My initial supervisor at the Centre for Family Medicine, Professor Peter Engfeldt, helped to design our first walking study. Thank you Peter for conveying to me valuable basic knowledge of the principles of medical research.

Professor Per Wändell has been my principal supervisor after Peter Engfeldt left CeFAM. Thank you Per, for always being there whenever I needed backup in one way or another. I am also grateful for all your informal lessons on statistics. I no longer feel like stepping into quicksand when making statistical calculations.

Professor Hans Åberg has been my co-supervisor at CeFAM. Hans, I am deeply grateful for your outstanding ability to analyse, and improve, my attempts at scientific writing. Your structural overview of the different sections of a scientific paper, your ability to find elegant solutions and your very observant eyes, which discover even the tiniest misspellings, are abilities that I have been lucky to profit from. I have also enjoyed discussing clinical matters with you and the several opportunities to learn from your lifelong medical experience.

Professor Juleen Zierath, Department of Molecular Medicine and Surgery, Integrative Physiology, has fulfilled a mitochondrial-like powerplant function in our group, with her never-ending energy. Juleen, I thank you for inviting me to look into the world of muscle cell metabolism and genetics, and thereby substantially widening my area of knowledge. You have also given me plenty of useful advice on how to communicate with editors and reviewers of scientific journals. It has been a true privilege to be accepted as one of your co-workers.

I am deeply grateful to Professor Kenneth Caidahl, Department of Molecular Medicine and Surgery, Clinical Physiology. Kenneth, you have always been ready to offer a helping hand with all the practical details that emerge when running a research project in collaboration with four different departments. I also appreciate that you enabled us to conduct the ergometer cycle tests with professional assistance, and all the hands-on guidance in scientific writing that you have contributed.

Professor Claes-Göran Östenson, Department of Molecular Medicine and Surgery, Endocrine and Diabetes Unit, and Professor Anna Krook, Department of Physiology and Pharmacology, Integrative Physiology; I thank you two for your valuable contributions during the planning of Study 2, and for always being available and willing to contribute your knowledge whenever I needed your help. Claes-Göran, you also provided for us to invite the former participants of the Stockholm Diabetes Preventive Project to our study. We were thereby able to include people with known Impaired Glucose Tolerance in Study 2, a category of people otherwise difficult to find.

Docent Megan Osler, Department of Molecular Medicine and Surgery, Integrative Physiology; thank you for being such a good friend and for your patience in guiding me through all the steps in the laboratory work involved in the mRNA assessments of our muscle biopsy material. I have also appreciated your never-ending enthusiasm in analysing our data, and your patience in helping a foreigner to choose the correct English words and expressions.

I thank Docent Alexander Chibalin, Department of Molecular Medicine and Surgery, Integrative Physiology, for introducing me to the basics of muscle protein analysis.

Assistant Nurse Pia Odenblad, Gustavsberg Primary Health Care Centre; without your outstanding ability to organize and keep track of people and things, we would not have managed to bring Study 2 to an end. You made sure that blood samples were collected, that materials and utensils were in their proper place when needed, and that study participants were scheduled for all the tests and examinations involved in this study.

Assistant Nurses Annika Eriksson, Ylva Persson and Riita Väliharju, Gustavsberg Primary Health Care Centre; I thank you for your enthusiasm in organizing the walking groups for study 1, and for joining the study participants during their walks in rain and sunshine.

I am most grateful to Mrs. Ann Nikolausson, Centre for Family Medicine and Mrs. Margareta Svedlund, Department of Molecular Medicine and Surgery, Integrative Physiology for being most helpful with secretarial functions during the years. You have saved me from many hours of work.

My special thanks to Biomedical Technologists Susanne Krogstadholm-Stjernfelt, Jacob Lindberg, Sara Persson, Kamel Ramak and Amira Soulios, Department of Clinical Physiology, Karolinska University Hospital and Doctor Fredirick Mashili, Department of Molecular Medicine and Surgery, Integrative Physiology, for your professional assistance during all the ergometer cycle exercise tests, usually after office hours.

SVENSK SAMMANFATTNING/SWEDISH SUMMARY

Bakgrund och mål

Typ 2-diabetes (T2DM) är en sjukdom som är starkt kopplad till en ökad risk för allvarliga blodkärlskomplikationer i hjärta, hjärna och ett flertal andra organ i människokroppen. Risken för att utveckla T2DM är relaterad till individens genetiska arv, en låg grad av fysisk aktivitet, övervikt och socioekonomiska faktorer.

Mitt mål har varit att studera effekterna av ökad fysisk aktivitet på kliniska riskfaktorer för hjärt-/kärlsjukdom, hälsorelaterad livskvalitet och på det genetiska uttrycket för mitokondriella enzymer som är centrala i glukos- och fettmetabolismen, hos överviktiga personer med T2DM, nedsatt eller normal glukostolerans.

Metod

Två olika motionsstudier genomfördes. I båda studierna omfattade studieperioden fyra månader.

I studie 1, som publicerades i artikel I, inkluderades endast personer med T2DM; 27 i interventionsgruppen och 31 i kontrollgruppen. De var 60,0±6,5 år med diabetes sedan 6,0±5,5 år tillbaka, kroppsmasseindex (BMI) 32,0±5,3 kg/m^2 och långtidssockervärde (HbA1c, Mono S standard) 6,3±0,9 %. Angivna mätvärden är medelvärde±SD. Motionsgruppen rekryterades från en vårdcentral medan kontrollgruppen rekryterades från en annan, näraliggande, vårdcentral. Denna studie var alltså inte randomiserad. Motionsgruppen instruerades att öka sin fysiska aktivitet med 45 minuter rask promenad tre dagar per vecka, medan kontrollgruppen instruerades att fortsätta sitt dagliga liv som vanligt. Vid studiestart och efter fyra månader mättes systoliskt och diastoliskt blodtryck, BMI och åldersjusterad fysisk arbetsförmåga. Samtidigt mättes blodkoncentration för glukos, insulin, HbA1c och blodfetter, och insulinkänsligheten beräknades.

I studie 2, som har legat till grund för artiklarna II, III och IV, inkluderades totalt 213 överviktiga personer som inte hade några fysiska eller medicinska funktionsnedsättningar, i åldern 60±5,3 år, med BMI 30,2±3,8 kg/m^2. Motionsgruppen instruerades att öka sin fysiska aktivitet med fem timmars stavgång per vecka, medan kontrollgruppen instruerades att fortsätta sitt dagliga liv som vanligt. Ett glukostoleranstest (s.k. sockerbelastning) vid studiestart möjliggjorde uppdelningen av deltagarna i personer med normal glukostolerans (NGT), nedsatt glukostolerans (IGT) eller typ 2-diabetes (T2DM). Deltagarna lottades sedan till en kontrollgrupp (n=126), eller till en motionsgrupp (n= 87). Mätningar av kroppsmått, blodtryck och blodkemiska variabler utfördes vid studiestart och efter fyra månader. Vid dessa båda tillfällen besvarade deltagarna även ett frågeformulär med frågor om hälsorelaterad livskvalitet (SWED-QUAL) och konditionstest med mätning av syreupptagningsförmåga genomfördes. De deltagare som accepterade detta fick även lämna ett muskelvävnadsprov i form av en muskelbiopsi från lårmuskeln vid studiestart och efter fyra månader. Muskelprovet analyserades sedan för uttrycket av ett antal mitokondriella gener, som reglerar syntesen av enzymer inblandade i cellernas glukos-

och fettförbränning. Deltagarna i motionsgruppen ombads även att föra en dagbok över sina stavgångspromenader.

Resultat

I studie 1 visade intention-to-treat-analysen ingen signifikant förbättring av kroppsmått, fysisk kondition, glukosmetabolism, blodfettsnivåer eller blodtryck. De 17 deltagare i motionsgruppen som uppnådde 80 % eller mer av föreskriven ökning av motionsgraden förbättrade däremot signifikant BMI, systoliskt/diastoliskt blodtryck och total kolesterolnivå i blodet.

I studie 2 ökade den självrapporterade fysiska aktiviteten för deltagare med NGT, IGT och T2DM i motionsgruppen. Hälsorelaterad livskvalitet vid studiestart var lika god som, eller bättre än ett ålders- och könsmatchat svenskt befolkningsurval, för 12 av de 13 skalorna som ingår i SWED-QUAL. Sömnkvaliteten förbättrades för motionsgruppens deltagare med NGT efter fyra månader med stavgång. I denna grupp förbättrades även kroppsvikt, BMI och midjeomfång, och i motionsgruppen med IGT förbättrades den fysiska konditionen. Smärta från rörelseapparaten, relaterat till stavgångsaktiviteten, rapporterades i liten eller ingen omfattning. De deltagare i motionsgruppen som uppnådde 80 % eller mer av föreskriven stavgång förbättrade sin fysiska kondition såväl i NGT- som i IGT- och T2DM-gruppen.

Muskelvävnadsprov från 79 män med NGT och 33 män med T2DM, alla i motionsgruppen, analyserades för ett antal mitokondriella gener, som kodar för syntesen av enzymer som spelar en viktig roll för förbränningen av glukos respektive fria fettsyror i muskelcellernas mitokondrier. Vid studiestart var uttrycket för tre mitokondriella gener, mätt som messengerRNA (mRNA), ökade i T2DM-gruppen. I NGT-gruppen ökade PDK4-uttrycket efter motionsperioden, men inte i T2DM-gruppen.

Slutsatser

Regelbundna promenader 45 minuter, tre gånger per vecka under fyra månader, förefaller inte medföra en tillräcklig mängd motion för att påverka de kliniska parametrar som vi har studerat. Genom att öka motionsgraden till stavgång fem timmar per vecka kunde vi visa att kroppsvikt, BMI, midjeomfång och sömnkvalitet förbättrades i NGT-gruppen och att den fysiska konditionen förbättrades i IGT-gruppen. Stavgång utgör möjligen en miniminivå av motion som krävs för att förbättra kardiovaskulära riskfaktorer och mitokondriella genuttryck med bibehållen livskvalitet. Promenader och stavgång kan rekommenderas i primärvård som en säker introduktion till regelbunden motion till låg kostnad, till personer med T2DM eller förstadierna till den sjukdomen. Genuttryck för enzymer som är involverade i glukos- och fettförbränning, särskilt enzymet PDK4, var ökat i gruppen med T2DM, men ej i NGT-gruppen, vid studiestart. Efter en period av regelbunden stavgång ökade uttrycket för PDK4 i NGT-gruppen men inte i T2DM-gruppen. Dessa resultat kan vara ett uttryck för att personer med T2DM har en något förhöjd förbränning av fria fettsyror, som en kompensation för den försämrade glukosförbränningen som utgör ett grundproblem vid T2DM. Å andra sidan kunde vi notera tecken till förbättrad fettförbränning i NGT-gruppen, i form av ökat PDK4-uttryck efter en period av stavgång. Detta skulle kunna

vara ett tecken på att personer med T2DM inte kan tillgodogöra sig de positiva metabola effekterna av motion, i samma utsträckning som personer med NGT.

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APPENDICES

Errata

Paper I – results section – “3 min × 45 min increment” should be “3 × 45 min increment”.

Paper III – Table 1 – Results for LDL cholesterol have by mistake been inserted in the line for HDL cholesterol and vice versa.

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Diabetes Research and Clinical Practice 71 (2006) 21–27
DIABETES RESEARCH AND CLINICAL PRACTICE
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Walking for exercise—does three times per week influence risk factors in type 2 diabetes?

Tomas Fritz [a,*], Per Wändell [a], Hans Åberg [a], Peter Engfeldt [b]

[a] *Center of Family Medicine, Karolinska Institutet, Alfred Nobels Allé 12, SE 141 83 Huddinge, Sweden*
[b] *Department of Clinical Medicine, Family Medicine Research Centre, Örebro University, Sweden*

Accepted 16 May 2005
Available online 6 July 2005

Abstract

In order to assess the effects of regular walking on metabolic control and cardiovascular risk factors in type 2 diabetes 26 patients from one primary care clinic, aged 60.0 ± 7.3 years, participated in a walking program during 4 months. Prescribed exercise was walking for 45–60 min three times weekly. A control group of 26 patients from a neighboring primary care clinic, aged 59.3 ± 6.2 years, received no exercise instructions. Thus, randomization was not performed.

There were no improvements of blood pressure, body mass index, physical fitness, glycated hemoglobin A1c, fasting plasma glucose or insulin by intention-to-treat analysis. Seventeen patients in the intervention group increased their physical activity and improved systolic blood pressure; −7.6 mmHg (−15 to −0.2), diastolic blood pressure; −4.3 mmHg (−7.4 to −1.2), body mass index; −0.6 kg/m^2 (−1.1 to −0.1) and total plasma cholesterol; −0.6 mmol/l (−0.9 to −0.3), (mean difference, with 95% CI). We could observe no effects on glucose metabolism in either group.

Our results suggest that an increase of regular physical activity equivalent to 45 min of walking 3 days/week may suffice to improve systolic and diastolic blood pressure, lipid metabolism and BMI in patients with type 2 diabetes.

Keywords: Type 2 diabetes; Exercise; Risk factors

1. Introduction

Cardiovascular morbidity is the major contributory factor to mortality in type 2 diabetes. In the United Kingdom Prospective Diabetes Study (UKPDS) systolic hypertension, dyslipidaemia and hyperglycaemia were significantly associated with coronary artery disease [1] and pharmacological treatment of hypertension was demonstrated to reduce cardiovascular mortality and morbidity [2]. Furthermore, UKPDS results show that glycaemic control is more crucial to the risk of developing micro vascular complications of type 2 diabetes, i.e. retinopathy and nephropathy, than macro vascular complications, i.e. stroke and coronary heart disease [3]. A subgroup analysis of the Scandinavian Simvastatin Survival Study (4S) suggests

* Corresponding author. Tel.: +46 8 524 887 28; fax: +46 8 524 887 06.
E-mail address: tomas.fritz@klinvet.ki.se (T. Fritz).

0168-8227/$ – see front matter
doi:10.1016/j.diabres.2005.06.002

that the improvement of blood lipid levels was beneficial also to patients with type 2 diabetes, with respect to coronary heart events [4].

Impaired insulin sensitivity is a central metabolic disturbance in type 2 diabetes with its strongest expression in skeletal muscle. Physical exercise is known to enhance insulin sensitivity and is therefore regarded as a cornerstone in the management of type 2 diabetes [5].

A meta-analysis of 14 controlled trials of physical exercise concluded that post-intervention HbA1c levels were lower in the exercise groups compared with the control groups [6]. Other studies have indicated that regular walking [7] or more strenuous exercise programs [8] are beneficial to insulin sensitivity in type 2 diabetes. Regular exercise, combined with dietary restrictions, has also been shown to prevent the development of impaired glucose tolerance into overt type 2 diabetes [9,10]. Improved glycaemic control, blood pressure, cholesterol levels and body weight have been demonstrated in patients with type 2 diabetes after a lifestyle modification program [11].

Several authors have reported discouraging difficulties in implementing long-term exercise programs for patients with type 2 diabetes 60 years or older [12–14]. Many patients are unaccustomed to exercising [15] and attempts at introducing habits of regular physical activities may also be limited by other co-existing ailments. Contrary to this a high attendance to a long-term exercise program was observed in younger persons with type 2 diabetes or impaired glucose tolerance [16].

Current research thus indicates that physical exercise is associated with improved glycaemic control, blood pressure and blood lipid levels in type 2 diabetes, as well as a reduced risk of developing this disease in persons with impaired glucose tolerance.

In Sweden most patients with type 2 diabetes are cared for in primary health care and one central aspect of diabetes care ought to concern matters of lifestyle. There is a need for more detailed knowledge of the effects of various lifestyle interventions to be utilized in individual counseling and goal setting in diabetes care.

The aim of this study was to describe the effects on metabolic control and cardiovascular risk factors in type 2 diabetes after a period of a low intensity exercise program feasible to most patients and to the resources of a primary health care center.

2. Materials and methods

2.1. Patients

Patients with type 2 diabetes were enrolled from two different primary care practices in two well-defined suburban communities outside Stockholm, Sweden. Exclusion criteria were severe angina pectoris or other severe disability and insulin treatment. Public meetings, advertised in the local press, were arranged in the two communities and attendants were invited to participate. In addition, 67 patients with type 2 diabetes were invited to participate when visiting their physician and 100 by a personal letter of invitation.

Twenty-seven patients consented to participate in the intervention group and 31 in the control group. One patient in each group declined further participation shortly after inclusion in the study. Four patients in the control group were lost to follow-up despite repeated reminders. Thus, the intervention group and the control group both consisted of 26 patients.

We chose to invite the patients to the two groups from different communities in order to diminish the possibility that the control group patients would start exercising as they learned about the activities in the intervention group. This was therefore not a randomized study.

2.2. Study design

The patients in the intervention group were instructed to increase their exercise by 45 min of brisk walking, three times weekly, during 4 months. We expected this to be achievable to most patients. Four months was considered to be a sufficient period to allow for changes of the parameters under study. Walking groups were provided four times per week and the patients were asked to register every walk in a diary, whether they were taken in a supervised group or individually. No recommendations were given concerning changes of dietary habits and medication remained unaltered in both groups. The patients in the control group were informed that their results were to be used as reference values for diabetes patients over a period of 4 months when no alterations of life style or medication were undertaken.

2.3. Methods

At the time of inclusion and after 4 months resting systolic (SBP) and diastolic (DBP) blood pressure in the supine position and body mass index (BMI) were assessed. Since the patients were examined at two different primary care units different blood pressure tonometers were used (Speidel and Keller^{TM}). There were no differences in the blood pressures recorded with the four different tonometers that were used, when tested on the same patient for control.

Blood samples, obtained in the fasting state were analyzed for plasma glucose, insulin, glycated hemoglobin (HbA1c), and lipid levels (total cholesterol, HDL and LDL cholesterol and triglycerides). According to Swedish national diabetes guidelines HbA1c $< 6.5\%$ is regarded as good control.

Insulin resistance was obtained as HOMA2-IR (Homeostasis Model Assessment) from fasting serum insulin and plasma glucose levels by the use of the computerized HOMA calculator as described by Wallace et al. [17]. Serum insulin levels were analyzed at a central hospital laboratory using the Auto DELFIA method (Perkin-Elmer^{TM}).

Blood samples and measurements of other variables in this study were obtained within 24–48 h after the last exercise walk.

Age adjusted physical fitness was determined by bicycle ergometry as a submaximal exercise test. The patients performed six minutes of cycling at a resistance (50–150 W) that resulted in a steady heart rate. The patient's heart rate, sex and age were used in the Åstrand nomogram to calculate the predicted maximum oxygen uptake ($VO_{2\ max}$). A Monark 839E ergometer cycle was used for the exercise tests (MONARK EXERCISE AB^{TM}).

The patients answered a questionnaire that focused on daily physical activity. The questionnaires and walking diaries made it possible to identify those individuals in the intervention group who did increase their actual level of exercise.

2.4. Statistics

Paired t-tests were used to analyze differences within the intervention group and the control group, respectively. For analysis of differences between groups two-sided *t*-tests were used.

The ethics committee of Karolinska Institutet, Stockholm, approved this study.

3. Results

Baseline characteristics and results after 4 months are shown in Table 1. The exercise group increased their total amount of weekly exercise according to walking diaries and exercise questionnaires. However, only 17 patients attained 80% or more of the stipulated 3 min $\times$ 45 min increment. The predominant mode of exercise reported was walking. Aerobics, swimming, cycling, gardening, golfing and weight lifting were activities reported by few patients in each group. At baseline 11 patients in the intervention group and 15 in the control group reported habitual exercise exceeding 3×45 min of walking.

There were no significant effects on parameters reflecting glucose metabolism, i.e. HbA1c, plasma glucose and plasma insulin levels. HDL and LDL cholesterol levels were improved in both groups. In the intervention group there was a tendency towards a decrease in systolic and diastolic blood pressure. In the control group the tendency was towards a rise in systolic as well as diastolic blood pressure. Physical fitness when measured as predicted $VO_{2\ max}$ was average to low in both groups and did not improve during the study.

In a separate analysis of the 17 patients in the exercise group that achieved 80% or more of the prescribed increment of exercise systolic and diastolic blood pressure, BMI and total plasma cholesterol were significantly reduced.

Nine patients in the intervention group did not alter their exercise habits. Six of them remained on a low level of physical activity and three maintained their previous level of exercise, which was higher than what was stipulated for the study.

A number of patients experienced ailments that limited their participation in physical activities to some degree. In the intervention group 12/26 and in the control group 9/26 patients reported such physical obstacles in the physical activity questionnaire.

4. Discussion

The main results of this study indicate that brisk walking for 45 min 3 days/week may not provide a

Table 1
Basic characteristics (T0) and differences at 4 months (dT4) for type 2 diabetes patients

	I ($n = 26$)		C ($n = 26$)		Incr ($n = 17$)	
	T0	dT4	T0	dT4	T0	dT4
Age (years)	60.0 ± 7,3		59.3 ± 6.2		60.4 ± 8.5	
Sex M/F	11/15		15/11		8/9	
Diabetes duration (years)	5.2 ± 4.3		5.8 ± 4.2		5.2 ± 3.5	
Glucose lowering treatment Diet/Tablets	11/15	11/15	14/12	14/12	8/9	8/9
Antihypertensive treatment	19	19	13	13	11	11
Lipid-lowering treatment	8	8	9	9	6	6
Exercise (h/week)	2.5 ± 1.6	1,2 (0.7 to 1.7)*	3.3 ± 2.1	0.2 (−0.6 to 1.0)	2.6 ± 1.8	2.6 (1.9 to 3.3)*
Systolic BP (mmHg)	146.9 ± 13.5	−4.3 (−9.6 to 1.0)	154.8 ± 18.6	2.6 (−4.7 to 9.9)	146.2 ± 12.2	−7.6 (−15.0 to −0.2)*
Diastolic BP (mmHg)	85.2 ± 5.3	−2.6 (−5.3 to 0.1)	85.0 ± 8.9	1.4 (−1.8 to 5.0)	85.2 ± 5.6	−4.3 (−7.4 to −1.2)*
BMI (kg/m^2)	32.2 ± 5.0	−0.3 (−0.8 to 0.2)	30.9 ± 5.4	0.1 (−0.1 to 0.3)	31.8 ± 5.2	−0.6 (−1.1 to −0.1)*
HbA1c (%)	6.3 ± 0.9	0,0 (−0.2 to 0.2)	6.0 ± 0.7	0.1 (−0.1 to 0.3)	6.3 ± 0.9	−0.1 (−0.4 to 0.2)
Fasting glucose (mmol/l)	9.0 ± 2.6	0.2 (−0.3 to 0.7)	8.2 ± 1.8	−0.2 (−0.7 to 0.3)	9.2 ± 2.8	0.0 (−0.7 to 0.7)
Fasting insulin (pmol/l)	99.6 ± 61.2	−6.4 (−23.1 to 10.3)	79.5 ± 50.9	−4.2 (−20.9 to 12.5)	98.2 ±69.1	−15.1 (−38.4 to 8.2)
HOMA2-IR	1.2 ± 0.4	0.0 (−0.1 to 0.1)	1.0 ± 0.3	0.0 (−0.1 to 0.1)	1.2 ± 0.5	0.0 (−0.04 to 0.04)
Total cholesterol (mmol/l)	5.5 ± 1.0	−0.3 (−0.6 to 0.0)	5.6 ± 1.1	−0.3 (−0.6 to 0.0)	5.7 ± 1.1	−0.6 (−0.9 to −0.3)*
HDL cholesterol (mmol/l)	1.15 ± 0.32	0.14 (0.10 to 0.18)*	1.19 ± 0.33	0.14 (0.10 to 0.18)*	1.14 ± 0.31	0.15 (0.09 to 0.21)*
LDL cholesterol (mmol/l)	3.3 ± 1.1	−0.4 (−0.7 to −0.1)*	3.4 ± 1.0	−0.4 (−0.7 to −0.1)*	3.4 ± 1.2	−0.6 (−1.0 to −0.2)*
Triglycerides (mmol/l)	2.4 ± 1.2	−0.1 (−0.5 to 0.3)	2.2 ± 1.1	0.0 (−0.3 to 0.3)	2.5 ± 1.2	−0.3 (−0.7 to 0.1)
$VO_{2\ max}$ (l/min)	2.0 ± 0.5	0.0 (−0.1 to 0.1)	2.0 ± 0.5	0.0 (−0.1 to 0.1)	2.0 ± 0.6	0.0 (−0.2 to 0.2)

I: the intervention group; C: the control group; and Incr: the patients in the intervention group that achieved 80% or more of the increment of exercise postulated in the study (walking 3 × 45 min weekly during 4 months). Data are means ± S.D. or means (95%CI). HOMA2-IR: computerized homeostasis model assessment of insulin resistance.
* $P < 0.05$.

sufficient increase of physical activity to improve the degree of glycaemic control, lipid homeostasis or blood pressure in patients with type 2 diabetes.

Various factors in our study might account for the absence of improved glycaemic control. The degree of physical activity obtained by the patients may have been insufficient. Our patients were in comparatively good glycaemic control when included in the study. This implies that the participating patients might have been a highly motivated group. Other investigators have demonstrated that diabetes patients with higher HbA1c levels respond with a more pronounced decrease in this respect following regular exercise [11]. With increasing age the glucose metabolism may react less favourably to physical activity. This may account for the lack of effect on glucose metabolism in our study. Furthermore, the number of patients in our study may have been too small to detect minor effects on glucose metabolic control in patients who already had a relatively good metabolic control.

A comparison of our results to those of another study comprising a similar number of patients with comparable degree of blood glucose control may be justified. Yamanouchi et al. [7] studied two groups of patients with type 2 diabetes, aged 23–59 years. A ''diet and exercise'' group (DE) walked ∼13.5 km daily and a ''diet only'' group (D) walked ∼3.2 km daily during a 6–8 weeks period of hospitalization, according to pedometer readings. In the DE group ($n = 14$) fasting blood glucose improved (6.1–5.2 mmol/l) as well as insulin sensitivity (euglycaemic insulin clamp). In the D group ($n = 10$) fasting blood glucose improved to a similar degree (5.9–5.1 mmol/l) whereas insulin sensitivity was not significantly altered. The dietary restrictions (reduction of daily caloric intake by 1000 kcal from the patient's ordinary intake) may explain the improved glycaemic control of both groups and the exercise may explain the improved insulin sensitivity of the DE group. The authors do not specify the actual increase of walking in the DE group but a reasonable assumption is that it amounted to approximately 10.3 km daily, i.e. the difference between the two groups. This comparison implies that the relatively small amount of exercise, rather than the small number of patients or their degree of glycaemic control, accounts for the absence of effects on glycaemic variables in our study. It deserves mention that the patients studied by Yamanouchi et al. were hospitalized during the study. The amount of walking applied in our study may better reflect the amount of exercise feasible in everyday life and especially in an elderly diabetes population.

Few studies have described the effects of exercise in type 2 diabetes patients over 55 years of age. Zierath and Wallberg-Henriksson conclude in a review article that there is ''a difference in the metabolic response to regular exercise between younger and older type II diabetic patients ... none of the older patient groups (57–61 years) respond to exercise training with improved HbA1c levels.'' [18]

The absence of metabolic response to exercise in elderly type 2 diabetes patients may be due to concomitant ailments that prevent more vigorous exercise. If an age-dependant ''exercise resistance factor'' should exist, this would diminish the value of recommending exercise to elderly patients. At the same time it seems likely that such an age-dependant decrement of response to exercise would be subject to individual variations.

The age of the patients in this study is well representative of the age of diabetes patients listed to Swedish primary health care. According to current data from the National Diabetes Registry of Sweden 23% of the 65,000 patients registered in primary health care are aged 30–59 years and 77% are 60 years or older.

$VO_{2\ max}$ did not change in any of our groups. Walker et al. have reported improved fitness, expressed as estimated $VO_{2\ max}$, following a period of walking for 1 h/day on 5 days weekly during 12 weeks, in postmenopausal women with and without type 2 diabetes [19]. The amount of exercise, in our study, may have been insufficient for increasing respiratory fitness. The exercise levels required to improve glycaemic control may be lower than those required for improved respiratory fitness. This does not necessarily exclude the possibility of improved glycaemic control even though respiratory fitness did not improve.

Most walks were not supervised and the patients recorded only the time spent on each exercise session, not the intensity. This lack of exactness concerning exercise intensity is a weakness of our study. At the same time it reflects the conditions and resources available in primary health care, where most Swedish type 2 diabetes patients receive counseling on lifestyle matters.

HDL and LDL cholesterol were equally improved in the exercise group and in the control group. This could not be explained by alterations in medication. Some factor, other than exercise, probably accounts for this improvement. Our study was conducted between April–September and the improved HDL and LDL cholesterol levels may have been a result of a seasonal change. Other authors have reported improved lipid levels during the summer months [20].

The SBP difference between the exercise and the control groups on inclusion in the study raised the question whether this difference was a reflection of differing traditions of treatment for hypertension between the two primary care practices where the patients were listed. A survey of the records of 280 and 290 patients, respectively at the two primary care practices, revealed no significant differences in blood pressure levels between the two. Mean SBP (±S.D.) were 148.6 (19.9) and 146.3 (19.0), respectively (unpublished data).

The nine patients in the intervention group who did not increase their level of physical activity probably illustrate the difficulties of obtaining adherence to exercise programs pointed out by other authors [12–14]. To three of those patients three walks per week was less exercise than they did before the study started and they had not altered their exercise habits after 4 months. They could therefore not be expected to improve their risk factors in this study. Thus, 6 out of 26 patients did not reach the relatively modest exercise goal of this study, which was conducted in a primary care setting at short distances from the homes of the patients. No special utensils were required for exercising. We believe that exercise alternatives that are easily available would provide the best opportunities for the establishment of regular exercise habits.

A separate analysis of the 17 patients in the intervention group who increased their amount of exercise seems justified. It resembles a clinical situation where some patients manage to adhere to lifestyle recommendations and some do not. No improvement of glucose control could be discerned in this group, as was also the case for the entire intervention group. However, systolic and diastolic blood pressures and BMI were decreased to statistically significant degrees.

The United States Centers for Disease Control and Prevention recommend every US adult to accumulate the equivalent of 30 min of brisk walking on most, preferably all, days of the week [21]. Further studies are needed to elucidate whether or not this recommendation is consistent with improved glucose metabolism in patients with type 2 diabetes.

The intensity and frequency of exercise, at which effects on diabetes risk factors can be discerned, may not be possible to define in terms of absolute threshold values. It is also likely that the effect of increased regular exercise depends on previous exercise habits. The necessary amount of exercise can be expected to differ between individuals and to appear on a sliding scale between ‘‘no effect’’ and ‘‘maximum effect’’. Mortality studies have demonstrated that great health benefits can be achieved if unfit persons can improve their degree of fitness by participating in moderately vigorous exercise programs [22,23]. A sedentary person that starts exercising can be expected to improve his/her risk factors relatively more than a well-trained person who makes a corresponding increment in exercise intensity.

Motivation plays a central role in any medical consultation concerning lifestyle alterations. This is particularly true for the question of exercise in type 2 diabetes. The acute blood glucose reduction following a 30 min walk, demonstrated in a previous study [24], might provide motivational information to diabetes patients, many of whom may not be accustomed to exercising at all. When counseling a person with type 2 diabetes it may be tempting to suggest low intensity exercise for psychological reasons. It might be relevant to argue that ‘‘a little exercise is better than none and more is better than a little’’. At the same time it is important to provide the patient with such substantial information that he or she can set realistic goals for sustainable exercise habits.

Our results suggest that different levels of physical activity may be required for the improvement of the various risk factors in type 2 diabetes. Thus, in the patients who increased their exercise levels a significant decrease of SBP and DBP as well as BMI and total plasma cholesterol levels occurred, without a corresponding improvement of glycaemic control. More detailed knowledge of the specific effects on diabetes risk factors that can be obtained from regular physical activities adds to the credibility of clinical advice concerning the role of regular physical training as an important part of the management of type 2 diabetes.

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DIABETICMedicine

DOI: 10.1111/j.1464-5491.2011.03348.x

Article: Treatment

Effects of Nordic walking on health-related quality of life in overweight individuals with Type 2 diabetes mellitus, impaired or normal glucose tolerance

T. Fritz, K. Caidahl*, M. Osler*, C. G. Östenson*, J. R. Zierath* and P. Wändell

Center for Family and Community Medicine, Karolinska Institutet, Huddinge and *Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden

Accepted 31 May 2011

Abstract

Aims To assess the effects of 4 months of increased physical activity on health-related quality of life in overweight individuals with Type 2 diabetes mellitus, normal or impaired glucose tolerance.

Methods We included 212 individuals without severe physical or cardiovascular impairments aged 61 (57–64) years, with BMI of 29 (27.5–32) kg/m^2. Numbers are median (25th–75th percentile). Subjects were stratified based on normal glucose tolerance (n = 128), impaired glucose tolerance (n = 34) or Type 2 diabetes mellitus (n = 50). They were randomized into either a control group (n = 125), who maintained unaltered habitual lifestyle, or an exercise intervention group (n = 87), who were directed to engage in Nordic walking with walking poles, 5 h per week over 4 months. Self-reported physical activity and health-related quality of life was assessed at the time of inclusion and after 4 months.

Results Baseline health-related quality of life of this study cohort was similar to, or better than, an age- and sex-matched Swedish population sample, for 12 of 13 scales. Quality of sleep and BMI were improved for participants with normal glucose tolerance after 4 months of Nordic walking, with little or no musculoskeletal pain as compared with control subjects. No correlation was evident between improved quality of sleep and improved BMI.

Conclusions Quality of sleep improved in the group with normal glucose tolerance following 4 months of Nordic walking. BMI reduction did not account for this improvement. Nordic walking can be introduced in a primary health care setting as a low-cost mode of exercise that promotes weight loss and improved health satisfaction.

Diabet. Med. 28, 1362–1372 (2011)

Keywords exercise, heath-related quality of life, primary health care, sleep, walking

Abbreviations SDPP, Stockholm Diabetes Prevention Program; SWED-QUAL, Swedish health-related quality of life questionnaire

Introduction

A sedentary lifestyle has become a major threat to public health worldwide. Physical inactivity predisposes individuals to obesity and Type 2 diabetes mellitus and often leads to premature death. People diagnosed with Type 2 diabetes are typically middle-aged or older, with a sedentary lifestyle. Daily exercise has been considered a cornerstone in the lifestyle management of Type 2 diabetes and numerous studies have provided evidence for reduced mortality and risk of cardiovascular disease in physically active people, irrespective of the diabetic state [1–4]. Exercise intervention promotes beneficial outcomes and positively influences morbidity and mortality even in middle-aged participants [5–7]. Moreover, regular walking, combined with reduced caloric intake, has a diabetes-preventive effect in individuals with impaired glucose tolerance [8–10].

Promoting a physically active daily life should have high priority in primary healthcare counselling. A proactive

Correspondence to: Tomas Fritz, Center for Family and Community Medicine, Karolinska Institutet, Alfred Nobels Allé 12, SE 141 83 Huddinge, Sweden. E-mail: t.fritz@telia.com

(Clinical Trials Registry No; NCT 00852982)

intervention by primary care physicians could be a valuable approach to reduce premature cardiovascular morbidity and mortality and to delay, or even prevent, the development of impaired glucose tolerance and Type 2 diabetes. However, an older sedentary population with Type 2 diabetes is more susceptible to painful conditions of the musculoskeletal system [11]. Introducing physical activity as part of a daily regimen for the treatment of Type 2 diabetes must therefore be initiated with caution. Moreover, attention should be given to quality-of-life aspects in assessing the benefits of an exercise programme on an older, sedentary population with Type 2 diabetes.

The aim of this study was to determine if increasing daily physical activity, in the form of Nordic walking with walking poles, would influence health-related quality of life in overweight individuals with normal glucose tolerance, impaired glucose tolerance or Type 2 diabetes.

Subjects and methods

Patient recruitment

This investigation was a randomized, controlled study. Recruitment was achieved through newspaper advertisements and personal letters of invitation to 447 former participants in the Stockholm Diabetes Prevention Program (SDPP) [12], living in the catchment area of the primary healthcare centre of Gustavsberg, a suburb outside Stockholm, Sweden. The study spanned three consecutive summer seasons (2006–2008) and occurred between May and September. New participants were recruited each year. Two hundred and twelve individuals were included (118 females and 94 males). Ninety participants were recruited by advertisements and 122 were former SDPP participants. The participants were stratified for state of glucose tolerance (normal, impaired glucose tolerance and Type 2 diabetes) and subsequently randomized to either a control or exercise-intervention group. Each year, new entries into the study were randomized into control or intervention groups. For the randomization procedure, blinded labels with the participants' names were drawn from a box and assigned to either the control or intervention group. Subjects randomized to the control group were invited to participate in the intervention group the following year. The subjects who joined the study in both control (first entry) and intervention (second entry) groups are represented only once in this work, as control subjects, skewing the control/exercise group numbers. The control group consisted of 125/212 individuals. The intervention group consisted of 87/212 individuals. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm.

Biochemistry and anthropometry

At the time of inclusion and after 4 months, an oral glucose tolerance test was performed. Plasma glucose was first determined in the fasting state, prior to the ingestion of 75 g of glucose in water solution, and again after 2 h, and calculated as the mean of two capillary blood samples. We used a portable HemoCue B-Glucose analyser (Ängelholm, Sweden) for this purpose [13]. Glucose tolerance was classified as either normal glucose tolerance < 8.9, impaired (impaired glucose tolerance) 8.9–12.1 or Type 2 diabetes ≥ 12.2 mmol/l, according to the 2-h level after one oral glucose tolerance test. Of the 212 subjects, 50 were classified as 'Type 2 diabetes', 34 as 'impaired glucose tolerance' and 128 as 'normal glucose tolerance'. The mean duration (SD) of diabetes was 5 (4) years for subjects with Type 2 diabetes. Subjects who were previously diagnosed with diabetes were classified as Type 2 diabetes regardless of the oral glucose tolerance test outcome on inclusion in this study.

Body weight and height were assessed at the time of inclusion and after 4 months. BMI was calculated by the formula body weight (kg), divided by height (m) squared. A BMI of 18.5–24.9 kg/m^2 was considered normal, 25–29.9 kg/m^2 overweight and ≥ 30 kg/m^2 obese. Systolic and diastolic blood pressures were determined in the seated position by the use of a Speidell and Keller tonometer (Jungingen, Germany). All measurements were made in the morning, in conjunction with the oral glucose tolerance test.

Inclusion criteria

Inclusion criteria were as follows: age 45–69 years, BMI > 25 kg/m^2 and, for people with Type 2 diabetes, HbA_{1c} between 57 and 78 mmol/mol [International Federation of Clinical Chemistry (IFCC) standard], comparable with 7.4–9.3% [National Glycohemoglobin Standardization Program (NGSP) standard]. Exclusion criteria were physical impairment, symptoms of angina pectoris (chest pain on physical strain), atrial fibrillation determined by electrocardiogram systolic or diastolic blood pressure > 160 or > 100 mmHg, respectively, and insulin treatment.

Assessment of physical activity and intervention protocol

Exercise in this study was unsupervised. Self-reported exercise was recorded at baseline and after 4 months. The participants were asked to estimate their physical activity level during the 6 months prior to the study and during study participation using a visual analogue scale to rank the frequency of physical activity from 0 mm (none) to 100 mm (intensive daily activity).

The participants in the exercise group were instructed to increase their weekly level of physical activity by 5 h of Nordic walking for 4 months. Nordic walking is a unique fitness technique that utilizes walking poles to involve the upper body in the exercise, in addition to providing extra support. Participants received verbal instructions for Nordic walking by a physiologist/personal trainer. The exercise group participants were provided with a diary and asked to record date and number of minutes for each bout of Nordic walking. To compare self-reported physical activity with an objective assessment, 25 participants—11 control subjects and 14 intervention

participants—agreed to wear a uniaxial accelerometer on the hip, for 7 days during waking hours (ActiGraph model GT1M; ActiGraph, Pensacola, FL, USA). This type of accelerometer records physical activity as total activity counts per min and min per day of inactivity, low, moderate or vigorous activity and is considered a valid and reliable tool for measuring physical activity in adults [14]. No instructions regarding eating habits or nutrition were provided and no dietary intervention was administered. The participants in the control group were directed to continue their habitual daily activity.

Health-related quality of life assessment

A quality-of-life questionnaire was provided at the time of inclusion and after 4 months. The Swedish health-related quality of life questionnaire (SWED-QUAL) [15], adapted from the Medical Outcomes Study [16], was used. Medical Outcomes Study assessments are generic (as opposed to disease specific) health-related quality of life questionnaires that present results as a health profile. The 66 items of the SWED-QUAL provide the basis for 13 scales that represent the following aspects of health-related quality of life: physical functioning, satisfaction with physical health, pain, role limitation attributable to physical health, role limitation attributable to emotional health, positive affect, negative affect, cognitive functioning, sleep, general health perception, family functioning, marital functioning and sexual functioning. The multi-item scales are set between 0 and 100 points, where 0 indicates the lowest possible score and 100 the highest. Inter-group differences of the SWED-QUAL results are expressed in terms of statistical significance. Effect size is a complementary method for identifying minimally important differences of patient-reported outcomes. The minimally important difference is defined as the smallest change in a patient-reported outcome that is perceived by the patient as beneficial. The effect size is defined by the formula: effect size = $(M_{treatment}–M_{comparison})/SD_{pooled}$, M = 'mean' i.e. 'M treatment' = mean value after treatment/intervention. 'M comparison' = mean value before treatment/intervention. An effect size of 0.2 is considered small, 0.5 moderate and 0.8 is a large change [17]. The SWED-QUAL has been used in previous studies including patients with Type 2 diabetes [18,19]. A random sample of 2500 Swedish men and women, aged 18–85 years, has previously answered the SWED-QUAL. Health status and anthropometric data are not known for this Swedish population sample. The SWED-QUAL results of each study participant were matched, and compared with, the aggregated results of all Swedish population sample individuals of the same age and sex.

Statistical methods

The original power calculation, based on the assumed differences in SWED-QUAL scales of 0 vs. 5 between the control and intervention groups, with $\alpha = 0.05$ and power = 0.8, indicated that 63 participants would be required in each group. With the skewed randomization, explained above, a power = 0.8 would be obtained by 77/54 participants. In the group with normal glucose tolerance, we included 75/53 individuals (= power 0.7957). The study was underpowered for participants with impaired glucose tolerance and those with Type 2 diabetes.

Stata statistical software (StataCorp., College Station, TX, USA) was used to calculate differences. The score for each scale was calculated as the median (25th–75th percentile) of the items (questions) included in each of the 13 scales. Differences within and between groups were calculated by non-parametric tests: Wilcoxon's signed-rank test for within-group comparisons and Wilcoxon's rank-sum test for between-group comparisons. Sex differences between groups were calculated by χ^2-test. *P*-values < 0.05 were considered statistically significant. Spearman's rho was determined for the assessment of correlation.

Results

Baseline data in Table 1 are presented separately for participants with normal glucose tolerance, impaired glucose tolerance and Type 2 diabetes. The 212 participants were 61 (57–64) years of age, with a BMI of 29 (27.5–32) kg/m^2. Numbers are median (25th–75th percentile). Fifty-three per cent of the study participants were overweight (BMI 25.0–29.9 kg/m^2) and 47% were obese (BMI ≥ 30 kg/m^2). ANOVA reflected a disease-state dependency for baseline levels of HbA_{1c} ($P < 0.001$), systolic blood pressure ($P = 0.019$) and BMI ($p = 0.0024$). Diastolic blood pressure levels were, however, similar between groups of varying glucose tolerance ($P = 0.85$). The 50 participants with Type 2 diabetes reported baseline health-related quality of life scores at the same level as the 128 participants with normal glucose tolerance, for all 13 SWED-QUAL scales, when compared with Wilcoxon's rank-sum test (statistics not shown). Of the 50 participants classified as Type 2 diabetes, 45 were previously known to have Type 2 diabetes and 21 were taking anti-diabetic medication. Of the remaining five individuals classified as Type 2 diabetes, two were again classified as Type 2 diabetes according to the oral glucose tolerance test at the end of the study. Two were classified as having impaired glucose tolerance and one as having normal glucose tolerance according to this second oral glucose tolerance test. Ten participants withdrew from the study prior to the conclusion. The principle of last-observation-carried-forward was applied in those cases and their initial results were thus included in the data analysis.

Following a 4-month period of either sustained (control group) or increased physical activity (Nordic walking, 5 h per week), BMI was reduced by –1.0 (–1.0 to 0.0) kg/m^2 in the intervention group with normal glucose tolerance and by 0.0 (–1.0 to 1.0) kg/m^2 in the control group with normal glucose tolerance ($P = 0.0019$). BMI did not change significantly in the groups with impaired glucose tolerance or Type 2 diabetes (Table 2).

Visual analogue scale estimates of physical activity increased significantly in the intervention groups with normal glucose tolerance and Type 2 diabetes, by 30 (10–40) and 24 (10–40)

Table 1 Baseline characteristics of participants with normal glucose tolerance, impaired glucose tolerance and Type 2 diabetes by control and intervention groups*

	NGT Control group	NGT Intervention group	*P*	IGTControl group	IGT Intervention group	*P*	Type 2 diabetes Control group	Type 2 diabetes Intervention group	*P*
n	75	53		20	14		30	20	
Age, years	61 (55–64)	60 (57–64)	0.9555	62.5 (59.5–64)	60 (56–63)	0.1698	60.5 (58–64)	63 (59–64)	0.6261
Sex, male/female %†	36/64	38/62	0.770	45/55	36/64	0.195	67/33	65/35	0.765
BMI, kg/m^2	29 (27–31)	29 (27–31)	0.7663	30.5 (28–34)	31.3 (28–38)	0.6602	29.5 (28–34)	30.5 (27.5–34)	0.8265
HbA_{1c}‡									
mmol/mol	38 (36–41)	37 (36–40)	0.1604	40 (40–42)	41 (37–34)	0.9718	50 (45–54)	53 (47–60)	4164
%	5.7 (5.5–5.9)	5.6 (5.5–5.8)	0.1604	5.8 (5.8–6.0)	5.9 (5.6–6.1)	0.9577	6.7 (6.2–7.1)	7.0 (6.4–7.7)	0.38280.
SBP, mmHg	140 (130–150)	140 (130–145)	0.7363	135 (132.5–150)	140 (130–155)	0.5138	145 (135–150)	145 (132.5–152.5)	0.9046
DBP, mmHg	85 (80–90)	85 (80–90)	0.8593	85 (80–90)	85 (75–90)	0.8577	85 (75–90)	85 (77.5–90)	0.5368
Physical activity estimate by VAS, mm	50 (20–60)	35 (20–50)	0.0267	35 (20–50)	26.5 (10–50)	0.5425	40 (20–50)	30 (30–60)	0.4892

Values are median (25th–75th percentile).
*P for intervention/control group difference by Wilcoxon's rank-sum test.
†P for sex distribution by χ^2-test.
‡Percentage [National Glycohemoglobin Standardization Program (NGSP) standard] and mmol/mol [International Congress of Clinical Chemistry and Laboratory Medicine (IFCC) standard].
DBP, diastolic blood pressure; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; SBP, systolic blood pressure; VAS, visual analogue scale.

mm, respectively, median,(25th–75th percentile), as shown in Table 2. The calculated effect sizes were 1.0 and 0.8. Walking diaries were obtained from 91% of the participants in the intervention group and 78% of the participants with normal glucose tolerance reported ≥ 80% (4 h/week) of prescribed Nordic walking. Corresponding figures for participants with impaired glucose tolerance and Type 2 diabetes were 67 and 50%, respectively. Median values (25th–75th percentile) of hours per week of Nordic walking were 4.7 (4.1–5.2), 4.6 (3.7–6.0) and 3.8 (3.1–4.7) for the groups with normal glucose tolerance, impaired glucose tolerance and Type 2 diabetes, respectively. ANOVA did not indicate any significant difference between the three groups (P = 0.1468). Comparison by Wilcoxon's rank-sum test showed a significant difference between participants with normal glucose tolerance and those with Type 2 diabetes (P = 0.0218), whereas comparisons between participants with normal–impaired glucose tolerance and impaired glucose tolerance–Type 2 diabetes showed no significant differences in hours of Nordic walking per week (P = 0.8702 and 0.1557, respectively).

Total counts per min, recorded by accelerometers, were 348 (220–528) for the control group (n = 11) and 466 (348–623) for the intervention group (n = 14), P < 0.001, Wilcoxon rank-sum test. Data are median (25th–75th percentile).

Quality of life was assessed with the SWED-QUAL questionnaire before and after 4 months of control or exercise intervention. Results of the intention-to-treat analysis of the SWED-QUAL are presented in Table 2, parallel with corresponding data from an age- and sex-matched Swedish population sample. At baseline, the group with normal glucose tolerance including both control and intervention participants scored significantly higher for eight SWED-QUAL scales [physical functioning (effect size = 0.4), satisfaction with physical functioning (0.4), role limitation because of physical health (0.2), positive affect (0.2), cognitive functioning (0.2), sleep (0.2), general health ([0.3) and sexual functioning (0.4)], compared with the Swedish population sample. For the remaining five scales, the baseline scores did not differ between the group with normal glucose tolerance and the Swedish population sample. The group with impaired glucose tolerance, including both control and intervention participants, scored significantly higher than the Swedish population sample for physical functioning (0.4), cognitive functioning (0.3), family life (0.6) and sexual functioning (0.5). For the other nine scales, there were no significant differences between participants with impaired glucose tolerance and the Swedish population sample. The group with Type 2 diabetes scored significantly higher for sexual functioning (0.3) than their Swedish population sample counterparts.

Quality of sleep and general health improved significantly in the normal glucose tolerance intervention group, compared with the normal glucose tolerance control group; effect size 0.4 and 0.1, respectively. In the Type 2 diabetes intervention group, satisfaction with physical functioning and sleep improved significantly; effect size 0.7 and 0.5, respectively.

Table 2 SWED-QUAL intention-to-treat analysis of changes after 4 months (ΔT4) of Nordic walking; data for participants with (a) normal glucose tolerance (NGT), (b) impaired glucose tolerance (IGT) and (c) Type 2 diabetes (T2DM), by control and intervention groups*

(a)

	NGT	ΔT4	NGT	ΔT4	*P*‡	NGT	SPS compared with control + intervention	*P*§
	Control, *n* = 75	NA	Intervention, *n* = 53	NA	NA	All, *n* = 128	*n* = 128	1.000
Age, years	61 (55–64)	NA	60 (57–64)	NA	0.9555	60.5 (55–64)	NA	NA
Sex, male/female %	36/64	NA	38/62	NA	0.770	37/63	37/63	1.000
BMI, kg/m^2†	29 (27–31)	0 (−1 to 1)	29 (27–31)	−1 (−1 to 0)	**0.0019**	29/27–31)	Unknown	NA
Physical functioning	95.3 (85.7–95.3)	0 (0:4.7)	95.3 (85.7–95.3)	0 (0–4.7)	0.7188	95.3 (85.7–95.3)	86.4 (81.7–88.7)	**< 0.0001**
Satisfaction with physical health	67 (33–67)	0 (0–33)	67 (33–67)	0 (0–33)	0.4143	67 (33–67)	65.9 (60–70.6)	**0.0030**
Pain	79.3 (64–100)	0 (−9.7 to 15.1)	79.3 (64.2–100)	0 (0–6.8)	0.1876	79.3 (64.1–100)	77.2 (74–82.2)	0.6799
Role limitation attributable to physical health	77.7 (44.3–100)	0 (−11 to 22.3)	78 (55.7–100)	0 (0–22)	0.3541	78 (50–100)	62.2 (60.5–71.2)	**0.0180**
Role limitation attributable to emotional health	100 (55.7–100)	0 (0–22)	89 (66.7–100)	0 (−11 to 11)	0.2018	94.5 (55.7–100)	74.3 (68.2–81.8)	0.0794
Positive affect	87.5 (75–95.8)	0 (−4.2 to 4.2)	87.5 (70.8–95.8)	0 (−8.3 to 12.5)	0.4218	87.5 (72.9–95.8)	76.1 (70.5–81.2)	**0.0003**
Negative affect	83.3 (62.5–95.8)	4.2 (0–12.5)	87.5 (62.5–95.8)	0 (−4.2 to 8.4)	0.2234	87.5 (62.5–95.8)	73.3 (68.3–79.7)	0.0700
Cognitive functioning	87.5 (66.7–95.8)	0 (−4.2 to 8.3)	83.3 (62.5–91.7)	0 (−4.2 to 8.4)	0.7666	83.3 (66.7–95.8)	74.6 (69.7–79.7)	**0.0099**
Sleep	82.1 (60.7–96.4)	0 (−10.7 to 3.6)	75 (46.4–92.9)	3.6 (−3.6 to 14.3)	**0.0091**	78.6 (57.1–94.7)	67.1 (62.8–74.2)	**0.0089**
General health perception	87.5 (75–93.8)	0 (0–6.2)	81.3 (65.6–93.8)	3.2 (0–12.5)	**0.0302**	84.4 (71.9–93.8)	73.6 (69.9–78.8)	**0.0005**
Family functioning	91.8 (73–100)	0 (0–0)	100 (79.3–100)	0 (0–6.3)	0.5179	93.8 (73–100)	84.3 (79.3–88.1)	0.0899
Marital functioning	83.3 (66.7–100)	0 (−4.1 to 4.2)	95.8 (70.8–100)	0 (−12.5 to 8.3)	0.8978	85.4 (68.8–100)	83.1 (77.4–88.3)	0.6352
Sexual functioning	90 (60–100)	0 (−5 to 5)	90 (65–100)	0 (0–0)	0.6014	90 (62.5–100)	64.8 (58.7–72.5)	**< 0.0001**
Physical activity estimated by VAS, mm	50 (20–60)	0 (−10 to 10)	35 (20–50)	30 (10–40)	**< 0.0001**	NA	NA	NA

(b)

	IGT	ΔT4	IGT	ΔT4	*P*‡	IGT	SPS compared with control + intervention	*P*§
	Control, *n* = 20	NA	Intervention, *n* = 14	NA	NA	All, *n* = 34	*n* = 34	1.000
Age, years	62.5 (59.5–64)	NA	60 (56–63)	NA	0.1698	61.5 (58–64)	NA	NA
Sex, male/female %	45/55	NA	36/64	0.195	NA	41/59	41/59	1.000
BMI, kg/m^2†	30.5 (28–34)	0 (−1 to 0)	31.3 (28–38)	0 (−1 to 0.5)	0.4301	30.8 (28–34)	Unknown	NA
Physical functioning	95.3 (85.7–95.3)	0 (0–4.7)	92.95 (85.7–95.3)	2.3 (0–4.7)	0.6196	95.3 (85.7–95.3)	84.9 (82.1–87.9)	**0.0122**
Satisfaction with physical health	67 (33–67)	0 (0–0)	67 (33–67)	0 (0–33)	0.5876	67 (33–67)	63 (60–70.6)	0.2415
Pain	79.3 (61.1–100)	0 (−0.1 to 20.7)	89.7 (69.7–100)	0 (0–11.3)	0.9710	79.3 (64–100)	76.7 (74–81.8)	0.3879
Role limitation attributable to physical health	78 (44.3–100)	0 (0–16.5)	61.2 (22–100)	5.5 (0–33.3)	0.6470	78 (33–100)	62.2 (62–67.8)	0.6753
Role limitation attributable to emotional health	100 (77.9–100)	0 (−5.5 to 0)	67 (33–100)	0 (0–33)	0.2550	100 (67–100)	74.9 (70.8–81.8)	0.2701

Table 2 *(Continued)*

(b)

	IGT	ΔT4	IGT	ΔT4	P‡	IGT	SPS compared with control + intervention	P§
	Control, *n* = 20	NA	Intervention, *n* = 14	NA	NA	All, *n* = 34	*n* = 34	1.000
Positive affect	91.7 (83.3–97.9)	0 (−4.2 to 4.2	83.4 (45.8–95.8)	0 (−4.2 to 4.2)	0.9859	91.7 (79.2–95.8)	77.1 (70.6–81.2)	0.0588
Negative affect	87.5 (79.2–100)	2.1 (−6.2 to 12.5)	81.3 (50–95.8)	6.3 (0–37.5)	0.2115	85.4 (70.8–100)	74 (70.6–80.2)	0.1741
Cognitive functioning	93.8 (75–100)	0 (−4.2 to 6.3)	83.4 (62.5–100)	0 (−12.5 to 8.3)	0.5310	91.7 (70.8–100)	75.8 (70.2–79.7)	**0.0205**
Sleep	82.2 (60.7–94.7)	−1.8 (−9.0 to 7.1)	73.2 (57.1–89.3)	0 (−7.2 to 10.7)	0.5389	76.8 (57.1–92.9)	67.7 (60.2–75.7)	0.1303
General health perception	78.1 (64.1–90.7)	6.2 (0–17.2)	86.0 (56.3–96.9)	3.1 (0–12.5)	0.3081	84.4 (59.4–93.8)	74.5 (70.1–77.9)	0.2415
Family functioning	100 (88.7–100)	0 (0–0)	92.8 (79.3–100)	0 (−6.2 to 0)	0.7431	100 (79.3–100)	84.9 (83–87.5)	**0.0049**
Marital functioning	97.9 (75–100)	0 (0–4.2)	85.4 (54.2–100)	4.2 (0–16.7)	0.2452	87.5 (70.8–100)	83.7 (77.1–87.8)	0.4622
Sexual functioning	100 (75–100)	0 (0–0)	92.5 (45–100)	−2.5 (−10:0)	0.1752	95 (65–100)	64.1 (58.7–72.2)	**0.0039**
Physical activity estimated by VAS, mm	35 (20–50)	8.5 (0–20)	26.5 (10–50)	20 (8.5–50)	0.0784	NA	NA	NA

(c)

	T2DM	ΔT4	T2DM	ΔT4	P‡	T2DM	SPS compared with control + intervention	P§
	Control, *n* = 30	NA	Intervention, *n* = 20	NA	NA	All, *n* = 50	*n* = 50	1.000
Age, years	60.5 (58–64)	NA	63 (59–64)	NA	0.6261	62 (58–64)	NA	NA
Sex, male/female %	67/33	NA	65/35	NA	0.765	66/34	66/34	1.000
BMI, kg/m²†	29.5 (28–34)	0 (−1 to 0)	30.5 (27.5–34)	0 (−1 to 0)	0.1867	30 (28–34)	Unknown	NA
Physical functioning	90.6 (81–95.3)	0 (−4.6 to 4.7)	90.6 (74.2–100)	0 (0–11.5)	0.5057	90.6 (76.4–95.3)	83.5 (81.4–86.4)	**0.0441**
Satisfaction with physical health	67 (33–67)	0 (0–0)	50 (33–67)	0 (0–33.5)	**0.0332**	67 (33–67)	62.5 (58.3–66.7)	0.0566
Pain	100 (73.7–100)	0 (0–4.1)	84.8 (52.9–100)	0 (−6.9 to 11.2)	0.8450	92.4 (62.7–100)	76.7 (74.6–80.1)	0.1975
Role limitation attributable to physical health	67 (44.3–100)	0 (0–22.3)	78 (50–100)	0 (0–0)	0.3203	67 (44.3–100)	62.2 (60.7–67.3)	0.2487
Role limitation attributable to emotional health	67 (44.3–100)	0 (−11 to 33)	100 (38.7–100)	0 (0–0)	0.6142	83.5 (44.3–100)	73.4 (66.1–76.5)	0.9884
Positive affect	83.3 (75–95.8)	−4.2 (−12.5 to 12.5)	87.5 (66.7–95.8)	0 (−8.3 to 0)	0.9603	83.3 (66.7–95.8)	73.6 (69.8–80.5)	0.0604
Negative affect	81.3 (62.5–91.7)	2.9 (0–16.7)	83.4 (50–100)	0 (−2.1 to 12.5)	0.7950	81.3 (54.2–100)	73.2 (68.3–78.1)	0.3566
Cognitive functioning	83.3 (58.3–95.8)	0 (−8.3–8.3)	77.1 (33.3–93.8)	0 (0–8.3)	0.1915	81.7 (58.3–95.8)	72.5 (69.4–82.5)	0.7318

Table 2 (*Continued*)

(c)

	T2DM	ΔT4	T2DM	ΔT4	*P*‡	T2DM	SPS compared with control + intervention	*P*§
	Control, *n* = 30	NA	Intervention, *n* = 20	NA	NA	All, *n* = 50	*n* = 50	1.000
Sleep	75 (64.3–96.4)	−5.3 (−14.3 to 7.2)	69.7 (51.8–85.7)	5.3 (−3.5 to 16.1)	**0.0312**	75 (60.7–96.4)	69.8 (60.2–76.3)	0.4901
General health perception	82.9 (65.6–90.6)	0 (−9.3 to 6.2)	75 (59.4–89.1)	3.1 (−3.2 to 11.0)	0.4567	81.3 (65.6–90.6)	72.8 (70.9–78.2)	0.1410
Family functioning	80.3 (73–100)	0 (0–6.3)	89.7 (57.4–100)	0 (0–27)	0.3930	83.4 (60.5–100)	84.7 (79.3–87.5)	0.2949
Marital functioning	77.1 (62.5–95.8)	0 (−8.4 to 9.4)	75 (58.3–97.9)	0 (−6.3 to 6.3)	0.8090	75 (58.3–95.8)	83.7 (77.4–87.8)	0.0703
Sexual functioning	85 (55–95)	0 (−3.8 to 5)	85 (70–95)	0 (−10 to 0)	0.2781	85 (70–95)	69.2 (59.3–75.8)	**0.0207**
Physical activity estimated by VAS, mm	40 (20–50)	0 (−1 to 15)	30 (30–60)	24 (10–40)	**0.0047**	NA	NA	NA

Data are median (25th–75th percentile).
*Baseline data for the entire NGT, IGT, and T2DM groups, respectively, are compared with the results of sex- and age-matched individuals from a healthy Swedish population sample (SPS) who have previously answered the SWED-QUAL questionnaire.
†BMI for Swedish population sample subjects not available.
‡*P* for intervention effect by Wilcoxon's rank-sum test.
§*P* for comparison between study participants and Swedish population sample by Wilcoxon's signed-rank test and *P* for sex distribution by χ^2-test.
NA, not available; VAS, visual analogue scale.
Bold numbers signify statistically significant differences, i.e. P < 0.05.

Correlation analysis showed no significant relationship between change of quality of sleep and change of BMI (Spearman's rho –0.12).

Discussion

The findings of this study provide evidence that overweight Swedish individuals with normal glucose tolerance, impaired glucose tolerance or Type 2 diabetes report health-related quality of life at the same level as, or better than, an age- and sex-matched Swedish population sample. The participants in this study did not suffer from any severe physical or cardiovascular impairment that limited daily activity. Moreover, their blood pressure was within, or near normal range and glucose metabolic control was within the acceptable range for the participants with Type 2 diabetes (Table 1). Other studies have reported impaired health-related quality of life in people with Type 2 diabetes [20]. The most prominent factors that negatively affected health-related quality of life in people with Type 2 diabetes were macrovascular disease (coronary heart disease and stroke), psychiatric disorders (depression) and musculoskeletal disorders. In the absence of co-morbidity or diabetes complications, Type 2 diabetes does not directly impair health-related quality of life. It is noteworthy that the participants with normal glucose tolerance in our study scored significantly higher than the Swedish population sample cohort for eight SWED-QUAL scales, whereas the participants with impaired glucose tolerance scored significantly higher than the Swedish population sample cohort for four scales and the participants with Type 2 diabetes for one scale. This might imply that health-related quality of life is impaired by a worsened state of glucose tolerance. However, the number of participants with impaired glucose tolerance and with Type 2 diabetes in this study was rather low compared with the group with normal glucose tolerance. This result should therefore be interpreted with caution.

Although quality of sleep at baseline was at the same level, or better, in our study group compared with the Swedish population sample, this variable improved significantly after 4 months of increased physical activity, whereas it tended to worsen in the control group. The control group with Type 2 diabetes reported a pronounced worsening of quality of sleep. We have no satisfactory explanation for this. There were no indications of a worsened state of diabetes during the time of study that might otherwise have offered an explanation. BMI was significantly lowered in the intervention group, but there was no correlation between improved quality of sleep and lowered BMI. An increase of physical activity may therefore have a positive effect on the quality of sleep.

A report on the treatment of insomnia in adult Swedish citizens has recently been published by the Swedish Council on Health Technology Assessment [21]. Insomnia, defined as difficulty in falling asleep or early awakening more than three times per week, was reported by 24% of study participants. Moreover, 11% reported impaired daytime quality of life as a result of disturbed night-time sleep. Furthermore, a questionnaire focusing on insomnia was randomly distributed to Swedish primary care physicians. The responding physicians (n = 352) reported seeing two or more patients per week who requested care for insomnia, more than half of whom were over 65 years of age. Medication for sleeping disorders was prescribed for approximately 8% of adult Swedish citizens in 2008 and to elderly people, often for long-term use. There is concern that the persistent use of certain hypnotic drugs may lead to problems of adverse effects and addiction over time. Thus, Nordic walking and other modes of exercise may provide a less costly and safer alternative to medication as a means of alleviating some sleep disorders. The Swedish Council on Health Technology Assessment report concludes that the evidence for physical activity and other alternative methods for treating insomnia are insufficient and therefore further research is warranted.

Quality of sleep appears to be a sensitive marker of holistic well-being, possibly reflecting the influence of somatic disease and various factors of psychosocial stress. Individuals with Type 2 diabetes whose medical health deteriorated over 4 years have shown deteriorating quality of sleep [22]. A study of psychological distress identified insomnia as one psychological stress factor contributing to the increased risk of developing pre-diabetes and Type 2 diabetes in Swedish middle-aged men, but not in women [12]. A reduced risk of sleep disorders in men and women who participated in physical activity at least once a week, and in men who walked at a brisk pace for more than six blocks daily has been reported [23]. Studies measuring sleep physiology in individuals with or without daily exercise have been contradictory, as assessed in a meta-analytic review. Total sleep time and slow-wave sleep seemed to increase as a result of exercise, whereas rapid eye movement sleep and sleep onset latency was decreased [24]. The associations between short sleep duration and increased prevalence of Type 2 diabetes and hypertension have been described [25,26]. It has been suggested that the common mechanism may be an over-secretion of adrenocorticotrophic hormone and cortisol, and that activation of the hypothalamic–pituitary-adrenal axis in these patients may play a role [24,25]. Hypothalamic–pituitary-adrenal axis activation is regarded as an effect of various stress factors, and hypothalamic–pituitary-adrenal hyperactivity has been suggested to promote the development of insulin resistance, central obesity, dyslipidaemia, hypertension and Type 2 diabetes [27].

An association of excess weight and impaired health-related quality of life has been described [28,29]. The authors provide evidence that physical aspects of health-related quality of life are negatively affected by excess weight or obesity. The results of our study do not indicate that overweight per se, in the absence of other disabling conditions, impairs health-related quality of life.

The participants with Type 2 diabetes did not report increased pain in comparison with Swedish population sample counterparts (Table 2). This finding is highly relevant, as painful conditions of the musculoskeletal system are more prevalent in people with Type 2 diabetes than in those without Type 2 diabetes [11]. Musculoskeletal pain was a primary reason for subject withdrawal from a Dutch study of a medical fitness

programme for patients with Type 2 diabetes [30]. Reported pain did not increase in exercise participants with normal glucose tolerance, impaired glucose tolerance or Type 2 diabetes in our study. No participant left the study for reasons involving musculoskeletal pain. Nordic walking may thus be a safe mode of introductory exercise, even for individuals with Type 2 diabetes.

Limitations of this study

Four months' intervention is potentially too brief for measurable effects to occur on some of the aspects of health-related quality of life under study. Long-term studies of similar exercise intensity and frequency are warranted to elucidate whether Nordic walking may be a sustainable mode of exercise, and whether it produces a more pronounced effect on health-related quality of life than in this brief intervention study.

The daily physical activity of participants in this study, prior to entry and during the study, was not uniform or controlled or matched. Self-reported level of physical activity at baseline varied considerably. This factor may have diminished the perceived exercise effects on health-related quality of life. The addition of Nordic walking may not have a substantial effect on quality of life to a person who was moderately physically active before inclusion in this study. In future studies of this kind, basal exercise exclusion criteria will be considered.

Exercise was not controlled and self-reporting of daily exercise, as carried out in this study, is not as accurate as supervised activities. Moreover, the visual analogue scale, utilized for estimating the frequency of daily physical activity, has not been validated as a measure of physical activity. In addition, some physical exercise normally undertaken may have been replaced by Nordic walking, thus diminishing the intended increase of physical activity during the time of study.

The numbers of participants with impaired glucose tolerance and Type 2 diabetes were too low to allow conclusions of statistical significance for these groups. The results of this study, indicating lower SWED-QUAL scores for people with impaired glucose tolerance and Type 2 diabetes, compared with participants with normal glucose tolerance must therefore be interpreted with caution.

The fact that more than half of the participants had previously been involved in the SDPP could theoretically have created some bias in our findings. The SDPP was not, however, an interventional study on the individual level and comprised no elements of lifestyle intervention. Moreover, the SWED-QUAL questionnaire was not used in the SDPP survey. Awareness of being overweight was an expressed concern and reason for the majority of individuals to participate in this study. It would seem unlikely that the former participation in the SDPP could somehow influence the results of this study.

Baseline classification of normal glucose tolerance, impaired glucose tolerance or Type 2 diabetes was based on one oral glucose tolerance test only. The diagnosis of asymptomatic diabetes in clinical practice would require the confirmation by a second oral glucose tolerance test. For the 45 participants with a diagnosis of Type 2 diabetes established prior to the time of inclusion in this study, a second oral glucose tolerance test would be redundant. Five participants were classified as 'previously unknown Type 2 diabetes' and, for two individuals in this group, the diagnosis was confirmed by a second oral glucose tolerance test at the end of the study. The remaining three individuals were classified as 'impaired glucose tolerance' (2) and 'normal glucose tolerance' (1) after the second oral glucose tolerance test.

Strengths of this study

This study was undertaken in a primary care setting. It can serve as an example of how exercise could be introduced to selected patients who might profit from a more physically active lifestyle. The participants were responsible for their own exercise schedules and the study did not involve costly supervision of exercise sessions. The exercise participants received instructions from a personal trainer for Nordic walking at baseline and one supportive telephone call after 2 months from an assistant nurse.

Although not a very strenuous mode of exercise, Nordic walking is more strenuous than brisk walking as the use of walking poles also activates the muscles of the upper limbs. It may therefore suit people who are unaccustomed to exercising. Our experience was that Nordic walking did not cause adverse reactions of musculoskeletal pain.

Three different means of reporting physical activity were used in this study: visual analogue scales, exercise diaries and accelerometers. All methods indicated that the intervention group was more physically active than the control group. Although the intensity of exercise was not well established, we can reasonably assume that the self-reported amount of exercise has been a rather accurate account of actual exercise performed, based on the subjects who also used accelerometer reporting.

Future research

The implementation of sustainable, regular exercise habits is a challenging undertaking. Exercise on prescription is presently practised in Swedish primary health care, and has been shown to promote increased self-reported physical exercise and improved quality of life [31]. Our results imply that a small increment of regular physical activity does not impair health-related quality of life. In our study, health-related quality of life tended to improve following exercise intervention. This experience could be used in clinical efforts of motivating patients to assume a more physically active life, a factor of utmost importance in health counselling of people with excess weight, disturbed glucose metabolism, hypertension and other cardiovascular risk factors. Introducing regular exercise to individuals unaccustomed to exercise training or suffering from cardiovascular disease should be done with care, taking into account the risks of cardiovascular or musculoskeletal complications.

Future studies of health-related quality of life in relation to physical activity should involve supervised exercise and participants with a somewhat uniform, and low, level of habitual exercise habits at inclusion. Elucidating whether people with impaired glucose tolerance or Type 2 diabetes are less responsive to the effects of regular exercise is clinically relevant and sufficient numbers of participants with normal glucose tolerance and Type 2 diabetes should be included.

Competing interests

Nothing to declare.

Acknowledgements

This study was supported by the Strategic Research Programme in Diabetes at Karolinska Institutet, the Stockholm County Council, the Swedish Heart Lung Foundation and the Swedish Research Council, Stockholm, Sweden. The authors thank Pia Odenblad for secretarial assistance and collection of blood samples and Petra Lundström for instructing the study participants in Nordic walking techniques. We also thank Sanofi-Aventis for unconditionally providing the study participants with walking poles and HemoCue AB for providing equipment for analysing plasma glucose levels.

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III

Effects of Nordic walking on cardiovascular risk factors in overweight individuals with type 2 diabetes, impaired or normal glucose tolerance

T. Fritz[1], K. Caidahl[2], A. Krook[3], P. Lundström[2], F. Mashili[2], M. Osler[2], F.L.M. Szekeres[2], C.G. Östenson[2], P. Wändell[1], and J.R. Zierath[2].

[1]Center for Family and Community Medicine, Karolinska Institutet, Huddinge, Sweden [2]Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden [3]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden

Running Title: Randomized control Nordic walking study

Clin trial no: NCT 00852982
Word count: Abstract 229, body text 3176; 26 references

Correspondence:

Professor Juleen R. Zierath

Section for Integrative Physiology

Department of Molecular Medicine and Surgery

Karolinska Institutet

SE 171 77 Stockholm, Sweden

e-mail: Juleen.Zierath@ki.se

Abstract

The effects of Nordic walking on cardiovascular risk factors were determined in overweight individuals with normal or disturbed glucose regulation. We included 213 individuals, aged 60±5.3 years, with BMI 30.2±3.8 kg/m^2 and normal glucose tolerance (NGT, n=128), impaired glucose tolerance (IGT, n=35) or type 2 diabetes mellitus (T2DM, n=50). Participants were randomized to unaltered physical activity or to five hours per week of Nordic walking with poles, for a four-month period. Dietary habits were unaltered. BMI, waist circumference, blood pressure, glucose tolerance, clinical chemistry, maximal oxygen uptake (peak VO_2) and self-reported physical activity (questionnaire) were assessed at the time of inclusion and after four months. The intervention participants kept a walking diary. In the NGT exercise group, self-reported physical activity increased markedly and body weight (–2.0±3.8 kg), BMI (–0.8±1.4 kg/m^2) and waist circumference (–4.9±4.4 cm), (mean±SD) decreased. Exercise power output (12.9±9.9 W) and peak VO_2 (2.7±2.8 ml × kg^{-1} × min^{-1}) increased in the IGT exercise group. More cardiovascular risk factors were improved in people with NGT compared with IGT or T2DM. Exercise capacity improved significantly in all three groups among participants at least 80% compliant with scheduled exercise time. Nordic walking improved anthropometric measurements and exercise capacity. However, unsupervised Nordic walking may not provide sufficient exercise intensity to achieve ultimate health-promoting benefits on the cardiovascular parameters assessed in this study, particularly for those with disturbed glucose regulation.

Keywords: Cardiovascular Risk Factors, Exercise, Primary Health Care, Walking

INTRODUCTION

Lifestyle changes involving exercise and food intake constitute conventional treatment of type 2 diabetes mellitus (T2DM). Regular walking, combined with reduced caloric intake, can prevent T2DM in people with impaired glucose tolerance (IGT) [1-3]. Prospective cohort studies of people with [4-6] and without [7] T2DM have provided evidence for reduced mortality in physically active, compared with more sedentary individuals. Regular physical exercise has beneficial effects on cardiovascular risk factors in people with T2DM [8]. However, adherence remains an issue, since patients with T2DM often fail to comply with exercise programs, due to musculoskeletal complications and poor motivation [9].

A meta-analysis of 27 studies, comprising 1,003 individuals with T2DM, provided evidence for a 0.8±0.3% reduction in HbA1c following aerobic and/or resistance training lasting for ≥12 weeks [10]. Many lifestyle intervention studies involve relatively intense exercise programs and expensive supervision, employing highly trained personnel. Thus, studies aimed at establishing low-level exercise programs to prevent cardiovascular risk are warranted.

In a previous study of people with T2DM, we provided evidence that four months of walking intervention, 45–60 minutes three times per week, was insufficient to improve cardiovascular risk factors [11]. We speculated that an exercise prescription of greater frequency and intensity than three hours per week of walking would be necessary to induce health benefits in overweight T2DM patients. Nordic walking with poles activates the muscles of the upper limbs and increases oxygen consumption, caloric expenditure and heart rate [12]. Applying this type of exercise, we studied a cohort of individuals with varying degrees of glucose regulation, including people with normal glucose tolerance (NGT), IGT and T2DM. The study participants increased not only the exercise intensity, but also the number of individual exercise sessions compared to our previous study.

PATIENTS AND METHODS

Patients

Inclusion criteria were age 45 to 69 years, BMI >25 kg/m2, and HbA1c for individuals with T2DM between 7.4 and 9.3% NGSP standard (57 to 78 mmol/mol International Federation of Clinical Chemistry (IFCC) standard). Exclusion criteria were physical impairments, symptoms of angina pectoris, i.e. chest pain on physical strain, atrial fibrillation determined by ECG, systolic or diastolic blood pressure >160 or >100 mmHg respectively and insulin treatment. Insulin treatment was an exclusion criterion since it would interfere with the calculation of insulin resistance. Recruitment was achieved by newspaper advertising and letters of invitation to 447 former participants in the Stockholm Diabetes Prevention Program (SDPP) [13] living in the catchment area of the primary health care center of Gustavsberg, a suburb outside Stockholm, Sweden. Examinations of the participants were performed

at the primary health care center and at the Department of Clinical Physiology, Karolinska University Hospital, Stockholm.

A total of 213 individuals were included, 123 former SDPP participants and 90 newly recruited participants from advertisements. Upon inclusion in the study, the participants were classified on the basis of one oral glucose tolerance test (OGTT) into either T2DM (n=50), IGT (n=35), or NGT (n=128). Forty-five individuals were previously classified as T2DM. The duration of diabetes was 5.1±3.7 years for people with T2DM (mean ±SD). The participants were stratified for state of glucose tolerance (NGT, IGT, or T2DM) and randomized to a control or exercise-intervention group. For the randomization procedure, blinded labels with the participants' names were drawn from a box and assigned to either the control or intervention group. The time of study spanned three consecutive summer seasons (2006–2008), between May and September. Each year new entries into the study were randomized into control (unaltered physical activity) or intervention (Nordic walking) group. Individuals randomized to the control group were invited to participate in the intervention group the following year. Those who joined the study in both control (first entry) and intervention (second entry) groups are represented only once in this work, as control subjects, thus skewing the control/exercise group numbers. The control group consisted of 126/213 individuals. The intervention group consisted of 87/213 individuals.

Study Protocol

This was a randomized controlled study. The participants in each category (NGT, IGT or T2DM) were randomized to the exercise or control group and asked to maintain their usual eating habits. The participants in the exercise group were instructed to increase their weekly level of physical activity by 5 hours of walking with poles (Nordic walking) for 4 months. An exercise physiologist provided instructions for Nordic walking. Walking intensity was prescribed as a pace that caused slight shortness of breath and perspiration. After two months the participants in the intervention group received a supportive telephone call from an assisting nurse. Written informed consent was obtained from all participants. The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm.

Basic characteristics

Body weight was measured with an electronic scale (Hugin, Mustelia, EB5011). Systolic (SBP) and diastolic (DBP) blood pressures were determined, to the nearest 5 mm Hg, in the seated position (Speidell & Keller Tonometer).

Clinical Chemistry

At the time of inclusion, one OGTT was performed. Plasma glucose was determined in the fasting state as the mean of two capillary blood samples prior to the ingestion of 75 g of glucose in water solution and again after 2 hours. Glucose was assessed with a HemoCue B-Glucose analyzer [14]. The 2-hour OGTT level was used to

classify the participants as NGT (<8.9 mmol/l), IGT (8.9 to 12.1 mmol/l), or T2DM (≥12.2 mmol/l). Participants previously diagnosed with diabetes were classified as T2DM regardless of the OGTT outcome on inclusion in this study. Venous blood samples were obtained in the fasting state and analyzed for total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and HbA1c at the Laboratory of Clinical Chemistry at Karolinska University Hospital, Stockholm. Fasting serum insulin was determined using an adipokine (HADK2-61K-B09) assay kit from Linco Research (Electra-Box Diagnostica AB, Tyresö, Sweden), according to the manufacturer's protocol. Plasma samples from each subject were extracted from whole blood and analyzed in duplicate. Results were quantified using the Luminex Bio-Plex 200 system (Bio-Rad, Stockholm, Sweden). Insulin resistance was calculated, using the HOMA IR model [15], as fasting insulin (μU/ml) × fasting glucose (mM)/22.5.

Exercise test

Bicycle exercise tests were performed using a Rodby ergometer RE 820/830. Before the exercise test, the subjects sat on the bicycle for one minute and thereafter pedaled without load for another minute to obtain a steady baseline. The initial work load was set at 50 W and was increased by 10 W/min. A SensorMedics ergospirometer Vmax Encore (SensorMedics Corporation; Yorba Linda, USA) was used to measure gas exchange. The gas analyzers were calibrated with two calibrated gases containing 16% O_2, 4% CO_2 and 26% O_2, 0% CO_2. Oxygen uptake (VO_2) was calculated breath by breath, and the mean value for the latest 30 seconds was used. The VO_2 at peak exercise (peak VO_2) was determined at the point of subjective exhaustion.

Self-reported physical activity

Self-reported exercise was registered for control and intervention participants at baseline and after four months. The participants were asked to indicate, in a questionnaire, an estimate of low, medium and high intensity physical activity. The question was: How often do you perform physical activities, more than 30 minutes at a time? Low, medium and high intensity physical activities were specified as low = not strenuous (e.g. bowling, golf, slow walk, slow bicycle ride, light gardening), medium = strenuous but still allowing you to continue a conversation (e.g. tennis, dancing, horseback riding, brisk walk, strenuous gardening) and high = strenuous enough to cause sweating, increased heart rate and pronounced shortness of breath (e.g. aerobics, workout, jogging, fast bicycle ride). Alternative answers were: never, irregularly, once a week, twice a week, > 2 times a week. The participants in the exercise group were also asked to record in the exercise diary the duration and date of each Nordic walking bout. The extent to which the intervention participants fulfilled the prescribed amount of Nordic walking was determined from analysis of the exercise diaries.

Accelerometer

To compare self-reported physical activity with an objective assessment, 25 consecutive participants (n=11 from the control group and n=14 from the intervention group) agreed to wear an accelerometer (ActiGraph model GT1M; ActiGraph, Pensacola, Florida, USA), in a belt around the waist, for seven days from morning to bedtime, shortly after randomization [16]. Physical activity was recorded as total activity counts per minute, minutes per day of inactivity, low, moderate, or vigorous activity.

Statistics

A paired t-test was used to analyze within-group differences and two-sample t-test for between-group differences. Differences of sex distribution, medication and physical activity levels were analyzed by χ^2 or Fisher's exact test. A p value of ≤ 0.05 was regarded as statistically significant. For statistical analyses Stata statistical software (Stata Corporation, College Station, Texas, USA) was used. Follow-up data were missing for 10 participants and the principle of last-observation-carried-forward was applied in those cases. Analysis was performed as intention-to-treat.

Power calculation

The power calculation was based on the number of study participants required to meet the statistical needs of a Quality of Life questionnaire, not reported in this article. The assumed differences in HbA1c of 0 versus –0.5%, between control and intervention T2DM participants, with $\alpha=0.05$ and power 0.8, indicated that 17 individuals with T2DM would be required in each group.

RESULTS

Baseline characteristics and medication

Baseline parameters are presented in Table 1. Self-reported high-intensity physical activity was significantly more frequent in the NGT control group than in the NGT intervention group. The total cholesterol level was lower and lipid-lowering statin medication was more frequent in the NGT intervention group, compared with the NGT control group. The triglyceride levels were lower in the T2DM control group but statin medication did not differ from that in the T2DM intervention group. There were no other significant differences between the control and intervention groups.

Medication was altered for four participants, all allocated to intervention; one NGT, two IGT, and one T2DM participants. Two were started on anti-hypertensive treatment and two altered their anti-hypertensive treatment. Blood pressure levels at the end of the study were not included for those four participants. Baseline SBP and DBP readings were carried forward according to intention-to-treat practice.

Effects of Nordic walking

Body weight, BMI, waist circumference, and self-reported physical activity were improved (p=0.0042, 0.0051, 0.0006, and <0.001 respectively), in the NGT exercise group compared with the NGT control group (Table 2). Exercise capacity was improved in the IGT exercise group; power output (p=0.0011) and peak VO_2 (p=0.0338), compared with the IGT controls. Self-reported high-intensity physical activity was improved (p=0.012) in the T2DM exercise group, compared with the T2DM control group. Blood pressure, blood lipids, HbA1c, and HOMA insulin resistance were unaltered between the NGT, IGT, and T2DM exercise and control groups. In the T2DM exercise group, however, the HbA1c, the 2h post OGTT plasma glucose levels and exercise power output improved in the intra-group analysis (p=0.0359/0.0391, 0.0062 and 0.0118 respectively). The results presented in Tables 1 and 2 were not altered by a multivariate linear regression analysis, adjusting for age, sex, and glucose tolerance (NGT, IGT, and T2DM), data not shown.

Reported exercise time and effects of high adherence

Hours per week of Nordic walking, according to exercise diaries, were: 4.7±1.5, 4.8±1.8, and 3.9±1.4 for the NGT, IGT and T2DM exercise groups respectively (p=0.1468 by ANOVA). Among the 25 persons wearing accelerometers, the exercise group (n=14) was more physically active than the control group (n=11). Total activity counts per minute were 496±210 for the exercise group versus 382±196 for the control group (p=0.0004 by two-sample t-test). Corresponding data for total activity counts per day were 439376±197888 and 349580±193073 (p=0.0035), inactive minutes per day 494±104 and 529±109 (p=0.0322), and vigorous activity minutes per day 7.9±17.8 and 1.4±6.3 (p=0.0028). Data from diaries and accelerometers are not included in Table 2.

Adherence to the exercise program varied within the intervention group. Therefore we performed a separate analysis for the 55 participants in the exercise group who reported ≥80% of the prescribed time of Nordic walking in their exercise diaries. In this group, exercise capacity (power output and/or peak VO2) significantly improved for the NGT, IGT and T2DM participants, compared with their respective control groups (Table 2). Improvement of body weight, BMI and waist circumference was similar to that of the entire NGT, IGT and T2DM exercise groups.

Withdrawal Rate

Ten individuals left the study before follow-up examination; 7 female (5 NGT, 1 IGT, 1 T2DM, 5 intervention and 2 control) and 3 male (1 IGT, 2 T2DM, 1 intervention, 2 control). Their baseline data were included in the intention-to-treat analysis. The motives for leaving were personal reasons/lack of time (4), medical reasons (4), and unknown (2).

DISCUSSION

Our primary finding is that a four-month unsupervised Nordic walking program improved body weight, BMI, and waist circumference in people with NGT (Table 2). Moreover, exercise capacity was improved in participants who reported ≥80% of the prescribed exercise.

Obesity was one risk factor common to all participants. The exercise intervention reduced weight, BMI and waist circumference in people with NGT, but not in those with IGT or T2DM. However, the IGT and T2DM groups were rather small compared with the NGT group. Visceral fat is a predictor of mortality [17] and waist circumference may be one simple measure of cardiovascular risk factors to gauge improvement following a period of physical activity. Our findings suggest that exercise intervention has a more pronounced effect on the anthropometric risk factors in people who do not have derangements in glucose metabolism.

Individuals in the IGT and T2DM ≥80% exercise groups (Table 2) did not report increased frequency of medium, nor high, intensity physical activity, while the NGT participants did. Exercise power output, however, improved in people with IGT or T2DM, but not in people with NGT, in the intention-to treat-analysis. The discrepancy between reported physical activity and exercise power output may be due to other physical activities being replaced by Nordic walking, or recall bias. Furthermore, the increased power output among the NGT control participants was substantial, which can explain the non-significant difference between NGT control and intervention groups with respect to exercise capacity. A habitual increase in physical activity during the summer months among the NGT control group participants may account for this difference. Data from the physical activity questionnaires, Nordic walking diaries, accelerometers, and exercise capacity tests indicate a pronounced increase in physical activity in all three intervention groups.

Due to the increasing number of individuals with, or at risk of developing T2DM [18], cost-effective healthy lifestyle programs are warranted. Improving or maintaining a high level of physical activity or physical fitness, reduces all-cause and cardiovascular death [7, 19]. The identification of key cardiovascular risk factors that are improved following a physical activity program is therefore of relevance. Exercise-referral and exercise on prescription have been practiced in the USA, the United Kingdom, and Scandinavia over the past 10–20 years, often in primary health care, and this may have led to increased physical activity levels [20-22].

The exercise intensity prescribed in this study may have been too mild, or of insufficient structure to achieve robust improvements in blood pressure, lipid levels or glucose metabolic control. Notably in the intra-group analysis of the T2DM exercise participants, the 2-hour OGTT p-glucose, HbA1c and exercise power output improved, compared with the control group, although the difference did not reach statistical significance (Table 2). The exercise protocols reported in a recent meta-analysis of 27 studies [10] included supervised exercise sessions, providing aerobic or resistance training programs that were more structured and strenuous than the Nordic walking program described in this study. Moreover the meta-analysis only included T2DM patients [10], whereas our study included individuals with NGT and IGT, which may explain the lack of improvement in overall glucose metabolic control (HbA1c) in this study. Nordic walking, five hours per week, may constitute a

minimum of exercise required for positive effects on cardiovascular risk factors studied here.

The reduced waist circumference noted in the NGT, IGT and T2DM control groups (Table 2) may reflect the fact that this study was performed during the summer months when people tend to be more physically active. The improved fasting glucose and 2-hour post-OGTT glucose values in the T2DM control group may possibly be explained by improved dietary habits (e.g. more vegetables and fruit), since neither exercise capacity nor self-reported physical activity were improved in that group.

Strengths and limitations of this study

We employed a low-cost model of exercise feasible for primary health care. While exercise protocols of greater intensity such as aerobics and/or resistance training [10, 23, 24], or home-based bicycle training [25], can yield a stronger beneficial outcome, the relatively low level of physical activity prescribed in this study could be achieved without occurrence of musculoskeletal complications in the NGT, IGT or T2DM participants, and without reported pain, which is more prevalent with T2DM [26]. Thus, Nordic walking may offer a safe mode of introductory exercise, even for people with T2DM.

Reported levels of physical activity at baseline varied considerably. Thus, the pre-existing (high) exercise levels of some participants may have limited the adaptive response to the Nordic walking. This study involved unsupervised, self-reported exercise. Whether the participants in the intervention group added Nordic walking to their pre-study daily activities, or replaced former exercise habits by Nordic walking, is unknown.

A greater number of participants would have strengthened the statistical power of this study, particularly in the IGT and T2DM groups. The participants in this study were mainly recruited from the catchment area of one primary care center during three consecutive years. IGT participants are particularly difficult to identify, since impaired glucose tolerance is not a common diagnosis in primary health care.

Food intake was not addressed in this study, but changes in dietary habits may have influenced the outcome. Indeed the increase in physical activity may alter food intake to some degree. However, the focus of this investigation was on the effects of exercise *per se*, and consequently we did not estimate the daily calorie intake of the study participants.

Future implications

Nordic walking is feasible for individuals unaccustomed to regular exercise. The improvement of body composition and physical fitness achieved following a relatively short period of moderately intense exercise could act as an incentive for overweight people to adopt a more physically active lifestyle. With supervised exercise, accompanied by motivational counseling focused on behavior changes, adherence to the program and clinical parameters may be enhanced. With an initial “trial” or “run-in” period it may be possible to identify, and exclude, individuals who are unable to adhere to an exercise program. The results of such a study would thereby be a more accurate reflection of the effects of the mode of exercise under

analysis. An improved study model for probing the effects of low/moderate physical activity should strive to include participants with more pronounced cardiovascular and metabolic risk factors to achieve a true representative picture of a primary care patient cohort. Our results may be used to support public health efforts to promote regular exercise. Physical activity on prescription issued at primary health care units may provide a tool for such support. Further studies are necessary to arrive at more precise exercise prescriptions that fully benefit individuals with obesity and/or metabolic disturbances.

ACKNOWLEDGMENTS

This study was supported by the Strategic Research Program in Diabetes at Karolinska Institutet, the Stockholm County Council, the Swedish Heart Lung Foundation and the Swedish Research Council, Stockholm. The authors thank Pia Odenblad, Primary Health Care Center, Gustavsberg, for secretarial assistance and blood sample collection; Susanne Krogstadholm-Stjernfelt, Sara Persson, Amira Soulios, Kamel Ramak and Jacob Lindberg from the Department of Clinical Physiology, Karolinska University Hospital, Stockholm, for assistance in peak VO_2 testing; Sanofi Aventis for unconditionally providing walking poles and HemoCue AB for providing equipment for the p-glucose analysis.

DISCLOSURES

Conflicts of interest: none.

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Table 1. Baseline characteristics for NGT, IGT and T2DM participants, by control/intervention.

	NGT		IGT		T2DM	
	Control	Intervention	Control	Intervention	Control	Intervention
n	75	53	21	14	30	20
Age (years)	59.3±5.9	59.4±5.4	61.8±3.4	59.1±6.2	61.0±4.7	61.4±4.6
Sex (M/F) n	27/48	20/33	10/11	5/9	20/10	13/7
Medication %						
Oral antidiabetic	0	0	0	0	63	65
Anti-hypertensive	23	21	48	43	63	60
Statin	1	11*	10	7	50	55
Weight (kg)	84.4±10.4	85.2±13.7	87.8±10.8	92.5±14.7	93.1±15.7	91.9±13.1
BMI (kg/m^2)	29.3±2.7	29.6±3.8	30.8±3.5	32.0±5.2	31.1±3.9	31.7±5.2
Waist (cm)	97.4±8.6	99.4±11.3	102.1±9.7	103.7±10.5	103.7±10.8	105.6±10.3
SBP (mmHg)	137±15.0	138±12.5	141±13.0	141±14.0	144±12.6	143±13.2
DBP (mmHg)	84±8.8	85±7.9	86±9.4	84±7.8	83±7.4	85±7.6
P glucose (mmol/l)	5.5±0.5	5.4±0.4	5.7±0.7	6.0±08.	7.8±1.7	7.9±1.4
OGTT 2h (mmol/l)	7.1±1.1	7.2±0.9	10.3±0.8	10.1±1.0	15.1±4.1	15.8±3.6
HbA1c (%)	5.7±0.3	5.6±0.3	5.9±0.3	5.9±0.4	6.9±0.9	7.1±0.9
HbA1c (mmol/mol)	39±3.8	38±3.9	41±3.3	41±3.4	52±9.5	54±9.2
$HOMA_{IR}$	2.3±1.5	2.7±2.0	2.1±1.2	2.9±2.1	3.5±1.9	2.7±1.5
Cholesterol (mmol/l)	5.7±0.9	5.4±0.9*	6.0±0.9	5.5±0.9	4.5±0.8	4.8±0.9
HDL (mmol/l)	1.7±0.4	1.6±0.4	1.6±0.4	1.5±0.4	1.3±0.3	1.4±0.3
LDL (mmol/l)	3.4±0.8	3.2±0.8	3.7±0.9	3.2±0.8	2.6±0.7	2.7±0.8
TG (mmol/l)	1.3±0.9	1.2±0.5	1.7±0.9	1.7±1.2	1.3±0.4	1.7±0.7*
Power output (W)	162.6±45.8	151.5±33.4	156.5±38.8	150.7±39.1	165.3±39.2	147.4±31.9
Ph act low	52/75	37/53	12/20	9/14	21/30	14/20
Ph act medium	39/75	23/53	5/20	7/14	13/30	10/20
Ph act high	28/75	3/53***	3/20	2/14	6/30	2/20

Values are mean±SD. P for Intervention/Control group difference by unpaired t-test, χ^2 or Fisher's exact test for sex distribution, medication and physical activity. P <0.05*; P <0.001***

NGT= normal glucose tolerance, IGT= impaired glucose tolerance. T2DM= type 2 diabetes mellitus. SBP= systolic blood pressure. DBP= diastolic blood pressure. OGTT= oral glucose tolerance test. HbA1c: %= NGSP standard, mmol/mol= IFCC standard. $HOMA_{IR}$= homeostasis model assessment of insulin resistance. Peak VO_2 = peak oxygen uptake.
Ph act = physical activity ≥2 times/week (questionnaire). Low intensity e.g. bowling, golf, slow walk, slow bicycle ride, light gardening. Medium intensity e.g. brisk walk, gardening, dancing. High intensity e.g. jogging, aerobics, workout.

Table 2. Intention-to-treat analysis of anthropometric and clinical measurements, by Control/Intervention group for NGT, IGT and T2DM participants. Separate analysis of exercise participants who achieved ≥80% of prescribed exercise. Results after four months of Nordic walking/unaltered level of exercise.

	Δ Control group	Δ Intervention group	p for inter-group effect	Δ ≥80% exercise group	p for inter-group effect
NGT	n =75	n =53		n=38	
Weight (kg)	–0.3±3.0	–2.0±3.8***	0.0042	–2.4±4.2**	0.0077
BMI (kg/m^2)	–0.1±1.0	–0.8±1.4****	0.0051	–0.9±1.5**	0.0094
Waist (cm)	–2.3±4.0****	–4.9±4.4****	0.0006	–5.2±3.9****	0.0004
SBP (mm)	–2.1±11.9	2.0±12.2	0.0602	2.0±11.9	0.0850
DBP (mm)	–0.3±8.0	1.1±8.0	0.3336	0.9±7.8	0.4454
P-glucose fasting (mmol/l)	–0.1±0.3**	–0.1±0.5	0.4854	0.0±0.5	0.1983
P-glu 2h post-load (mmol/l)	0.2±1.3	–0.1±1.1	0.1125	–0.2±1.1	0.1078
HbA1c (%)	0.0±0.2	0.0±0.2	0.4945	0.0±0.2	0.2426
HbA1c (mmol/mol)	–0.4±2.8	0.0±1.0	0.3571	–0.3±2.5	0.1475
HOMA $_{IR}$	0.02±1.8	–0.4±2.2	0.2923	–0.1±0.4	0.6679
Chol (mmol/l)	–0.04±0.5	–0.04±0.6	0.9505	–0.1±0.6	0.6140
HDL (mmol/l)	–0.1±0.2****	–0.02±0.2	0.0345	0.01±0.2	0.0103
LDL (mmol/l)	0.04±0.4	–0.04±0.6	0.3938	–0.1±0.6	0.1655
TG (mmol/l)	0.05±0.6	–0.04±0.3	0.2564	–0.03±0.3	0.3257
Power output (W)	5.0±15.0**	8.1±11.6****	0.2109	10.0±11.6****	0.0562
Peak VO_2 (ml/kg/min)	0.8±3.4$^{\#}$	2.2±5.2**	0.0891	2.3±3.2***	0.0331
Ph act medium	–1	13*	<0.001	10*	<0.001
Ph act high	–4	12**	<0.001	9**	<0.001

IGT	n=21	n=14		n=8	
Weight (kg)	–0.9±2.5	–0.5±2.2	0.6395	–0.4±2.6	0.6335
BMI (kg/m^2)	–0.3±0.9	–0.1±0.9	0.4665	–0.1±1.0	0.5329
Waist (cm)	–2.5±4.1*	–2.4±3.0*	0.8963	–3.3±2.1**	0.6382
SBP (mm)	1.6±11.1	–0.7±20.0	0.5745	–13.1±10.7*	0.0033
DBP (mm)	0.5±7.3	1.8±11.2	0.6878	–3.8±9..1	0.1983
P-glucose fasting (mmol/l)	–0.1±0.6	0.0±0.6	0.9434	–0.1±0.4	0.6871
P-glu 2h post-load (mmol/l)	–0.8±2.1$^{\#}$	–0.7±1.0*	0.8398	–1.0±0.9*	0.6910
HbA1c (%)	0.1±0.2	–0.1±0.2	0.0437	0.0±0.2	0.2452
HbA1c (mmol/mol)	0.5±2.2	–0.9±1.9	0.0607	–0.6±2.4	0.2416
HOMA $_{IR}$	0.04±1.4	–0.5±1.5	0.2632	–0.1±0.4	0.2005
Chol (mmol/l)	–0.1±0.7	0.04±0.5	0.4337	0.1±0.4	0.4352
HDL (mmol/l)	–0.02±0.1	0.04±0.1	0.2643	0.03±0.2	0.4784
LDL (mmol/l)	–0.1±0.6	0.03±0.5	0.4197	0.03±0.5	0.4905
TG (mmol/l)	–0.04±0.5	–0.07±0.5	0.8519	0.03±0.6	0.8023
Power output (W)	2.0±7.7	12.9±9.9***	0.0011	13.8±9.2****	0.0082
Peak VO_2 (ml/kg/min)	0.2±3.6	2.7±2.8**	0.0338	3.2±3.7$^{\#}$	0.0751
Ph act medium	4	0	0.088	–2	0.057
Ph act high	1	2	1.000	0	0.439

T2DM	n=30	n=20		n=9	
Weight (kg)	–0.6±2.2	–1.0±2.1*	0.4716	–1.2±2.5	0.4912
BMI (kg/m^2)	–0.1±1.0	–0.4±0.8*	0.2818	–0.6±0.7#	0.1773
Waist (cm)	–0.7±2.7	–1.3±2.7*	0.4158	–2.0±2.8	0.2026
SBP (mm)	–3.8±15.2	0.3±15.5	0.4251	3.9±13.9	0.1824
DBP (mm)	–1.7±8.7	–1.3±11.9	0.8871	–3.3±13.5	0.6621
P-glucose fasting (mmol/l)	–0.4±0.7**	–0.3±1.0	0.5128	0.0±1.2	0.3599
P-glu 2h post-load (mmol/l)	–0.8±2.0*	–1.8±2.6**	0.1596	–1.7±3.3	0.4444
HbA1c (%)	0.0±0.3	–0.3±0.6*	0.0714	–0.3±0.5	0.0960
HbA1c (mmol/mol)	–0.3±3.6	–3.3±6.6*	0.0779	–2.7±5.1	0.1275
HOMA $_{IR}$	–0.6±2.7	1.0±3.3	0.0823	–0.04	0.1193
Chol (mmol/l)	0.3±0.7*	–0.1±0.5	0.0341	0.1±0.3	0.4082
HDL (mmol/l)	–0.04±0.2	–0.005±0.2	0.5456	0.03±0.1	0.3070
LDL (mmol/l)	0.2±0.5*	–0.04±0.4	0.0746	0.08±0.3	0.4076
TG (mmol/l)	0.1±0.4	–0.1±0.4	0.0548	–0.03±0.5	0.4631
Power output (W)	0.0±16.4	7.4±11.5*	0.0941	11.3±6.4**	0.0054
Peak VO_2 (ml/kg/min)	–1.0±5.1	0.8±7.6	0.3768	3.0±3.2*	0.0430
Ph act medium	–1	3	0.220	0	0.485
Ph act high	–4	5	0.012	2	0.050

Values are mean±SD. Paired t-test for within-group differences, unpaired t-test for between-group differences. ^{2} or Fisher's exact test for Ph act medium and high. P <0.1$^{\#}$; P < 0.05*; P <0.01**; P <0.001***; P <0.0001****

Hba1c: % = NGSP standard, mmol/mol = IFCC standard. The Δ values for the ≥80% exercise sub-groups in the NGT, IGT and T2DM groups were compared with the Δ values of the NGT, IGT and T2DM control groups respectively, for between-group effects.
Ph act = physical activity ≥2 times/week (questionnaire). Medium intensity e.g. brisk walk, gardening, dancing. High intensity e.g. jogging, aerobics, workout.

IV

METABOLISM CLINICAL AND EXPERIMENTAL 61 (2012) 175–185

Available at www.sciencedirect.com

Metabolism

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Mitochondrial regulators of fatty acid metabolism reflect metabolic dysfunction in type 2 diabetes mellitus

Sameer S. Kulkarni[a], *Firoozeh Salehzadeh*[a], *Tomas Fritz*[b], *Juleen R. Zierath*[a,c], *Anna Krook*[c,*], *Megan E. Osler*[a]

[a] *Department of Molecular Medicine and Surgery, Integrative Physiology, Karolinska Institutet, Stockholm, Sweden*
[b] *Center for Family and Community Medicine, Karolinska Institutet, Huddinge, Sweden*
[c] *Department of Physiology and Pharmacology, Integrative Physiology, Karolinska Institutet, SE-171 77 Stockholm, Sweden*

ARTICLE INFO

Article history:
Received 29 March 2011
Accepted 20 June 2011

ABSTRACT

The delicate homeostatic balance between glucose and fatty acid metabolism in relation to whole-body energy regulation is influenced by mitochondrial function. We determined expression and regulation of mitochondrial enzymes including pyruvate dehydrogenase kinase (PDK) 4, PDK2, carnitine palmitoyltransferase 1b, and malonyl–coenzyme A decarboxylase in skeletal muscle from people with normal glucose tolerance (NGT) or type 2 diabetes mellitus (T2DM). Vastus lateralis biopsies were obtained from NGT (n = 79) or T2DM (n = 33) men and women matched for age and body mass index. A subset of participants participated in a 4-month lifestyle intervention program consisting of an unsupervised walking exercise. Muscle biopsies were analyzed for expression and DNA methylation status. Primary myotubes were derived from biopsies obtained from NGT individuals for metabolic studies. Cultured skeletal muscle was exposed to agents mimicking exercise activation for messenger RNA (mRNA) expression analysis. The mRNA expression of PDK4, PDK2, and malonyl–coenzyme A decarboxylase was increased in skeletal muscle from T2DM patients. Methylation of the PDK4 promoter was reduced in T2DM and inversely correlated with PDK4 expression. Moreover, PDK4 expression was positively correlated with body mass index, blood glucose, insulin, C peptide, and hemoglobin A_{1c}. A lifestyle intervention program resulted in increased PDK4 mRNA expression in NGT individuals, but not in those with T2DM. Exposure to caffeine or palmitate increased PDK4 mRNA in a cultured skeletal muscle system. Our findings reveal that skeletal muscle expression of PDK4 and related genes regulating mitochondrial function reflects alterations in substrate utilization and clinical features associated with T2DM. Furthermore, hypomethylation of the PDK4 promoter in T2DM coincided with an impaired response of PDK4 mRNA after exercise.

Author contributions: All authors contributed extensively to this paper. SSK performed experiments, analyzed data, and contributed to discussion. FS performed experiments and analyzed data. TF recruited patients and performed clinical analysis. JRZ designed the study and contributed to discussion and writing of the manuscript. AK contributed to discussion and data analysis and wrote the manuscript. MEO performed experiments, analyzed data, and contributed to discussion and writing of the manuscript.

* *Corresponding author*. Tel.: +46 8 524 87824; fax: +46 8 33 54 36.
E-mail address: Anna.Krook@ki.se (A. Krook).

0026-0495/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2011.06.014

1. Introduction

Skeletal muscle substrate metabolism plays a fundamental role in whole-body nutrient utilization and homeostasis. Metabolic flexibility, defined by the ability to switch between lipid and glucose oxidation in response to a meal or after insulin stimulation, is impaired in skeletal muscle from insulin-resistant (type 2 diabetes mellitus [T2DM]) patients [1-3]. A number of key mitochondrial genes determine the balance between glucose and fatty acid metabolism [4], including pyruvate dehydrogenase kinases (PDKs), carnitine palmitoyltransferase (CPT1b, also known as *CPT1 muscle, CPT I, CPT1-M, CPTI-M*), and malonyl–coenzyme A (CoA) decarboxylase (MCD). The PDK enzymes phosphorylate the pyruvate dehydrogenase complex, which leads to inhibition of the complex and reduced carbohydrate oxidation. Thus, pyruvate dehydrogenase complex inhibition is recognized as a flux point at which fuel selection in skeletal muscle can be shifted toward fat oxidation [5]. Four isoforms of PDK have been identified in the human genome [6,7], and the primary isoforms expressed in skeletal muscle are PDK2 and PDK4 [8]. Malonyl CoA decarboxylase is a key enzyme regulating cellular malonyl CoA levels [9], and malonyl-CoA is an allosteric inhibitor of CPT1b [10]. Carnitine palmitoyltransferase 1b is located at the outer mitochondrial membrane and controls the transfer of long-chain fatty acid–CoA molecules from the cytosol to the mitochondria where they are oxidized [10]. Like the PDK enzymes, MCD and CPT1b also play a pivotal role in fuel selection and drive increases in fatty acid oxidation.

The role of PDK4 in skeletal muscle has been examined with various metabolic challenges. Pyruvate dehydrogenase kinase 4 expression is increased in response to short-term fasting [11] or high-fat diet [12], two circumstances that increase the supply of lipids to skeletal muscle. Pyruvate dehydrogenase kinase 4 transcription and PDK4 messenger RNA (mRNA) are markedly increased in human skeletal muscle during exercise of acute high intensity or prolonged low intensity [13]. Increased PDK activity in skeletal muscle suppresses glucose oxidation and thus may cause or exacerbate hyperglycemia. Mice with a targeted deletion of PDK4 have lower blood glucose levels and slightly improved glucose tolerance as compared with wild-type mice after an 18-week high-fat diet [14], highlighting a role of PDK4 in the development of hyperglycemia. Furthermore, insulin exposure during the euglycemic-hyperinsulinemic clamp reduces PDK4 mRNA expression in normal glucose-tolerant (NGT), but not insulin-resistant, people, indicating that elevations in PDK4 expression and activity may further increase plasma glucose levels [15-17].

Malonyl-CoA decarboxylase and CPT1b also coordinate fuel balance during exercise and states of metabolic disease. Exercise reduces skeletal muscle malonyl-CoA [18,19] and increases expression of MCD1 [19], which may contribute to the increase in lipid oxidation at the onset of exercise [18]. Increased levels of malonyl-CoA have been reported in insulin-resistant animal models [20]. However, MCD knockdown protects against the development of dietary-induced whole-body insulin resistance in mice [21] and enhances insulin-stimulated glucose uptake in cultured human muscle cells [9]. Carnitine palmitoyltransferase 1b expression and function have been linked to the regulation of insulin sensitivity. Chemical inhibition of CPT1b with the pharmacological agent etomoxir increases lipid deposition and exacerbates insulin resistance in rats fed a high-fat diet [22], whereas overexpression of CPT1b in rat hind limb muscle by electrotransfer prevents the dietary-induced insulin resistance on glucose uptake [23]. Whether MCD or CPT1b levels are altered or related to the metabolic phenotype in T2DM is unknown.

The aim of this study was to profile the expression pattern of PDK4, PDK2, MCD1, and CPT1b in human skeletal muscle from T2DM and matched NGT volunteers. We hypothesized that lifestyle modification resulting in weight loss and improved glycemic control would be accompanied by normalization of these regulators of metabolic flexibility.

2. Materials and methods

2.1. Subjects

Type 2 diabetes mellitus (n = 33) patients and NGT (n = 79) male and female volunteers were matched for age (61 ± 5 years) and body mass index (BMI) (30 ± 5 kg/m^2). The ethics committee at Karolinska Institutet approved all study protocols. Patients on insulin treatment and with symptomatic coronary heart disease were excluded. The clinical characteristics of the patients are presented in Table 1. All individuals provided a muscle biopsy upon entry into the study.

2.2. Intervention program

This was a randomized controlled study. Volunteers were randomized to the exercise or control group and asked to maintain their usual eating habits. The participants in the exercise group were instructed to increase their weekly level of physical activity by 5 hours of walking with walking poles (Nordic walking) for 4 months. They received instructions for Nordic walking from a physiologist/personal trainer. Of the total number of volunteers, a select group of male participants in each category (n = 23, NGT; n = 17, T2DM) underwent an exercise intervention and provided muscle biopsies before and after exercise training.

2.3. Skeletal muscle biopsies

Skeletal muscle biopsies were obtained from subjects in the morning, following an overnight fast. Local anesthesia (lidocaine hydrochloride, 5 mg/mL) was administered, and an incision (5 mm long/10 mm deep) was made in the skin and skeletal muscle fascia. A biopsy (20-100 mg) was obtained from the vastus lateralis portion of the quadriceps femoris using a conchotome tongue. Biopsies were immediately frozen and stored in liquid nitrogen until analysis.

2.4. Human skeletal muscle cells

Skeletal muscle biopsies (rectus abdominis) were obtained during scheduled abdominal surgery with informed consent from the donors. The subjects were 61 ± 5 years of age, were of

Table 1 – Anthropometric measurements and metabolic parameters in NGT and T2DM subjects

	NGT	T2DM	Total	NGT vs T2DM
N	79	33	112	P value
Sex (M/F)	30/49	24/9	66/69	
Age (y)	60 ± 1	62 ± 1	61 ± 0.5	.165
Height (cm)	169.8 ± 1.0	174.2 ± 1.6	171.1 ± 0.9	.024
Waist circumference (cm)	98.4 ± 1.1	105.0 ± 1.6	100.3 ± 0.9	.001*
SBP (mm Hg)	136 ± 1.62	141.52 ± 2.26	137.63 ± 1.34	.060
DBP (mm Hg)	82.66 ± 0.89	81.82 ± 1.39	82.41 ± 0.75	.611
BMI (kg/m^2)	29.8 ± 0.4	30.7 ± 0.6	30.1 ± 0.3	.246
FBG (mmol/L)	5.54 ± 0.05	8.08 ± 0.36	6.29 ± 0.16	<.001*
2-h BG (mmol/L)	7.20 ± 0.11	15.53 ± 0.59	9.66 ± 0.41	<.001*
HbA_{1c} (%)	4.7 ± 0.0	6.1 ± 0.2	5.1 ± 0.1	<.001*
Insulin (pmol/L)	61.78 ± 4.04	69.71 ± 9.43	63.99 ± 3.91	.366
Cholesterol (mmol/L)	5.50 ± 0.09	4.71 ± 0.15	5.27 ± 0.09	<.001*
LDL (mmol/L)	1.63 ± 0.09	1.28 ± 0.14	1.52 ± 0.08	.002*
HDL (mmol/L)	3.32 ± 0.05	2.79 ± 0.06	3.17 ± 0.04	<.001*
C-peptide (nmol/L)	0.83 ± 0.03	1.05 ± 0.07	0.89 ± 0.03	<.001*
TG (mmol/L)	1.23 ± 0.06	1.38 ± 0.09	1.28 ± 0.05	.173
Apo A-I (g/L)	1.60 ± 0.03	1.38 ± 0.04	1.54 ± 0.03	<.001*
Apo B (g/L)	1.01 ± 0.02	0.93 ± 0.04	0.99 ± 0.02	.063
Vo_2 (L/min)	2.01 ± 0.07	2.07 ± .08	2.03 ± 0.06	.654
RQ	1.10 ± 0.01	1.08 ± 0.01	1.09 ± 0.01	.333

Data are presented as means ± SEM. SBP indicates systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglycerides; Vo_2, oxygen consumption; RQ, respiratory quotient. *Significant at $P < .01$ level.

average weight (BMI, 26 ± 0.5 kg/m^2), and had no known metabolic disorders. The ethics committee at Karolinska Institutet approved the study protocols. Satellite cells were isolated from the biopsies by trypsin and collagenase digestion and grown to confluent myoblasts that were differentiated to myotubes as described [24,25]. Dulbecco modified Eagle medium, Ham F-10 medium, fetal bovine serum, penicillin, streptomycin, and Fungizone were obtained from Gibco BRL (Invitrogen, Stockholm, Sweden). Unless specified, all reagents were purchased from Sigma (Stockholm, Sweden). Radioactive reagents were purchased from Amersham.

2.5. *mRNA expression analysis in muscle biopsies*

Messenger RNA was prepared from vastus lateralis muscle biopsies for gene expression analysis. Pure mRNA was extracted from 30 mg of frozen tissue using a standard Trizol extraction method (Invitrogen). Complementary DNA was generated from 1 μg of mRNA using the High-Capacity cDNA RT kit (Applied Biosystems, Stockholm, Sweden). Gene expression analysis was carried out using a TaqMan-based multifluidic card (MFC) gene expression assay (Applied Biosystems, Foster City, CA). The following primer and probe sets from Applied Biosystems were lyophilized in the MFC well: MFC internal endogenous control 18S; Hs99999907_m1 (β2-microglobulin); Hs99999905_m1 (glyceraldehyde-3-phosphate dehydrogenase [GAPDH]); Hs00176865_m1 (PDK2); Hs00176875_m1 (PDK4); Hs03046298_s1 (CPT1B [muscle]); Hs00201955_m1 (MCD-1, MLYCD). Samples were applied into the MCF well, and polymerase chain reaction (PCR) amplification was performed using an Applied Biosystems Prism 7900HT sequence detection system (Applied Biosystems, Stockholm, Sweden). Messenger RNA was quantified according to technical documents supplied by the manufacturer, and the relative abundance of target transcripts was calculated after normalization of the data against the housekeeping genes.

2.6. *Immunoblot analysis*

Aliquots of human skeletal muscle lysates (20 μg) were resuspended in Laemmli buffer. Proteins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes (Bio-Rad, Danvers, MA), blocked with 7.5% nonfat milk, washed with TBST (10 mmol/L Tris-HCL, 100 mmol/L NaCl, and 0.02% Tween 20), and incubated with primary antibodies overnight at 4°C. Membranes were washed with TBST and incubated with appropriate horseradish peroxidase–conjugated secondary antibodies (Bio-Rad, diluted 1:25 000). Proteins were visualized by enhanced chemiluminescence (GE Healthcare, Stockholm, Sweden). A rabbit monoclonal antibody against PDK2 (catalog no. ab76152) was purchased from Abcam (Cambridge, UK), and mouse monoclonal antibody against MCD (catalog no. H00023417-B01P) was purchased from Abnova (Taipei City, Taiwan).

2.7. *mRNA expression analysis in cultured cells*

Myotubes, prepared as described above, were washed 3 times with RNase-free phosphate-buffered saline and then harvested directly for RNA extraction (RNeasy Mini Kit, Qiagen, Sollentuna, Sweden). Total RNA concentration was measured and reverse transcribed with random hexamers using the high-capacity reverse transcription kit (Applied Biosystems). Reactions were performed in duplicate in a 96-well format using Prism 7000 Sequence Detector and TaqMan-based technology (Applied Biosystems). TaqMan probes were purchased from Applied Biosystems. Relative quantities of target

transcripts were calculated after normalization of the data using the standard curve method or comparative CT method.

2.8. Bisulfite sequencing

A subset of muscle biopsies derived from NGT and T2DM subjects was selected for gDNA methylation analysis (age- and BMI-matched male donors; n = 4, NGT; n = 4, T2DM). Bisulfite treatment was performed as described [26], with the following adaptations: 1 μg of genomic DNA was embedded in a 2% low–melting point agarose solution, and 10 agarose beads were formed. A freshly prepared bisulfite solution (4 mol/L sodium β-bisulfite, Sigma; 250 mmol/L hydroquinone, Sigma; pH 5.0) was added to each reaction tube containing one single bead. The reaction mixtures were incubated for 4 hours at 50°C under exclusion of light. Treatment was stopped by equilibrations against 1 mL of Tris-EDTA (4× 15 minutes) followed by desulfonation in 500 μL of 0.2 mol/L NaOH (2× 15 minutes). The reaction was neutralized, and beads were washed with 1 mL Tris-EDTA (2× 15 minutes). Before the PCR analysis, the beads were equilibrated against 1 mL of H_2O (2× 30 minutes). For the amplification of regions +160 to +446 of the PDK4 promoter, the following primers were used: sense 5′-GGT ATT TTT AAA TTT TAG TTT AGG T-3′, antisense 5′-ATC CAA TAA CTA CTT CAT AAA CAA C-3′. The PCR fragments were purified from an agarose gel using MinElute Gel Extraction Kit (Qiagen) and cloned into pDrive vector using a PCR cloning kit (Qiagen) according to the manufacturer's protocol. Individual clones were grown, and plasmids were purified using QIAprep Spin Miniprep Kit (Qiagen). For each condition, 10 to 50 clones were sequenced using T7 promoter primer on an ABI 3730xl DNA Analyzer platform at Cogenics (Hope End, UK).

2.9. Statistical analysis

All data are presented as mean ± SEM. Baseline differences in gene expression between NGT subjects and T2DM patients were analyzed using an independent *t* test. Pearson correlations were calculated for baseline measurements of gene and clinical parameters. Regression models controlled for covariates (age, sex, and BMI) were used to examine clinical predictors of gene expression. Computation of the fold change following exercise intervention was performed using a paired *t* test. Comparisons were considered statistically significant at $P < .05$. Natural log transformation was applied when data were not normally distributed. Analyses were performed using SPSS version 17.0 (SPSS, Chicago, IL).

3. Results

3.1. PDK4, PDK2, and MCD1 mRNA expression is increased in skeletal muscle from T2DM patients

We measured skeletal muscle mRNA expression of PDK4, PDK2, CPT1b, and MCD1 in T2DM and in age- and BMI-matched NGT subjects (clinical characteristics are reported in Table 1). Skeletal muscle mRNA expression of PDK4 was increased approximately 70% ($P < .01$) in T2DM patients as compared with BMI- and age-matched NGT volunteers (Fig. 1A). Messenger RNA expression of PDK2 and MCD1 was increased approximately 50% in skeletal muscle from T2DM patients (Fig. 1B-C, $P < .05$). In contrast, mRNA expression of CPT1b was similar between cohorts. Protein expression of PDK2 and MCD was determined in a subset of subjects where material permitted (Fig. 1E). Protein levels support the observed mRNA expression patterns. Antibody detection of protein levels of PDK4 and CPT1b using commercially available antibodies was unsuccessful, with antibodies detecting several nonspecific bands.

3.2. Skeletal muscle mRNA expression of PDK4 correlates with BMI and with PDK2, CPT1, and MCD mRNA expression

Skeletal muscle PDK4 mRNA expression was positively correlated with BMI (represented graphically in Fig. 2B), waist circumference, fasting and 2-hour blood glucose, hemoglobin A_{1c} (HbA_{1c}), insulin, and circulating C peptide in the entire cohort of study participants (n = 112, Table 2). The differential clustering pattern of NGT and T2DM subjects may suggest a tighter relationship between PDK4 mRNA and BMI in the presence of maintained glucose regulation. Pyruvate dehydrogenase kinase 4 mRNA expression was negatively correlated ($P < .05$) with cholesterol and low-density lipoprotein (LDL). Pyruvate dehydrogenase kinase 2 mRNA was positively correlated with fasting and 2-hour blood glucose measurements ($P < .05$), and negatively correlated with diastolic blood pressure, high-density lipoprotein (HDL) and apolipoprotein (apo) A-I. Malonyl-CoA decarboxylase was inversely correlated with HDL and apo A-I (Table 2, $P < .05$). Messenger RNA expression of the mitochondrial genes was positively and highly correlated ($r = 0.246$-0.889, $P < .001$), with the strongest correlation observed for expression of PDK2 and MCD (Fig. 2A, $r = 0.889$, $P < .0001$).

Multiple regression analysis revealed that BMI is associated with PDK4 gene expression after adjustment for age, sex, and diabetes status (Table 3). Regression models demonstrate that the relationship between PDK4 and BMI was significant, independent of age, sex, cohort, and glucose tolerance. After correcting for age, sex, and cohort, the expression of PDK4 remained independently determined by BMI ($r = 0.348$, $P = .002$). Although the expression of the genes correlated highly, similarly strong relationships did not exist between other genes and the clinical factors after controlling for age, sex, and cohort.

3.3. PDK4 promoter methylation is reduced in skeletal muscle from T2DM patients

Using bisulfite sequencing, we determined the methylation status of cytosines in the +160 to +446 region of the PDK4 promoter in skeletal muscle from T2DM patients and NGT volunteers. The increase in PDK4 mRNA expression noted in the T2DM patients was accompanied by a parallel reduction in PDK4 promoter methylation (Fig. 3).

3.4. Effect of lifestyle modification on skeletal muscle mRNA expression

We further determined the effect of a 4-month lifestyle intervention involving approximately 4 hours of Nordic

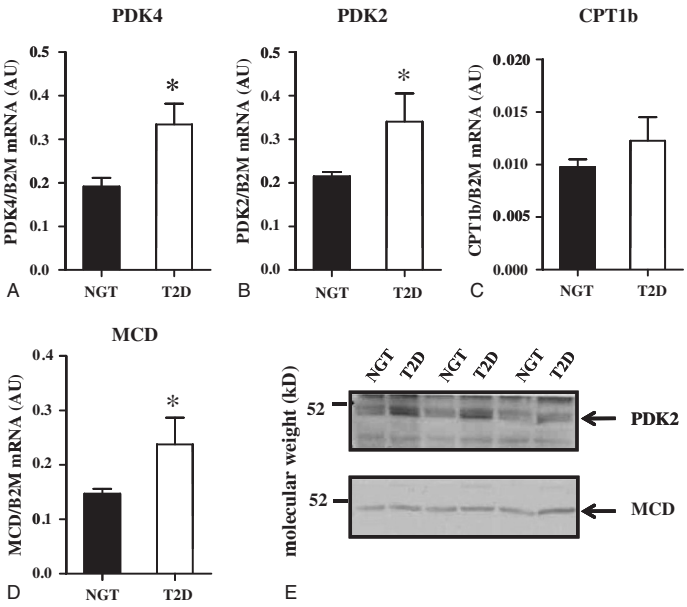

Fig. 1 – Gene expression analysis. Pyruvate dehydrogenase kinase 4 (A), PDK2 (B), CPT1b (C), and MCD (D) mRNA expressions were measured in skeletal muscle of age- and BMI-matched subjects with NGT (n = 79) and T2DM (n = 33). The mRNA expression of PDK4, PDK2, and MCD was significantly elevated in T2DM patients (*P < .05). The mRNA expression is reported as a relative expression related to ß2 microglobulin (B2M) mRNA. Values are means ± SE arbitrary units (AU) for subjects in each group. E, Representative immunoblot analysis of skeletal muscle protein expression of PDK2 and MCD.

walking a week on skeletal muscle mRNA expression in a subgroup of this population. Walking exercise was associated with modest weight loss in NGT and T2DM subjects ($P < .05$; Table 4). In the T2DM patients, weight loss was accompanied by a reduction in 2-hour plasma glucose levels, whereas in the NGT subjects, the fasting plasma insulin level was reduced. Skeletal muscle PDK4 mRNA expression was increased in response to lifestyle intervention, but only in NGT subjects (Fig. 4A). Although PDK4 expression in skeletal muscle was elevated in T2DM before the exercise intervention (Fig. 1A), no further increase was noted upon the completion of the intervention program. Skeletal muscle mRNA expression of PDK2, MCD1, or CPT1b was unaltered by the lifestyle intervention program (Fig. 4 B-D).

3.5. *Regulation of mRNA expression of PDK4, PDK2, CPT1b, and MCD1 in cultured human muscle cells*

We determined mRNA expression of PDK4, PDK2, CPT1b, and MCD1 in cultured skeletal muscle following 48 hours of incubation in the presence of 3 mmol/L caffeine, 1 mmol/L AICAR, or 200 μmol/L palmitate. These agents were selected to mimic metabolic conditions generated in skeletal muscle during exercise, such as increased Ca^{2+} (induced in cultured muscle following exposure to caffeine [27]), activation of AMPK [28], or increased lipids. Caffeine and palmitate independently increased mRNA expression of PDK4 approximately 40-fold and 15-fold, respectively (Fig. 5A). Caffeine exposure increased mRNA expression of PDK2 approximately 50% ($P < .05$; Fig. 5B). Palmitate exposure increases CPT1 mRNA, whereas MCD1 expression increased in response to both caffeine and palmitate (Fig. 5C, D).

4. Discussion

Type 2 diabetes mellitus is associated with abnormal substrate metabolism, raising the possibility that alterations in the expression of mitochondrial enzymes controlling lipid uptake and metabolism may by altered. Here we determined skeletal muscle expression of key mitochondrial genes that orchestrate the switch of substrate utilization between glucose and lipid sources. We show that mRNA expression of PDK4, PDK2, and MCD1 is upregulated in human skeletal muscle from T2DM patients. We have previously demonstrated that reduction of MCD1 enhances glucose and reduces lipid metabolism in cultured human muscle cells [9]. Thus, increased MCD1 expression may play a role in reducing skeletal muscle glucose utilization in glucose-intolerant individuals.

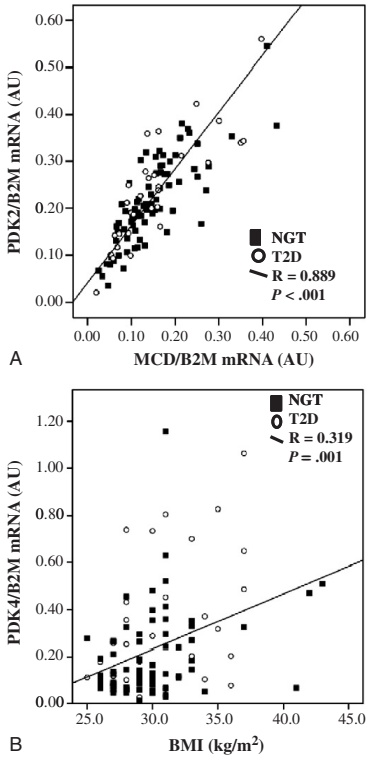

Fig. 2 – Baseline correlation analysis. A, Gene expression of PDK4, PDK2, CPT1b, and MCD is highly correlated in the entire cohort of subjects examined. Correlations between PDK2 and MCD represent the strongest correlation (n = 112, r = 0.889, P < .0001). The mRNA expression is reported as a relative expression related to B2M mRNA. B, The PDK4 gene expression correlates weakly with BMI (r = 0.319, P = .001). NGT (n = 79, black, filled squares) and T2DM (n = 33, open circles).

Elevated PDK4 gene expression corresponds with various clinical phenotypes. Circulating lipid levels have been highlighted as a key factor determining PDK4 expression [5]. In rats, a 4-week high-fat diet increases PDK4 mRNA expression [29]. In our study, plasma triglyceride levels were unaltered between NGT volunteers and T2DM patients; and thus, PDK4 mRNA expression was unrelated to triglyceride levels. A subset of the T2DM patients in the present study regularly used cholesterol-lowering medications or other pharmacological interventions, which may mask such relationships. Messenger RNA expression of PDK4 is also acutely regulated by insulin [17,30]; and thus, the increased PDK4 mRNA in the T2DM patients may be a consequence of hyperinsulinemia and insulin resistance at the level of the enzyme, such that expression is not appropriately suppressed. Reductions in skeletal muscle glycogen is known to increase PDK4 mRNA [31]. Although muscle glycogen content was not determined, PDK4 was positively correlated with fasting glucose levels. However, a significant correlation between PDK4 mRNA expression and BMI highlights the relationship between the expression of mitochondrial genes related to lipid metabolism and body mass. Examination of gene-gene correlations revealed positive relationships between PDK4, PDK2, CPT1b, and MCD in the entire study cohort (Table 2), further supporting that this cluster of genes is similarly coordinated and regulated in healthy and diseased individuals. The inverse correlation noted between PDK4 methylation and PDK4 expression in skeletal muscle suggests the possibility that epigenetic modification orchestrates the regulation of mitochondrial genes involved in substrate switching. Although the verification of PDK4 regulation at the protein level would further strengthen this finding, we were unable to reliably detect PDK4 protein using currently available antibodies. Our analysis of PDK2 and MCD protein reflects the increase noted in mRNA levels. Because the elevation of these genes at the mRNA level correlates highly (Fig. 2A), this suggests that an increase in PDK4 transcript may consequently result in an end-point increase in protein expression also, as has been reported for the majority of mammalian genes [32]. Collectively, our findings provide evidence that the level of PDK4 gene expression reflects glucose/lipid metabolism, which is possibly potentiated by epigenetic control.

A 4-month lifestyle modification program increased skeletal muscle PDK4 mRNA expression in NGT subjects, but was without effect on PDK4 expression in T2DM patients. This differential response could be due to a higher compliance with the exercise program in the NGT subjects or to an inability of the T2DM patients to appropriately respond to the exercise training program. Pyruvate dehydrogenase kinase 2, CPT1b, or MCD gene expression was unaltered following exercise intervention in our study, indicating a set level of stability and/or a resistance of fluctuation in response to this particular exercise stimulation. However, the understanding of the overall metabolic regulation of PDK4 is complicated by seemingly conflicting results, as an elevation is observed both in T2DM and following exercise intervention. The apparent paradox of skeletal muscle lipid metabolism in states of insulin resistance vs exercise training has been discussed previously [33].

Conditions of obesity and insulin resistance are strongly associated with impaired rates of fatty acid oxidation in skeletal muscle [34]. Further indication of high fatty acid availability is a buildup of intramyocellular lipids (IMCL), which reflects an elevated fatty acid flux into the muscle [35,36]. Thus, our finding that PDK4 mRNA is elevated in T2DM and correlates with BMI further supports that an elevated lipid flux in an insulin-resistant state requires an upregulation of gene pathways involved in the switch from carbohydrate to lipid oxidation. However, the molecular pathways involved in lipid uptake and storage are not entirely transparent, as the muscle of highly trained athletes also contains excess IMCL [33]. The observation that PDK4 is elevated after exercise, concomitant with an observed decrease in BMI, may appear

Table 2 – Correlation analysis between skeletal muscle mRNA expression and clinical parameters in NGT and T2DM subjects

	PDK4 mRNA		PDK2 mRNA		CPT1b mRNA		MCD mRNA	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
PDK4 mRNA	–	–	0.278†	.003	0.246†	.009	0.374†	<.001
PDK2 mRNA	0.278†	.003	–	–	0.637†	<.001	0.889†	<.001
CPT1b mRNA	0.246†	.009	0.637†	<.001	–	–	0.689†	<.001
MCD mRNA	0.374†	<.001	0.889†	<.001	0.689†	<.001	–	-
Height (cm)	−0.066	.489	0.061	.520	−0.117	.221	−0.046	.631
Waist circumference (cm)	0.328†	<.001	−0.033	.730	−0.141	.140	−0.065	.496
SBP (mm Hg)	0.057	.550	0.005	.956	0.009	.925	0.025	.792
DBP (mm Hg)	−0.045	.641	−0.203*	.032	−0.087	.364	−0.192*	.042
BMI (kg/m^2)	0.319†	.001	−0.010	.919	0.006	.950	0.030	.752
FBG (mmol/L)	0.248†	.008	0.198*	.037	0.149	.120	0.185	.050
2-h BG (mmol/L)	0.284†	.002	0.227*	.016	0.149	.120	0.198*	.036
HbA_{1c} (%)	0.251†	.008	0.165	.083	0.089	.352	0.147	.122
Insulin (pmol/L)	0.263*	.011	0.107	.306	0.085	.422	0.190	.068
Cholesterol (mmol/L)	−0.241	.022	−0.105	.270	−0.030	.757	−0.057	.548
LDL (mmol/L)	−0.186*	.050	−0.029	.761	0.045	.641	0.007	.939
HDL (mmol/L)	−0.155	.103	−0.224*	.018	−0.216*	.022	−0.156	.102
C-peptide (nmol/L)	0.328†	<.001	0.053	.580	0.077	.420	0.081	.395
Apo A-I (g/L)	−0.104	.274	−0.230*	.014	−0.243*	.010	−0.185	.051
Apo B (g/L)	−0.067	.482	−0.069	.472	0.055	.563	−0.012	.898
Vo_2 (L/min)	−0.048	.622	0.080	.410	0.156	.107	0.052	.588
RQ	−0.076	.435	−0.183	.056	0.033	.737	−0.115	.232

* Significant at $P < .05$ level.
† Significant at $P < .01$ level.

contradictory given the positive correlation between PDK4 and BMI observed at baseline. However, reports examining the effects of exercise on skeletal muscle fatty acid transport and oxidation, particularly low-intensity exercise, demonstrate a high reliance of the muscle on lipid sources of fuel [37,38]. The initiation of exercise mobilizes FFAs from adipose tissue as a fuel source [39], and subsequent oxidation in muscle mitochondria requires increased participation of relevant players involved in the metabolic switch [40]. Thus, as with states of insulin resistance, exercise intervention elicits an increase in PDK4 expression in NGT subjects, reflecting a metabolic adjustment to prioritize lipid fuel handling. We did not observe a PDK4 elevation in T2DM following exercise, which

Table 3 – Multiple regression analysis between age, sex, BMI, and PDK4 mRNA expression in NGT and T2DM subjects

	PDK4	
	β-Coefficient	*P* value
Model 1		
Age	0.023	.132
Sex	−0.065	0.685
Cohort	0.208	.020*
BMI	2.296	.002†
Model 2		
Age	0.022	.154
Sex	−0.066	.683
Cohort	0.143	.443
BMI	2.285	.002†
2-h BG	0.176	.689
Model 3		
Age	0.018	.305
Sex	0.006	.972
Cohort	0.227	.016*
BMI	1.731	.029*
Insulin	0.342	.026*

* Significant at $P < .05$ level.
† Significant at $P < .01$ level.

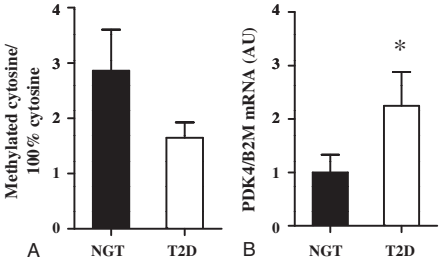

Fig. 3 – Methylation analysis. A, Methylation analysis of the PDK4 promoter by bisulfite sequencing. The PDK4 promoter methylation is expressed as a percentage of cytosine methylation per 100% of total cytosine in subjects with NGT (n = 4) and T2DM (n = 4). Results are mean ± SEM. B, Skeletal muscle PDK4 mRNA expression in the same set of subjects. Expression is reported as a relative expression related to B2M mRNA. Values are means ± SE AU for subjects in each group (*P < .05).

Table 4 – Anthropometric measurements and metabolic parameters in NGT and T2DM men before and after exercise training

		Pre ± SEM	Post ± SEM	Δ ± SEM	P value		
					Pre vs post	Pre, NGT vs T2DM	Δ, NGT vs T2DM
N	NGT	23					
	T2DM	17					
Age	NGT	61 ± 1					
	T2DM	62 ± 1				.253	
Weight (kg)	NGT	89.9 ± 2.04	88.5 ± 2.16	−1.39 ± 0.43	.003†		
	T2DM	94.1 ± 2.74	92.5 ± 2.71	−1.54 ± 0.59	.018*	.220	.830
BMI (kg/m^2)	NGT	28.7 ± 0.43	28.1 ± 0.46	−0.57 ± 0.15	.001†		
	T2DM	29.9 ± 0.81	29.5 ± 0.78	−0.44 ± 0.20	.042*	.184	.628
Waist circumference (cm)	NGT	101.57 ± 1.63	98.00 ± 1.64	−3.57 ± 0.54	<.0001†		
	T2DM	105.11 ± 2.05	103.06 ± 2.07	−2.06 ± 0.63	.005†	.178	.076
FBG (mmol/L)	NGT	5.57 ± 0.11	5.48 ± 0.11	−0.09 ± 0.11	.434		
	T2DM	8.32 ± 0.52	8.01 ± 0.52	−0.32 ± 0.24	.207	<.0001†	.407
2-h BG (mmol/L)	NGT	7.07 ± 0.22	6.84 ± 0.27	−0.23 ± 0.26	.392		
	T2DM	15.11 ± 0.82	13.37 ± 1.00	−1.73 ± 0.55	.006†	<.0001†	.021*
Insulin (pmol/L)	NGT	65.82 ± 6.91	44.76 ± 4.01	−17.33 ± 5.02	.003†		
	T2DM	88.19 ± 12.93	87.07 ± 13.35	10.06 ± 12.91	.917	.139	.066
Vo_2 (L/min)	NGT	2.46 ± 0.14	2.61 ± 0.12	0.15 ± 0.12	.249		
	T2DM	2.13 ± 0.12	2.22 ± 0.12	0.03 ± 0.15	.872	.102	.540
Nordic walking (h/wk)	NGT		4.895 ± 0.34				
	T2DM		4.061 ± 0.38			.110	

* Significant at $P < .05$ level.
† Significant at $P < .01$ level.

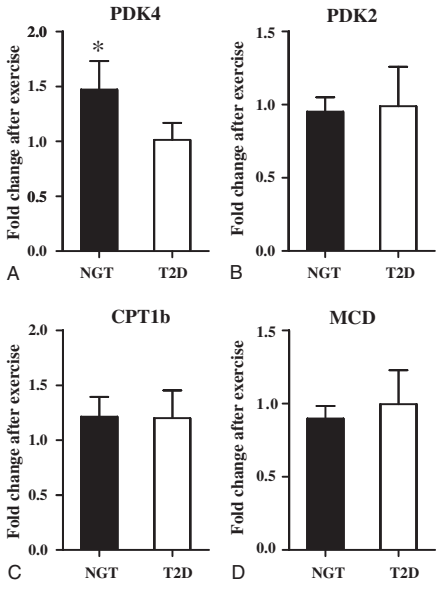

Fig. 4 – Effects of exercise on gene expression. The relative change in skeletal muscle mRNA expression of PDK4 (A), PDK2 (B), CPT1b (C), and MCD (D) mRNA in age- and BMI-matched NGT and T2DM subjects following a 4-month lifestyle intervention program. Values are means ± SEM (*$P < .05$).

may signify that this exercise intervention was not of sufficient intensity to elicit a response in insulin-resistant participants. An examination of IMCL was not performed in this study.

The expression of the FAT/CD36 transporter in skeletal muscle reflects a similar pattern of regulation as we report for PDK4. Increases in FAT/CD36 mRNA and/or protein have been observed in human obesity, in T2DM [41], and after a high-fat diet [42,43]. Elevation in FAT/CD36 transporter levels in skeletal muscle is also observed following exercise training, through a proposed metabolic adaptation of greater fuel utilization [44]. Thus, an increase in expression of genes involved in the switch of fuel sources could be expected in two contrary circumstances, both of which require similar molecular orchestration to balance an elevated fatty acid influx.

DNA methylation alters gene transcription by regulating the accessibility of the transcription factors and transcription machinery to the DNA strand. Whether the differential exercise-dependent mRNA change in PDK4 is related to the difference in PDK4 promoter methylation noted between NGT and T2DM patients remains to be investigated. Increased promoter methylation of peroxisome proliferator-activated receptor γ coactivator 1α (PGC1α), another key regulator of mitochondrial activity, has been reported in skeletal muscle from people with impaired glucose tolerance or T2DM [45] and in subjects with low birth weight [46]. Furthermore, exposure of cultured muscle cells to fatty acids [45] or to a high-fat diet in low-birthweight men [46] increases PGC1α promoter methylation. Whether PDK4 promoter methylation is similarly regulated remains to be determined. However, here we provide evidence that reduced PDK4 promoter methylation is associated with metabolic disease, whereas our previous study established that PGC1α promoter methylation is

increased [45]. This suggests that altered methylation patterns associated with metabolic disease may be promoter specific.

Weight loss is accompanied by a marked reduction in skeletal muscle PDK4 expression following surgery-induced weight reduction [47,48], and the depression in PDK4 expression has been correlated to improvements in insulin sensitivity [47]. Here we note that BMI is associated with PDK4 expression independent of age, sex, or state of glucose tolerance. A diet-mediated weight reduction program, resulting in a stabilized 10% weight loss, was associated with a nonsignificant tendency for reduced skeletal muscle PDK4 expression [49]. The weight loss achieved following lifestyle intervention in the present study was relatively modest (1.5 kg), yet PDK4 mRNA was elevated in NGT subjects after exercise training. This finding perhaps reflects a modulation of fuel selection. Although PDK4 has been highlighted as a point of dysregulation in the context of T2DM and obesity [17,50], the overall impact of a loss of PDK4 is surprisingly slight. Mice lacking PDK4 have a modest reduction in fasting glucose and are slightly more glucose tolerant than wild-type mice after 16 weeks on a high-fat diet [14]. Clearly, PDK4 is only recognized in certain circumstances as a key regulator of glucose vs lipid utilization. For example, we have recently reported that in response to exposure to glucocorticoids, skeletal muscle cultures increase lipid oxidation and reduce glucose metabolism; and a key mediator of this response is induction of PDK4 expression [51].

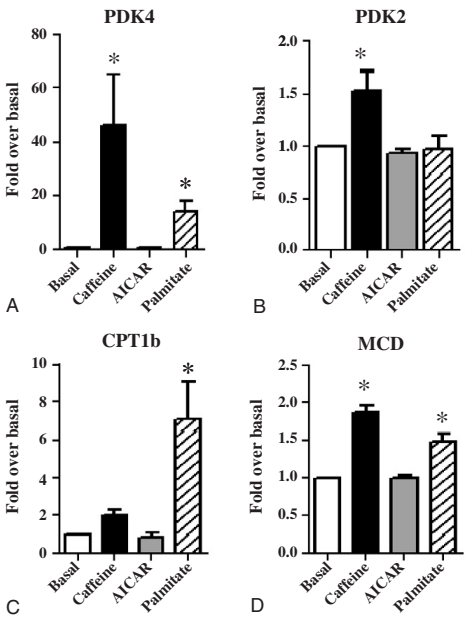

Fig. 5 – Effect of exercise mimetics on gene expression. Effect of caffeine, AICAR, and palmitate treatment on mRNA expression of PDK4, PDK2, CPT1b, and MCD (A, B, C, and D, respectively) in cultured human skeletal myotubes. Differentiated myotubes were incubated in the absence or presence of caffeine (3 mmol/L, 48 hours), AICAR (1 mmol/L, 18 hours), or palmitate (200 μmol/L, 48 hours) before the extraction of total RNA. Results are reported as a relative expression related to B2M mRNA. Values are means ± SEM (*P < .05).

The role of mitochondria in metabolic disease, and whether increasing fatty acid flux into muscle mitochondria reduces or exacerbates insulin sensitivity, is the subject of debate [52-55]. One crucial role in muscle mitochondrial function may be the ability to adapt to or show a preference for different fuel substrates, that is, the flexibility of the muscle to adapt to altered environments [1]. Because the same lifestyle modification program in NGT subjects increased skeletal muscle PDK4 expression, the final PDK4 expression noted in T2DM patients may be appropriate for the level of substrate demand placed on the muscle as a result of the increased exercise performed. To dissect the relative impact of different exercise-associated factors, we exposed cells in culture to agents that increase intracellular Ca^{2+} (caffeine [27]), activate AMPK [28], or change the lipid supply to mimic exercise-dependent increase in circulating fatty acids. Activation of AMPK (in response to AICAR) was insufficient to induce alterations in mRNA expression of these genes. In fact, no single agent resulted in a coordinated increase in expression of the genes under study; however, caffeine exposure led to a profound increase in PDK4. Indeed, caffeine exposure resulted in increased expression of PDK4, PDK2 and MCD, which were also increased in skeletal muscle from the T2DM patients. Whether intracellular Ca^{2+} signaling is enhanced in T2DM is unknown. However, because caffeine exposure in cultured muscle resulted in a profound (40-fold) increase in PDK4 mRNA, the lack of exercise-dependent induction of PDK4 in diabetic muscle would argue against enhanced Ca^{2+} signaling in diabetic skeletal muscle.

In conclusion, we provide evidence that expression of key enzymes regulating mitochondrial function in skeletal muscle is altered in T2DM patients. Increased expression of PDK4 mRNA was coincident with decreased PDK4 promoter methylation, indicative of epigenetic regulation. In addition, a lifestyle intervention involving low-intensity exercise resulted in increased expression of PDK4 mRNA in NGT volunteers but not T2DM patients, possibly implicating resistance to the appropriate adaptive responses to exercise and changes in metabolic demands in skeletal muscle associated with insulin resistance.

Funding

The Swedish Research Council, European Foundation for the Study of Diabetes, European Research Council, Swedish Diabetes Association, Strategic Research Foundation, Knut and Alice Wallenberg Foundation, Stockholm County Council, Novo Nordisk Research Foundation, Johan and Jakob Söderbergs Foundation, and diabetes research program at Karolinska Institutet and the Swedish Society of Medicine supported this research.

Conflict of interest

The authors declare that they have no conflict of interest as pertains to the data presented in this paper.

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