Exercise therapy in Type 2 Diabetes

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Dissertation

The study presented in this thesis was performed within the Nutrition and Toxicology Research Institute Maastricht (NUTRIM) which participates in the Graduate School VLAG (Food Technology, Agrobiotechnology, Nutrition and Health Sciences), accredited by the Royal Netherlands Academy of Arts and Sciences.

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We must walk before we run
- George Borrow, *Lavengro* (1851)

Voor mijn ouders

Contents

Chapter 1 General introduction and outline of the thesis

1.1 TYPE 2 DIABETES: A MODERN, BUT NOT MODEST, HEALTH THREAT

Already in the first century the ancient Greek physician *Aretaeus of Cappadocia* described the term diabetes (*diabaínein)* as '...a wonderful affection, not very frequent among men...'[1]. However, it was not until 1675 that Thomas Willis, an English physician, added the word *mellitus*, as a reference to the sweet taste of a diabetes patient's urine. Whether this condition referred to Type 1 (autoimmune disease) or Type 2 (relative insulin deficiency) diabetes mellitus is unknown. However, it lasted another 200 y before the French physician Lanceraux made the distinction between diabetes in lean and obese men: *diabete gras* and *diabete maigre* [2]. In the 1930s, the diabetologist Joslin c.s. noted that the incidence of diabetes in lean individuals was relatively constant in each decade of life, while diabetes in the obese was related to age [3]. Already in those days he attributed the increasing prevalence of diabetes in the 1930s to increasing obesity [3].

The sharp rise in Type 2 diabetes prevalence during the second half of the 20th century first occurred in developing countries, parallel to the rapid socio-economic development and dramatic changes in lifestyle in these countries [4]. In traditionally more affluent societies, the prevalence of Type 2 diabetes showed a clear rise in the early 90s [5,6], almost parallel to the increase in the prevalence of obesity [7]. Although certain combinations of gene variants appear to predispose to the development of insulin resistance and/or Type 2 diabetes [8], obesity and lack of physical activity are regarded as the most important risk factors, both independently associated with diabetes and diabetes-related co-morbidities [9].

According to the International Diabetes Federation (IDF), the disease now affects 246 million people worldwide and is expected to affect some 380 million by 2025, representing as much as 7.1% of the global adult population [10]. As such, the associated health burden in terms of cardiovascular disease, kidney failure, blindness, amputations and premature death will increase progressively, unless more effective primary and secondary pharmaceutical and/or life-style interventional strategies become widely available.

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1.2 METABOLIC DISTURBANCES ASSOCIATED WITH TYPE 2 DIABETES

Hyperglycemia in the insulin resistant state

The main feature of Type 2 diabetes is formed by the relative resistance to peripheral insulin action, resulting in impaired glycemic control. According to WHO criteria someone is considered to have Type 2 diabetes if fasting plasma glucose levels are equal or above 7.0 mmol/L or if 2 h following a 75 g oral glucose tolerance test (OGTT) plasma glucose concentration is ≥11.1 mmol/L [11]. Although still subject of intense debate, these diagnostic cut-off points have been based on epidemiological studies that have examined the risk of developing retinopathy over a range of plasma glucose levels [12]. However, even in 'high-risk' obese subjects without blood glucose abnormalities during an OGTT, real-life hyperglycemia was already detectable for almost 14% throughout the day [13]. This indicates that even in the absence of formal intermediate hyperglycemia, as defined by the IDF/World Health Organisation (WHO) [12], so-called post-prandial hyperglycemic spikes are probably an early feature of the insulin resistant state. It has been suggested that hyperglycemic spikes may contribute to the development of macro- and/or microvascular complications in pre-diabetic states [14 15]. However, many different pathophysiological pathways may be simultaneously activated (see below). Therefore, its separate contribution to macro- or microvascular complications is currently unknown.

Hyperglycemia and β-cell failure

The pathophysiological basis for aforementioned post-prandial hyperglycemic spiking lies in a disturbed first-phase insulin response of the pancreatic β-cell, which normally suppresses endogenous glucose production. Subsequently, β-cell function further deteriorates and endogenous insulin production is insufficient to fully compensate for the peripheral insulin insensitivity in muscle-, liver- and/or fat cells [16] (Fig. 1). This glucose stimulated insulin-secretory defect appears to have a genetic origin, but only becomes apparent in the context of peripheral insulin resistance [17]. Once the β-cell fails, post-prandial hyperglycemia may induce large amounts of reactive oxygen species (ROS) that can cause further damage to cellular components of insulin production and induce apoptosis in β-cells [18]. In addition, lipotoxicity [19] and possibly also amyloid deposits [20] may contribute to further deteriorate β-cell function. Certain drugs, such as sulfonylurea (SU) derivatives, are still widely applied to stimulate glucose dependent insulin release. Although these drugs temporarily improve glucose homeostasis, they do not restore β-cell function and may accelerate loss of long-term glycemic control [21]. In a quest to improve long-term diabetes outcome, a whole new line of drugs has become available that try to mimic the release of specific gut hormones [22]. Interestingly, these incretin mimicking drugs not only reduce post-prandial hyperglycemia and (to some extent) body weight, but also seem to restore β-cell mass [23]. Although promising, long-term efficacy of incretin mimic-

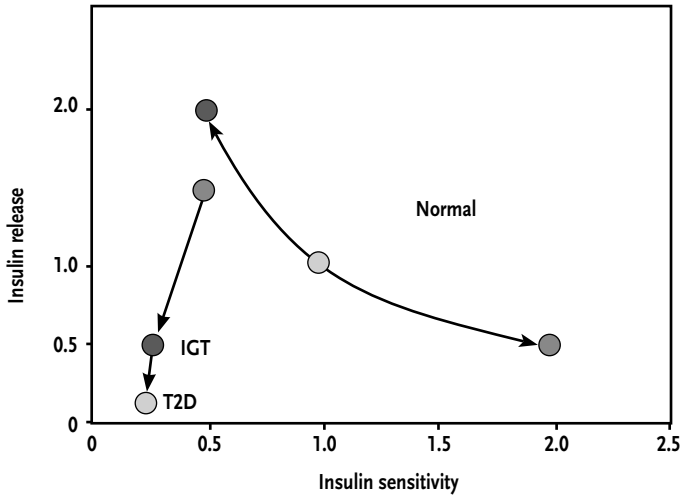

Fig.1 Hyperbolic relationship between insulin sensitivity and insulin release in health and disease (adapted from Kahn et al. 2006 [30])

king drugs as a monotherapy are still under investigation. Besides medication, dietary measures such as slowly digestible carbohydrates [24] and the application of amino-acid induced insulin secretion [25-27] and/or high protein diets [28] can modulate post-prandial hyperglycemia as well. As such, structured life style interventions combined with metformin remain the first treatment of choice. If oral dose adjustment is not sufficient to meet therapeutic targets, early exogenous insulin therapy should be initiated [29].

Obesity and insulin resistance

Over the past three decades, the etiology of insulin resistance and β-cell dysfunction has been subject to intense study [16 30]. Obesity, as a result of inactivity in combination with overeating, plays a key role in the development of pancreatic β-cell dysfunction as well as insulin resistance. Several mechanisms mediating this interaction have been identified. It is now well established that a number of circulating hormones, cytokines, and metabolic fuels, such as non-esterified fatty acids (NEFAs), are being released by adipose tissue and can modulate insulin action. An increased mass of stored triglyceride, especially in visceral or deep subcutaneous adipose depots, leads to large adipocytes that are themselves resistant to the ability of insulin to suppress lipolysis. This results in increased release and circulating NEFA- and glycerol levels. Both aggravate insulin resistance in skeletal muscle [31 32] and likely also the liver [33].

Adipokines and chronic inflammation

Besides increased concentrations of NEFA, expanded visceral adipose tissue also releases pro-inflammatory cytokines (e.g. tumour necrosis factor α (TNF-α), (IL-6), monocyte chemoattractant protein-1 (MCP-1)). Pathways regulating suppression of cytokine signaling (SOCS) proteins [34] and inducible nitric oxide synthase (iNOS) [35] may be involved in mediating cytokine-induced insulin resistance. Secretion of these cytokines, particularly MCP-1 by adipocytes, endothelial cells and monocytes, increases macrophage recruitment and subsequently amplifies cytokine-induced insulin resistance in a feed forward manner [36]. TNF-α and IL-6 act through classical receptor mediated processes, resulting in upregulation of potential mediators of systemic inflammation that can lead to insulin resistance.

More recently, a new adipokine, named retinol binding protein-4 (RBP-4), has been discovered that is directly linked to the level of obesity induced insulin resistance, both in cross-sectional [37 38] and longitudinal studies[37 39-41]. Another adipokine, subject to intense study, is adiponectin. Low adiponectin levels have been correlated with visceral obesity and whole-body insulin sensitivity [42]. This fat cell hormone acts as an insulin sensitizer, inhibiting triglyceride formation in liver and stimulating fatty acid oxidation in muscle in an AMP-activated protein kinase (AMPK) and peroxisome proliferators activated receptor α (PPAR-α) dependent way [43]. Despite their apparent importance in the insulin resistance syndrome, aforementioned adipokines are just examples of a family of adipocyte derived factors that modulate insulin resistance and systemic inflammation. Besides new adipokines, also certain myokines now appear to affect insulin sensitivity and inflammatory responses [44]. As such, the list of insulin sensitizing proteins and cytokines is likely not complete.

Ectopic fat causes insulin resistance in muscle and liver

In the context of a low habitual physical activity level and low oxidative capacity, excess intramyocellular lipid (IMCL) storage has been associated with skeletal muscle insulin resistance [45]. In accordance, longitudinal intervention studies indicate that insulin sensitivity can change independently of IMCL contents [46-51]. Indeed, not IMCL content itself, but rather the peroxidation of inactive pools of IMCL and intracellular fatty acids may explain the apparent correlation between IMCL content and insulin resistance [52]. In addition, intracellular lipid metabolites such as diacylglycerol (DAG), long chain fatty-acyl CoA and ceramides have been shown to interfere with the insulin signaling pathway [53]. These metabolites activate a protein kinase leading to the phosphorylation of serine/threonine sites on the insulin receptor substrate 1, subsequently hampering glucose transport activity and insulin-stimulated myocellular glucose uptake [54].

Ectopic fat storage in hepatocytes, so called intrahepatic lipids (IHL), has also been related to the development of hepatic insulin resistance [55] and hepatic inflammation, initiating non-alcoholic fatty liver disease [56]. In rodents, 3 days of a high fat

diet induces hepatic insulin resistance, while no significant changes in fat content in muscle or visceral tissue could be detected [57]. Experimental research now suggests that hepatic insulin resistance arises from DAG-induced activation of protein kinase Cε, which directly binds to and inhibits insulin receptor tyrosine kinase activity [58]. As such, fat induced hepatic insulin resistance and hepatic inflammation are considered important etiological factors in the development of systemic insulin resistance.

Glucolipotoxicity and long-term complications in Type 2 diabetes

Besides inhibiting intra-cellular insulin signaling, afore mentioned metabolic disturbances in glucose and fat metabolism, increase the formation of Amadori-glycated proteins and advanced glycation end-products (AGE), impairs receptor function for AGE (RAGE) and increases exposure to reactive oxygen species (ROS) in almost any organ system [59-61]. Chronic exposure to Amadori-products, AGE and ROS, so-called glucolipotoxicity, can cause vasculopathy [59], glomerulopathy [62 63] and potentially also induce nerve cell damage [64]. In accordance, hyperglycemia induced AGE and ROS formation provide a unifying model for the high incidence of microvascular disease, retinopathy, nephropathy [63 65], and possibly also neuropathy [66] prevalent in long-term Type 2 diabetes [67]. In accordance, certain pharmaceutical [68 69], nutritional [64] and/or exercise interventions [70-72] that modulate AGE, RAGE and/or ROS formation have been reported to improve insulin sensitivity in experimental rodent models. In humans both structured exercise and alpha lipoic acid have been suggested to reduce neuropathic symptoms [73 74]. However, it is unknown whether such a combined and long-term approach can modulate glucolipotoxicity and prevent diabetes related complications [75].

Reduced oxidative capacity and mitochondrial function in insulin resistant states

It has been well established that most patients with Type 2 diabetes have a significantly lower oxygen uptake capacity ($\dot{V}O_{2peak}$) than age-matched controls [76-78]. Whether this lower oxygen uptake capacity is attributed to a low habitual physical activity level, reduced mitochondrial content or an intrinsic mitochondrial defect is a topic of intense debate [79-87]. Several lines of evidence indicate that oxidative stress and a reduced capacity to oxidize fatty acids, might lead to IMCL accretion, lipid peroxidation and subsequent development of skeletal muscle insulin resistance [88 89]. Despite the wealth of data suggesting that mitochondrial dysfunction plays a key role in the development of progression of the Type 2 diabetes state, [80-82 84 90-98], recent experimental evidence indicates that mitochondrial respiration is not abnormal when normalized for mitochondrial content [85 99]. Environmental factors play an important role in regulating skeletal muscle oxidative capacity, and impairments in oxidative metabolism in Type 2 diabetes patients might simply be the result of a more sedentary lifestyle [87]. Furthermore, mitochondrial dysfunction in Type 2 diabetes might be secondary to impaired insulin signaling [83 86 100 101] and/or abnormal blood glucose, insulin [102 103] and

non-esterified fatty acid (NEFA) [86 101] levels. Therefore, the debate continues as to whether mitochondrial dysfunction represents either cause and/or consequence of insulin resistance and/or Type 2 diabetes.

Hyperinsulinemia, autonomic dysregulation and cardiovascular disease

Above mentioned metabolic disturbances in oxidative capacity, glucose homeostasis and fat metabolism, not only affect systemic insulin resistance, but also appear to influence long-term energy homeostasis [104]. Animal studies indicate that long-term energy balance is coordinated through the combined action of insulin and leptin in the brain [105]. Interestingly, these studies have suggested that insulin action in certain hypothalamic centers reduces food intake while increasing sympathetic nervous system (SNS) outflow to brown adipose tissue to produce heat from fatty acid oxidation as a mechanism to increase energy expenditure [105]. As such, these chronically elevated insulin [106 107] and leptin concentrations [108] further contribute to obesity-associated hypertension through activation of the SNS and release of catecholamines in the basal state [109]. Indeed, early-stage insulin resistance appears to cause sympathovagal imbalance in normoglycemic, insulin resistant offspring of Type 2 diabetes patients [110]. Also in more advanced insulin resistant states, aforementioned increases in sympathetic tone have been associated with changes in cardiac and vascular function that lead to hypertension, left ventricular dysfunction, and/or cardiac autonomic neuropathy [111]. Such changes set the stage for arrhythmia, silent infarction, and sudden death [112 113]. Because potentiation of atherogenesis and cardiac dysfunction occurs in the presence of early diabetic symptoms as well as in the established disease [114 115], early implementation of strategies to reduce cardiovascular risk factors and to attenuate diabetes progression may help to improve long-term outcomes for at-risk individuals. Such interventions may include well-established pharmaceutical treatments for hypertension and dyslipidemia, dietary modulation and/or energy restriction, weight loss, and exercise intervention [113].

1.3 EXERCISE THERAPY TO REVERSE OR STABILIZE PATHOPHYSIOLOGICAL CHANGES

As stated above, much effort is currently put into the discovery of novel pharmacological solutions that may improve metabolic control and prevent diabetes related co-morbidities. Although intense blood pressure and blood glucose lowering therapy has been shown to reduce (microvascular) complications [116 117], blood glucose control with either sulfonylureas, glitazones or exogenous insulin therapy does not seem to prevent the development of macrovascular disease [117 118]. As such, the current increase in Type 2 diabetes incidence and concomitant cardiovascular co-morbidities can only be reversed by dramatic changes in our lifestyle. In accordance, lifestyle intervention programs consisting of regular exercise with [119 120] or without [119 121 122] dietary modula-

tion and/or oral blood glucose lowering medication [123 124] have been proven an effective therapeutic strategy in Type 2 diabetes .

Current guidelines from the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD) or the American College of Physicians (ACP) all firmly recognize the therapeutic strength of exercise intervention [125-127]. The ADA states that *´to improve glycemic control, assist with weight maintenance, and reduce risk of CVD, at least 150 min/week of moderate-intensity aerobic physical activity is recommended and/or at least 90 min/week of vigorous aerobic exercise, ... distributed over at least 3 days/week and with no more than 2 consecutive days without physical activity.'* [127]. Since 2006, the ADA guidelines explicitly mention and recognize that *'in the absence of contraindications, people with Type 2 diabetes should be encouraged to perform resistance exercise 3 times a week, targeting all major muscle groups, progressing to 3 sets of 8-10 repetitions at a weight that can not be lifted more than 8-10 times'* [125]. However, these clinical guidelines generally do not include detailed information on the preferred type and intensity of exercise that should be applied to maximize the benefits of exercise for different subgroups of Type 2 diabetes patients.

1.4 PHYSIOLOGICAL BENEFITS OF EXERCISE IN TYPE 2 DIABETES

Short versus long-term exercise effects

Both a single bout of endurance [128] as well as resistance type exercise [129] have been shown to improve whole-body insulin sensitivity and/or oral glucose tolerance. Therefore, both types of exercise are of therapeutic use in an insulin resistant state [130]. The acute effects of exercise on skeletal muscle insulin sensitivity are attributed to the prolonged activation of the skeletal muscle glucose transporter system [131 132], depletion of muscle and liver glycogen stores [133-135], and/or increased skeletal muscle blood flow following the cessation of exercise [136]. The glucoregulatory benefits of either type of exercise training is represented by the sum of the effects of each successive bout of exercise [135]. In addition, more prolonged exercise training is accompanied by a more structural adaptive response. For instance, endurance training may upregulate mitochondrial enzyme activity in skeletal muscle and subsequently improve whole-body oxygen uptake capacity [137 138]. On the other hand, resistance type exercise is able to induce muscle protein synthesis [139 140] and, as such, an effective intervention to increase lean body mass in Type 2 diabetes patients [141 142]

Type of exercise determines physiological response

In terms of metabolic adaptations, apparent differences exist in the long-term adaptive response to endurance or resistance type exercise training. Prolonged endurance type exercise training has been shown to improve insulin sensitivity in both young [143], elderly [144] and/or insulin resistant subjects [135 145-147]. The latter is attributed to the concomitant induction of weight loss, the upregulation of skeletal muscle GLUT4

expression, improved nitric oxide-mediated skeletal muscle blood flow [148], reduced hormonal stimulation of hepatic glucose production [149], and the normalization of blood lipid profiles [150]. Long-term resistance type exercise interventions have also been reported to improve glucose tolerance and/or whole-body insulin sensitivity [129 141 151]. Besides the consecutive effects of each successive bout of exercise in acutely reducing glycogen stores [152 153], resistance type exercise training has been associated with a substantial gain in skeletal muscle mass, thereby improving whole-body glucose disposal capacity [129]. Besides the attenuation of the loss of muscle mass with aging, resistance type exercise training also improves muscle strength and functional capacity, thereby allowing a healthier, more active lifestyle. Some studies report even greater benefits of resistance as opposed to endurance type exercise training on glycemic control and insulin sensitivity in long-term Type 2 diabetes patients [154]. As such, it has been firmly established that both endurance and resistance type exercise training can be applied to improve metabolic control and quality of life in Type 2 diabetes patients [130].

1.5 EXERCISE IN LONG-TERM TYPE 2 DIABETES PATIENTS

Another expanding Type 2 diabetes subpopulation is formed by the long-term, insulin-treated, Type 2 diabetes patients [155]. These patients generally suffer from severe exercise intolerance due to low oxidative capacity [156], neuropathy-related muscle weakness [157-160], sarcopenia [161], and/or micro- and macrovascular disease [160 162]. As generic exercise intervention programs are too demanding for most of these patients, it is of utmost importance to implement intermediate exercise intervention programs. Such intermediate programs are needed to bring the patient to a level at which they are able to participate in more generic diabetes intervention programs. Such intermediate programs should implement short, relatively high-intensity, exercise bouts applied in an intermittent fashion with the intention to increase muscle strength and functional performance. These so-called short 'in-and-out' exercises do not produce feelings of dyspnoea or discomfort and have been proven safe and effective in cardiac [163 164]. The efficacy and safety of such intermediate programs in long-term Type 2 diabetes patients with high cardiovascular risk profile remains to be established, since exercise intervention studies have generally excluded this specific subgroup of Type 2 diabetes patients.

1.6 LONG-TERM PROGRAM ADHERENCE

Despite the growing body of scientific evidence showing the health benefits of exercise intervention in Type 2 diabetes, several meta-analyses show a general lack of structured, long-term exercise intervention studies [130 165-169]. Most exercise intervention studies consist of endurance and/or resistance type of exercise, often supervised by a physical therapist. The long-term adherence to these (so-called) medical fitness (MF) programs generally ranges between 10 and 80% [124 170 171], and are accompanied

by considerable costs per patient. Brisk walking exercise, when of sufficient volume and intensity, has been proposed to be equally effective [172-174], especially under conditions of frequent counseling by highly motivated and physically active physicians [172]. However, the long-term efficacy of such intervention programs remains to be evaluated.

1.7 OUTLINE OF THE THESIS

As mentioned in the introduction, epidemiological studies and preliminary intervention studies have shown that postprandial hyperglycemia appears a direct and independent risk factor for the development of cardiovascular disease. To define abnormal postprandial blood glucose excursions and relate this to the pathogenesis of diabetic vascular complications, it is important to have more detailed information on normal postprandial blood glucose profiles in a non-insulin resistant population under exactly the same dietary ambulatory conditions. Therefore, in **chapter 2**, we investigated 24 h blood glucose profiles in Type 2 diabetes patients on oral blood glucose lowering medication and healthy, normoglycemic controls under strictly standardized, but free-living conditions.

Most patients with Type 2 diabetes have a significantly lower oxidative capacity than age-matched controls. Whether this lower oxygen uptake capacity is attributed to a low habitual physical activity level, reduced mitochondrial content or an intrinsic mitochondrial defect is a topic of intense debate. In this context, skeletal muscle mitochondrial function is now frequently being studied in Type 2 diabetes patients using various experimental paradigms, each having its own specific advantages and disadvantages. In **chapter 3**, we applied an *in vivo* method using nuclear magnetic resonance (NMR) spectroscopy to assess mitochondrial function and compared the outcome with *in vitro* markers of muscle oxidative capacity in a group of long-term insulin-treated Type 2 diabetes patients.

The associated co-morbidity, and deconditioned state of both the skeletal muscle and cardiovascular apparatus severely limits the intensity of endurance or resistance exercise program in long-term insulin-treated Type 2 diabetes patients. Since this population deconditioned Type 2 diabetes patients on exogenous insulin therapy is growing progressively, it is of utmost importance to establish whether exercise can effectively modulate glycemic control and reduce episodes of glucotoxic hyperglycemia in this Type 2 diabetes subpopulation. Therefore, in **chapter 4** we applied continuous glucose monitoring to evaluate whether a single bout of exercise improves 24 h glycemic control in patients with long-term, insulin-treated, Type 2 diabetes. Besides a low oxidative capacity, these long-term Type 2 diabetes patients on exogenous insulin therapy generally have multiple other complications that prevent them from participating in more generic exercise intervention programs. In accordance, few studies have assessed the efficacy of exercise intervention in this patient population. Therefore, in **chapter 5 and 6**, respectively, we assessed short- and medium-term

benefits of a specifically designed exercise program for this category patients on exogenous insulin treatment.

Despite the growing body of scientific evidence on the health benefits of exercise, most meta-analyses report a lack of studies that have tried to assess the long-term efficacy of exercise prescription in Type 2 diabetes. Long-term adherence to such supervised fitness programs is generally poor, and financial costs are high. Instead, a cheap and group-based exercise program consisting of brisk walking may represent an attractive alternative. However, its long-term efficacy as an addendum to a primary diabetes care program remains to be established. As such, in **chapter 7** we investigated the health benefits of the prescription of 12 months of supervised group-based brisk-walking versus a more individualized medical fitness program in a large population Type 2 diabetes patients in a primary health care setting.

In **chapter 8** the results and conclusions of the previous chapters are integrated and the clinical implications of the work presented in this thesis are discussed. Finally, therapeutic guidelines for tailored exercise interventions in Type 2 diabetes patients are proposed and suggestions for future research are provided.

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Chapter 2 Glycemic instability is an underestimated problem in Type 2 diabetes

ABSTRACT

OBJECTIVE: To assess the level of glycemic control by the measurement of 24 h blood glucose profiles and standard blood analyses under identical nutritional and physical activity conditions in Type 2 diabetes patients and healthy, normoglycemic controls.

RESEARCH DESIGN AND METHODS: A total of 11 male, Type 2 diabetes patients and 11 healthy, matched controls participated in a 24 h continuous subcutaneous glucose monitoring (CGMS) assessment trial under strictly standardized dietary and physical activity conditions. In addition, fasting plasma glucose, insulin and HbA_{1c} concentrations were measured, and an oral glucose tolerance test was performed to calculate indices of whole-body insulin sensitivity, oral glucose tolerance and/or glycemic control.

RESULTS: In the healthy control group, hyperglycemia (blood glucose concentration >10 $mmol.l^{-1}$) was hardly present (2±1 % or 0.4±0.2 / 24 h). However, in the Type 2 diabetes patients hyperglycemia was experienced for as much as 55±7% of the time (13±2 h / 24 h) while using the same standardized diet. Breakfast-related hyperglycemia contributed most (46±7%, ANOVA, $P<0.01$) to the total amount of hyperglycemia and postprandial glycemic instability. In the diabetes patients, blood HbA_{1c} contents correlated well with the duration of hyperglycemia and the postprandial glucose responses ($P<0.05$).

CONCLUSIONS: CGMS measurements show that standard measures for glycemic control underestimate the amount of hyperglycemia prevalent during real-life conditions in Type 2 diabetes. Given the macro- and microvascular damage caused by postprandial hyperglycemia, CGMS provides an excellent tool to evaluate alternative therapeutic strategies to reduce hyperglycemic blood glucose excursions.

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2.1 INTRODUCTION

Over the last 15 years, improvements in microdialysis and biosensor technology have enabled clinicians to reliably monitor plasma and/or interstitial glucose concentrations in an ambulatory and continuous way [1 2]. These, so-called, continuous subcutaneous glucose-monitoring systems (CGMS) have proven quite useful to optimize individual exogenous insulin administration in diabetes patients [3], since they provide information on ambulatory postprandial [4] and/or nocturnal glucose excursions [5]. Moreover, both in children and adults with type 1 diabetes it has been shown that average 24 h blood glucose concentrations strongly correlate with HbA_{1c} concentrations [6 7]. However, the inter- and intra-individual day-to-day variation in glycemic load, meal composition [8] and daily physical activity [9] can complicate therapeutic decision-making based on these 24 h blood glucose profiles [10 11]. Therefore, in order to compare CGMS results between normoglycemic and diabetic subjects, standardization of both diet [8] and physical activity [9] is essential.

Epidemiological studies and preliminary intervention studies have shown that postprandial hyperglycemia is a direct and independent risk factor for the development of cardiovascular disease (CVD) [12]. Importantly, the postprandial rapid increase in blood glucose concentrations or 'hyperglycemic spikes' seem to be even more relevant to the onset of cardiovascular complications than merely elevated fasting plasma glucose [13]. Therefore, therapeutic targets should be aimed at reducing postprandial blood glucose excursions. Although scientific studies on the prevalence of hyperglycemic spikes in Type 2 diabetes are still scarce [13], recommendations on proper glycemic control have recently been redefined [14 15]

To define abnormal postprandial blood glucose excursions and relate this to the pathogenesis of diabetic vascular complications, it is important to have more detailed information on normal postprandial blood glucose profiles in a non-insulin resistant population under exactly the same dietary ambulatory conditions. Therefore, in the present study, we investigated 24 h blood glucose profiles in Type 2 diabetes patients on oral blood glucose lowering medication and healthy, normoglycemic controls under strictly standardized, but free-living conditions. As such, this study provides a frame of reference for future studies on the role of real-life postprandial hyperglycemia in the pathogenesis of diabetic complications.

2.2 SUBJECTS AND METHODS

Subjects

A total of 11 long-term diagnosed male Type 2 diabetes patients and 11 healthy, age and BMI matched, normoglycemic control subjects were selected to participate in this study. Subjects' characteristics are presented in Table 1. Exclusion criteria were impaired renal or liver function, severe obesity (BMI>35 $kg.m^{-2}$), cardiac disease, hypertension, diabetic complications, and exogenous insulin therapy. All Type 2 diabe-

Table 1 Subjects' characteristics

	Controls	Diabetes patients
Age (yrs)	59 ± 2	58 ± 1
Body Mass Index ($kg.m^{-2}$)	27.8 ± 1.4	27.9 ± 1.2
Years Type 2 diabetes	NA	8.1 ± 2.1
HbA_{1c} (%)	5.5 ± 0.1	7.4 ± 0.3*
FPG ($mmol.L^{-1}$)	5.7 ± 0.2	10.6 ± 1.0*
HOMA-IR	3.5 ± 0.5	8.0 ± 1.4†
OGIS	374 ± 18	256 ± 19*

BMI, body mass index; HbA_{1c}, glycated hemoglobin; Fasting plasma glucose (FPG) was determined after 48 hour abstinence of blood glucose lowering medication and 15 min before the oral glucose tolerance test (OGGT); HOMA-IR, homeostasis model assessment insulin resistance index as desribed by Matthews et al. [18] ; OGIS, oral glucose insulin sensitivity-index for a 2 h OGTT as described by Mari et al. [17] *: significantly different between groups; P<0.01, †: P<0.001.

tes patients were treated with oral plasma glucose lowering medication (metformin only (n=3), or in combination with sulfonylureas (n=8)). All medication was continued during the trials. All subjects were informed about the nature and the risks of the experimental procedures before their written informed consent was obtained. The study was approved by the local Medical Ethical Committee.

Screening

Before inclusion, all subjects first performed an oral glucose tolerance test (OGTT). Blood glucose lowering medication was withheld prior to the screening. After an overnight fast, subjects reported at the laboratory at 8.00 a.m. A catheter (Baxter BV, Utrecht, the Netherlands) was inserted into an antecubital vein and a resting blood sample was drawn after which a bolus of 75 g glucose (dissolved in 250 ml water) was ingested (t= 0 min). After the bolus was consumed, blood was sampled every 30 min until t=120 min. Plasma glucose concentrations were measured to determine glucose intolerance and/or Type 2 diabetes according to the World Health Organisation criteria of 1999 [16]. In addition, plasma glucose and insulin concentrations were used to assess insulin sensitivity using the oral glucose insulin sensitivity (OGIS)-index for a 2 h OGTT as described by Mari *et al* [17] and whole-body insulin resistance using the homeostasis model assessment insulin resistance index (HOMA-IR) [18].

Blood sample analysis

Blood (10 ml) was collected in EDTA containing tubes and centrifuged at 1,000 *g* and 4°C for 10 min. Aliquots of plasma were immediately frozen in liquid nitrogen and stored at -80°C until analyses. Glucose concentrations (Uni Kit III, Roche, Basel) were analyzed with the COBAS FARA semi-automatic analyzer (Roche). Plasma insulin was determined in duplicate and averaged by radioimmunoassay (HI-14K, Linco research Inc, St. Charles, USA). To determine HbA_{1c} content a 3 ml blood sample was collected in EDTA containing tubes and analyzed by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany).

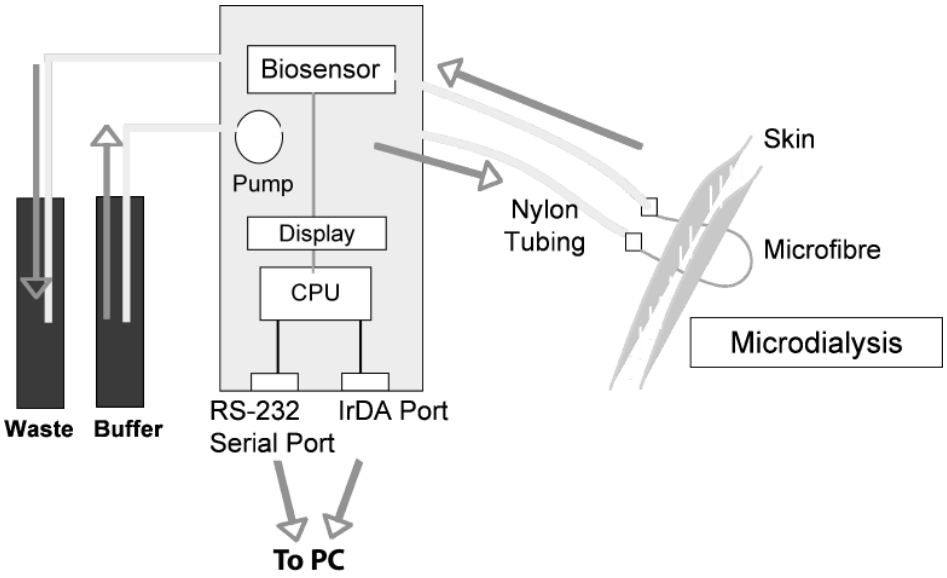

Fig 1. Schematic drawing of Glucoday® microdialysis system. (courtesy of A. Menarini Diagnostics). The device consists of a programmable micropump with 10 to 100 µl.min-1 flow rate, a nylon fluid line/ microdialysis fibre 1.5-2 cm inserted under the skin, a wall jet flow cell and glucose sensor with immobilized glucose oxidase enzyme, a micocontroller for pumpspeed programming, alphanumeric display, RS232 and IrDA interface for PC-connection and a 9 volts battery for continuous >48 h recording during patient monitoring. For further explanations please see Methods section.

Study protocol

All experimental trials described in this study are part of a greater project investigating the effects of nutritional interventions to improve glycemic control in Type 2 diabetes patients. On the first day subjects reported to the laboratory in the afternoon and were instructed about their diet, and on the use of the food intake and physical activity diaries. Next, subjects received instructions in the use of the capillary blood sampling method (Glucocard Memory PC, A. Menarini Diagnostics, Firenze, Italy) used for the calibration of the continuous glucose monitoring system. All subjects were instructed to measure capillary blood glucose concentrations before every meal. After the subjects were fully instructed, a microdialysis fibre (Medica, Medolla, Italy) with an internal diameter of 0.17 mm and a cut-off weight of 18 kD was inserted in the peri-umbilical region, without anesthesia, using an 18-gauge Teflon catheter as a guide, as described previously [19]. For the measurements the micro-fibre was then connected to a portable CGMS (GlucoDay®*S*, A. Menarini Diagnostics, Firenze, Italy), which consists of a peristaltic pump that pumps Dulbecco's solution at 10 µL/ min through the microdialysis fibre. A more detailed description of the device has been published earlier [1]. Briefly, the subcutaneous interstitial fluid is taken up by the

Fig 2. Example of subject wearing Glucoday® microdialysis system which is contained in a small pouch and comfortably worn as a belt.

microdialysis fibre and is transported to the measuring cell. (Fig. 1) The glucose sensor, consisting of immobilized glucose oxidase, measures the glucose concentration every sec and stores an average value every 3 min for a total of 48 h. The entire device weighs about 250 g and is worn in a pouch under the subjects' clothes. (Fig. 2) After the CGMS was checked for proper function, subjects were provided with their diet (pre-weighed and packaged meals, drinks and snacks) and were allowed to return home and resume all their normal activities. CGMS data of the second test day (from 7.00 am to 7.00 am) were used for data analysis. The first period was used to familiarize subjects with the equipment and, therefore, not used in the data analyses.

Diet and physical activity

All subjects maintained their normal physical activity patterns throughout the entire experimental period. Subjects refrained from heavy physical labor and exercise training for at least 3 d prior to and on the day of the trial. Subjects were asked to keep a comprehensive record of time spent performing all activities (to the nearest 10 min) including sleeping, eating sitting, standing, watching television, occupational activity and household tasks, as well as information on the duration and relative intensity (e.g. light, moderate) of all structured activities. The rate of energy expenditure for each ac-

tivity was then determined using the Compendium of Physical Activities [20]. Daily energy expenditure did not differ between groups and averaged 13.6±0.7 and 13.3±0.6 MJ day^{-1} in the Type 2 diabetes patients and normoglycemic controls, respectively. All meals, snacks and beverages were provided in pre-weight packages and ingested at predetermined time points to ensure fully standardized dietary modulation. On the evening prior to the 24 h analyses period, all subjects received the same standardized meal (43.8 kJ kg BW^{-1}; consisting of 60 Energy % (En%) carbohydrate, 28 En% fat and 12 En% protein). The following day the subjects were instructed to ingest their designated meals, drinks and snacks at set time-points. Throughout this 24 h test period subjects received a standardized diet (3 meals and 3 snacks per day) representing an energy intake of 121 kJ kg BW^{-1} per day consisting of 64 En% carbohydrate, 25 En% fat and 11 En% protein. Before and after consuming a meal (i.e. breakfast, lunch and dinner) subjects were asked to obtain a capillary blood glucose sample (Glucocard Memory PC). The following day the subjects reported back to our laboratory to obtain a non-fasting venous blood glucose measurement and to remove the CGMS. The acquired data were then downloaded from the device to a personal computer with GlucoDay® software (V3.0.5). Values reported by the CGMS were converted into glucose values using the capillary glucose measurements as calibration values.

Statistics and data analyses

Data are expressed as means±SEM. Glucose responses were calculated as mean glucose area under the curve (AUC) up to 6 h after each meal. Since the CGMS device provides an average glucose value every 3 min, AUC is expressed as mmol/L * 3 min. To quantify and compare the glucose excursions in the control and diabetes population, AUC and the amount of time during which glucose concentrations were above 10.0 $mmol.L^{-1}$ or below 3.9 $mmol.L^{-1}$ were calculated. On the first and second study day, fasting glucose was determined from the calibrated CGMS curves 10 min before breakfast and averaged. The non-fasting venous blood glucose measurement was used to calculate the coefficient of variation (CV) of the CGMS data. Relationships between CGMS parameters and standard measures of insulin sensitivity were calculated using linear regression models.

To assess intra-day glycemic variability, continuous overall net glycemic action (CONGA), a novel method recently described by McDonnell *et al*, was used [21]. CONGA*n* has been defined as the standard deviation of the differences in glucose concentration using varying time differences of *n* hours. We used CONGA1, CONGA2 and CONGA4, indicating intra-day glycemic variability based on 1 h, 2 h and 4 h time differences, respectively. In normal non-diabetic subjects CONGA values vary between 0.4 and 1.2, while values above 1.5 indicate glycemic lability [21].

Before pooling data from all 22 subjects, homogeneity of regression was tested using ANCOVA in order to exclude significant interaction. Time dependent variables were

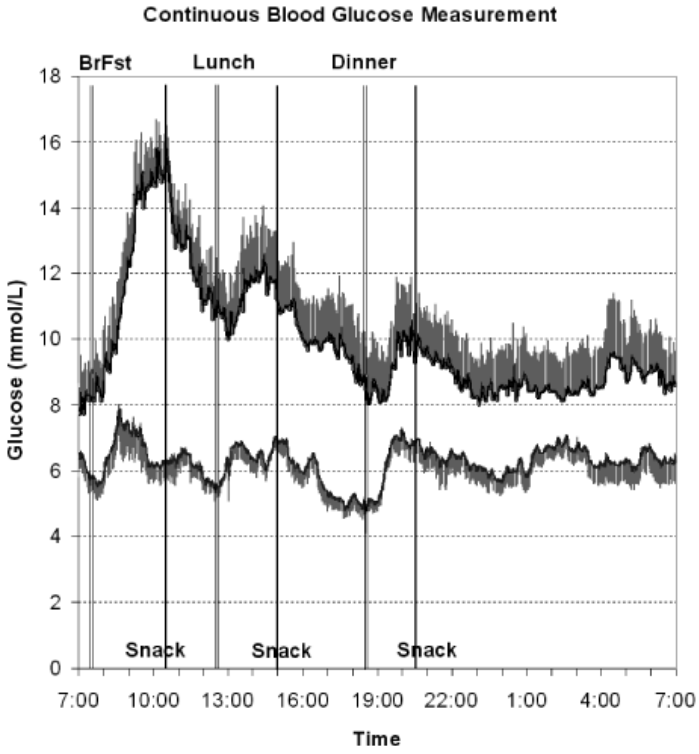

Fig. 3 Mean±SEM glucose concentrations from 07:00 till 07:00 h using CGMS in respectively, eleven healthy, control subjects (lower curve) and eleven Type 2 diabetes patients (upper curve). The SEM is indicated by the grey bars. The vertical lines indicate the time that subjects were consuming their standardized dietary components, consisting of breakfast (BrFst) (07:00-07:30), morning snack (10:30-11:00), lunch (12:30-13:00), afternoon snack (15:30-16:00), dinner (18:30-19:00) and evening snack (20:30-21:00), respectively.

tested using repeated-measures ANOVA with a Tukey-Kramer post-hoc test when applicable. For non-time dependent variables, a Student's t-test for unpaired observations was applied. Significance was set at the 0.05 level of confidence. All statistical calculations were performed using the SPSS 12.0.1 software package (SPSS Inc, Chicago, IL, USA).

2.3 RESULTS

Baseline and postprandial blood glucose responses are provided in Table 2. Total 24 h blood glucose responses in both diabetes patients and healthy controls are illustrated in Fig. 3. Basal and mean glucose concentrations were significantly greater in the Type 2 diabetes patients vs the normoglycemic controls (t-test, $P<0.05$). In the Type 2 diabetes patients, the prevalence of hyperglycemia (>10.0 mmol.L^{-1}) was 55±7% of

Table 2 CGMS measurements

24 h analysis (7-7 am)	Controls	Diabetes patients
Mean 24h glucose (mmol.L^{-1})	6.3 ± 0.2	10.8 ± 0.5*
Hyperglycemic episodes (h)	0.4 ± 0.2	13.3 ± 1.7*
Hypoglycemic episodes (h)	0.5 ± 0.2	0.1 ± 0.05
FPG (mmol.L^{-1})	5.9 ± 0.4	8.6 ± 0.6*
Mean nocturnal glucose (mmol.L^{-1})	7.0 ± 0.9	9.3 ± 0.8*
CONGA1	1.5 ± 0.1	2.5 ± 0.1*
CONGA2	1.7 ± 0.1	3.4 ± 0.1*
CONGA4	1.8 ± 0.2	4.2 ± 0.2*
Post-prandial analyses		
AUC PP breakfast	778 ± 38	1559 ± 75*
AUC PP lunch	713 ± 20	1419 ± 80*
AUC PP dinner	736 ± 39	1158 ± 82*
Glycemic variability		
CONGA1 PP breakfast	1.8 ± 0.3	3.4 ± 0.3#
CONGA1 PP lunch	1.5 ± 0.2	2.2 ± 0.2$
CONGA1 PP dinner	1.5 ± 0.2	2.0 ± 0.3$
CONGA1 nocturnal fasting	1.0 ± 0.2	1.2 ± 0.3

Data presented are means±SEM.; * significant group difference, P<0.001, # = P<0.01, $ = P<0.05, ANOVA; Hyperglycemic episodes, total time during which [glucose] levels are above 10.0 mmol.L^{-1}; Hypoglycemic episodes, total time during which [glucose] levels are below 3.9 mmol. L^{1}; FPG, fasting glucose was determined from the calibrated CGMS curves 10 min before breakfast on the the first and second day; Mean nocturnal glucose, average glucose concentration between 24:00 and 07:00 h; CONGA1,2,4: continuous overall net glycemic action describing intra-day glycemic variability between respectively 1, 2 and 4 h time periods over 24 h ; AUC PP, area under the curve 6 h postprandial (mmol. L* 3 min); CONGA1 glycemic variability between 1 h time periods. CONGA1 PP breakfast: 07:00 –12:00; PP lunch:13:00-18:00 ; PP dinner: 19:00 – 24:00; Nocturnal fasting: from 01:00 till 6:00 h under fasting conditions

the 24 h period. In contrast, in the normoglycemic controls, hyperglycemia was evident in 1.6±1 %. As such, hyperglycemia was present for 13.3±1.7 h and 0.38±0.2 h, respectively.

The postprandial AUCs above 10.0 mmol.L^{-1} following breakfast, lunch and dinner contribute, respectively 46±7%, 29±3%, 11±3% to the total amount of hyperglycemia in our Type 2 diabetes patients present during the 24 h monitoring period. This breakfast-related hyperglycemia was significantly greater (ANOVA, P<0.01) compared to the amount of hyperglycemia during the evening or during the night.

Both in Type 2 diabetes patients and healthy controls the average CONGA1 values following breakfast were significantly raised compared to the 6 h following lunch and dinner (Table 2, ANOVA, P<0.01). CONGA1 values were lowest during the night (ANOVA, P<0.01) and did not differ between groups from 01.00 - 06.00 h (ANOVA, P>0.05, Table 2).

In this study the coefficient of variation (CV) between interstitial CGMS glucose values and venous blood glucose was on average 8.0±1.3%.

Correlations between CGMS parameters and our standard measures for glycemic control are presented in Table 3. In the diabetes patients, HbA_{1c}-levels correlated well with the average 24 h blood glucose concentrations (R=0.81, P<0.01), the time dur-

Table 3 Pearson's correlation matrix between standard insulin sensitivity measures and CGMS measures in both diabetes patients and control subjects

Variable	mean 24 h glucose	% hyper-glycemia	mean noct. glucose	AUC PP breakfast	AUC PP lunch	AUC PP dinner
Diabetes patients (n=11)						
FPG (mmol.L^{-1})	0.61*	0.50	0.70*	0.01*	0.35	0.41
HbA_{1c} (%)	0.81†	0.70*	0.30	0.37	0.80†	0.87†
Control Subjects (n=11)						
FPG (mmol.L^{-1})	0.84†	0.37	0.86†	0.45*	0.40	0.71*
HbA_{1c} (%)	0.05	-0.18	0.03	-0.17	0.32	0.01*

FPG, fasting plama glucose was determined from the calibrated CGMS curves 10 min before breakfast on the second day; HbA_{1c}, glycosylated hemoglobin; mean noct. glucose, average glucose concentration between 00:00 am and 07:00 h; AUC PP, area under the curve 6 h postprandial (mmol/L* 3 min); *significant correlations P<0.05, † P<0.01;

ing which blood glucose levels were >10 mmol.L^{-1} (R= 0.70, P<0.05), and postprandial AUC following lunch (R=0.80, P<0.01) and dinner (R=0.87, P<0.01). In a subgroup of diabetes patients with apparent acceptable glycemic control ($HbA_{1c} \leq 7.0$, n=6), hyperglycemia was present for 46±8% of the day (11.0±1.9 h).

Both in the diabetes and control group, mean 24 h and nocturnal blood glucose concentrations correlated strongly with fasting plasma glucose levels (R between 0.61-0.86, P<0.05). In both groups, no significant correlations were reported between the 24 h CONGA indices and HbA_{1c} content, however, in the diabetes patients a significant correlation was found between postprandial CONGA1 values and AUC in the 6 h following a meal (R=0.47, P<0.01). When pooling the data from both groups, the 24 h CONGAn values correlated significantly with blood HbA_{1c} content (R=0.53-0.66, P<0.01), mean 24 h glucose concentrations (R=0.73-0.77, P<0.001) and to a lesser extent with mean fasting plasma glucose concentrations (R=0.50-0.52, P<0.05). Also, a significant correlation was found between postprandial CONGA1 values and AUC 6 h following a meal (R=0.60, P<0.001)

2.4 DISCUSSION

The present study shows that under normal, standardized dietary conditions, Type 2 diabetes patients using oral blood glucose lowering medication experience a substantial amount of hyperglycemia for more than 13 h within a 24 h period. This disturbance in blood glucose homeostasis is predominantly present following breakfast. After comparing 24 h blood glucose profiles between healthy, normoglycemic controls and Type 2 diabetes patients under usual medical care by a general practitioner, it seems clear that standard treatment schemes with oral blood glucose lowering drugs appear to have insufficient therapeutic strength to normalize postprandial hy-

perglycemia. Given the clinical relevance of the hyperglycemic spikes [13], CGMS provides an excellent tool to evaluate the level of glycemic stability in Type 2 diabetes patients.

The concept that oral blood glucose lowering therapy provides inadequate protection against hyperglycemia is not new [22-24]. Epidemiological studies and preliminary intervention studies have shown that postprandial hyperglycemia is a direct and independent risk factor for the development of cardiovascular disease [12]. However, the postprandial rapid increase in blood glucose concentrations seems to be more relevant to the onset of cardiovascular complications than merely elevated fasting plasma glucose concentrations [13]. Therefore, more detailed information on 24 h blood glucose profiles in a diabetic state is essential to increase our understanding of the relationship between hyperglycemia, glucotoxicity and cardiovascular morbidity. In an attempt to assess postprandial glycemic instability in Type 2 diabetes, we applied CGMS in diabetes patients and compared this with blood glucose profiles of normoglycemic subjects under strict nutritional and exercise standardization, but otherwise free-living conditions. In most of our normoglycemic subjects, hyperglycemia or glycemic instability was not detectable. In contrast, despite healthy dietary conditions and continued use of oral blood glucose lowering medication according to standard primary care [25] and international guidelines [15], the Type 2 diabetes patients were hyperglycemic during more than 13 h per day, while using exactly the same diet as the normoglycemic controls. In accordance with earlier observations by Monnier et al [26], this study shows that postprandial hyperglycemia was most prominent following breakfast, and less evident during the night. As we provided a healthy, balanced diet (43.8 kJ/kg BW^{-1}; consisting of 60 En%), carbohydrate, 28 En% fat and 12 En% protein), it could be speculated that the total amount of hyperglycemia may even be worse under normal, unrestricted dietary conditions. The observed levels of hyperglycemia during the day (13±2 h / 24 h) are unacceptable and likely cause the excess formation of advanced glycation end-products [27], causing the macro- and microvascular damage [28].

In line with earlier studies [29-31], our findings emphasize the need for different types of interventional strategies in Type 2 diabetes patients. It should be noted that there is a weak, non-significant, correlation between fasting blood glucose and the percentage of hyperglycemia (R^2=0.25, P>0.05, Table 3). The latter indicates that FPG is unlikely to be of sufficient sensitivity to successfully evaluate new treatment strategies that focus on reducing postprandial hyperglycemia. For more long-term evaluation purposes, changes in blood HbA_{1c} concentrations have generally been assessed, since blood HbA_{1c} content correlates relatively well with both mean 24 h [32 33] and postprandial glucose levels [6 7]. In accordance, in the present study we observed strong correlations between HbA_{1c} and mean 24 h glucose and postprandial glucose levels following lunch and dinner (Table 3). It should be mentioned here that even under clinically acceptable HbA_{1c} levels (i.e. $HbA_{1c} \leq 7.0$ in 6 out of 11 diabetes patients)

hyperglycemia can still be unacceptably large at 11±2 hours of blood glucose excursion >10.0 mmol.L^{-1} per 24 h. Therefore, these results extend on earlier findings [24 34 35], and strongly suggest that the ability of HbA_{1c} to monitor postprandial hyperglycemia is debatable. Moreover, the measurement of prospective changes in blood HbA_{1c} content only has sufficient sensitivity to detect changes in glucose homeostasis during middle to long-term interventions [36]. Therefore, the present study underlines the notion that CGMS is a promising tool when evaluating short-term (<3 months) changes in blood glucose homeostasis following pharmacological, dietary and/or exercise interventions [1].

Another benefit of the CGMS approach, that has potential clinical application as well, is the possibility to calculate the level of glycemic instability in insulin resistant states. This so-called Continuous Overall Net Glycemic Action (CONGA*n*) is probably a more appropriate measure to assess short-term changes in glucose homeostasis throughout the day [21]. This CGMS measure reflects the standard deviation of the differences in glucose concentration using varying time windows [21]. Therefore, we determined CONGA*n* values in both our diabetes patients and normoglycemic controls (Table 2). The proposed sensitivity of CGMS to detect subtle variations in glycemic control was confirmed in our normoglycemic control group. Interestingly, 2 of our control subjects appeared to have rather high postprandial CONGA1 values that almost approached values observed in the Type 2 diabetes patients (i.e. average postprandial CONGA1 >2.1). These 2 subjects also showed the highest insulin values during the oral glucose tolerance test, and were the only 'normoglycemic' persons who showed some hyperglycemia throughout the day (data not shown). Altogether, our results suggest that more advanced CGMS analyses techniques provide promising measures to assess glycemic instability in diabetes patients [21]. Research is warranted to investigate the diagnostic value of CGMS in other diabetes related populations, like patients in a prediabetic and/or insulin resistant state.

In conclusion, detailed analyses of 24 h blood glucose profiles show that standard measures for glycemic stability grossly underestimate the amount of hyperglycemia during real-life conditions in Type 2 diabetes patients. Given the macro- and microvascular damage caused by postprandial hyperglycemia, CGMS provides an excellent tool to more directly evaluate additional therapeutic strategies to reduce the amount of glycemic instability and risk of cardiovascular complications in Type 2 diabetes patients.

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Chapter 3 ^{31}P MR spectroscopy and in vitro markers of oxidative capacity in Type 2 diabetes patients

ABSTRACT

BACKGROUND: Skeletal muscle mitochondrial function in Type 2 diabetes is currently being studied intensively. In vivo ^{31}P magnetic resonance spectroscopy (^{31}P MRS) is a non-invasive tool used to measure mitochondrial respiratory function in skeletal muscle tissue. However, microvascular co-morbidity in long-term Type 2 diabetes can interfere with the ^{31}P MRS methodology.

AIM: To compare ^{31}P MRS-derived parameters describing *in vivo* mitochondrial respiratory function with an *in vitro* assessment of muscle respiratory capacity and muscle fibre-type composition in Type 2 diabetes patients.

METHODS: ^{31}P MRS was app lied in long-standing, insulin-treated Type 2 diabetes patients. ^{31}P MRS markers of mitochondrial respiratory function were measured in the *M. vastus lateralis*. Muscle biopsy samples were collected from the same muscle and analyzed for succinate dehydrogenase activity (SDH) and fibre-type distribution.

RESULTS: Several ^{31}P MRS parameters of mitochondrial respiratory function showed moderate to good correlations with the percentage of type-I fibres and type-I fibre-specific SDH-activity (Pearson's R between 0.70-0.75). *In vivo* and *in vitro* parameters of local mitochondrial respiration also correlated well with whole-body fitness levels ($\dot{V}O_{2peak}$) in these patients (Pearson's R between 0.65-0.90).

CONCLUSION: Good correlations exist between *in vivo* and *in vitro* measurements of mitochondrial respiratory function in long-standing, insulin-treated Type 2 diabetes subjects, which are qualitatively and quantitatively consistent with previous results measured in healthy subjects. This justifies the use of ^{31}P MRS to measure mitochondrial respiratory function in relation to Type 2 diabetes.

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3.1 INTRODUCTION

The presence of skeletal muscle mitochondrial dysfunction in ageing, insulin resistance and/or Type 2 diabetes is currently a topic of intense debate [1-6]. Even though recent studies seem to support the concept that mitochondrial dysfunction plays a key role in the pathogenesis of skeletal muscle insulin resistance [2 4 6], it remains to be established whether mitochondrial dysfunction represents either cause or consequence [7]. In this context, skeletal muscle mitochondrial function is now frequently being studied in Type 2 diabetes patients using various experimental paradigms, each having its own specific advantages and disadvantages.

^{31}P MR spectroscopy (^{31}P MRS) is a commonly applied method allowing *in vivo* measurement of mitochondrial function in exercising muscle [8-14]. Previous studies in healthy subjects showed ^{31}P MRS to be an effective non-invasive method to measure mitochondrial oxidative capacity based on moderate to good correlations between ^{31}P MRS derived parameters and *in vitro* measurements of oxidative capacity [15 16]. ^{31}P MRS can be used to measure the time constant of phosphocreatine (PCr) recovery after exercise. During recovery from exercise, PCr is resynthesized purely as a consequence of oxidative ATP synthesis and, therefore, analysis of PCr recovery provides information about mitochondrial respiratory function. Alternative measures as the recovery of adenosine diphosphate (ADP), the initial PCr recovery rate and the maximal mitochondrial aerobic capacity can all be inferred from the PCr recovery data. ^{31}P MRS studies have shown that mitochondrial function is impaired in mitochondrial myopathy [17 18] and in chronic disease such as cardiac failure [19], peripheral arterial occlusive disease [20]. However, tissue pH [8 21-24], local muscle blood flow [25] and concomitant tissue oxygenation [26] might affect the ^{31}P MRS measurement of mitochondrial respiration. Both impaired vascular function [27 28], and low capillary density [29] have been associated with Type 2 diabetes. However, recently it has been postulated that a wide intersubject variability exists in the level of diabetes-related complications [30]. Given the potential confounding influence of an impaired microcirculation and tissue oxygenation [25 26] on PCr-recovery kinetics, we questioned whether PCr recovery kinetics can be reliably used as an index of mitochondrial function in long-term Type 2 diabetes patients on exogenous insulin therapy. It was hypothesised that the earlier described correlations between ^{31}P MRS and *in vitro* markers of mitochondrial function [15 16] are weaker or even entirely absent in a population of long-term diagnosed Type 2 diabetes patients on exogenous insulin treatment.

In the present study, we evaluate the relationship between ^{31}P MRS-derived parameters describing mitochondrial function, maximal succinate dehydrogenase activity (SDH) as an *in vitro* marker of muscle oxidative capacity and muscle fibre type composition in a group of long-term insulin-treated Type 2 diabetes patients. As such, this study provides additional insight in the applicability of ^{31}P MRS to investigate *in vivo* skeletal muscle mitochondrial respiratory function in Type 2 diabetes.

3.2 MATERIALS AND METHODS

Subjects

Eleven male Type 2 diabetes patients were selected to participate in this study. Subjects had been diagnosed with Type 2 diabetes for over 5 years, established by a fasting blood glucose larger than or equal to 6.1 mmol.L^{-1} at the time of diagnosis as defined by the World Health Organisation [31]. All subjects were on exogenous insulin treatment for at least 24 months and had been on a stable regimen of diabetes medication over the last 3 months before being recruited. Patients using thiazolidinediones and/or ß-blockers less than 6 months, and subjects with impaired liver function, renal failure, severe retinopathy or a history of severe cardiovascular problems, were excluded from participation. The nature and the risks of the experimental procedures were explained to the subjects and all gave their written informed consent to participate in the study, which was approved by the local Medical Ethical Committee of the Máxima Medical Center, Veldhoven, The Netherlands.

Body composition

Body mass index and waist circumference were measured using an analog weight scale and standard measuring tape. Segmental and whole-body bone mass and fat free mass (FFM) were determined using whole-body dual energy X-ray absorptiometry (DEXA) (Hologic QDR-4500 Discovery A, software version 12.3:3, Hologic Inc. Bedford, MA, USA).

Whole-body oxygen uptake capacity

Two weeks before the muscle biopsy and MRS measurements, maximal whole-body oxygen uptake capacity ($\dot{V}O_{2peak}$) and maximal workload capacity (W_{max}) were measured during an incremental exhaustive exercise test until exhaustion, performed on a cycle ergometer (Medifit Ergometer, Medifit systems, Maarn, The Netherlands) using a ramp protocol [32]. After 4 min of unloaded cycling the load was increased linearly until exhaustion. The aim was to apply a load rate that would cause exhaustion within 8-12 min as recommended by Zhang et al. [32]. Subjects were requested to abstain from exercise and caffeinated beverages on the day of the test and were tested more than 2 h after a light meal. Gas exchange measurements were performed continuously by using a computerized metabolic cart (Ergostar II, PMS Professional Medical Systems, Basel, Switzerland) that was calibrated before each study. Maximal whole-body oxygen uptake capacity was defined in the present study as $\dot{V}O_2$ remaining unchanged or increasing less than 1 ml.min^{-1}.kg^{-1} for 30 sec or more despite an increment in work load [33]. Cardiac function was monitored using a 12-lead electrocardiogram with heart rate (HR) being recorded continuously (Polar Electro, Kempele, Finland) and sampled at 1 kHz through a data log device (Co2ntrol™, Tildesign, Zeewolde, The Netherlands). A picture of the experimental set up is shown in Fig. 1.

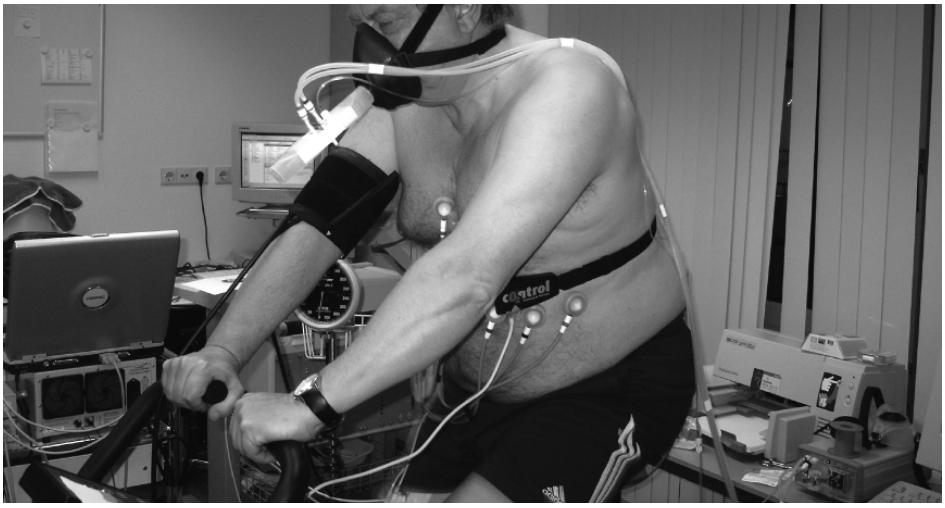

Fig 1. Experimental setup to assess maximum heart rate, peak oxygen uptake and maximum workload capacity of our patients during a ECG-stress test on a cycle ergometer.

^{31}P Magnetic resonance spectroscopy

^{31}P MRS of the *M. vastus lateralis* was performed by using a 1.5-Tesla whole-body magnet (Gyroscan S15/ACS, Philips Medical Systems, Best, The Netherlands). Subjects were measured in a supine position. After collecting transversal and sagittal scout images (Fig. 2), the magnetic field homogeneity was optimized by localized shimming on the proton signal using the body coil. The ^{31}P signals were collected using a 6-cm diameter surface coil placed over the *M. vastus lateralis*. Data were acquired fol-

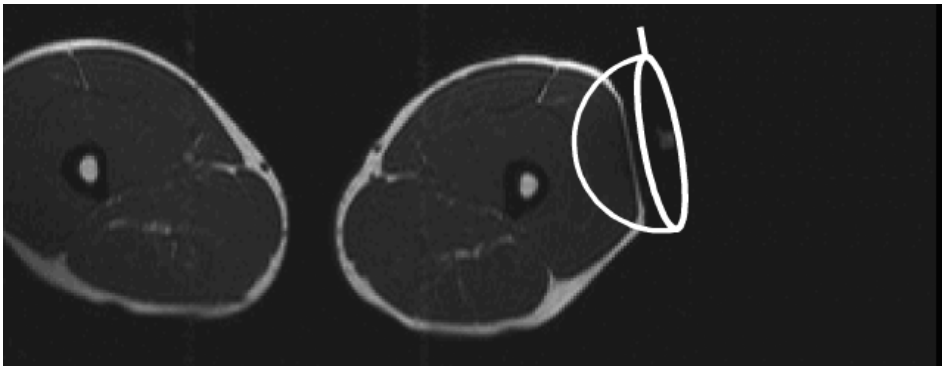

Fig. 2. After collecting transversal and sagittal scout images of the upper limb, the magnetic field homogeneity was optimized by localized shimming on the proton signal using the body coil. Its position is indicated through the white oval. The white semi-circle projects the theoretical MRS volume of the coil on the quadriceps muscle.

lowing a 90° adiabatic excitation pulse with a sweep width of 2 kHz and 1024 data points. A fully relaxed spectrum was measured at rest with a repetition time of 30 s and 24 scans. Then, spectra were acquired using a repetition time of 3 s at rest (60 scans/spectrum) and during a rest-exercise-recovery protocol (2 scans/spectrum yielding a time resolution of 6 s, total of 150 spectra/15 min). For the latter time series, the first 20 spectra (2 min) were measured at rest, after which the subjects started the exercise (see below). The duration of the exercise varied per subject, but never exceeded 8 min, so that at least 5 min of recovery were recorded. From the dimension of the coil and the size and geometry of a typical upper leg, it was estimated that the majority of the signal in the unlocalized ^{31}P MRS measurements originates from the *M. vastus lateralis*, with minimal contaminations from the adjacent *M. rectus femoris* and underlying *M. vastus intermedius*.

Exercise protocol inside magnet

All subjects performed a single leg extension exercise in the supine position inside the magnet, which has been shown to be limited to the four muscles of the quadriceps [34]. The exercise was conducted by rhythmically lifting a lever (resting on the lower leg, proximal of the foot) connected to an ergometer. The upper leg was supported with the hip joint in a 30 degrees ante flexed position and immobilised with two 3 cm wide Velcro straps (Fig. 3). One contraction was performed every 1.5 s acoustically guided by a digital metronome. The workload was set at 2.5 W for the first min and then increased by 2.5 W each min (except for one subject, whose work load was increased by 5 W each min). To get subjects acquainted to the in-magnet exercise protocol subjects visited the laboratory twice within a 7 day period. During the first

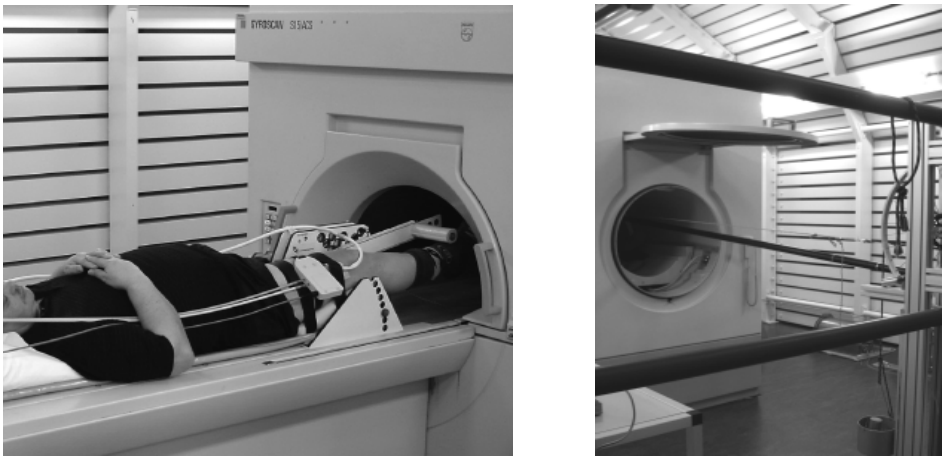

Fig. 3 ^{31}P MRS of the M. vastus lateralis was performed by using a 1.5-Tesla whole-body magnet. After entering the magnet, subjects were measured in the same supine position, while rhythmically extending the knee against an increasing load applied through a custom-made ergometer.

visit, subjects performed a test run and exercised their left leg until fatigued. The data were then used to estimate the duration of the exercise for the second visit. During the second visit, subjects exercised their right leg until phosphocreatine depletion was sufficient (~ 50%) to measure the recovery, aiming to avoid intracellular pH to drop below 6.8.

Analysis of ^{31}P MR spectra

Zero and first order phase corrections were determined from the rest spectra and then also applied to the time series. Spectra were fitted in the time domain by using a non-linear least squares algorithm (AMARES) in the jMRUI software package [35]. PCr, inorganic phosphate (P_i), adenosine triphosphate (ATP) and phosphodiesters (PDE) signals were fitted to Lorentzian line shapes. The three ATP peaks were fitted as two doublets and one triplet, with equal amplitudes and line widths and prior knowledge for the J-coupling constant (17 Hz). For the time series, the PCr line width during recovery was constrained to the average PCr line width during recovery (excluding the first 10 data points), obtained from a prior, unconstrained fit.

Absolute concentrations of the phosphorylated metabolites were calculated after correction for partial saturation and assuming that [ATP] is 8.2 mM at rest [36]. Saturation correction factors determined from the fully relaxed spectra were 1.93±0.05, 1.60±0.05, 1.54±0.06 and 2.14±0.06 (mean±SD, respectively, for PCr, P_i, ATP and PDE. Intracellular pH was calculated from the chemical shift difference between the P_i and PCr resonances (δ; measured in parts per million) using the following formula [37]:

$$\mathrm{pH} = 6.75 + \log\left(\frac{\delta - 3.27}{5.63 - \delta}\right) \quad [1]$$

Free cytosolic [ADP] was calculated from pH and [PCr] using a creatine kinase equilibrium constant (K_{eq}) of 1.66×10^9 M^{-1} [38] and assuming that 15% of the total creatine is unphosphorylated at rest [39], using the equation:

$$[\mathrm{ADP}] = \frac{[\mathrm{ATP}][\mathrm{Cr}]}{[\mathrm{PCr}][\mathrm{H}^+]K_{\mathrm{eq}}} \quad [2]$$

During recovery from exercise, PCr is resynthesized purely as a consequence of oxidative ATP synthesis and therefore analysis of PCr recovery provides information about mitochondrial respiratory function. Recoveries of PCr and ADP were fitted to mono-exponential functions using Matlab (version 6.1, Mathworks, Natick, Massachusetts, USA). Results are expressed as the metabolite's time constant of recovery, i.e. τ_{PCr} and τ_{ADP}.

Calculation of the initial rate of PCr recovery (V_{PCr}) was based on the PCr recovery rate ($1/\tau_{PCr}$) and the difference between the resting and the end-exercise PCr concentrations (ΔPCr) [40]:

$$V_{PCr} = \frac{1}{\tau_{PCr}} \cdot \Delta PCr \qquad [3]$$

Calculation of the maximum aerobic capacity (Q_{max}) was based on V_{PCr}, the end-exercise ADP concentration ($[ADP]_{end}$), and the assumption that oxidative ATP synthesis is regulated by the ADP concentration according to Michaelis-Menten kinetics with a K_m of 30 mM [40]:

$$Q_{max} = V_{PCr} \cdot \left(1 + \frac{K_m}{[ADP]_{end}} \right) \qquad [4]$$

Blood and muscle biopsy samples

On the evening before the blood sample and muscle biopsy collection, subjects received a standardized meal (35.2±1.8 kJ per kg BW, containing 53 energy% (En%) fat, 10 En% protein, and 37 En% carbohydrate) after which subjects remained fasted and were allowed to drink water only. Subjects reported at the laboratory at 08.00 a.m. After 5-10 min of supine rest, a venous blood sample was collected from an antecubital vein. Blood plasma samples were collected into EDTA containing tubes and centrifuged for 10 min at 4°C. Aliquots of plasma were frozen immediately in liquid nitrogen and stored at -80°C until further analyses. Plasma glucose and HbA_{1c} measurements were performed on a Modular P analyzer (Roche Diagnostics, Basel, Switzerland) using the Hexokinase and Tina-quant assays, respectively. After blood collection and local anesthesia, a percutaneous muscle biopsy was collected from the middle region of the *M. vastus lateralis*, using a modified Bergstrøm needle to increase sample size (Maastricht Instruments, Maastricht, The Netherlands). Muscle samples were dissected carefully, freed from any visible non-muscle material, embedded in Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands) and rapidly frozen in liquid nitrogen-cooled isopentane to its melting point. Multiple serial sections (5 µm) from each biopsy sample were thaw-mounted together on uncoated, pre-cleaned glass slides. The proportion of Type I, IIa, and IIx muscle fibres was determined using antibodies raised against human myosin heavy chain (MHC) Type I (A4.840) and type IIa (N2.261), developed by Dr Blau et al. [41]. Muscle fibre-type specific oxidative capacity was estimated by measuring SDH-activity in the muscle cross-sections using histochemical analyses [42]. After 24 h, glass slides were examined using a Nikon E800 fluorescence microscope (Uvikon, Bunnik, The Netherlands) coupled to a Basler A113 C progressive scan color CCD camera, with a Bayer color

filter. Epifluorescence signal was recorded using a fluorescein isothiocyanate (FITC) excitation filter (465-495 nm) for muscle fibre-type, and a 4',6-diamidino-2-phenylindole (DAPI) UV excitation filter (340-380 nm) for laminin. Digitally captured images (240x magnification) with a minimum of six fields-of-view per muscle cross-section, were processed and analyzed using Lucia 4.8 software (Nikon, Düsseldorf, Germany). SDH stained sections were captured in full color using bright field light microscopy. Digitally captured images (120x magnification), were processed and analyzed using the Lucia 4.8 software package. The bright-field images of the SDH stains were converted *post hoc* to 8-bit grayscale values. The mean optical density of the SDH-raised signal per individual fibre was quantified by averaging the optical density measured in every pixel in the cell, corrected for the mean optical density of the background stain measured in a field-of-view containing no muscle fibres.

Statistics

All data are expressed as means±SEM. Student's t-test as well as simple and multiple stepwise regression analyses were applied on the data on whole-body oxygen uptake capacity, ^{31}P MRS, and muscle biopsy measurements using the SPSS 12.0.1 software package (SPSS Inc, Chicago, IL, USA). Level of statistical significance was set at $P<0.05$.

3.3 RESULTS

Subjects' characteristics

The characteristics of our 11 male subjects are shown in Table 1. Mean duration of Type 2 diabetes was 12.1±2.1 years since diagnosis. Subjects had been on exogenous insulin therapy for 7.0±2.4 years, and seven patients combined this with oral blood

Table 1 Subjects' characteristics

n=11	Mean ± SEM	Range
Age (yrs)	59.0 ± 2.5	49 - 69
Body Mass Index (kg.m^{-2})	32.2 ± 1.2	25.5 - 38.7
Body Weight (kg)	97.6 ± 4.9	78.0 - 123.0
FFM (kg)	68.9 ± 2.9	56.3 - 85.2
Waist circumference (cm)	110.6 ± 3.7	94.5 - 131.5
HbA_{1c} (%)	7.6 ± 0.3	6.3 - 9.5
FPG (mmol.L^{-1})	10.4 ± 0.9	4.0 - 16.4
Years of Type 2 diabetes	12.1 ± 2.1	6 - 29
Years of insulin therapy	7.0 ± 2.4	2 - 29
Daily insulin requirement (I.U.)	92.5 ± 11.1	16 - 150
$\dot{V}O_{2peak\ per\ kg\ BW}$ (ml.min^{-1}.kg^{-1})	24.3 ± 1.4	15.0 - 29.6
$\dot{V}O_{2peak\ per\ kg\ FFM}$ (ml.min^{-1}.kg^{-1})	34.2 ± 1.9	21.7 - 40.5

BMI, body mass index; FFM, fat free mass; HbA_{1c}, glycosylated hemoglobin; FPG: fasting plams glucose; $\dot{V}O_{2peak}$ maximal oxygen uptake per kg bodyweight / fat free mass

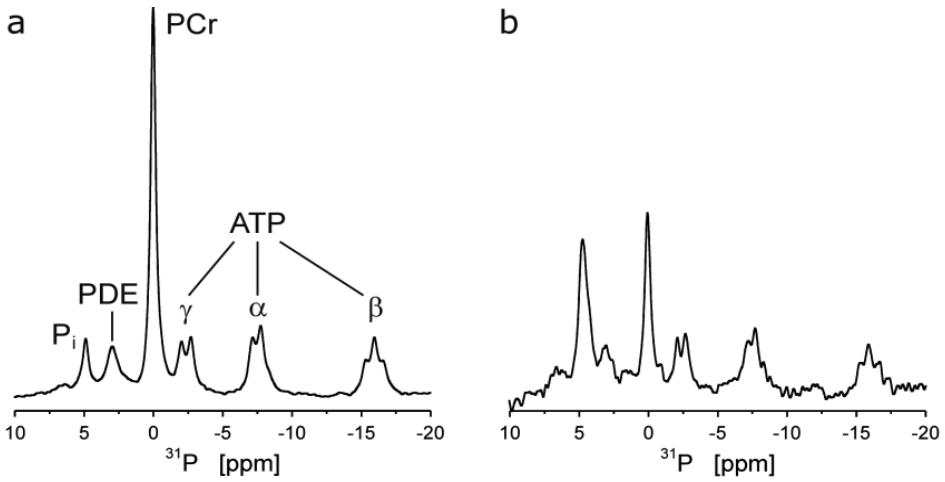

Fig. 4 Typical M. vastus lateralis 31P spectra for one subject at rest (panel a, number of scans = 60) and at the end of exercise (panel b, number of scans = 2). P_i indicates inorganic phosphate; PDE, phosphodiesters; PCr, phosphocreatine; and α, β, γ indicate the three phosphate groups of ATP. For this subject the PCr depletion at the end of exercise was 47.6% and the corresponding pH was 6.95.

glucose lowering medication. All subjects were using blood pressure lowering medication, and 8 patients were receiving cholesterol-lowering therapy. Whole-body maximal oxygen uptake ($\dot{V}O_{2peak}$) was 24.3±1.4 ml per min per kg body weight.

MRS measurements

Figure 4a and b show typical examples of 31P MR spectra from a subject's *vastus lateralis* muscle at rest and at the end of exercise, respectively. Figure 5 shows a stack plot of similar 31P MR spectra during a rest-exercise-recovery protocol. Table 2 summarizes the baseline and end-exercise 31P MRS results for the 11 subjects. At the end of exercise, the average PCr depletion was 45.5±2.3% and the pH was 6.90±0.04, which was significantly different from the resting condition (t-test, $P<0.01$). Among all subjects, the end-exercise pH ranged between 6.75-7.05.

Figure 6 illustrates both the raw data and mono-exponential fits of the PCr and ADP recoveries of one subject. Table 2 summarizes the PCr and ADP recovery time constants and the other estimates of mitochondrial respiratory function, V_{PCr} and Q_{max}. All 31P MRS estimates of mitochondrial respiratory function displayed a wide range of values. For instance τ_{PCr} ranged from 27.2 to 86.6 s. All 31P MRS variables correlated significantly with each other (Pearson's R between 0.77 and 0.92, $P<0.05$).

Histochemical analyses

Table 3 summarizes muscle fibre-type distribution and both mixed and fibre-type specific SDH-activity. No significant differences in SDH-activity between type I and

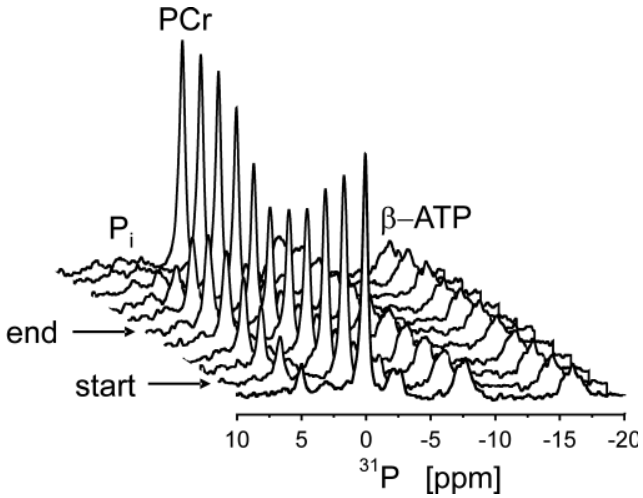

Fig. 5 Stack plot of ^{31}P MR spectra from a time series measured during a rest-exercise-recovery protocol in human muscle. Peaks are from inorganic phosphate (P_i), phosphocreatine (PCr) and ATP, the β-ATP group is indicated on the right side, ppm=parts per million (courtesy of dr. J.J. Prompers)

Table 2 ^{31}P MRS parameters

n=11	Rest	End-exercise
[PCr] (mM)	36.9 ± 1.2	20.2 ± 1.3
[P_i] (mM)	4.7 ± 0.2	19.8 ± 1.2
[ADP] (μM)	10.3 ± 0.1	46.8 ± 2.8
pH	7.07 ± 0.004	6.90 ± 0.04
MRS markers of mitochondrial respiratory function	**Mean ± SEM**	**Range**
τ_{PCr} (s)	49.4 ± 5.5	27.2 - 86.6
τ_{ADP} (s)	22.5 ± 2.9	14.9 - 45.3
V_{PCr} (mM.s^{-1})	0.37 ± 0.03	0.20 - 0.54
Q_{max} (mM.s^{-1})	0.61 ± 0.05	0.31 - 0.82

Data presented are means ± SEM. PCr, phosphocreatine; P_i, inorganic phosphate; ADP, adenosine diphosphate; pH, intracellular muscle pH; τ_{PCr}, PCr recovery time constant; τ_{ADP}, ADP recovery time constant; V_{PCr}, initial rate of PCr recovery based on the PCr recovery rate ($1/\tau_{PCr}$) and the difference between the rest and end-exercise PCr concentrations; Q_{max}, maximum rate of oxidative ATP synthesis calculated from V_{PCr} in relation to end-exercise ADP concentration and an assumed K_m of 30 μM.

type IIa fibres were observed (t-test, NS). Type IIx muscle fibre SDH-activity was significantly lower compared to the type-I and IIa muscle fibres (t-test, $P<0.05$).

Correlations

Correlations between *in vivo* and *in vitro* estimates are presented in Table 4 and Figures 7 and 4. All ^{31}P MRS markers of mitochondrial respiratory function revealed a

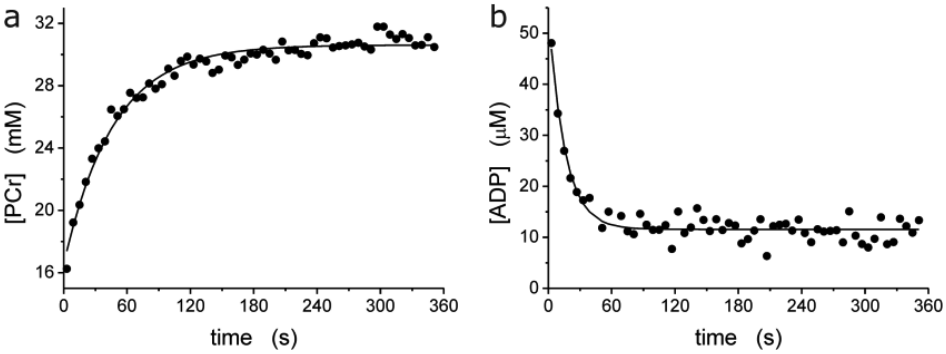

Fig. 6 PCr (panel a) and ADP (panel b) recovery curves for an individual subject (the same subject as in Fig.4). Mono-exponential functions (dark lines) were fit to the actual data (filled circles) obtained every 6 s. The time constants for PCr and ADP recovery were 46.9 and 15.6 s, respectively.

Table 3 Skeletal muscle tissue histochemistry analyses

Characteristic	Mean ± SEM	Range
Type I fibre distribution (%)	35.9 ± 3.4	13.9 to 50.8
Type IIa fibre distribution (%)	43.6 ± 3.6	28.7 to 69.4
Type IIx fibre distribution (%)	20.5 ± 2.6	14.5 to 43.8
Total muscle SDH activity (AU)	53.7 ± 8.5	24.5 to 114.4
SDH activity type I fibres (AU)	29.0 ± 5.2	5.5 to 68.7
SDH activity type IIa fibres (AU)	20.0 ± 3.9	4.8 to 40.4
SDH activity type IIx fibres (AU)	4.8 ± 1.3	0.4 to 13.1

SDH, succinate dehydrogenase activity in arbitrary units as measured by immunohistochemistry

strong and significant correlation with $\dot{V}O_{2peak}$ (Fig. 7 and Table 4) and became somewhat stronger when $\dot{V}O_{2peak}$ was expressed per kg fat free mass (Table 4). A comparison between whole-body $\dot{V}O_{2peak}$ and *in vitro* estimates of mito-chondrial respiratory function showed strong positive correlations for both SDH-activity in the type I muscle fibres (Fig. 8 a, Pearson's R: 0.77, P<0.05) and SDH-activity in the type IIa muscle fibres (Fig. 8 b, Pearson's R: 0.62. P<0.05).

Significant relationships were observed for most of the ^{31}P MRS markers of mitochondrial function when compared with *in vitro* estimates such as type I SDH-activity (Fig. 8 c), total muscle SDH-activity (Fig. 8 d) and percentage of type I muscle fibres (Fig. 8 e). An inverse correlation was observed between ^{31}P MRS parameters and %type IIa fibres (Fig. 8 f). The type I SDH-activity was co-linear with the type I fibre content. Type I fibre content was the best predictor in linear stepwise regression models with ^{31}P MRS estimates set as a dependent variable in the model (Pearson's R: 0.76, P<0.01).

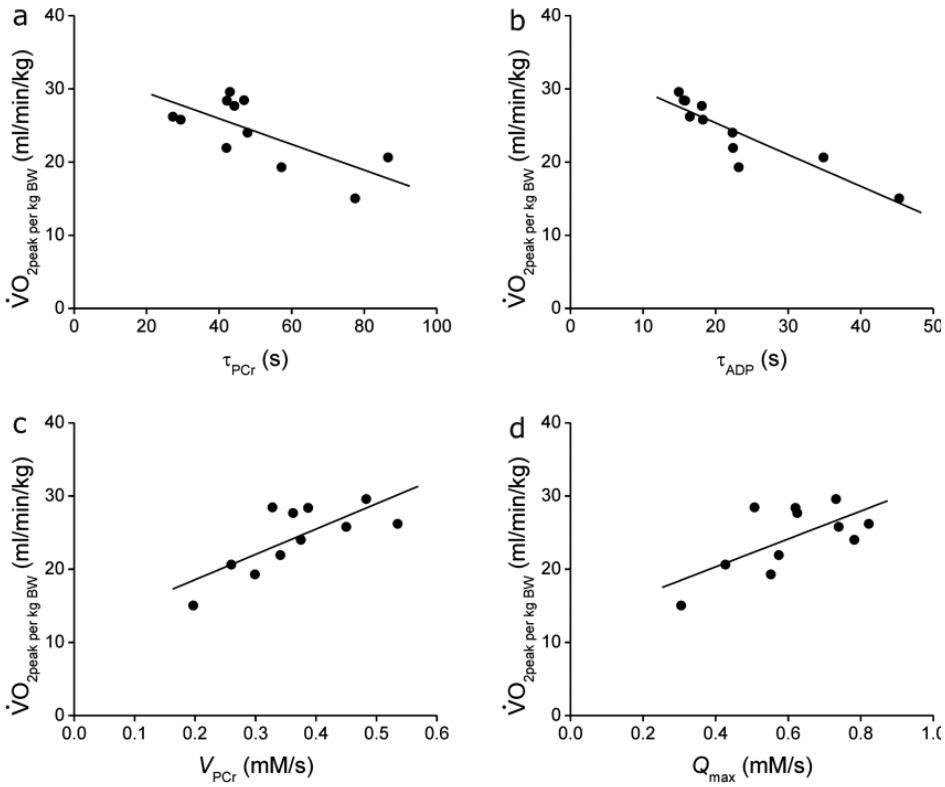

Fig. 7 Relationships between peak whole-body oxygen uptake ($\dot{V}O_{2peak}$ per kg BW) and the ^{31}P MRS parameters of mitochondrial respiratory function (panel a: τ_{PCr} (R=-0.70, P<0.05), panel b: τ_{ADP}, (R=-0.90, P< 0.01), panel c: V_{PCr} (R=0.74, P<0.05) and panel d: Q_{max} (R=0.65, P<0.05).

Table 4 Pearson's correlation matrix among body composition, ^{31}P MRS markers of mitochondrial respiratory function, whole-body oxygen uptake and histochemical muscle fibre analysis

Variable	$\dot{V}O_{2peak\ per\ kg\ BW}$	$\dot{V}O_{2peak\ per\ kg\ FFM}$	SDH Type I	SDH Type IIa	Total SDH	%Type I	%Type IIa
τ_{PCr} (s)	- 0.70*	- 0.78**	- 0.70*	- 0.03	- 0.48	- 0.75**	0.83**
τ_{ADP} (s)	- 0.90**	- 0.91**	- 0.76**	- 0.38	- 0.70*	- 0.46	0.57
V_{PCr} (mM/s)	0.74*	0.76**	0.59	0.18	0.47	0.61*	- 0.58
Q_{max} (mM/s)	0.66*	0.70*	0.51	0.07	0.38	0.64*	- 0.67*
$\dot{V}O_{2peak\ per\ kg\ BW}$	-	-	0.77**	0.62*	0.84**	0.22	- 0.42
$\dot{V}O_{2peak\ per\ kg\ FFM}$	-	-	0.76**	0.47	0.76**	0.35	-0.55

Muscle fibre type composition (%type I, IIa) based on number of fibres

* P<0.05, **P<0.01, all correlations are based on n=11

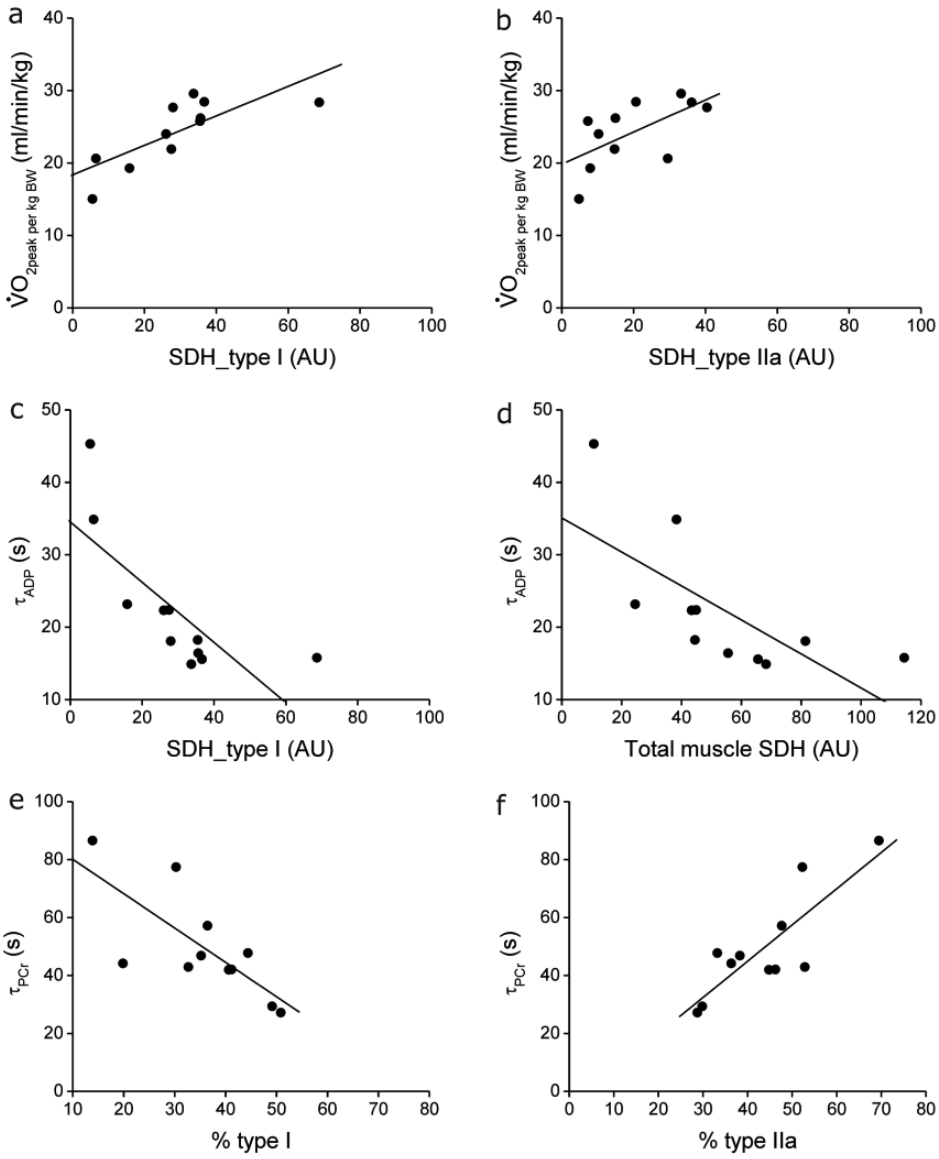

Fig. 8 Relationships between peak whole-body oxygen uptake ($\dot{V}O_{2peak}$) and fibre-type specific mitochondrial enzyme activity (panel a: $\dot{V}O_{2peak}$ vs SDH_type I, (R: 0.77, P<0.01); panel b: $\dot{V}O_{2peak}$ vs SDH type IIa, (R= 0.62, P<0.05)), between ^{31}P MRS recovery kinetics and fibre-type specific mitochondrial enzyme activity: τ_{ADP} vs. SDH_type I (panel c: R= -0.76, P<0.01) and τ_{ADP} vs. total muscle SDH (panel d, R= -0.70 P<0.05). The lower two panels show τ_{PCr} versus percentage of type I fibres (panel e, R=-0.75, P<0.05) and τ_{PCr} versus percentage of type IIa fibres (panel f, R=0.83, P<0.01).

3.4 DISCUSSION

The present study shows that in a group of long-term, insulin-treated, Type 2 diabetes patients commonly used ^{31}P MRSmarkers of *in vivo* mitochondrial respiratory function are strongly correlated with muscle fibre type-specific SDH-activity and type-I muscle fibre content. These data imply that ^{31}P MRS represents an appropriate methodology to study *in vivo* skeletal muscle mitochondrial respiratory function in long-term Type 2 diabetes patients.

The non-invasive quantification of the post-exercise PCr recovery time constant using ^{31}P MRS provides us with an approach that can be used to measure in vivo mitochondrial respiration. However, several studies have shown that cytosolic pH has a strong influence on PCr recovery kinetics [8 21-24]. The exercise protocol in the MR scanner resulted in a small, but significant drop in intracellular pH from 7.07±0.004 at rest to 6.90±0.04 at the end of the exercise ($P<0.01$). However, the variation within this end-exercise pH was relatively small, ruling out end-exercise pH as a major source of variation within τ_{PCr}. Moreover, τ_{PCr} showed good correlations with the type I fibre specific SDH activity as well as with the type I muscle fibre content (Table 4, Fig. 8e). As opposed to the PCr recovery time constant, the ADP recovery time constant has been proposed to be a pH-independent marker of mitochondrial respiratory function [23 43]. Correlations between τ_{ADP} and type I fibre specific and total SDH activity were indeed stronger than for τ_{PCr} (Table 4, Fig. 8c and 8d). The initial PCr recovery rate (V_{PCr}) and maximal aerobic capacity (Q_{max}) have also been shown to be independent of end-exercise pH [23 24]. However, both V_{PCr} and Q_{max} were not significantly correlated with SDH activity. All ^{31}P MRS derived parameters showed good correlations with whole-body oxygen uptake (Table 4, Fig. 7). V_{PCr} is a measure of the actual mitochondrial ATP synthesis rate and therefore does not represent an absolute measure of mitochondrial function. According to the kinetic control model, V_{PCr} has a hyperbolic dependence on the end-exercise ADP concentration (Equation 4). As such, the correlation of V_{PCr} with $\dot{V}O_{2peak}$ can be explained by the small variation in end-exercise [ADP] (46.8±2.8 μM).

Local muscle blood flow [25] and tissue oxygenation [26] can influence local skeletal muscle metabolism and, as such modulate the ^{31}P MRS parameters. Since only a relatively small amount of muscle tissue is recruited in the single leg extension exercise and the exercise intensity is rather low, cardiac output does not reach maximal rates. Therefore, this exercise paradigm allows us to study maximal *in vivo* respiration within the *vastus lateralis* muscle in the absence of any limits set by the potential oxygen supply dictated by a finite cardiac output [44]. However, in long-standing, insulin-treated Type 2 diabetes patients it has been reported that microvascular flow in the *vastus lateralis* muscle is likely impaired during dynamic exercise [28 45]. As such, even in our human dynamic single leg-extensor model, impaired PCr recovery caused by impaired vascular blood flow and oxygen supply or intramuscular oxygen diffusion would represent a potential confounding factor. Since there might be a

rather wide intersubject variability of vascular impairments in long-term Type 2 diabetes patients, we hypothesized that the correlations between ^{31}P MRS and *in vitro* markers of mitochondrial function, as described in healthy populations [15 16], might be weaker or even entirely absent in this population. Instead, the quality of the correlations between *in vivo* and *in vitro* parameters is strikingly similar to that found in healthy subjects. The pathological implications of these findings suggest that impairments of vascular oxygen supply or intramuscular oxygen diffusion were either not present or not severe enough to modify/alter the relationship between *in vivo* and *in vitro* markers of mitochondrial function in these Type 2 diabetes patients. Therefore, PCr recovery kinetics can be used to assess mitochondrial function in long-term Type 2 diabetes patients on exogenous insulin therapy.

It could be argued that the correlations measured between *in vivo* and *in vitro* markers of mitochondrial respiration can only explain ~ 50% of the variance (r^2 ranged from 0.38 to 0.59) of these relationships. This could point towards other mechanisms contributing to the variance of the measurements. As discussed by Larson-Meyer *et al.* [15], maximal enzyme activities under ideal conditions are significantly higher than *in vivo* maximal metabolic flux rates. However, the comparison between *in vivo* and *ex vivo* conditions can be rather complex and e.g. depends on temperature factors that adjust for the in vitro respiratory rates of intact mitochondria [46]. In addition, substrate availability and, as such, allosteric regulation of all enzymes involved is generally different as well. Therefore select marker enzyme activity may not completely reflect metabolic capacity of intact muscle. Besides the conceptual differences between *in vitro* and *in vivo* conditions, the difference in the muscle volume sampled has been suggested as an important factor that explains a large part of the variance [15 16].

We compared the ^{31}P MRS quantitative results with previous studies in healthy, elderly subjects published in the literature. For this purpose, PCr recovery time constants from the literature were recalculated at an end-exercise pH of 6.90 by using a correction factor of -45 s per pH unit [22-24]. For studies in M. gastrocnemius [47], M. tibialis anterior [48], M. vastus lateralis [49] and M. rectus femoris [50], τ_{PCr} ranged between 43 and 53 s, which is well in line with the average τ_{PCr} of 49.4±5.5 s in our long-term Type 2 diabetes patients. Scheuermann-Freestone et al. [47] investigated the calf muscle of Type 2 diabetes patients and age-, sex- and body mass index-matched control subjects using dynamic ^{31}P MRS. The end-exercise energy and pH status was similar to that in our study. However, τ_{PCr} was longer (75 s) and V_{PCr} was lower (0.25 mM/s) in their Type 2 diabetes patients than in the present study. In fact, the results in our Type 2 diabetes patients resemble more closely their findings in healthy controls. Scheuermann-Freestone et al. [47] ascribe the slower PCr recovery in their Type 2 diabetes patients to microvascular disease. We speculated (above) that in our group of Type 2 diabetes patients microvascular blood flow is probably not significantly impaired, which could explain the 'normal' PCr recovery in our patients. However, a direct comparison is not warranted as the two studies are performed in different muscle

groups. It should be noted that even in our homogeneous group of patients large inter-individual differences exist (Table 2). Nevertheless, it is striking to note that most of our long-term diagnosed Type 2 diabetes patients show a rather normal PCr recovery time constant and as such appear to have a rather normal mitochondrial respiratory function. Therefore, our results indicate that some of the earlier described deficits of *in vitro* [4 6] or *in vivo* [2] mitochondrial function parameters in insulin resistant states might in fact be a consequence of subclinical microvascular disease, detectable using near infra red spectroscopy [47]. However, since no carefully matched control group was available for the present experimental set-up, further studies are needed to provide a more definitive answer to this question.

On theoretical grounds there might have been a confounding effect of the blood pressure lowering medication as used by the majority of our patients. It is known that the inhibition of the renin-angiotensin system (RAS) with angiotensin converting enzyme inhibitors (ACEIs) produces vasodilatation and enhance blood flow through the microcirculation of skeletal muscles [51], For angiotensinogen II receptor blockers (ARBs) this effect on skeletal muscle blood flow is still a topic of debate, but rather points towards another insulin mediated mechanism [52]. Although it is likely that RAS inhibition improves tissue oxygenation of hypertensive diabetes patients during resting conditions, a rat study showed that ACE inhibition caused only minor effects on mitochondrial function and was clearly not associated with improved endurance time and maximal oxidative capacity [53]. Besides ACEIs and ARBs 3 subjects were also using β-adrenoceptor antagonists. In rat skeletal muscle it has been shown that acute administration of β-adrenoceptor antagonists affects muscle bioenergetics (lower cyclic-AMP and free ADP concentrations), but does not seem to modulate PCr resynthesis kinetics [54]. Therefore, a confounding influence of blood pressure lowering medication on our *in vivo* or *in vitro* markers for mitochondrial respiration is rather unlikely.

In conclusion, the present study shows moderate to good correlations between *in vivo* and *in vitro* measurements of oxidative capacity in a population of long-standing, insulin-treated Type 2 diabetes patients. Overall, the results are both qualitatively as quantitatively consistent with previous results measured in healthy subjects. As such, the results of the present study suggest that ^{31}P MRS is an appropriate means to assess *in vivo* mitochondrial respiratory capacity in Type 2 diabetes.

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Chapter 4 Influence of acute exercise on hyperglycemia in insulin-treated Type 2 diabetes

ABSTRACT

INTRODUCTION The impact of exercise on blood glucose homeostasis has not been assessed in long-term, insulin-treated, Type 2 diabetes patients. Because of a high level of co-morbidity insulin mediated glucose uptake may be impaired in this subcategory Type 2 diabetes patients.

PURPOSE To study the effects of an acute bout of resistance exercise on the subsequent 24 h blood glucose excursions under free-living conditions in Type 2 diabetes patients on stable exogenous insulin therapy and oral medication.

METHODS Eleven male Type 2 diabetes patients (59±2 yrs) performed an acute bout of exercise. One day prior to the exercise bout, a continuous glucose monitoring system (GlucoDay®, A. Menarini Diagnostics) was inserted subcutaneously in the peri-umbilical region. The glucose sensor measured glucose concentrations in the dialysate over a 48-h period.

RESULTS The prevalence of hyperglycemic excursions was reduced by 39% over a 24 h period (equivalent to 3 h) following an acute bout of exercise ($P<0.05$). Average glucose concentrations 24 h before and after the exercise bout did not differ significantly. Mean glucose concentrations and the prevalence of hyperglycemic periods correlated strongly with baseline blood HbA_{1c} concentration (Pearson's $R=0.69$, $P<0.05$).

CONCLUSION An acute bout of exercise effectively reduces the prevalence of hyperglycemia over a 24 h period under free-living conditions in long-term Type 2 diabetes patients on exogenous insulin therapy.

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4.1 INTRODUCTION

Skeletal muscle tissue accounts for most of the insulin-stimulated glucose disposal in humans. The prolonged application of either endurance or the combination of resistance and endurance exercise training has been shown to improve whole-body glucose tolerance and/or insulin sensitivity in Type 2 diabetes patients [1-3]. However, even an acute bout of endurance [4] or resistance exercise [5] has been shown to improve insulin sensitivity and/or glucose tolerance. These effects have been reported to persist for a period ranging from 2 h [6], 4-6 h [7], 12-16 h [4], 24 h [5] up to 48 h following cessation of exercise [6].

So far, no studies have investigated the effects of an acute bout of exercise in long-standing, insulin-treated Type 2 diabetes patients. The associated co-morbidity, and deconditioned state of both the skeletal muscle and cardiovascular apparatus severely limits the intensity of an endurance or resistance exercise program. Since the population Type 2 diabetes patients on exogenous insulin therapy is growing progressively [8], it is of utmost importance to establish whether exercise can effectively modulate glycemic control and reduce episodes of glucotoxic hyper-glycemia (defined as blood glucose concentrations >10 mmol.L^{-1}) in these patients.

A novel way to evaluate changes in blood glucose concentrations under free-living conditions is provided by the use of continuous subcutaneous glucose-monitoring systems (CGMS). CGMS can be used to obtain continuous information on ambulatory postprandial [9] and/or nocturnal dialysate glucose excursions [10], and have been shown to represent a sensitive tool to study the impact of dietary modulation on 24 h blood glucose profiles [11]. Moreover, glucose tolerance tests are generally not applicable in insulin-treated diabetes patients, which is likely one of the many reasons why intervention studies generally exclude Type 2 diabetes patients on exogenous insulin therapy with evident co-morbidities. As such, CGMS represents a good alternative to the use of glucose tolerance tests as means to monitor short-term changes in glycemic control, without interfering with daily living activities.

In the present study, we applied CGMS to evaluate whether the implementation of a single bout of exercise can improve 24 h glycemic control in patients with long-term, insulin-treated, Type 2 diabetes. Since both resistance [12 13] and intermittent endurance exercise [14] have been shown to improve peripheral insulin sensitivity and/or glucose disposal in Type 2 diabetic subjects, we hypothesized that a single session of a combination of these types of exercise would reduce the presence and/or duration of hyperglycemic dialysate glucose excursions.

4.2 METHODS

Subjects

Eleven male, Type 2 diabetes patients were selected to participate in this study. Subjects had been diagnosed with Type 2 diabetes for over 5 yrs, and had been on exog-

Table 1 Subjects' characteristics

n=11	Mean±SEM	Range
Age (yrs)	59.1 ± 2.3	49 to 68
Body Mass Index (kg m^{-2})		25.5 to 38.7
Body Weight (kg)	97.6 ± 4.9	78.0 to 123.0
FFM (kg)	68.9 ± 2.9	56.3 to 85.2
Waist circumference (cm)	110.6 ± 3.7	94.5 to 131.5
Fat percentage (%)	27.0 ± 0.8	21.2 to 30.7
HbA_{1c} (%)	7.6 ± 0.3	6.3 to 9.5
FPG ($mmol.L^{-1}$)	10.4 ± 0.9	4.0 to 16.4
Adiponectin ($\mu g.L^{-1}$)	5.4 ± 0.8	2.9 to 11.3
NEFA ($\mu mol.L^{-1}$)	459 ± 73	130 to 946
Total Cholesterol/HDL ratio	5.1 ± 0.3	3.9 to 7.3
Triacylglycerol ($mmol.L^{-1}$)	2.3 ± 0.4	0.8 to 3.2
C-peptide ($\mu mol.L^{-1}$)	0.77 ± 0.17	0 to 1.7
Years since diagnosis with Type 2 diabetes	12.1 ± 2.1	6 to 29
Years of exogenous insulin therapy	7.0 ± 2.4	2 to 29
Daily insulin requirements (I.U.)	92.5 ± 11.1	16 to 150
$\dot{V}O_{2peak\ per\ kg\ BW}$ ($ml.min^{-1}.kg^{-1}$)	24.3 ± 1.4	15.0 to 29.6
$\dot{V}O_{2peak\ per\ kg\ FFM}$ ($ml.min^{-1}.kg^{-1}$)	34.2 ± 1.9	21.7 to 40.5
1RM leg press (kg)	153 ± 9	119 to 195
1RM leg press ($kg.BW^{-1}$)	1.60 ± 0.1	1.0 to 2.3
1RM leg extension (kg)	49 ± 3	28 to 74
1RM leg extension ($kg.BW^{-1}$)	0.51 ± 0.04	0.29 to 0.79

BMI, body mass index; FFM, fat free mass; HbA_{1c}, glycosylated hemoglobin; FPG: fasting plasma glucose; NEFA: Non-esterified fatty acids ($\dot{V}O_{2peak}$) oxygen uptake per kg bodyweight / fat free mass; 1RM; 1 repetition maximum

enous insulin treatment for 7.0±2.4 yrs. They had no history of participating in any regular exercise program for over 10 yrs. All subjects had been on a stable regimen of diabetes medication for at least 3 months before being recruited. Seven subjects were using short (Novorapid®, n=6) or rapid (Humulin®, n=1) acting insulin before each meal (n=7) either in combination with NPH insulin (Insulatard®, n=5), premixed biphasic isophane insulin (Mixtard 30/70® in combination with metformin, n=1), or a very long-acting insulin analogue (insulin glargine, n=1), administered before bedtime. Three subjects were using premixed biphasic isophane insulin twice a day (Mixtard 30/70®, n=3) in combination with metformin. One subject used NPH insulin (Humulin NPH®, once a day before breakfast in combination with metformin and a sulfonylurea (glimepiride). Subjects were explicitly instructed to keep oral medication and exogenous insulin schemes constant throughout the entire study period. Patients using thiazolidinediones and/or β-blockers for less than 6 months, and subjects with impaired liver function, renal failure, severe retinopathy or a history of severe cardiovascular problems were excluded from participation. Subjects' characteristics are shown in Table 1. The nature and the risks of the experimental

procedures were explained to the subjects and all gave their written informed consent to participate in the study, which was approved by the local Medical Ethical Committee of the Máxima Medical Center, Veldhoven, The Netherlands.

Body composition

Body weight and waist circumference were measured using an analog weight scale and standard measuring tape, respectively. Segmental and whole-body bone mass and fat free mass (FFM) were determined using whole-body DEXA (Hologic QDR-4500 Discovery A, software version 12.3:3, Hologic Inc. Bedford, MA, USA).

Peak whole-body oxygen uptake capacity

Peak whole-body oxygen uptake capacity ($\dot{V}O_{2peak}$) and maximal workload capacity (W_{max}) were measured during an incremental exhaustive exercise test until volitional exhaustion, performed on a cycle ergometer (Medifit Ergometer, Medifit systems, Maarn, The Netherlands) using a ramp protocol [15]. Gas exchange measurements were performed continuously (Ergostar, PMS Professional Medical Systems, Basel, Switzerland). Cardiac function was monitored using a 12-lead electrocardiogram with heart rate (HR) being recorded continuously (Polar Electro, Kempele, Finland) and sampled at 1 kHz through a data log device (Co2ntrol™, Tildesign, Zeewolde, The Netherlands).

Strength testing

At least one wk before the experimental exercise session, subjects participated in 2 exercise trials to become familiarized with the exercise protocol and the equipment. Proper technique was demonstrated and practiced for each of the 3 lower-limb exercises (leg press, leg extension and lunges) and for the 3 upper-body exercises (vertical traction, vertical row, upright row). Maximum strength was estimated using the multiple repetitions testing procedure and at least 1 wk before the experimental trial, subjects' 1 repetition maximum (1RM) was determined. After warming up, the load was set at 90-95% of the estimated 1RM, and increased after each successful lift until failure. A 5 min resting period between subsequent attempts was allowed. A repetition was valid if the subject was able to complete the entire lift in a controlled manner without physical assistance.

EXPERIMENTAL TRIALS

Standardization of diet and activity prior to and post exercise

After the strength testing, subjects received instructions regarding the use of food intake and physical activity diaries. Subjects were asked to maintain a stable diet, constant medication schemes, and to record their food intake during 3 days starting one day before the CGMS device was attached. Immediate after the 3-day monitoring

period a clinical dietician analysed and discussed the food records together with the subjects in order to obtain a good reliability of the food intake. Intake, doses and timing of oral blood glucose lowering medication and exogenous daily insulin therapy was kept constant throughout the study. Total daily exogenous insulin doses averaged 1.0±0.1 I.U. $kg^{-1}.day^{-1}$. Energy intake averaged 77.7±5.7 kJ $kg^{-1}.day^{-1}$ (with 75.1±6.3, 77.9±8.3 and 80.0±6.0 $kJ.kg^{-1}.day^{-1}$ on Day 0, 1, and 2, respectively. Macronutrient composition of the diet averaged 18.9±1.1 Energy% (En%) protein, 42.7±1.4 En% fat and 38.4±1.4 En% carbohydrate on respectively Day 0, 1, and 2. Energy intake and meal composition did not differ between days, even though subjects were free to increase or decrease portion size of all meals, drinks and snacks. All subjects were instructed to refrain from any sort of heavy physical labour or exercise during the entire period except for the exercise session. -According to our protocol patients were explicitly instructed not to change their exogenous insulin doses over a 3 day period to exclude that this factor would interfere with the glycemic control. As such, Day 1 can be regarded as the best non-exercising control condition possible in this specific population. Energy expenditure during the exercise bout was not assessed in this study. Based on indirect calorimetry measurements performed during circuit resistance training both in older [16] and in younger [17] adults, we estimate that energy expenditure ranged between 11.5 and 15.7 ml O_2. min^{-1} in the performed exercise regimen. This corresponds to 3.3 to 4.3 metabolic equivalents (METs). However, since our subjects were more obese than the subjects in the above-mentioned studies the intensity will be more towards the 4 METs. As such, our acute bout of exercise can be considered to be of moderate intensity [18]. The caloric expenditure during such exercise would be equivalent to 10-15% of total energy intake per day in our subjects.

CGMS measurements

On the first day subjects reported to our laboratory at 09.30 in the morning and received a short training in the use of the capillary blood sampling method (Glucocard Memory PC, A. Menarini Diagnostics, Firenze, Italy) used for the calibration of the CGMS. All subjects were instructed to measure capillary blood glucose concentrations before every meal. After the subjects were fully instructed, a microdialysis fibre (Medica, Medolla, Italy) with an internal diameter of 0.17 mm and a cut-off weight of 18 kD was inserted in the peri-umbilical region, without anesthesia, using an 18-gauge Teflon catheter as a guide, as described previously [19]. For the measurements the micro-fibre was then connected to a portable CGMS (GlucoDay®*S*, A. Menarini Diagnostics, Firenze, Italy), which consists of a peristaltic pump that pumps Dulbecco's solution at 10 µL min^{-1} through the microdialysis fibre. A detailed description of the device has been described earlier [20]. In brief, the subcutaneous interstitial fluid is taken up by the microdialysis fibre and is transported to the measuring cell. The glucose sensor, consisting of immobilized glucose oxidase, measures the glucose concentration every sec and stores an average value every 3 min for a total measuring

time of 48 h. After analysis, the dialysate is then pumped from the glucose sensor into a waste-bag. The lag time between subcutaneous glucose values and venous plasma glucose concentration has been estimated to vary in vivo between less than 3 to 7 min [20 21]. Interstitial measured glucose was calibrated using 1 capillary and 1 venous blood sample obtained at least 12 h apart from each other. The venous blood sample was obtained immediate prior to the acute exercise bout. Interstitial measured glucose has been shown to correlate well with venous blood glucose levels [20], and show a clinical accuracy of 100 and 97.8 % in the normo- and hyperglycemia range, respectively [21]. However, the clinical accuracy in the hypoglycemic range (blood glucose < 3.9 mmol.L^{-1}) drops to 57-60% [21], therefore individual sensor values in the low range should be interpreted with some cautiousness. The entire device, including the perfusion solution and the waste-bag, weighs about 250 g and is worn in a pouch under the subjects' clothes. After the CGMS was checked for proper function, subjects were allowed to return home and resume all their normal activities.

Exercise protocol

The day after the CGMS device was attached, subjects reported at the hospital following transport by car or public transportation at 11:00 am. Subjects performed a general warm-up procedure of 5 min cycling on a bicycle ergometer at 40% of their individual W_{max}, followed by 2 sets of 10 repetitions on 3 resistance exercise machines targeting the upper-body (vertical traction, vertical row, upright row as well as 2 sets of floor exercises (push up and abdominal crunch) and 2 sets of 20 alternate left/right lunges without additional weight. The latter were included to provide a whole-body warm-up and to reduce the risk of injury. Thereafter, the resistance exercise session targeted at the legs, with 2 sets of 10 repetitions on the horizontal leg press machine (Life Fitness (Atlantic) BV, Barendrecht, The Netherlands) and 2 sets of 10 repetitions on the leg extension machine (Life Fitness) with ~2 min rest intervals between sets. All exercises were performed at 50% of the subjects' individual 1RM, and averaged 148±6 and 81±3 kg for the leg press and leg extension, respectively.

The highly deconditioned status of long term Type 2 diabetes patients [22] complicates the use of sufficiently intense endurance exercise that produces a proper metabolic response. Therefore, the resistance exercise was followed by 4 bouts of 30 s high intensity interval exercise on a bicycle ergometer, alternated with 60 s of 15 W recovery, aimed to stress the working leg muscles, without overloading the cardiovascular system [23]. Work rate for the interval modes was 50% (30/60 s) of the maximum achieved W_{max} during a steep ramp test (increments of 25 W per 10 s, as desribed by Meyer et al. [23], corresponding to 137±9 W. The total training regimen required ~45 min to complete. The form and intensity of the different exercises was chosen to recruit sufficient muscle mass without causing delayed onset muscle soreness (DOMS) or feelings of dyspnoea in this rather deconditioned group of diabetes patients. All subjects were verbally encouraged during the test to complete the entire protocol.

Analyses

On a separate occasion, 2 weeks before the experiment, after a fasting period of 10 h following a standardized meal 35.2±1.8 kJ kg BW^{-1}, containing 53 energy% (En%) fat, 10 En% protein, and 37 En% carbohydrate), fasting blood samples (4 ml) were collected in tubes containing a glycolytic inhibitor (sodium fluoride) and anticoagulant (potassium oxalate), immediately centrifuged at 1000·*g* and 4°C for 10 min, after which aliquots of plasma were frozen immediately in liquid nitrogen and stored at -80°C until analyses. Plasma glucose (Glucose 125 Hexokinase kit, ABX Diagnostisc, Montpellier, France), serum cholesterol (CHOD-PAP, ABX Diagnostics), HDL-cholesterol (543004, Roche Diagnostics, Basel, Switzerland), non-esterified fatty acids (NEFA) (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany) and triacylglycerol (GPO-Tinder 337B: Sigma Diagnostics, St Louis, MO) concentrations were analyzed with the COBAS FARA semi-automatic analyzer (Roche). To determine basal fasting blood HbA_{1c} content a 3 ml blood sample was collected in EDTA containing tubes and analyzed by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany). The serum concentration of adiponectin was quantified using a commercially available Human Adiponectin ELISA (#HADP-61K, Linco Research Inc. St. Charles, MO). C-peptide was analysed through a electrochemiluminiscent immunoassay (Nr 03184897, Elecsys Module, Roche GmbH, Mannheim).

CGMS parameters

To quantify and compare the CGMS glucose excursions 24 h before and after the exercise bout, mean dialysate glucose and the amount of time during which glucose concentrations reside at a level above 10.0 $mmol.L^{-1}$ or below 3.9 $mmol.L^{-1}$ were calc6ulated. Except for the calibration values, all other capillary blood glucose measurements performed by our subjects were used to calculate the coefficient of variation (CV) of the CGMS data.

To assess intra-day glycemic variability pre- and post-exercise, continuous overall net glycemic action (CONGA), a novel method recently described by McDonnell *et al*, was used [24]. CONGA*n* has been defined as the standard deviation of the differences in dialysate glucose concentration using varying time differences of *n* hours. We used CONGA1, CONGA2 and CONGA4, indicating intra-day glycemic variability based on 1 h, 2 h and 4 h time differences, respectively. In normal non-diabetic subjects CONGA values vary between 0.4 and 1.2, while values above 1.5 indicate glycemic lability [24].

Statistics

All data are expressed as means±SEM. Repeated measures ANOVA was used to compare food intake on the 3 consecutive days. Student's t-test was applied to the CGMS parameters determined over the 24 h periods before and after the bout of exercise.

Relationships between CGMS parameters and HbA_{1c} were calculated using Pearson's correlation analyses. Statistical significance was set at $P<0.05$.

4.3 RESULTS

CGMS measurements

Mean continuous dialysate glucose concentrations before and after exercise are illustrated in Fig. 1. During exercise dialysate glucose levels declined and remained lower for ~3 h following cessation of exercise (Fig. 2, panel B). Average 24 h dialysate glucose concentrations before and after the exercise bout did not differ significantly ($P=0.26$). The duration of hyperglycemic dialysate glucose excursions (>10.0 mmol.L^{-1}) averaged 31.7±6.0 % of the day. The latter is equivalent to 7.6±1.4 h per day. The duration of hyperglycemia (>10 mmol.L^{-1}) was significantly lower on Day 2 when compared to Day 1 (4.6±1.1 vs 7.6±1.4 h, respectively; $P<0.05$, Fig. 3). Mild hypoglycemia occurred in only incidentally in 6 out 11 subjects throughout the 48 hours. The average amount of hypoglycemic episodes, here defined as blood glucose concentrations below 3.9 mmol.L^{-1}, remained unchanged and averaged 51±19 and 36±16 min on Day 1 and 2, respectively. Total additive amount of hypoglycemia did only exceed 100 min per 24 h in 2 of these 6 subjects, explaining why average glucose curves in Fig. 2 does not drop below 3.9 mmol.L^{-1}. The CONGA*n* values, which can be regarded as a measure of the variability of the circulating dialysate glucose concentrations during the day, were not different between Days 1 and 2 ($P=0.34$-0.61, Table 2).

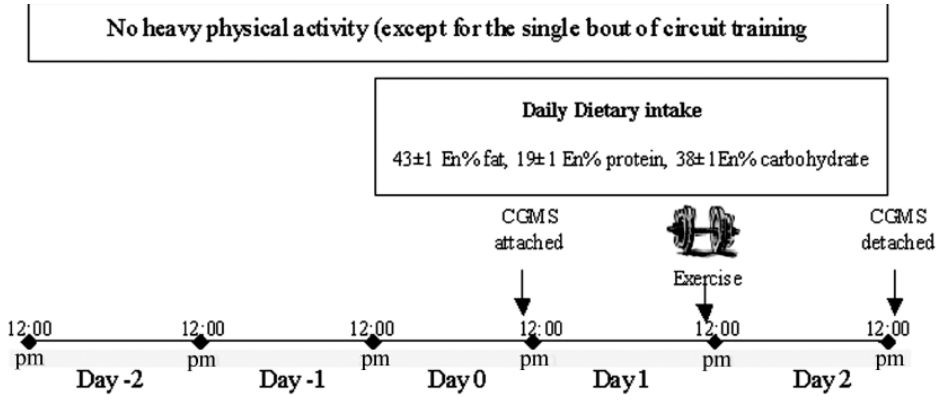

Fig. 1. Overview of the study design. All subjects followed the same dietary pattern and exogenous insulin therapy during the entire study period; prior to the attachment of the CGMS, on the control day (Day 1) and following the exercise bout (Day 2) which was performed at 11:00 am in the morning. All subjects were instructed to refrain from any sort of heavy physical labour and/or exercise during the entire period (5 days) except for the exercise session.

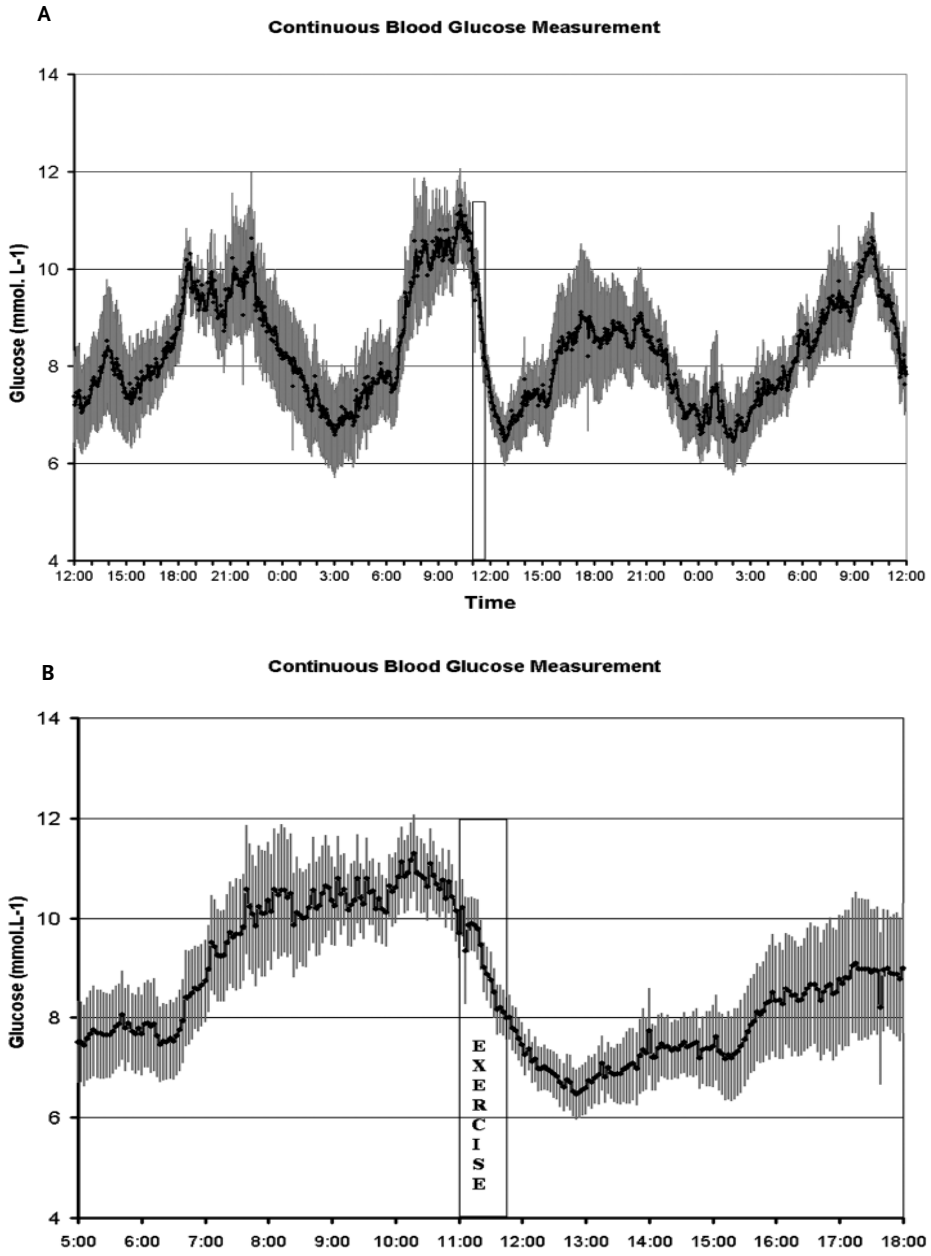

Fig. 2. Panel A: Mean±SEM glucose concentrations over respectively 48 h using CGMS. The SEM is indicated by the grey bars. The vertical open bar indicates the time that subjects were performing the 45 min circuit training exercise. Panel B is similar to panel A, but shows the glucose concentrations 6 h before and after the exercise bout in more detail.

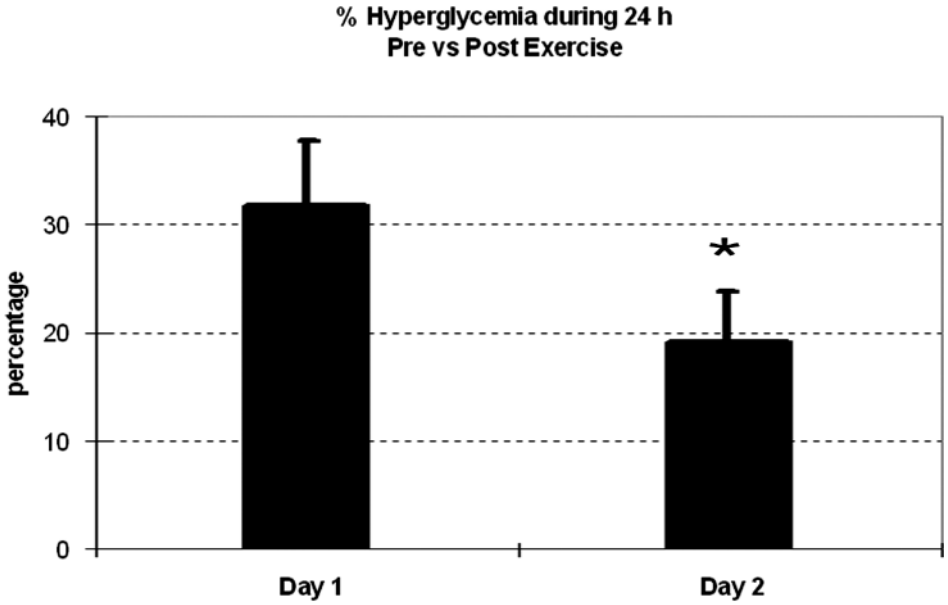

*Fig. 3. The duration of hyperglycemia, (i.e. percentage of time [glucose] above 10.0 mmol.L^{-1}, 24 h before and after 45 min of circuit training. Values are expressed as means±SEM. *: significantly different from values observed on Day 1 (pre-exercise) (P<0.05).*

CGMS measures vs blood HbA_{1c} concentration

Mean dialysate glucose concentrations over 48 h as well as the prevalence of hyperglycemic periods over 48 h both correlated significantly with HbA_{1c} concentration, measured one week before the CGMS measurements (Pearson's R=0.69, P<0.05). Mean CONGA1, 2 and 4 values over 48 h did not show a significant correlation with HbA_{1c} (Pearson's R= 0.46 - 0.51, P>0.05).

4.4 DISCUSSION

In the present study, we show that an acute bout of moderate intensity exercise significantly modulates dialysate glucose concentrations over a 24 h period under free-living conditions in long-term, insulin-treated, Type 2 diabetes patients. An acute bout of exercise reduces the total additive duration of hyperglycemic episodes within a time frame of 24 h after cessation of exercise in these patients. The latter finding is of clinical relevance, as it shows that daily exercise represents an effective strategy to modulate 24 h glucose homeostasis in this subgroup of Type 2 diabetes patients.

Both endurance and strength exercise have been shown to improve blood glucose homeostasis in uncomplicated Type 2 diabetes patients [2]. The improvement in glucose disposal capacity following resistance and/or endurance exercise is attribut-

Table 2 CGMS measurements over 24 hours before and after an acute bout of exercise

n=11	Day 1 (Pre)	Day 2 (Post)
24 h analysis		
Mean 24h glucose (mmol.L^{-1})	8.5 ± 0.4	8.1 ± 0.4
CONGA1	2.0 ± 0.2	1.8 ± 0.2
CONGA2	2.6 ± 0.3	2.4 ± 0.3
CONGA4	3.3 ± 0.4	3.1 ± 0.5
Hyperglycemic episodes (h)	7.6 ± 1.4	4.6 ± 1.1*
Hypoglycemic episodes (h)	0.9 ± 0.3	0.6 ± 0.3

The acute exercise bout was performed during the last hour of Day 1. Data presented are means±SEM.; * significant difference, P<0.05, Student's t-test; CONGA1,2,4: continuous overall net glycemic action describing intra-day glycemic variability between respectively 1, 2 and 4 h time periods over 24 h [24]; Hyperglycemic episodes, total time during which [glucose] levels are above 10.0 mmol L^{-1}; Hypoglycemic episodes, total time during which [glucose] levels are below 3.9 mmol L^{-1};

ed to improved insulin signaling downstream of the insulin receptor, resulting in increased GLUT4 translocation [13]. In addition, more long-term resistance exercise training increases skeletal muscle mass [25]. As skeletal muscle tissue accounts for >75% of the insulin-stimulated whole-body glucose disposal, a greater lean body mass will also augment total blood glucose disposal capacity. As such, both strength and endurance exercise can substantially improve glucose tolerance and/or insulin sensitivity. In the present study, we implemented both types of exercise in a single exercise session. We investigated the beneficial effect of such an acute bout of exercise in insulin-treated, long-term diagnosed Type 2 diabetes patients. Information about the benefits of exercise in insulin-treated, long-term diagnosed Type 2 diabetes patients is generally lacking in the literature. Because of the progressive nature of the disease, these patients often have a complex spectrum of cardiovascular, neuromuscular and metabolic disorders. Their vulnerable health status, in combination with methodological difficulties, has withheld many scientists to investigate the therapeutic options of exercise in this subpopulation of Type 2 diabetes patients. To obtain more insight whether this subpopulation of Type 2 diabetes patients is still responsive to a moderate intensity exercise program, we assessed the effects of acute exercise on 24 h glucose homeostasis under free-living conditions. The exercise bout reduced the duration of periods of hyperglycemia by almost 40% over the subsequent 24 h. As such, the subjects experienced hyperglycemia (>10 mmol.L^{-1}) during 4.6±1.1 h instead of 7.6±1.4 h, as was observed in the 24 h period prior to the exercise session. This modulating effect is comparable to the reduction in postprandial hyperglycemia reported after energy intake restriction [26] or following administration of an insulinotropic agent [27].

In a recent study we [28] **(see Chapter 2)** and others [29] have shown that in non-insulin dependent Type 2 diabetes patients post-prandial hyperglycemia is most pronounced 1.5-2 h following breakfast. In non-insulin dependent Type 2 diabetes patients blood glucose values reached their maximum between 10:00-10:30 h and

subsequently showed a decline that lasted until lunch time (12:00-12:30 h) [28]. Interestingly, in our insulin-treated Type 2 diabetes patients glucose values show a rather similar pattern around this time of the day. For logistic reasons our 45 min exercise bout had to be scheduled between 11:00 and 12:00 h, which coincided with the down sloping part of the glucose curve. Since in Fig. 2 a similar down sloping can be observed on Day 2 this raised the question to what extent our exercise bout was responsible for the decline in glucose. As such, both on Day 1 and Day 2 a linear trend line analysis, based on a least square fit procedure, was performed for this part of the individual glucose curves. This post-hoc analysis showed that the average slope of the glucose curve from 10:00-12:00 was significantly steeper on Day 1 than on Day 2 (-1.9±0.3 vs. -1.1±0.3 $mmol.L^{-1}h^{-1}$, respectively, t-test, $P<0.05$), indicating that the acute exercise bout indeed contributed to a faster reduction of the post-prandial hyperglycemia following breakfast. Since hyperglycemia is directly related to the formation of advanced glycation end-products and the genesis of microvascular disease [30], our data show that even in this high-risk, insulin-treated, Type 2 diabetes population, exercise intervention represents an effective means to improve blood glucose homeostasis. Therefore, daily moderate intensity exercise can be applied to reduce glucotoxicity and subsequently prevent Type 2 diabetic complications, also in this group of patients.

The use of CGMS provides a safe and effective means to assess the modulating effects of acute exercise on blood glucose excursions under normal dietary conditions. Despite the fact that the deconditioned status of our patients forced us to apply only moderate-intensity exercise, our data show that even such marginal exercise intensities are sufficient to have a clinically relevant effect on blood glucose homeostasis. The latter is of great relevance to the design of exercise intervention programs for this category of Type 2 diabetes patients with co-morbidities, who often suffer from severe muscle deconditioning. In this population only moderate-impact anaerobic and interval type of exercise can be implemented to minimize muscle soreness and feelings of dyspnoea. In this respect, the measurement of cortisol and blood lactate levels following primarily anaerobic type of exercise would have been interesting in relation to 24 h glucose homeostasis. However, for a correct interpretation of these values baseline measurements on Day 1 (at exactly the same time as on Day 2) would have been necessary. Since, the main focus of this study was to measure the effect of a well defined but practically feasible exercise program on 24 h glucose homeostasis during free-living conditions, it was felt that additional blood samples to measure cortisol or blood lactate throughout the CGMS monitoring period would interfere too much with normal free-living or exercise conditions.

It should be noted that a large inter-subject variation was observed in the blood glucose response immediately preceding the bout of exercise and 3-4 hrs after exercise, as displayed in Fig. 2a and Fig. 2b. In fact, only in 7 out of 11 subjects postprandial hyperglycemia was prominent after breakfast, while in 4 other subjects post-

prandial glucose values stayed well below 10.0 mmol.L^{-1}. Moreover, 3 to 4 hrs following the acute bout of exercise in 2 subjects' blood glucose values approached the hypoglycemic range, while all other subjects remained normoglycemic. On a 24 h scale the variable response to exercise is also marked by the fact that in 7 out of 11 patients, CONGA*n*-values and the amount of hyperglycemic episodes over 24 h were clearly decreased, in 2 patients increased and in 2 others practically unchanged. This variability in glucose or insulin sensitivity response to an acute bout of resistance exercise is a phenomenon that has already been described in both healthy and insulin resistant subjects [31 32].

As described before [20], CGMS measurements have been reported to show a bias between -2.0 and 11.2% when compared to venous blood glucose measurements. In this study the mean CV between CGMS and 94 randomly taken capillary blood glucose measurements was 6.0%. In accordance, Bland-Altman plots and Clark's grid error analyses of CGMS using micro-dialysis bio-sensor technique have shown that CGMS measurements provide reliable information on glucose excursions in insulin resistant populations [20 21]. Clinical accuracy of the microdialysis sensor readings have been shown to be good to excellent in the normo- and hyperglycemic range, while in the hypoglycemic range the accuracy is still a matter of concern [21]. Nevertheless, since the total amount of hypoglycemic episodes was very little (3.0±1.2 %) and not different between Day 1 and Day 2, this type of error has minimal to no influence on our main findings.

In the present study we observed a strong and positive correlation (R=0.69) between the duration of hyperglycemia during the total 48 h period and blood HbA_{1c} concentration. Despite the fact that HbA_{1c} corresponds to a weighted 1-3 months average [33], the latter suggests that ~50% of the variation in blood HbA_{1c} content in this population of Type 2 diabetes patients is attributable to the hyperglycemic periods as measured by CGMS during free-living conditions. However, the measurement of prospective changes in blood HbA_{1c} content only has sufficient sensitivity to detect changes in glucose homeostasis or insulin sensitivity during middle to long-term interventions [34]. Therefore, the present study underlines the notion that CGMS is a promising tool when evaluating short-term (<3 months) changes in glucose homeostasis following pharmacological, dietary and/or exercise interventions [20].

In conclusion, an acute bout of exercise substantially reduces the duration of hyperglycemic episodes .in long-term, insulin-treated, Type 2 diabetes patients. Therefore, daily, moderate intensity exercise, represents a valuable adjunct to the therapeutical arsenal to improve glycemic control in a subpopulation of Type 2 diabetes patients that has been proven difficult to manage.

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Chapter 5 Long-standing, insulin-treated Type 2 diabetes patients with complications respond well to short-term resistance and interval exercise training

ABSTRACT

OBJECTIVE: To determine the feasibility and benefits of combined resistance and interval exercise training on phenotype characteristics and skeletal muscle function in deconditioned, Type 2 diabetes patients with diabetic polyneuropathy.

METHODS: Eleven male Type 2 diabetes patients (age: 59.1±7.5 y (mean±SD); BMI: 32.2±4.0 $kg.m^{-2}$) performed progressive resistance and interval exercise 3 times a wk for 10 wks. Besides primary diabetes outcome measures, daily exogenous insulin requirements, muscle strength, maximal workload capacity, whole-body and muscle oxidative capacity, intramuscular lipid and glycogen storage, and markers for systemic inflammation were determined before and after training. Daily exogenous insulin requirements and historic individualized insulin requirements were gathered and analysed.

RESULTS: Muscle strength and maximum workload capacity increased with 17% (90% confidence limits 9 to 24%) and 14% (6 to 21), respectively. Furthermore, mean arterial blood pressure declined with 5.5 mmHg (-9.7 to -1.4). Exogenous insulin requirements dropped with 5.0 $I.U.d^{-1}$ (-11.5 to 1.5) compared to baseline. A decline of respectively -0.7 $mmol.L^{-1}$ (-2.9 to 1.5) and -147 $\mu mol.L^{-1}$ (-296 to 2) in FPG and NEFA concentrations were observed following the intervention, but these were not accompanied by changes in oxidative capacity, intramyocellular lipid or glycogen content, blood HbA_{1c}, adiponectin, TNF-α and/or cholesterol concentrations.

CONCLUSION Short-term resistance and interval exercise training is feasible in deconditioned, Type 2 diabetes patients with polyneuropathy and is accompanied by moderate improvements in muscle function and blood pressure regulation. Such a specific exercise regimen may provide a better framework for future generic training programs in the treatment of deconditioned Type 2 diabetes patients.

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5.1 INTRODUCTION

Physical exercise has long been recognized as an effective interventional strategy in the treatment of Type 2 diabetes. The prolonged application of either endurance or the combination of resistance and endurance type exercise training has been shown to increase whole-body glucose tolerance and/or insulin sensitivity [1-3], and improve cardiovascular risk profile [1,3,4] in Type 2 diabetes patients. However, studies assessing the effects of exercise training in long-term, insulin-treated, Type 2 diabetes patients with complications are generally lacking. The latter is partly due to the many difficulties when trying to define an appropriate exercise program for these patients, who generally suffer from substantial weight gain [5], exercise intolerance, and diabetic polyneuropathy [6-8]. The level of diabetic polyneuropathy also appears to be associated with general muscle weakness [9,10], impaired physical performance [11], poor glycemic control [12] and a high cardiovascular risk profile [13]. As clinical evidence for the health benefits of exercise intervention is quite scarce in this diabetes subpopulation, many patients are generally not advised to participate in intense endurance exercise intervention programs.

It has been reported that the adaptive response to exercise training is strongly determined by the presence of clinical signs of (autonomic) neuropathy and/or cardiorespiratory deconditioning [14]. Therefore, the presence of co-morbidities should be taken into account when tailoring an exercise program for long-standing, insulin-treated Type 2 diabetes patients. To compensate for neuropathy-related muscle weakness and cardiorespiratory fitness levels, it would be advisable to focus on improving muscle strength [15].

Exercise intervention studies in chronic heart failure patients have taught us that short bouts of high intensity interval training (HIT) represents a safe and effective type of exercise regimen that may increase maximal workload and peak whole-body oxygen uptake ($\dot{V}O_{2peak}$) in deconditioned subjects [16,17]. Since exercise intensity and subsequent muscle fibre-type recruitment patterns during resistance exercise and HIT are rather similar. HIT on top of resistance exercise could represent an attractive style of exercise training in deconditioned Type 2 diabetes patients. By directly transferring gains in muscle strength into more functional movements, performance capacity for endurance type of activities may also show greater long-term benefits. As the population long-term diagnosed Type 2 diabetes patients on exogenous insulin therapy is vastly expanding [18], it is of clinical importance to establish whether the combined application of resistance type exercise and HIT represents a feasible training method in deconditoned Type 2 diabetes patients with polyneuropathy.

Exercise interventions generally aim to maximize the skeletal muscle adaptive response. The latter is based on the fact that skeletal muscle tissue is responsible for ~80% of whole-body blood glucose disposal [19]. Previous studies have shown that changes in muscle fibre type composition [20-23], muscle oxidative capacity [24], and intramyocellular lipid [25,26] and/or glycogen [25] content are associated with the develop-

ment of skeletal muscle insulin resistance. Although insulin sensitivity has been reported to improve following resistance [27-29] as well as endurance [2 30-32] type exercise training, the concomitant structural changes in resting intramyocellular lipid and/or glycogen content following exercise interventions in Type 2 diabetes patients remain controversial [33-38].

Over the past couple of years, it has been suggested that in the context of cardiorespiratory deconditioning [39] and ectopic fat accumulation [33 40 41-43], chronic low-grade inflammation plays an important role in the development of microvascular complications in the insulin resistant state [44 45]. Recent studies indicate that lifestyle interventions modulate circulating adipokines levels and reduce the level of systemic inflammation [46], thereby improving whole-body insulin sensitivity [47]. As we aim to investigate both the feasibility and impact of exercise training in long-standing type 2 diabetes patients with polyneuropathy, it would be appropriate to also assess various markers relevant to the inflammatory state (hsCRP, TNF-α , IL-6, adiponectin) before and after exercise intervention.

The present study aims to define the feasiblity and clinical benefits of 10 weeks of resistance and interval type exercise training in long-standing, insulin-treated Type 2 diabetes patients with diabetic polyneuropathy. Furthermore, this study aims to obtain more insight into the structural and metabolic changes that are associated with the skeletal muscle adaptive response to exercise training in these patients.

5.2 METHODS

Subjects

Eleven male, Type 2 diabetes patients were selected from an outpatient clinic to participate in this hospital based case-control intervention study. Subjects had been diagnosed with Type 2 diabetes for over 12.1±7.0 y, and had been on exogenous insulin treatment for 7.0±8.0 y. They had no history of participating in any regular exercise program for at least 10 y. All subjects had been on a stable regimen of diabetes medication for at least 3 months before being recruited. Patients using thiazolidinediones and/or ß-blockers shorter than 6 months, and subjects with impaired liver function (serum-aspartate amino transferase and/or gamma-glutamyltransferase >2 times standard value), macroalbuminuria, severe retinopathy or a history of severe cardiovascular problems were excluded from participation. Furthermore, patients were required to show clinical signs of diabetic polyneuropathy, which was initially determined through history taking and by quantitative sensory testing using a 10-g Semmes-Weinstein monofilament. Since diabetic polyneuropathy was one of our main inclusion criteria, a complete electrodiagnostic evaluation of 4 motor (peroneal, tibial, median, and ulnar) and 3 sensory (sural, median, and ulnar) nerves using electromyography (EMG) was performed by an independent clinical neurophysiologist. All conduction velocity and distal amplitude values for the nerve conduction studies (NCS) were given

a score of 0 for normal and 1 for abnormal [48]. The maximum NCS score if all parameters were abnormal was 28 points (16 motor and 12 sensory). The total NCS score was defined as the sum of the number of abnormal values and is considered abnormal if higher than three [48]. In accordance, an NCS-score of 4 or higher was a prerequisite for inclusion in the study.

Table 1 Univariate analyses of subjects' characteristics at baseline and change following 10 wks of exercise training

n=11	Baseline	Change	90% CI	P-value
Age (y)	59.1 ± 7.5	-		
Years since diagnosis T2D	12.1 ± 7.0	-		
NCS-score (AU)	15.8 ± 6.3	-		
Years of exogenous insulin therapy	7.0 ± 8.0	-		
Daily insulin requirements (I.U.)	92.5 ± 37.0	-5.0	-11.5 to 1.5	0.196
Body Mass Index ($kg.m^{-2}$)	32.2 ± 4.0	0.0	-0.2 to 0.2	0.870
Body Weight (kg)	97.6 ± 16.1	-0.1	-0.8 to 0.5	0.799
Waist circumference (cm)	112.6 ± 12.1	-1.1	-2.8 to 0.7	0.286
FFM (kg)	68.9 ± 9.6	-		
Fat percentage (%)	27.0 ± 2.8	-		
SBP (mmHg)	**147.4 ± 12.3**	**-7.6**	**-15.2 to 0.1**	**0.098**
MAP (mmHg)	**105.7 ± 7.3**	**-5.5***	**-9.7 to -1.4**	**0.036**
DBP (mmHg)	82.5 ± 7.1	-2.2	-7.2 to 2.8	0.446
Wmax RAMP test (W)	**152 ± 39**	**21‡**	**10 to 32**	**0.006**
$\dot{V}O_{per\ kg\ BW}$ ($ml.min^{-1}.kg^{-1}$)	24.3 ± 1.4	0.9	-0.2 to 2.1	0.171
%pred $\dot{V}O_{2peak}$	79.2 ± 15.1	3.1	-0.4 to 6.7	0.138
W_{max} Steep RAMP (W)	**275 ± 62**	**41‡**	**27 to 56**	**0.000**
1RM strength LowerB (kg)	**100.6 ± 23.5**	**18.0 †**	**8.9 to 27.1**	**0.005**
1RM strength UpperB (kg)	**60.9 ± 7.3**	**9.8***	**3.2 to 16.4**	**0.023**
C-peptide ($nmol.L^{-1}$)	0.77 ± 0.56	0.07	-0.12 to 0.26	0.520
HbA_{1c} (%)	7.63 ± 0.99	-0.18	-0.54 to 0.18	0.386
FPG ($mmol.L^{-1}$)	10.2 ± 3.1	-0.71	-2.9 to 1.5	0.568
Total-C/HDL-C ratio	5.1 ± 1.2	-0.1	-0.7 to 0.4	0.709
LDL-C ($mmol.L^{-1}$)	3.4 ± 0.4	0.1	-0.2 to 0.32	0.644
Triacylglycerol ($mmol.L^{-1}$)	2.3 ± 0.4	-0.2	-0.9 to 0.2	0.386
NEFA ($\mu mol.L^{-1}$)	459 ± 243	-147	-296 to 2	0.103
hsCRP ($mg\ L^{-1}$)	2.07 ± 1.77#	0.21	-0.38 to 0.80	0.532
Adiponectin (μgL^{-1})	5.4 ± 2.6	-0.1	-0.8 to 0.6	0.751
TNF-α ($ng\ L^{-1}$)	7.2 ± 1.5	0.1	-0.7 to 0.9	0.783

Numbers are Mean±standard deviation (SD). 90% CI: 90% confidence interval; T2D: type 2 diabetes; NCSs: Nerve Conductions Study-score using electromyography (EMG) in arbitrary units, abnormal results are defined as three or more abnormal parameters [48] . BMI, body mass index; FFM, fat free mass; MAP: mean arterial blood pressure mmHg; W_{max} RAMP test: maximum work load capacity during RAMP: linear incremental (15 or 20 W/min) cycling exercise, or Steep RAMP: cycling protocol of 25 W/10 s until exhaustion.; $\dot{V}O_{2peak}$ maximal oxygen uptake per kg bodyweight; Relative age-, height-, body weigth and sex-adjusted cardio respiratory fitness (%Pred. $\dot{V}O_{2peak}$) was based on the equation by Fairbarn et al. [49];1RM; average 1 repetition maximum (kg) for resp. 2 lower (LowerB) and 3 upper body (UpperB) exercises; HbA_1c, glycosylated haemoglobin; FPG: fasting plasma glucose; hsCRP: high sensitivity C-reactive protein; TNF- α : Tumor Necrosis Factor α ; * significant difference, P<0.05, † P<0.01; ‡ P<0.001, paired Student's t -test. # based on n=10

Subjects' characteristics are shown in Table 1. Out of the 11 participating subjects, 7 patients were treated with short (Novorapid®, n=6) or rapidly acting insulin (Humulin®, n=1) before each meal (n=7) either in combination with NPH insulin (Insulatard®, n=5), premixed biphasic isophane insulin (Mixtard 30/70® in combination with metformin, n=1), or a very long-acting insulin analogue (insulin glargine, n=1), all administered before bedtime. Three subjects were treated with premixed biphasic isophane insulin twice a day (Mixtard 30/70®, n=3) in combination with metformin. One subject used NPH insulin (Humulin NPH®) once a day before breakfast in combination with metformin and a sulfonylurea (glimepiride). Data on daily insulin requirements preceding the study were gathered by a retrospective search through the individual patient records from the department of Internal Medicine at the Máxima Medical Centre. The nature and the risks of the experimental procedures were explained to the subjects and all gave their written informed consent to participate in the study, which was approved by the local Medical Ethical Committee of the Máxima Medical Center, Veldhoven, The Netherlands.

Body composition

Body mass and waist circumference were measured using an analog weight scale and standard measuring tape, respectively. Segmental and whole-body bone mass and fat free mass (FFM) were determined using whole-body DEXA (Hologic QDR-4500 Discovery A, software version 12.3:3, Hologic Inc.).

Blood pressure recording

Before and after the 10 wks exercise program (Fig. 1), systolic and diastolic blood pressure were recorded on 2 separate occasions during a 15 min supine rest period using a Dinamap 1846SX automatic blood pressure measuring device (model 8262, Critikon, Tampa Florida, USA). Each measurement was performed under standardized supine rest. Mean arterial blood pressure (MAP) was calculated from the last 3

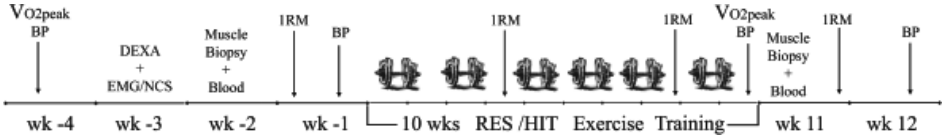

Fig 1. Overview of study protocol, including timing of the different tests before and after the 10 wks resistance (RES) and high intensity interval (HIT) exericse training program: $\dot{V}O_{2peak}$: exhaustive cycle ergometry test to determine maximum power output (W_{max}) and peak oxygen uptake $\dot{V}O_{2peak}$, ml. min^{-1}. kg BW^{-1}); BP: standardized blood pressure recording in supine position; EMG: standardized nerve conduction velocity study (NCS) to assess level of diabetic sensorimotor polyneuropathy; DEXA: dual energy x-ray absorptiometry to assess body composition and fat free mass; Muscle biopsy + Blood: percutaneous muscle biopsy and venous blood sample during fasting conditions, gathered 3 days following any form of intense physical exercise; 1RM: muscle strength testing for 2 lower and 3 upper body exercises

stable blood pressure measurements (i.e. mean arterial pressure difference < 4 mmHg) over a 10 min period during the 2 separate visits to minimize the influence of day-to-day variation and familiarization to the protocol. Intake and dosage of blood pressure lowering medication was maintained throughout the entire study period.

Peak whole-body oxygen uptake

Peak whole-body oxygen uptake ($\dot{V}O_{2peak}$) and maximal workload capacity (W_{max}) were measured during an incremental exhaustive exercise test until exhaustion, performed on a cycle ergometer using a linearly increasing (15 or 20 $W.min^{-1}$) ramp protocol. Gas exchange measurements were performed continuously (Ergostar, PMS Professional Medical Systems, Basel, Switzerland). Relative age-, height-, body weight- and sex-adjusted cardio respiratory fitness (%Pred.$\dot{V}O_{2peak}$) was based on the equation by Fairbarn et al. [49]. Cardiac function was monitored using a 12-lead electrocardiogram with heart rate (HR) being recorded continuously and sampled at 1 kHz through a data log device (Co2ntrol™).

Strength testing

At least one wk before the first exercise session, subjects participated in 2 exercise trials to become familiarized with the exercise protocol and the equipment. Proper lifting technique was demonstrated and practiced for each of the 2 lower-limb exercises (leg press, leg extension) and for the 3 upper-body exercises (shoulder press, horizontal pull and lat-pull down). Maximum strength was estimated using the multiple repetitions testing procedure and at least 1 wk before the experimental trial, subjects' 1 repetition maximum (1RM) was determined [50]. To individualize the training program to the level of co-morbidity and subsequently maximize the progress in muscle strength, 1RM strength testing was repeated 4 and 8 wks after the start of the training program after which the absolute exercise training intensity was adjusted accordingly (Fig. 1).

Blood sampling and analysis

Two wks before the start of the exercise program and 3 days after the last exercise session blood and muscle biopsy samples were collected (Fig. 1) to ensure that structural differences in skeletal muscle biochemical and morphological characteristics were not confounded by the acute effects of the last exercise bout [2]. On the evening before the blood sample and muscle biopsy collection, subjects received a standardized meal (35.2±6.0 kJ/per kg BW, containing 53 energy% (En%) fat, 10 En% protein, and 37 En% carbohydrate) after which subjects remained fasted till the next morning. Subjects reported at the laboratory at 08.00 am. Venous blood samples were collected, immediately centrifuged at 1000·*g* and 4°C for 10 min, after which aliquots of plasma were frozen immediately in liquid nitrogen and stored at –80°C until analyses. Fasting plasma glucose (FPG), serum cholesterol, HDL-cholesterol, LDL-Cho-

lesterol, non-esterified fatty acids (NEFA) and triacylglycerol concentrations were analyzed with the COBAS FARA semi-automatic analyzer (Roche Diagnostics). Blood HbA_{1c} content was determined through high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany). The serum concentration of adiponectin was quantified using a commercially available Human Adiponectin ELISA (#HADP-61K, Linco Research Inc.). TNF-α concentration was analysed using a solid-phase, chemiluminescent immunometric assay (IMMULITE TNF-α, DPC Biermann GmbH). HsCRP was measured by means of immunoephelometry (*Cardio*phase, Dade Behring GmbH). C-peptide was analysed through a electrochemiluminiscent immunoassay (#03184897, Roche GmbH).

Muscle biopsy and immunohistochemical analyses

After blood sample collection, a percutaneous muscle biopsy was collected from the *M. vastus lateralis*. Muscle samples were freed from any visible non-muscle material, mounted in embedding medium (Tissue-Tek, Sakura Finetek) and frozen in liquid nitrogen-cooled isopentane (-160 °C) and stored at -80 °C.

For histochemical analyses, multiple serial transverse cryosections (5 µm) from biopsy samples were collected and thaw-mounted together on uncoated, pre-cleaned glass slides for each subject. To permit the determination of muscle fibre IMCL content stained by oil red O (ORO) together with immunolabelled cellular constituents we used the protocol as previously described [51]. The proportion of type I, IIa and IIx muscle fibres was determined by ATPase staining [52] To assess intramyocellular glycogen content we used the modified periodic acid Schiff (PAS) stain as recently described [53], allowing direct, fibre-type-specific determination of muscle glycogen content. Muscle fibre-type-specific oxidative capacity was estimated by determining succinate dehydrogenase activity (SDH) in the muscle cross-sections using histochemical staining [54]. Figure 2 shows subsequently representative cross-sections of *M. vastus lateralis* obtained in the post-absorptive state with sections stained for laminin and myosin heavy chain I (panel a), SDH (panel b), PAS (panel c) and ORO (panel d).

Training procedures

The backbone of the exercise program was progressive resistance training (PRT), with high intensity interval training (HIT) protocol as a supplement. Four bouts of resistance type exercise targeting the upper-body were performed (2x 10 reps 50% of 1RM). Thereafter, resistance training was continued with horizontal leg press and leg extension (2x10 reps). After 5 wks, the intensity of the PRT was progressively slowly increased from 50 to 60% 1RM to accommodate for the generally deconditoned state of our patients and minimize the risk of musculoskeletal overuse injuries. In each session, PRT was followed by multiple bouts of HIT to predominantly stress type II muscle fibres of the working leg muscle without overloading the cardiovascular sys-

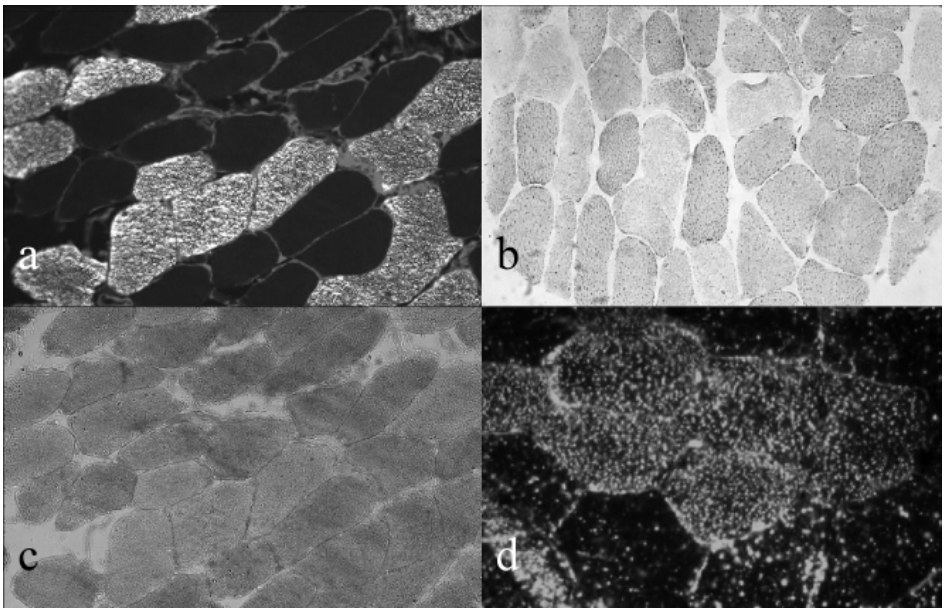

Fig 2. Images (120x magnification (a,b,c) and 240x magnification (d)) of representative cross-sections of M. vastus lateralis obtained in the post-absorptive state with sections stained for laminin and myosin heavy chain I (a), succinate dehydrogenase (b), glycogen content using PAS (c) and IMCL using oil-red-O (d). Further details are described in the Methods section.

tem [55]. In heart failure patients HIT exercise has been shown to increase $\dot{V}O_{2peak}$ and W_{max}, while exertion levels were perceived as moderate [16]. Both numbers of bouts and work rate for the interval modes were progressively increased. The HIT included 4-8 cycling bouts of 30 s, at 50-60% of the maximum achieved W_{max} during a steep ramp test (increments of 25 watts/10 s, [55]) alternated with 60 s of unloaded cycling. In total, a single training session required ~45 min to complete. All subjects were verbally encouraged during the trainings sessions to complete the entire protocol.

Postprandial blood glucose was monitored before and after exercise using a capillary blood glucose meter (Glucocard Memory PC, Menarini Diagnostics). If blood glucose was <6.0 mmol.L^{-1} before the start of the exercise session a snack with 30 g carbohydrate and 5 g protein was provided. Glucose monitoring log sheets were provided to subjects' diabetic nurse and diabetologist for follow up care. If hypoglycemia occurred more than 2 times following an exercise session, the diabetic nurse was consulted to adjust exogenous insulin dose.

Statistics

All data are expressed as means±SD. Repeated measures analyses of variance (ANOVA) and paired Student's t-test was applied to compare the physical, biochemical and

immunohistochemical test results before and after the 10 wks of exercise, using the SPSS 12.0.1 software package (SPSS Inc, Chicago, IL, USA). Level of statistical significance was set at $P<0.05$. Given the experimental nature of a small-scale feasibility study, 90% confidence limits (CI) of the changes were calculated to assess the chances of benefit and harm. In accordance with Snowling and Hopkins [3], clinically important changes of the between-subject standard deviation at baseline were interpreted using thresholds of 0.2, 0.6, and 1.2 for small, moderate, and large, respectively.

5.3 RESULTS

Subjects' characteristics

Subjects' characteristics are shown in Table 1. Relative baseline cardiorespiratory fitness (%pred $\dot{V}O_{2peak}$) was 79.2±15.1%. Mean duration of Type 2 diabetes was 12.1±7.0 y since diagnosis, and subjects had been on exogenous insulin therapy for 7.0±8.0 y. The average insulin doses over a 2 y period prior to the study was raised from 64.1±38.0 I.U. d^{-1} towards 92.5±37.0 I.U.d^{-1} before entering the study. This represents an average increase in insulin dose of +3.6±2.6 I.U.d^{-1} per 3 month period. Under normal clinical conditions the latter would have resulted in (virtual) daily exogenous insulin requirements of 96.3±37 I.U.d^{-1}. After 10 wk of training the average daily dose of exogenous insulin dropped to 87.7±37.0 I.U.d^{-1}, representing a virtual improvement of -8.6 I.U. d^{-1} (90% CI: -15.6 to -1.5 I.U.d^{-1}) from the expected trend of increasing exogenous insulin dose. All subjects finished the 10 wks training program. A total of 4 subjects reported mild and uncomplicated hypoglycaemia (capillary blood glucose 2.7-3.8 mmol.L^{-1}) following the 4^{th} (n=2) and 9^{th} (n=2) exercise session. Only 1 out of 4 subjects required multiple adjustments of exogenous insulin dosage to prevent recurrent exercise-induced hypoglycaemia.One subject developed an overload injury of the knee after 4 wks of training that limited further progression of the training intensity. Compliance to the program was good with a mean participation rate of 83±13 % of all available training sessions.

Anthropometry, blood pressure and physical performance measures

Table 1 shows the results of all physical and biochemical tests performed before and after 10 wks of training. Body weight and waist circumferences remained constant throughout the training period. Both systolic (SBP) and mean arterial blood pressure (MAP) were reduced by 7.6 mmHg (-15.2 to 0.1) and 5.5 mmHg (-9.7 to -1.4), respectively. Maximum power output during the ramp test on the cycle ergometer increased with 14% (6 to 21) or +21 W (10 to 32). Both maximum power output during a steep ramp test and overall muscle strength improved with 17 (9 to 24) and 17% (10 to 24), respectively over the 10 wk intervention period. Whole-body peak oxygen uptake ($\dot{V}O_{2peak}$) remained constant, with an average change of 0.9 ml.min^{-1}. kg BW^{-1} (-0.2 to 2.1).

Plasma analyses

Table 1 summarizes all biochemical analyses of the blood samples. Except for small changes of -0.71 mmol.L^{-1} (-2.9 to 1.5) in FPG and -147 µmol.L^{-1} (-296 to 2) in plasma NEFA concentration, no changes in blood hsCRP, TNF-α, C-peptide, HbA_{1c}, lipid profile, triglycerides or adiponectin concentrations were detected following the 10 wk exercise program.

Immunohistochemical analyses

Table 2 summarizes both mixed and fibre-type specific intramyocellular lipid (IMCL) and glycogen content and muscle fibre oxidative capacity before and after 10 wk of resistance exercise. The training program employed in the present study did not result in any significant changes in absolute IMCL, glycogen or SDH-content in type I, type IIa, type IIx or mixed muscle fibres (table 2, $P>0.05$). IMCL, glycogen or SDH-content of individual muscle fibres was significantly higher in type I than type IIa and type IIx muscle fibres ($P<0.05$) both before and after 10 wks of exercise. Muscle fibre-type distribution and muscle fibre cross sectional area did not differ between pre-training (type I: 47±7% / 867 ±3482 µm^2; type IIa,: 41±8 % / 7700±2225 µm^2; type IIx: 16±8% / 5250±1847 µm^2) and post-training conditions (type-I: 45±10 % / 7831±2667 µm^2; type IIa,: 45±9 % / 8400±3293 µm^2; type IIx: 10±3 % / 4704±2040 µm^2) after the 10 wk resistance exercise program ($P>0.05$).

5.4 DISCUSSION

In the present study, we show that 10 wks of supervised resistance and high-intensity interval training significantly improves muscle strength, workload capacity and blood pressure regulation in long-standing, insulin-treated Type 2 diabetes patients with diabetic polyneuropathy. Diabetic polyneuropathy is an important factor in the development of peripheral muscle weakness in diabetes patients [10]. The associated loss of muscle strength is an underestimated but disabling problem [9] that has been associated with impaired physical function [11] and poor glycemic control [12]. Due to the impaired functional capacity, generic exercise intervention programs designed to prevent and/or treat chronic metabolic disease are generally not applicable in long-standing, insulin-treated Type 2 diabetes patients with diabetic polyneuropathy. Alternatively, resistance training has been proposed to augment functional capacity before participation in the more generic endurance exercise intervention programs. Since the population long-standing, insulin-treated Type 2 diabetes patients with complications is vastly expanding [18], it is of important clinical relevance to assess the response to exercise training in these patients. In the present exercise intervention, we focus to improve muscle strength and exercise capacity to compensate for neuropathy-related muscle weakness in deconditioned Type 2 diabetes patients.

Following 10 wks of moderate intensity exercise training consisting of resistance and relative high-intensity interval exercise, we established a 17±14 and 14±13% in-

Table 2 Skeletal muscle tissue characteristics

Characteristic	Baseline	Change	90% CI	P-value
Mixed muscle SDH activity (AU)	54 ± 28	-5	-16 to 7	0.522
SDH activity type I fibres (AU)	26 ± 16	0	-10 to 10	0.995
SDH activity type IIa fibres (AU)	20 ± 11	0	-5 to 5	0.992
SDH activity type IIx fibres (AU)	8 ± 4	-4	-6 to -3	0.006
Mixed muscle ORO activity (AU)	12 ± 3	4	-2 to 9	0.327
ORO activity type I fibres (AU)	8 ± 2	2	-2 to 6	0.364
ORO activity type IIa fibres (AU)	3 ± 1	2	-0 to 4	0.185
ORO activity type IIx fibres (AU)	1 ± 0	0	-0 to 0	0.688
Mixed muscle PAS activity (AU)	28 ± 2	-1	-5 to 2.5	0.662
PAS activity type I fibres (AU)	16 ± 5	0	-3 to 3	0.934
PAS activity type IIa fibres (AU)	9 ± 1	1	-1 to 4	0.326
PAS activity type IIx fibres (AU)	3 ± 4	-3	-5 to 0	0.123

Skeletal muscle characteristics at baseline and absolute change following 10 wks of resistance and interval exercise training. 90% CI: 90% confidence interval. P-value for paired Student's t-test.

Muscle fibre type distribution was based on individual samples of >300 fibres used for the SDH, ORO and PAS stain analyses; SDH, succinate dehydrogenase stain activity (mean ± SD) in arbitrary units as measured by immunohistochemistry indicates the amount of of mitochondrial enzyme activity; ORO, oil-red-O stain activity (Mean ± SD) in arbitrary units as measured by immunohistochemistry as a measure of intramyocellular triglyceride concentration; PAS, periodic acid-Schiff stain activity in arbitrary units (Mean ± SD) as measured by immunohistochemistry indicates the glycogen content inside the muscle fibres. Fibre type specific content of SDH, ORO and PAS stain activity is corrected for area and number of fibres.

crease in muscle strength and workload capacity, respectively. Previous resistance type exercise intervention studies in uncomplicated Type 2 diabetes patients have applied higher-intensity exercise and reported strength increases between 25-75% [29 56-60]. Some of these intervention studies report significant improvements in HbA_{1c} [56 57], and glucose area under the curve (AUC) [58 61]. No such improvements in glycemic control were observed in this feasibility study. In line with recent findings by Sigal et al. [62] this might be related to the fact that resistance type exercise is insufficient to further improve glycemic control when baseline HbA_{1c} levels approach 7.5%. However, given the lack of a control group in our feasibility study, it is not expedient to speak of an attenuated training response. Besides baseline HbA_{1c}, numerous other factors such as, training volume, exercise intensity, [3], diet [63] or medication [15 64] prohibit us to compare the different studies. Nevertheless, future exercise intervention studies in long-term Type 2 diabetes patients should consider the level of neuropathy and muscle wasting. Our short-term exercise intervention study indicates that, despite these disabling co-morbidities, moderate improvements in muscle strength are feasible in long-term Type 2 diabetes patients with diabetic neuropathy. In accordance with other resistance type exercise training studies in Type 2 diabetes [57 60], improvements in muscle strength and workload capacity were accompanied by a moderate reduction in mean arterial blood pressure (Table 1). Although this is an

interesting finding, the underlying mechanisms cannot be deducted from our feasibility study and are likely multi-factorial [65]. Nevertheless, the potential cardiovascular benefits for patients with long-term Type 2 diabetes warrant further investigation.

Based on the experience with deconditioned heart failure patients [16], we implemented high intensity interval training (HIT) as a supplement to moderate-intensity progressive resistance training. HIT is considered an attractive training stimulus since it stresses the working leg muscles without overloading the cardiovascular system or causing feelings of dyspnoea [55]. Our combined short-term exercise intervention effectively improved maximal workload capacity (Table 1). Despite the improvement in maximal workload capacity, muscle oxidative capacity (SDH enzyme activity) and peak whole-body oxygen uptake ($\dot{V}O_{2peak}$) did not increase significantly. Compared to a training study with younger and early-diagnosed Type 2 diabetes patients [66], it could be speculated that the implemented exercise -intensity, -duration and -frequency in the present study may have restricted an upregulation of myocellular oxidative capacity and peak whole-body oxygen uptake. Although Meyer et al. reported improvements in whole-body oxygen uptake capacity in heart failure patients following HIT exercise training [16], the present study indicates that the regular application of 4 to 8 exercise bouts of 30 s, is insufficient to stress mitochondrial respiration in peripheral skeletal muscle (Table 2). However, this lack of response might also be attributed to our selected subject population, as it has been reported that genetic factors [67], older age [68] and diabetes related co-morbidities attenuate the adaptive response in peak whole-body oxygen uptake [14 61 64 69]. Therefore, larger-scale studies are needed to gain more insight in the muscle fibre type specific adaptation following resistance, interval, endurance or combined types of exercise training in different Type 2 diabetes subpopulations.

The present study supports the notion that intermediate exercise programs are warranted to bring more deconditioned patients with long-standing type 2 diabetes to a level at which they will be able to participate in more generic diabetes intervention programs. Our results indicate that a well-designed exercise regimen composed of short, relatively high-intensity, intermittent exercise bouts is both feasible and safe. Therefore, intermediate exercise intervention programs prescribing such an exercise regimen could be of great value to increase muscle strength and functional performance in deconditioned type 2 diabetes patients with polyneuropathy.

Insulin resistance, visceral adiposity [41-43] as well as low cardiorespiratory fitness [39] have been associated with a state of chronic inflammation and ectopic fat accumulation in liver [40] and muscle [33] tissue. Therefore, in the present study we investigated whether markers for systemic inflammation and lipid abnormalities would change following a short-term exercise intervention. In contrast to previous work [47 70], we did not observe changes in parameters for chronic inflammation (Table 1). The apparant discrepancy might be attributed to differences in type of exercise, exercise intensity, a more prolonged intervention period, the use of cholesterol low-

ering agents [71,72] or simply because of the selected Type 2 diabetes subpopulation with polyneuropathy. In accordance, comparative and more detailed exercise intervention studies will be required to study the complex metabolic interaction between muscle, liver and fat tissue [73].

We observed small, but clinically relevant improvements in fasting plasma glucose following the exercise intervention (Table 1). The latter was accompanied with an attenuated rise in exogenous insulin requirements (Table 1). In the present study we did not apply hyperinsulinemic euglycemic clamping to assess whole-body insulin sensivity as the latter are likely to interfere with the exogenous insulin requirements. Therefore, we can only speculate whether the observed trends in reduced fasting plasma glucose and attenuated rise in exogenous insulin requirements are the result of structural changes in hepatic and/or peripheral insulin sensitivity or exercise-induced improvements in β-cell function [74]. From a clinical perspective the feasibility of the combined application of resistance and interval training seems promising in more advanced stages of Type 2 diabetes, which is generally associated with a progressive worsening of glycemic control despite increasing exogenous insulin doses [75].

Lowered peak oxygen uptake and elevated intramyocellular lipid and glycogen contents have been associated with the development of insulin resistance and/or Type 2 diabetes [25]. Despite the significant gain in functional performance, we observed no structural changes in fibre-type specific IMCL, glycogen or SDH-content in type I, type IIa, type IIx or mixed muscle fibres in *M. vastus lateralis* (Table 2). Furthermore, fibre type composition had not changed after 10 wks of exercise intervention. In accordance, improvements in insulin sensitivity following resistance type exercise training [27-29] have been shown to occur independent of structural changes in skeletal muscle and/or intramyocellular lipid and/or glycogen contents [33]. Previous studies have either reported no change [76-78], a decrease [79], or even an increase [80,81] in IMCL content after exercise training. As such, we extend on previous findings that the reported improvements in physical fitness, muscle strength as well as the attenuated rise in exogenous insulin requirements are not necessarily accompanied by significant changes in muscle fibre type characteristics, muscle oxidative capacity and/or muscle lipid and/or glycogen contents.

Long-term adherence to resistance type exercise training in Type 2 diabetes patients has proven problematic [82]. Therefore, proper supervision is considered an important factor to maintain program adherence [82]. Even though we implemented 3 exercise sessions per wk, subjects showed excellent compliance (83±13 % attendance of the training sessions) and no dropout. The greater workload capacity and increased strength following the 10 wk intervention should be sufficient to enable these patients to participate in a more generic exercise intervention programs. As such, the applied exercise regimen might represent an effective interventional strategy to enable patients to pursue a more active, healthier life style.

In conclusion, the combined application of resistance and interval type exercise training improves physical work-load capacity, lowers resting blood pressure and attenuates the progressive rise in exogenous insulin requirements in long-standing, insulin-treated Type 2 diabetes patients with diabetic polyneuropathy. Such a specific exercise regimen may provide a better framework for future generic training programs in the treatment of deconditioned Type 2 diabetes patients.

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Chapter 6 Exercise training improves glycemic control in long-standing, insulin-treated Type 2 diabetes patients

ABSTRACT

OBJECTIVE To determine the health benefits of exercise intervention in deconditioned, long-term Type 2 diabetes patients on exogenous insulin treatment.

METHODS Eleven male, long-term Type 2 diabetes patients (12±2 y) on exogenous insulin treatment (92±11 I.U.d^{-1}) participated in an exercise intervention program combining both resistance and interval type exercise training. Glycemic control (HbA_{1c}), body composition (DEXA/MRI),intramyocellular lipid content (^{1}H MRS), and skeletal muscle oxidative capacity (^{31}P MRS and $\dot{V}O_{2peak}$) were assessed before and after 5 months of exercise intervention (3 exercise sessions per wk).

RESULTS All subjects completed the study and showed a high compliance (>83%) to the prescribed exercise sessions. Exercise training significantly reduced blood HbA_{1c} levels (7.6±0.3 to 7.2±0.2%: $P<0.05$) and tended to lower fasting plasma glucose concentrations (10.4±0.9 to 8.6±0.7 mmol.L^{-1}, $P=0.05$). This was associated with attenuated exogenous insulin requirements, increased exercise performance capacity and reduced fat mass. Also the cardiovascular risk was reduced as mean arterial pressure decreased from 105.8±2.3 to 98.1±3.1 mmHg ($P<0.05$).

CONCLUSION Long-standing, insulin-treated Type 2 diabetes patients should be stimulated to participate in specifically designed exercise intervention programs. A combination of low-impact resistance and high-intensity interval type exercise training improves glycemic control, augments exercise performance capacity, improves body composition and attenuates exogenous insulin requirements in these patients.

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6.1 INTRODUCTION

Besides diet and medication, exercise training forms one of the 3 cornerstones of Type 2 diabetes treatment. Over the years, it has been established that regular physical exercise represents an effective strategy to prevent and/or treat Type 2 diabetes [1-4]. Exercise training is now generally prescribed for newly diagnosed Type 2 diabetes patients to reduce insulin resistance, improve glycemic control, and prevent cardiovascular co-morbidities [1 3-7]. Clinical guidelines for the practical application of exercise intervention in the prevention and treatment of Type 2 diabetes prescribe that '*... at least 150 min/week of moderate-intensity aerobic physical activity is recommended and/or at least 90 min/week of vigorous aerobic exercise, ... distributed over at least 3 days/week and with no more than 2 consecutive days without physical activity.*' [8 9].

The increase in Type 2 diabetes prevalence can only partly be attributed to the changing population demographics, as epidemiological data show diabetes incidence to increase particularly in the young and middle-aged [10]. As a consequence, the population long-term, Type 2 diabetes patients on exogenous insulin treatment will expand considerably in the coming years [11]. This Type 2 diabetes subpopulation is characterized by a progressive rise in exogenous insulin requirements, the presence of co-morbidities, a high cardiovascular risk profile and substantial exercise intolerance [12 13]. The application of intense endurance exercise activities in this deconditioned group of Type 2 diabetes patients is not always feasible and often contraindicated. Therefore, generic exercise intervention programs designed to prevent and/or treat Type 2 diabetes are generally not applicable in this diabetes subpopulation. Furthermore, research studies investigating the health benefits of exercise intervention programs generally exclude long-standing, insulin-treated Type 2 diabetes patients with co-morbidities for obvious methodological considerations. As such, the potential benefits of exercise intervention in this specific Type 2 diabetes subpopulation with a high cardiovascular risk profile have not yet been established.

Besides endurance exercise, resistance type exercise has been recognized as a useful therapeutic tool for the treatment of Type 2 diabetes [3 4 14 15]. As intense endurance exercise is often difficult to adhere to for those who have been habitually sedentary [15 16], it has been suggested that progressive resistance training would be more feasible in a population of elderly, obese Type 2 diabetes patients [3 4 14 15]. In the present study, we aimed to investigate the feasibility and the benefits of a specifically designed low-impact exercise intervention program, combining both high-intensity interval and low-impact resistance type exercise, in a population of long-standing, insulin-treated Type 2 diabetes patients with a high cardiovascular risk profile. We assessed the impact of 5 months of exercise training on glycemic control, body composition, whole-body and skeletal muscle oxidative capacity and cardiovascular risk profile.

6.2 RESEARCH DESIGN & METHODS

Subjects

Eleven male, Type 2 diabetes patients were selected to participate in this study. Subjects had been diagnosed with Type 2 diabetes for 12±2 y and had been on exogenous insulin treatment for 7.0±2.4 y. All subjects were sedentary and had no history of participating in any regular exercise program for at least 10 y prior to the study. All patients had been on a stable regimen of diabetes medication for at least 3 months before being recruited. Type 2 diabetes patients using thiazolidinediones and/or ß-blockers shorter than 6 months and subjects with impaired liver function, macroalbuminuria, severe retinopathy or a history of severe cardiovascular problems were excluded from participation. Out of the 11 participating subjects, 7 patients were treated with short (Novorapid®, n = 6) or rapid acting insulin (Humulin®, n = 1) before each meal either in combination with NPH insulin (Insulatard®, n = 5), premixed biphasic isophane insulin (Mixtard 30/70® in combination with metformin, n = 1), or a very long-acting insulin analogue (insulin glargine, n = 1), all administered before bedtime. Three subjects were treated with premixed biphasic isophane insulin twice a day (Mixtard 30/70®) in combination with metformin. One subject used NPH insulin (Humulin NPH®) once a day before breakfast in combination with metformin and a sulfonylurea (glimepiride). The nature and the risks of the experimental procedures were explained to the subjects and all gave their written informed consent to participate in the study, which was approved by the local Medical Ethical Committee of the Máxima Medical Center, Veldhoven, The Netherlands.

Study design

All 11 subjects participated in a 5 month (22 wk) exercise intervention program. Before and after the exercise program the following variables were assessed: body composition, glycemic control, blood lipid profile, blood pressure, whole-body oxygen uptake and maximal workload capacity, intramyocellular lipid content (IMCL) and whole-body and skeletal muscle oxidative capacity.

Body composition

Body weight and waist circumference were measured using an analog weight scale and standard measuring tape, respectively. Segmental and whole-body fat mass (FM) and lean mass (LM) were determined using whole-body dual x-ray absorptiometry (DEXA) (Hologic QDR-4500 Discovery A, software version 12.3:3, Hologic Inc. Bedford, MA, USA). Magnetic resonance (MR) imaging was performed in the upper leg using a 1.5-Tesla whole-body MR scanner (Gyroscan S15/ACS, Philips Medical Systems, Best, The Netherlands). A stack of 42 transversal T_1-weighted spin-echo images (TR/TE = 500/12 ms) was acquired covering 48.3 cm of the upper leg. Muscle tissue was segmented from the images using EasyVision (5.1.1.2, 2001, Philips Medical

Systems, Best, The Netherlands) and the total upper leg muscle and *Musculus* (*M.*) *vastus lateralis* volumes were calculated.

Blood analyses

Two weeks before the start of the exercise program and 3 days after the last exercise session blood samples were collected. On the evening before the blood sample collection, subjects received a standardized meal (35.2±1.8 kJ per kg body weight (BW), containing 53 energy% (En%) fat, 10 En% protein, and 37 En% carbohydrate) after which subjects remained fasted. The following morning at 8.00 am, subjects arrived at the laboratory by car or public transportation. After 10 min of supine rest, a venous blood sample was collected from an antecubital vein. Blood samples (4 ml) were collected in tubes containing a glycolytic inhibitor (sodium fluoride) and an anticoagulant (potassium oxalate), immediately centrifuged at 1000·*g* and 4°C for 10 min, after which aliquots of plasma were frozen immediately in liquid nitrogen and stored at -80°C until analyses. Plasma glucose (Glucose 125 Hexokinase kit, ABX Diagnostisc, Montpellier, France), serum cholesterol (CHOD-PAP, ABX Diagnostics), HDL-cholesterol (543004, Roche Diagnostics, Basel, Switzerland), LDL-Cholesterol (LDL-C plus 2nd generation test kit, Roche), non-esterified fatty acids (NEFA) (Wako NEFA-C test kit, Wako Chemicals, Neuss, Germany) and triacylglycerol (GPO-Tinder 337B: Sigma Diagnostics, St Louis, MO) concentrations were analyzed with the COBAS FARA semi-automatic analyzer (Roche). To determine basal fasting blood HbA_{1c} content a 3 ml blood sample was collected in EDTA containing tubes and analyzed by high-performance liquid chromatography (Bio-Rad Diamat, Munich, Germany). The serum concentration of adiponectin was quantified using a commercially available Human Adiponectin ELISA (#HADP-61K, Linco Research Inc. St. Charles, MO). TNF-α concentration was analysed using a solid-phase, chemiluminescent immunometric assay (IMMULITE TNF-α, DPC Biermann GmbH, Bad Nauheim, Germany). HsCRP was measured by means of immunoephelometry (*Cardio*phase, Dade Behring GmbH, Marburg, Germany). C-peptide was analysed by an electrochemiluminescent immunoassay (Nr 03184897, Elecsys Module, Roche GmbH, Mannheim).

Blood pressure

Before and after the 22 wk exercise program systolic and diastolic blood pressure were recorded on 2 separate occasions during 15 min of supine rest using a Dinamap 1846SX automatic blood pressure measuring device (model 8262, Critikon, Tampa Florida, USA). Arterial blood pressure measures (mean arterial blood pressure, MAP, mean systolic, MSP and diastolic blood pressure, MDP) were calculated from the last 3 stable blood pressure measurements (i.e. mean arterial pressure difference < 4 mmHg) over a 10 min period. Blood pressure lowering medication was not changed during the study.

Whole-body oxygen uptake capacity

Peak whole-body oxygen uptake capacity ($\dot{V}O_{2peak}$) and maximal workload capacity (W_{max}) were measured during an incremental exercise test to exhaustion, performed on a cycle ergometer (Medifit Ergometer, Medifit systems, Maarn, The Netherlands) using a ramp protocol [17]. Gas exchange (VO_2) measurements were performed continuously (Ergostar, PMS Professional Medical Systems, Basel, Switzerland). Cardiac function was monitored using a 12-lead electrocardiogram with heart rate being recorded continuously (Polar Electro, Kempele, Finland) and sampled at 1 kHz through a data log device (Co2ntrol™, Tildesign, Zeewolde, The Netherlands).

Magnetic resonance spectroscopy

MR spectroscopy (MRS) measurements were performed using a 1.5-Tesla whole-body MR scanner (Gyroscan S15/ACS, Philips Medical Systems, Best, The Netherlands) following blood sampling, as desribed in **chapter 3**. Subjects received a standardized breakfast before the start of the MRS measurements. First ^{1}H MRS was applied to measure IMCL, which was followed by ^{31}P MRS to assess *in vivo* skeletal muscle oxidative capacity.

^{1}H MRS

Five single-voxel measurements were performed in the *M. vastus lateralis* using the PRESS sequence (TR/TE=1500/35 ms). The voxels (10 × 10 × 15 mm^3) were positioned in different regions of the *M. vastus lateralis*, avoiding subcutaneous and visible interstitial fat using standard T_1-weighted images. From each voxel a spectrum with (128 acquisitions) and without (32 acquisitions) CHESS water suppression was collected. Water and IMCL CH_2 peak areas were quantified from the unsuppressed and suppressed spectra, respectively, using a nonlinear least squares algorithm (AMARES) in the jMRUI software package [18]. IMCL content was expressed as percentage of the water signal and IMCL levels determined from the different voxels of one subject were averaged.

^{31}P MRS

Phosphocreatine (PCr) recovery after exercise was measured as described previously [19]. In short, subjects performed a single-leg extension exercise in an MR compatible ergometer. ^{31}P MRS was applied during the rest-exercise-recovery protocol to measure PCr recovery kinetics. To get subjects acquainted to the in-magnet exercise protocol, subjects visited the laboratory twice within a 7 day period. During the first visit, subjects performed a test run and exercised their leg until fatigue. These data were then used to determine the exercise duration for the second visit. During the second visit, subjects exercised their leg until PCr depletion was sufficient (~50%) to measure the recovery, while avoiding intramuscular pH to fall below 6.8. Spectra were fitted in the time domain by using a nonlinear least squares algorithm (AMARES) in

the jMRUI software package [18]. Recoveries of PCr and ADP were fitted to mono-exponential functions using Matlab (version 6.1, Mathworks, Natick, Massachusetts, USA). Results are expressed as the metabolite's time constant of recovery, i.e. τ_{PCr} and τ_{ADP}, representing skeletal muscle oxidative function [20].

Exercise training program

The exercise intervention program was designed specifically for long-term, deconditioned Type 2 diabetes patients suffering from exercise intolerance and with a high cardiovascular risk profile. The backbone of the exercise training program was progressive resistance training (PRT), with high intensity interval training (HIT) as a supplement. The modality and intensity of the different exercise routines was chosen to recruit sufficient muscle mass without causing delayed onset of muscle soreness or feelings of dyspnoea in this deconditioned group of Type 2 diabetes patients.

The training sessions started with a 5 min warm-up procedure on a cycle ergometer at 50% of the individuals pre-determined W_{max}, followed by 4 bouts of resistance exercise targeting the upper-body (vertical traction, vertical row, upright row and abdominal crunches), each bout consisting of 2 sets of 10 repetitions. The intensity of these exercises was set at 50% of the previously determined 1 repetition maximum (1RM) [21]. Two sets of 20 alternate left/right lunges without additional weights concluded the warm-up. Thereafter, the resistance training was continued with horizontal leg press and leg extension, each 2 sets of 10 repetitions with ~2 min rest between sets. Throughout the PRT the intensity was progressively increased from 50 to 80% of the subjects' individual 1RM. The 1RM was re-evaluated every 4 wk until week 11. In each training session, the PRT was followed by multiple bouts of HIT on a cycle ergometer aimed to stress the working leg muscles without overloading the cardiovascular system [17]. Both the number of bouts and work rate for the interval modes were progressively increased. The interval exercise included 4 - 8 bouts of 30/60 s at 50 - 60% of the W_{max} achieved during the previous steep ramp test (increments of 25W/10 s, described by Meyer *et al.* [17]). Similar to the 1RM, the steep ramp test was performed every 4 weeks to re-evaluate W_{max}. Each exercise session required ~45 min to complete and was carried out 3 times a week for 22 wk. All subjects were verbally encouraged during the training sessions to complete the entire protocol.

Statistical analyses

All data are presented as mean±standard error of the mean (SEM). Paired samples T-tests were applied to evaluate changes in body composition, glycemic control, blood lipid profile and blood pressure, MRS parameters, whole-body oxygen uptake capacity and W_{max} following the exercise training program. The test was performed two-sided and statistical significance was set at $P<0.05$. All statistical data processing was performed using SPSS 14.0 (SPSS Inc, Chicago, IL, USA).

6.3 RESULTS

Subjects

Subjects (age: 59.0±2.5 y; BMI: 32.2±1.2 kg.m^{-2}) had been diagnosed with Type 2 diabetes for 12±2 y and were treated with exogenous insulin for 7.0±2.4 y. At the start of the exercise intervention program daily insulin requirements averaged 92.5±11.1 I.U. All subjects completed the 5 months exercise training program. A total of 4 subjects reported mild and uncomplicated hypoglycemia (capillary blood glucose 2.7 - 3.8 mmol.L^{-1}) following the 4th (n = 2) and 9th (n = 2) exercise session. Only 1 of these 4 subjects required multiple adjustments of exogenous insulin dosage to prevent recurrent hypoglycemia. On average 55.1±2.6 (range 40 to 63) of the 66 available exercise sessions were attended, resulting in an overall compliance of 83±4%.

Body composition

The exercise program induced a significant change in body composition (Table 1). Truncal fat percentage declined from 30.1±1.1 to 28.8±1.3% (P<0.05). Leg lean muscle mass increased from 20.6±1.0 to 21.2±0.9 kg (P<0.05). MRI segmentation analyses of the upper leg showed an increase in muscle volume of the *M. vastus lateralis* from 0.50 to 0.54 L (P< 0.05) but no significant increase in total leg muscle volume (P=0.08). Total body weight (BW) (P=0.87), whole-body fat percentage (P=0.09), whole-body lean mass (P=0.25), waist circumference (P=0.78) and IMCL content (P=0.22) did not change significantly.

Glycemic control

Blood HbA_{1c} content declined from 7.6±0.3 to 7.2±0.2% during the exercise training program (P<0.05) (Table 1). Fasting plasma glucose concentrations showed a strong tendency to decline from 10.4±0.9 at the onset to 8.6±0.7 mmol/L following the 22 wk intervention period (P=0.05). Exogenous insulin requirements did not change significantly during the intervention period (from 92.5±11.1 to 85.4±12.3 I.U.; P=0.26). As exogenous insulin requirements had increased progressively from 60.7±12.7 to 92.5±11.1 I.U. over a period of 3 y (Jan 2002 - Jan 2005) prior to this intervention, exogenous insulin requirements were attenuated during the intervention. When calculating the slope of the insulin requirements over time, it changed from on average + 6.69 I.U. per 6 months in the 3 y prior to the exercise program to -1.6 I.U. per 6 months (P<0.01) following onset of the exercise program.

Blood pressure

A significantly lower MAP (from 105.8±2.3 to 98.1±3.1 mmHg; P=0.02) and lower MSP (from 147.4±3.7 to 137.9±5.1 mmHg; P=0.06) was observed following the exercise training, whereas MDP did not change (P=0.13).

Table 1 Body composition, muscle and whole-body oxidative capacity, functional performance and fasting plasma analyses (n = 11)

	BEFORE training			AFTER training			p
BW (kg)	97.5	±	4.9	97.5	±	4.8	0.95
WC (cm)	112.6	±	3.7	113.2	±	4.0	0.7
Total fat (%)	27.0	±	0.8	25.9	±	0.9	0.09
Truncal fat (%)	**30.1**	**±**	**1.1**	**28.8**	**±**	**1.3**	**0.04**
TBLM (kg)	68.9	±	2.9	69.6	±	2.7	0.25
LLMM (kg)	**20.6**	**±**	**1.0**	**21.2**	**±**	**0.9**	**0.03**
MRI tot. muscle (L)	3.55	±	0.17	3.65	±	0.15	0.08
MRI vast. lat. (L)	0.50	±	0.03	0.54	±	0.08	0.01
Fasting plasma glucose (mmol.L^{-1})	10.4	±	0.9	8.6	±	0.7	0.05
HbA_{1c} (%)	**7.6**	**±**	**0.3**	**7.2**	**±**	**0.2**	**0.04**
Daily insulin requirement (I.U.)	92.5	±	11.1	85.4	±	12.3	0.26
$\dot{V}O_{2peak}$ (ml..min^{-1}.kg BW^{-1})	24.3	±	1.4	24.2	±	1.5	0.87
W_{max} (W.kg BW^{-1})	**1.6**	**±**	**0.1**	**1.9**	**±**	**0.2**	**<0.01**
Ave. 1RM	**77**	**±**	**4**	**90**	**±**	**6**	**<0.01**
IMCL (% of water signal)	1.9	±	0.2	2.0	±	0.3	0.15
τ_{pcr} (s)	49.4	±	5.5	45.6	±	5.6	0.09
τ_{ADP} (s)	22.5	±	2.9	21.2	±	2.4	0.43
MAP (mmHg)	105.8	±	2.3	98.1	±	3.1	**0.02**
MSP (mmHg)	147.4	±	3.7	137.9	±	5.1	0.06
MDP (mmHg)	82.5	±	2.1	78.3	±	2.4	0.13
Total cholesterol (mmol.L^{-1})	4.24	±	0.17	4.37	±	0.24	0.55
HDL cholesterol (mmol.L^{-1})	0.87	±	0.07	0.91	±	0.07	0.55
LDL cholesterol (mmol.L^{-1})	3.44	±	0.13	3.53	±	0.21	0.57
Triacylglycerol (mmol.L^{-1})	2.31	±	4.26	2.26	±	3.31	0.86
NEFA (mmol.L^{-1})	0.459	±	0.073	0.367	±	0.044	0.07
Adiponectin (µg.L^{-1})	5.43	±	0.78	5.47	±	0.82	0.9
TNF-α (ng.L^{-1})	7.19	±	0.46	7.06	±	0.47	0.74
hsCRP (mg.L^{1})	2.1	±	0.6	2.08	±	0.5	0.4
C-peptide (nmol.L^{-1})	0.94	±	0.14	0.90	±	0.12	0.7

BW: Body weight, WC: Waist Circumference, TBLM: Total Body Lean Mass, LLMM: Leg Lean Muscle Mass, MRI tot. muscle and MRI vast. lat.: volume measurements based on MRI data for total upper leg muscle compartment and *M. vastus lateralis*, respectively, W_{max}: maximal power output on cycle ergometer, Ave. 1RM: Average weight lifted in 1 Repetition Maximum tests from 5 different resistance exercises, IMCL: intramyocellular lipids, τ_{PCr}: PCr recovery time constant, τ_{ADP}: ADP recovery time constant, MAP: Mean Arterial blood Pressure, MSP: mean Systolic blood Pressure, MDP: mean Diastolic blood Pressure, NEFA: Non-Esterified Fatty Acids, TNF: Tumor Necrosis Factor, hsCRP: high sensitivity C-reactive Protein.

Blood analyses

Blood analyses results are shown in Table 1. Blood lipid-related parameters did not change following the exercise training program. Plasma adiponectin, TNF-α, hsCRP and C-peptide also did not change after the exercise intervention program.

Whole-body and skeletal muscle oxidative capacity

Maximal whole-body oxygen uptake capacity was similar before and after exercise intervention (24.3±1.4 and 24.2±1.5 ml.min^{-1}.kg.BW^{-1}, respectively; P=0.87) (Table 1). Both ^{31}P MRS derived parameters reflecting *in vivo* muscle oxidative capacity, did not change following exercise intervention (τ_{PCr} and τ_{ADP}, P=0.09 and P=0.43, respectively).

Physical performance capacity

The exercise training induced a significant increase in maximal power output during the maximal exercise test on the cycle ergometer (1.6±0.1 to 1.9±0.2 W.kg.BW^{-1}; P<0.01). Also muscle strength improved significantly, indicated by an increase in the 1RM values of the different strength exercises. Leg press 1RM increased from 159±12 (pre-intervention), 162±14 (4 wk), 172±16 (8 wk) to 187±18 kg (11 wk). Leg extension 1RM increased from 42±15 (pre-intervention), 43±5 (4 wk), 47±6 (8 wk) to 51±6 kg (11 wk). Vertical traction 1RM increased from 85±4 (pre-intervention), 85±4 (4 wk), 90±4 (8 wk) to 96±5 kg (11 wk). Vertical row 1RM increased from 61±3 (pre-intervention), 62±3 (4 wk), 66±4 (8 wk) to 70±4 kg (11wk). Upright row 1RM increased from 37±2 (pre-intervention), 40±3 (4 wk), 43±3 (8 wk) to 47±3 kg (11 wk). Overall, the average of the weight lifted in the 5 different exercises increased from 77±4 kg at the start of the exercise program to 90±6 kg in wk 11 (P<0.01) (Table 1).

6.4 CONCLUSIONS

This study demonstrates that a combination of low-impact resistance and high-intensity interval type exercise training is feasible and well tolerated in deconditioned, long-term Type 2 diabetes patients on exogenous insulin treatment. Proper exercise intervention in these diabetes patients with a high cardiovascular risk profile is shown to improve glycemic control, improve body composition, decrease blood pressure and attenuate the progressive increase in exogenous insulin requirements.

It has been firmly established that regular physical exercise should form one of the main strategies for the prevention and treatment of Type 2 diabetes [3 4]. As such, newly diagnosed Type 2 diabetes patients are generally recommended to participate in exercise intervention programs [22]. However, the clinical relevance of exercise intervention in a vastly expanding group of long-standing, insulin-treated Type 2 diabetes patients suffering from co-morbidities is less evident. These patients are generally excluded from participation in exercise intervention studies for obvious methodological considerations. Furthermore, as these long-standing, insulin-treated Type 2 diabetes patients generally suffer from muscle weakness [12 23-25], cardiovascular co-morbidities [26-29] and/or exercise intolerance [12 13 30 31], it has been proven difficult or even impossible to have these patients adhere to an intense endurance type exercise intervention program [32 33].

In long-standing, insulin-treated Type 2 diabetes patients it would be preferred to implement a combination of low-impact resistance and high-intensity interval type exercise training, as this provides a much lower cardiovascular challenge [17] and improves functional performance capacity to a similar extent [34 35]. In the present study, we evaluated the efficacy of such a combined interval and resistance type exercise intervention program, which appeared to be well tolerated by these patients. All subjects completed the training program and participated in more than 83% of the supervised training sessions. The 5-month training program improved glycemic con-

trol in that both fasting blood glucose levels and blood HbA_{1c} content were lowered after 5 months of training (Table 1). The observed decline in HbA_{1c} content (-0.4%) is in line with previous studies reporting a reduction in HbA_{1c} content between 0.1 - 1.2% after combined endurance and resistance type exercise training in mild Type 2 diabetes patients [36]. The improved glycemic control in the insulin-treated Type 2 diabetes patients was accompanied by an attenuation of the progressive increase in exogenous insulin requirements.

The combined exercise training program resulted in significant changes in body composition in the obese Type 2 diabetes patients. Both total and truncal body fat percentage had declined by 1.1±0.4 and 1.2±0.6%, respectively, following 5 months of intervention (Table 1). The reduction in body fat mass was accompanied by an increase in leg lean muscle mass (Table 1). In contrast to endurance training programs [15], resistance type exercise training has been shown to increase lean muscle mass in Type 2 diabetes patients [14] as well as in the elderly [37]. The latter is an important advantage for the inclusion of resistance type exercise in an intervention program, as total muscle mass represents a key factor in determining whole-body blood glucose disposal capacity. The observed increase in muscle mass likely contributed to the observed improvements in glycemic control [38]. In accordance with the increase in lean muscle mass, muscle strength and workload capacity were substantially improved after the exercise training program (Table 1). This substantially enhances functional capacity, thereby contributing to a healthier, more active lifestyle. Improvements in workload capacity can be used to assess progress of the training program, and to decide on the subsequent possibility to enroll these patients in the more generic Type 2 diabetes exercise intervention programs.

As insulin resistance and/or Type 2 diabetes are presently being associated with mitochondrial dysfunction [39-41], we were interested in evaluating the effects of exercise training on whole-body oxygen uptake capacity and skeletal muscle mitochondrial oxidative capacity in these patients. Five months of combined interval and resistance type exercise training did not raise whole-body oxygen uptake capacity ($\dot{V}O_{2peak}$). In accordance, we observed no evidence of improvements in skeletal muscle oxidative capacity, τ_{PCr} and τ_{ADP}, in these patients. Next to these findings, localized single-voxel ^{1}H MRS did not reveal any differences in IMCL content in the *M. vastus lateralis* following exercise training (Table 1). Despite the reported correlations between insulin resistance and IMCL contents, improvements in glycemic control and/or insulin resistance are not necessarily mechanistically related to IMCL content [42]. In accordance, previous studies have either reported no change [43-45], a decrease [46], or even an increase [47 48] in IMCL content after exercise training. Finally, we observed a significant decline in resting mean arterial blood pressure (MAP - 7.7 mmHg; Table 1), which is likely to have a substantial health impact. It has been estimated that a reduction of 3 mmHg in systolic blood pressure reduces cardiac morbidity by 5 to 9%, stroke by 8 to 14% and all-cause mortality by 4% in an average population [49].

Therefore, the observed decline in MAP appears to represent another health benefit of exercise intervention in these patients.

In summary, relatively low impact resistance and high-intensity interval type exercise training is well tolerated in long-standing, insulin-treated Type 2 diabetes patients with a high cardiovascular risk profile. Five months of interval and resistance type exercise training improves glycemic control, improves body composition, reduces blood pressure, increases muscle strength and workload capacity, and attenuates the progressive increase in exogenous insulin requirements. Combined interval and resistance type exercise training should be prescribed in the vastly expanding population of long-standing, insulin-treated Type 2 diabetes patients.

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Chapter 7 Participation in a 12-month brisk walking or medical fitness program improves cardiovascular risk profile in Type 2 diabetes patients

ABSTRACT

AIMS Structured exercise is considered an important cornerstone in Type 2 diabetes treatment. Adherence to medical fitness (MF) programs is generally poor, and financial costs are high. A cheap, group-based exercise program consisting of brisk walking (BrW) may represent a more attractive alternative. However, its long-term efficacy as addendum to primary diabetes care remains to be established. Therefore, we compared the clinical benefits of a 12-month exercise intervention program consisting of either BrW or MF in Type 2 diabetes patients.

METHODS 92 Type 2 diabetes patients (60±1 y) were randomized to a structured exercise program, consisting of either 3 times a week 60 min BrW (n=43) or MF (n=49). Changes in metabolic profile, blood pressure, physical fitness, and body composition were monitored before, and 12 months after initiating the exercise intervention.

RESULTS After 12 months, only 40% of the participants were still actively participating. Besides motivational problems, 50% of the dropout was attributed to overuse injuries. Mean arterial blood pressure had changed with -7.9±1.2 mmHg ($P<0.001$), while glycemic control, lipid profile, physical fitness, body weight and quality of life (RAND36) remained unchanged in both groups. Post-hoc analyses revealed that patients with $HbA_{1c}>7.5\%$ prior to intervention significantly reduced HbA_{1c} levels with 0.9±0.3% ($P<0.05$), with no differences between groups. Overall, metabolic and cardiovascular improvements did not differ between BrW and MF.

CONCLUSIONS Group-based BrW represents a low-cost but equally effective interventional strategy to improve cardiovascular risk profile and glycemic control when compared to the implementation of more individualized MF.

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7.1 INTRODUCTION

Regular exercise has been identified along with diet and medication as one of the 3 components of good diabetes therapy [1]. Structured exercise intervention programs have been reported to be as effective as many pharmaceutical strategies when trying to improve glycemic control [2-5] and/or cardiovascular risk profile [6 7] in Type 2 diabetes patients. Despite the growing body of scientific evidence on the health benefits of exercise intervention, most meta-analyses report a lack of studies that have tried to assess the long-term efficacy of exercise intervention in Type 2 diabetes patients [3-5 7-9]. In general, exercise intervention studies generally implement endurance and/or resistance type exercise, supervised by a physical therapist [10-17]. The long-term adherence to these (so-called) medical fitness (MF) programs has been shown to range between 10-80% [11 18-20], and financial costs per patient are considerable in these programs. Brisk walking exercise has been proposed as cheaper alternative, with a good clinical outcome when patients are frequently counselled by motivated, supportive physicians [6 21 22]. However, the long-term efficacy of the prescription of structured group-based brisk walking regimen in patients with Type 2 diabetes remains to be evaluated.

In the present study, we compared the health benefits and diabetes care outcome control following the prescription of 12 months of either supervised group-based brisk-walking (BrW) or a more individualized medical fitness program in a large population Type 2 diabetes patients (n=92) in a primary health care setting.

7.2 METHODS

Subjects

From June till December 2005 a total of 96 Type 2 diabetes patients were selected to participate in either the brisk walking or medical fitness exercise intervention program. Type 2 diabetes patients were selected who had been diagnosed more than 3 months prior to screening according to the WHO criteria [23]. Exclusion criteria were defined that would preclude successful participation in the exercise programs, like the presence of (silent) cardiac or peripheral vascular disease, orthopedic limitations, and/or diabetic foot ulceration. Potential study candidates performed a graded exercise test on a calibrated cycle ergometer (Medifit Ergometer, Medifit systems, Maarn, The Netherlands) using a ramp protocol [24]. Cardiac function was monitored using a 12-lead electrocardiogram and blood pressure was measured to detect malignant hypertension. Out of the 96 selected patients, 4 subjects were excluded because of an abnormal stress-ECG. The remaining 92 patients were included in the study and randomized to participate in either the supervised brisk walking (BrW: n=49) or medical fitness (MF: n=43) program. Baseline subjects' characteristics are provided in Table 1. The nature and the risks of the exercise intervention were explained to all subjects before written informed consent was obtained. This study was approved by

Table 1: Subjects' baseline characteristics

	Total (n=92)	Brisk Walking (n=49)	Medical Fitness (n=43)
Age (y)	60 ± 1	61 ± 1	59 ± 1
Male/Female	47 / 45	27 / 22	20 / 23
Duration of Diabetes	5 ± 1	5 ± 1	4 ± 1
BW (kg)	94.3 ± 1.9	94.1 ± 2.6	94.5 ± 2.9
BMI ($kg.m^{-2}$)	32.3 ± 0.6	32.1 ± 0.7	32.5 ± 30.9
FPG ($mmol.L^{-1}$)	8.4 ± 0.3	8.7 ± 0.4	8.2 ± 0.4
HbA_{1c} (%)	7.1 ± 0.1	7.2 ± 0.2	7.1 ± 0.2
HOMA[a]	5.5 ± 0.5	5.9 ± 0.6	5.1 ± 0.7
W_{max}	156 ± 5	155 ± 7	157 ± 7
Est. $\dot{V}O_{2peak}$ ($L.min^{-1}$)	2.1 ± 0.1	2.1 ± 0.1	2.1 ± 0.1
%Pred.$\dot{V}O_{2peak}$	82 ± 1	82 ± 1	83 ± 2
Participants treated with:			
Diet only	11	5	6
OGLA	68	38	30
OGLA + insulin	13	6	7

Data are *n* or means ± SEM , OGLA: oral blood glucose lowering agents; a: based on n=42 (BrW) and n=36 (MF). Estimated $\dot{V}O_{2peak}$ was based on W_{max} during cycling ergometry and the equation according to Storer et al. [28]. Relative age-, height- and sex-adjusted cardio respiratory fitness (%Pred.$\dot{V}O_{2peak}$) was based on the equation by Fairbarn et al. [29];

[a] for all HOMA calculations exogenous insulin users (n=13) were excluded.

the local Medical Ethical Committee of the Máxima Medical Center, Veldhoven, The Netherlands.

Exercise intervention

Two different exercise intervention programs were implemented over a 12-month period, during which 3 brisk walking or medical fitness sessions were performed each week. The exercise load was progressive in nature and had components of both resistance and endurance type exercise according to the ADA/ACSM guidelines [25].

Brisk walking program

The weekly volume of brisk walking consisted of three 60 min exercise sessions. During the first 3 months participants were supervised by both a certified exercise trainer and a physical therapist. Group size varied between 15-25 patients. After 3 months the certified trainer guided and supervised the training sessions, the physical therapist was visited on a consultation basis. The endurance type exercise consisted of brisk walking (5-6 km/h), with a focus on interval type endurance exercise training. During the intervention period the intensity was gradually increased and averaged 75±5% of HF_{max}, as determined during the maximal cycle ergometer test. The resistance type exercise training consisted of resistance and floor exercises using individual body weight and/or elastic bands (The Hygenic Corporation, Akron, OH, USA).

Medical fitness program

The volume of medical fitness consisted of 3 exercise sessions per week. Endurance type exercise consisted of interval type exercise on a home trainer, an elliptical trainer or a rowing ergometer with an average intensity of 73±2% of HF_{max}. All training sessions were tailored to the performance capacity of each individual patient. Resistance type exercise consisted of 8 different exercises targeting both upper and lower body muscle groups. Over a period of 6 months the training volume of the medical fitness program was progressively increased from 3 times per week 30 min (90 min/wk) towards a total of 180-220 min per week.

Based on average heart rate and indirect calorimetry measurements performed during either brisk walking [26] or circuit resistance training in elderly [27] subjects, energy expenditure in both exercise interventions was estimated to range between 0.23-0.33 $kJ.kg^{-1}\ min^{-1}$. Given the subjects' body weight the exercise interventions should be regarded moderate intensity.

Outcome variables

Resting heart rate and blood pressure were determined before and 6 and 12 months after the start of the exercise program. Measurements were performed in a resting, supine position. Heart rate was measured, stored and averaged through a heart rate monitoring system (Co2ntrol, Tildesign, Zeewolde, the Netherlands). Average systolic (SBP) and diastolic blood pressure (DBP) (model HEM-907, Omron Health Care, Hoofddorp, The Netherlands) were determined from 5 successive measurements.

Physical fitness

Before and 12 months after the start of the exercise program, peak oxygen uptake capacity ($\dot{V}O_{2peak}$) was estimated based on W_{max} during cycling ergometry according to Storer et al. [28]. Relative age-, height- and sex-adjusted cardio respiratory fitness (%Pred.($\dot{V}O_{2peak}$) was defined as the ratio between estimated $\dot{V}O_{2peak}$ and predicted $\dot{V}O_{2peak}$ as determined in a healthy non-diabetic population [29].

Blood analyses

Two weeks before the start of the exercise program and 3, 6, 9 and 12 months after initiating the exercise program, fasting blood samples were collected. Blood samples collected before and after 3 and 9 months of intervention were analysed for HbA_{1c} content and basal glucose concentration. On the evening prior to blood sampling, subjects remained fasted from 00:00 onwards. Thereafter, subjects arrived at the laboratory at 8.00 am by car or public transportation. After 5-10 min of rest, a venous blood sample was collected from an antecubital vein. Blood samples (4 ml) were collected in tubes containing a glycolytic inhibitor (sodium fluoride) and an anticoagulant (potassium oxalate), immediately centrifuged at 1000·g and 4°C for 10 min, after which aliquots

of plasma were frozen immediately in liquid nitrogen and stored at -80°C until analyses. Plasma glucose (BG01, dehydrogenase assay), triacylglycerol (TRIG) (BT28, lipase/peroxidase assay) concentrations, total serum cholesterol (T-CHOL) (BC10, peroxidase/cholesterol-esterase assay), HDL-cholesterol (HDL-C) (BH33, peroxidase esterase assay) and LDL-cholesterol (LDL-C) (according to Friedewald formula if TRIG<4.5 mmol.L^{-1}, otherwise through direct peroxidase esterase assay) were analyzed with the Synchron LX20 analyzer (Beckman Coulter, Fullerton, CA, USA). Plasma insulin was determined in duplicate by electrochemiluminescence-immunoassay (ECLIA, Elecsys 2010, Roche, Mannheim, Germany). Because of cross-sensitivity in the latter assay, exogenous insulin users were excluded from this analysis. HOMA insulin resistance index [30] was assessed to monitor changes in insulin sensitivity [31]. To determine basal fasting blood HbA_{1c} content a 3 ml blood sample was collected in EDTA containing tubes and analyzed by high-performance liquid chromatography (HA8160 Menarini, A. Menarini Diagnostics, Firenze, Italy).

Diabetes Care Outcome control

The efficacy of both exercise intervention programs as part of a primary health care based diabetes treatment program was determined by the changes in European Union diabetes indicators (EUDIP) that define risk factors for complications in persons with diabetes [32]. In accordance, EUDIP outcome control was defined as the percentage of participating patients who achieved the following target values: $HbA_{1c} \leq 7.5\%$, SBP≤140 mmHg, DBP≤90 mmHg, T-CHOL≤5.0 mmol.L^{-1}, HDL-C>1.15 mmol..L^{-1}, LDL-C≤2.6 mmol.L^{-1} and TRIG≤2.3 mmol.L^{-1} [32].

Quality-of-Life Assessment

Health related quality-of-life (QoL) was measured with the RAND 36-Item Health Survey 1.0 [33] before and after 12 months of exercise intervention. This questionnaire has been shown a reliable and valid generic measure of QoL [34].

Statistical analyses

Data were analysed according to the intention-to-treat (ITT) principle, as such we did not exclude patients that failed to adhere to 70% of the scheduled exercise sessions or dropped out for any reason. End-point analyses were performed for missing values at 12 months follow-up for the continuous variables. Data are expressed as means±SEM. ANOVA with repeated measures was used to determine differences between baseline and status after 12 months of exercise intervention. For longitudinal analyses of non-normally distributed variables we used the non-parametric McNemar method. Mann-Whitney U test was used to test whether a change in QoL or outcome control variables differed between both intervention groups. Significance was set at the 0.05 level of confidence. All statistical calculations were performed using the SPSS 10.1 software package (SPSS Inc, Chicago, IL, USA).

7.3 RESULTS

Characteristics of the study population

Following randomization there were no significant differences in baseline characteristics, gender and anthropometry between the BrW and MF intervention groups (Table 1). A total of 71% of the predominantly (98%) Caucasian population was diagnosed with Type 2 diabetes for over 2 y with a mean duration of 5±1 y. Out of 92 randomized patients 12% of our subjects were being treated with dietary modulation only, 74% with oral blood glucose lowering agents (metformin and/or sulfonylurea and/or thiazolidiniones) and 14% were treated with exogenous insulin therapy in combination with oral blood glucose lowering agents. A total of 64% of the subjects were on lipid lowering medication, and 59% used one or more blood pressure lowering agents.

Program adherence and follow up

After 6 months of intervention, 45% of the subjects in the BrW group and 30% of the subjects MF group (Chi-Square, p=0.15) were no longer participating in the exercise intervention program. After 12 months, 40% of the participants (BrW: n=18, MF: n=19, P>0.05) were still actively participating with a mean adherence level of 71±3% of the total amount of 156 available exercise sessions throughout the 12-month intervention period. Besides motivational problems (25%), orthopedic related co-morbidities, such as overuse injuries and/or subclinical osteoarthritis of the lower extremities formed the main reason for dropout jn 48 and 50% of the patients in the BrW and MF group, respectively. After 12 months, respectively 15 and 26 subjects were lost from follow-up to obtain standardized blood pressure recordings and fasting blood samples. About 36% of our patients were unable or unwilling to perform the 12-month follow up cycle ergometry test. The distribution of missing data points was similar and not statistically different between groups (Chi-square test, P>0.05)

Glycemic control

According to the EUDIP outcome criteria, glycemic control, defined as HbA_{1c}<7.5%, was satisfactory in 76 and 72% of the subjects in the BrW and MF group prior to exercise intervention, respectively. After 12 months, 74 and 81% of the subjects in the BrW and MF group, respectively, achieved EUDIP glycemic outcome control (McNemar test, P>0.05). Blood HbA_{1c} content tended to decline by 0.2±0.1% following exercise intervention (Table 2, ANOVA, P=0.09), with no differences between groups. No significant changes in insulin sensitivity (as measured by HOMA index) were detected in either group (Table 2). Post-hoc analyses revealed that, independent of the type of exercise intervention, Type 2 diabetes patients with HbA_{1c}>7.5% (n=24) prior to intervention showed a significant -0.9±0.3 (90% CI: -0.44 to -1.40) percent point net decline in blood HbA_{1c} content following 12 months of exercise interven-

tion (ANOVA, P<0.001). A similar HbA_{1c} decline of -0.5±0.2 (90% CI:-0.21 to -0.90, P=0.029) percent point was obtained in the subgroup (n=40) with suboptimal baseline HbA_{1c} levels ≥7.0%, as proposed by the American Diabetes Association (ADA). A total of 12 patients had increased the doses of blood glucose lowering medication throughout the follow-up period. When these subjects were excluded from our post-hoc analysis, HbA_{1c} content still declined significantly from 9.1±0.3% towards 8.3±0.3% following 12 months of exercise intervention in the population Type 2 diabetes patients with suboptimal glycemic control (HbA_{1c}>7.5%; n=19, ANOVA, P<0.01).

Resting heart rate and blood pressure

Following the 12-month exercise intervention, resting heart rate dropped significantly with 5.1±1.9 bpm in BrW and 3.4±1.4 bpm in MF (ANOVA, p=0.001), with no differences between groups. At baseline, 71% of the BrW group and 65% of the MF group were classified as hypertensive according to EUDIP criteria [32]. About 55% of these hypertensive subjects were using one or more blood pressure lowering agents. Following the 12-month exercise intervention, mean arterial blood pressure was reduced by 7.9±1.2 mmHg, with no differences between groups (Table 2, ANOVA, P<0.001). Accordingly, we observed a 27 and 23% reduction in the number of hypertensive patients in the BrW and MF intervention group, respectively (McNemar test, P<0.05, Table 3). During the 12-month follow-up period 11% of the subjects received higher doses of anti-hypertensive medication. When we excluded these subjects from our analyses, mean arterial blood pressure was shown to be reduced from 104.0±2.1 mmHg to 96.4±1.6 mmHg in the BrW group (n=35, ANOVA, P<0.001) and from 103.8±2.0 to 96.3±1.5 mmHg in the MF group (n=32, ANOVA, P<0.001).

Blood lipid profile

Table 2: Intention to treat analysis of diabetes outcome control parameters

	Brisk Walking (n=49)		**Medical Fitness (n=43)**	
	T0	*T12*	*T0*	*T12*
BMI ($kg.m^{-2}$)	32.1 ± 0.7	31.9 ± 0.7	32.5 ± 0.9	31.7 ± 0.9
FPG ($mmol.L^{-1}$)	8.7 ± 0.2	8.3 ± 0.4	8.2 ± 0.4	8.1 ± 0.3
HbA_{1c} (%)	7.2 ± 0.2	7.0 ± 0.2	7.1 ± 0.2	6.9 ± 0.2
HOMA[a]	5.9 ± 0.6	5.8 ± 0.5	5.1 ± 0.7	5.4 ± 0.8
SBP (mmHg)	**150 ± 3**	**139 ± 2** #	**146 ± 3**	136 ± 2 #
DBP (mmHg)	**81 ± 2**	**76 ± 1** #	**83 ± 2**	77 ± 3 #
T-CHOL ($mmol.L^{-1}$)	4.8 ± 0.1	4.6 ± 0.1	4.5 ± 0.1	4.4 ± 0.1
HDL-C ($mmol.L^{-1}$)	1.1 ± 0.03	1.1 ± 0.04	1.1 ± 0.05	1.1 ± 0.004
LDL-C ($mmol.L^{-1}$)	2.9 ± 0.1	2.7 ± 0.1	2.7 ± 0.1	2.7 ± 0.1
TRIG ($mmol.L^{-1}$)	2.1 ± 0.1	2.1 ± 0.2	1.8 ± 0.2	1.7 ± 0.2

Data are means ± SEM; # P<0.01, rep. measures ANOVA with Bonferroni adjustment for multiple comparisons;
[a]For all HOMA calculations exogenous insulin users (n=13) were excluded.

Table 3: Intention-to-treat analyses of European Union diabetes indicators (EUDIP)

Intention-to-treat analysis	Overall (n=92)			Brisk Walking (n=49)			Medical Fitness (n=43)		
EUDIP Outcome	*T0*	*T12*		*T0*	*T12*		*T0*	*T12*	
	%	%		%	%		%	%	
Glycemic control									
$HbA_{1c} \leq 7.5\%$	74	77	NS	76	74	NS	72	81	NS
Blood pressure control									
SBP ≤ 140 mmHg	32	57	##	29	55	#	35	58	*
DBP ≤ 90 mmHg	77	96	##	80	96	*	74	95	*
Lipid profile									
T-CHOL ≤ 5.0 $mmol.L^{-1}$	69	73	NS	61	67	NS	77	79	NS
LDL-C ≤ 2.6 $mmol.L^{-1}$	41	50	NS	41	53	NS	42	47	NS
HDL-C ≤ 1.15 $mmol.L^{-1}$	35	41	NS	37	35	NS	33	49	*
TRIG ≤ 2.3 $mmol.L^{-1}$	75	77	NS	74	80	NS	77	75	NS
Combined EUDIP Outcome	1	9	*	0	8	NS	2	9	NS

Data are percentages of participating diabetes patients that adhered to EUDIP that define risk factors for complications in persons with diabetes [32] before and after 12 months of exercise intervention. EUDIP outcome control was achieved if $HbA_{1c} \leq 7.5\%$, SBP ≤ 140 mmHg, DBP ≤ 90 mmHg, T-CHOL ≤ 5.0 $mmol.L^{-1}$, HDL-C>1.15 $mmol.L^{-1}$, LDL-C ≤ 2.6 $mmol.L^{-1}$ and TRIG ≤ 2.3 $mmol.L^{-1}$ [32]; Non parametric statistics according to McNemar test: ## P<0.001; # P<0.01; * P<0.05; NS: not significant.

While at baseline total cholesterol was ≤5.0 $mmol.L^{-1}$ in 69% of our subjects, only 9% of our participating subjects met all combined EUDIP normolipidemia targets. After 12 months, the overall fasting lipid profile did not differ between the BrW and MF group, and 16% and 14% of the subjects achieved normolipidemia according to EUDIP criteria in the BrW and MF group, respectively (Table 3, McNemar test, P>0.05). The percentage of participants achieving HDL-cholesterol targets had increased significantly from 33 to 49% in the MF group (Table 3, McNemar test, P<0.05), which was significantly different from the unchanged HDL-C outcome in the BrW group (Table 3, Mann-Whitney U, p=0.015). When patients (n=16) who increased statin therapy doses throughout the 12-month follow up period were excluded from our outcome analysis, HDL-C outcome percentage was shown to increase significantly from 33 to 53% (p=0.016) in the subjects in the MF group, while in the BrW group outcome percentage was 35% and 33% at baseline and 12-month follow up, respectively (McNemar test, p=1.0).

EUDIP Outcome control

At baseline, only one Type 2 diabetes patient met all EUDIP criteria for glycemic control, blood pressure regulation and lipid profile (Table 3). After 1 y of intervention, 8 subjects (4 subjects in each group) met all EUDIP criteria (Table 3; McNemar test,

P=0.012). Per protocol analysis showed neither in the BrW or MF group significant improvements in the combined EUDIP targets (Table 3; McNemar test, P>0.05). Furthermore, no significant differences in EUDIP outcome were observed between the BrW and MF group (Table 3, Mann-Whitney U test, P>0.05).

Body composition and workload capacity

Body weight, BMI or waist circumference did not change following exercise intervention (ANOVA, P>0.05). Workload capacity as measured during cycle ergometry averaged 1.68±0.07 and 1.69±0.08 W. kg^{-1} in the BrW and MF group, respectively, and remained unchanged following 12 months of exercise intervention (ANOVA, P>0.05). Maximum heart rates during cycle ergometry were 146±3 and 152±3 bpm at baseline and 152±3 and 149±5 bpm following 12 months of exercise intervention, in the BrW and MF group, respectively (ANOVA, P>0.05).

Quality of Life assessment

A total of 89 and 58 subjects completed the RAND-36 questionnaire at baseline and after 12 months of intervention, respectively. Reliability and internal consistency was excellent with Crohbach's alpha of respectively 0.93 and 0.91. At baseline, total RAND-36 score averaged 70±3 and 75±3 and did not differ from post-intervention values (69±3 and 73±3), in the BrW and MF group, respectively (Mann-Whitney U test, P>0.05).

Financial cost of the intervention programs

The direct cost of the group-based brisk walking program, consisting of an ECG stress test, supervision and consultations by the physical therapist, as well as the salaries for the certified exercise trainers averaged 396 euro per patient per year. In the medical fitness group, total cost averaged 853 euro per subject per year.

7.4 DISCUSSION

The present study shows that a low-cost, group-based, brisk walking program is equally effective in improving glycemic control and cardiovascular risk profile when compared to more expensive and individualized medical fitness programs. The latter generally include both resistance and endurance type exercise performed under strict supervision by an exercise therapist. It has been firmly established that physical activity counselling [35] and participation in a long-term structured exercise intervention programs [15 19] improves glycemic control. In the present study, the group-based, brisk walking exercise program was shown to be equally effective when compared to the medical fitness program, which combined both resistance and endurance type exercise under strict personalized supervision. In line with previous exercise intervention studies in diabetes patients with average baseline HbA_{1c} contents exceeding 8.5% (see [3] for a review), we also observed a significant reduction in HbA_{1c} content in

diabetes patients with suboptimal glycemic control according to EUDIP or ADA targets. The observed improvements in glycemic control occurred independent of adaptations in blood glucose lowering medication. Therefore, our results seem to indicate that if standard blood glucose lowering and dietary therapy are insufficient to achieve glycemic outcome control, the prescription of either type of structured exercise intervention has substantial added therapeutic value. However, the absence of a significant decline in blood HbA_{1c} content in patients with a baseline HbA_{1c} value below 7.0 (ADA) or 7.5% (EUDIP) does not imply that the prescription of exercise has no therapeutic value in these patients [3 7 25 36-38]. In accordance, we observed significant improvements in blood pressure control following either type of exercise intervention (Table 2). The latter occurred independent of changes in antihypertensive medication and even resulted in a more than 75% increase in the number of patients achieving blood pressure targets (Table 3) according to European Union diabetes indicators (EUDIP) defining risk factors for complications in Type 2 diabetes [32]. Furthermore, in terms of the positive changes in the predefined diabetes outcome parameters, no significant differences were observed between the brisk walking program and the medical fitness intervention. Consequently, the structured application of either group-based brisk walking or supervised medical fitness tends to have the same impact on diabetes outcome control and cardiovascular risk profile.

Although structured and individualized exercise intervention in Type 2 diabetes has proven to represent an effective therapeutic strategy [3], its clinical implementation has been problematic due to the high cost, lack of reimbursement, low compliance, and/or absence of proper infrastructure [39]. In the present study, we aimed to assess the differences in the long-term outcome following the prescription of either BrW or MF as part of a comprehensive diabetes care program. We speculated that, compared to a personally supervised medical fitness program, an easily accessible exercise intervention program such as brisk walking would represent a cheaper alternative and a more feasible exercise intervention in a primary health care setting. Nevertheless, independent of the provided level of guidance and infrastructure, 60% of the patients had dropped out of the exercise intervention program during the 12-month intervention period, with no differences in program adherence and dropout pattern between the BrW and MF exercise program. Although motivational factors explained 25% of the dropout in both exercise programs, almost 50% of the dropout was attributed to orthopedic related co-morbidities and overuse injuries of the lower extremities. The latter indicates that, before prescribing therapeutic exercise, diabetes health care workers should pay ample attention to obesity- and diabetes-related musculoskeletal [40-42] deconditioning. In addition, it will be necessary to provide alternative and more individualized programs that allow circumventing such physical disabilities. Such programs should probably focus more on resistance type exercise activities [43 44] to bring the patient to a level at which they are able to participate and adhere to more generic diabetes intervention programs. Furthermore, it has been

suggested that psychological strategies such as motivational interviewing [45] or booster sessions [21 46] might help to further improve program adherence. Nevertheless, more long-term, tailored exercise intervention studies are warranted to further define the most effective and feasible exercise interventional strategies.

In conclusion, group-based brisk walking represents a relatively cheap and equally effective interventional strategy to improve cardiovascular risk profile and glycemic control in Type 2 diabetes patients when compared to more individualized medical fitness programs. General deconditioning, musculoskeletal overuse injuries and lack of motivation may limit the benefits of long-term exercise intervention as an addendum to a comprehensive diabetes care program. Future diabetes exercise intervention programs in primary health care settings should consider diabetes related comorbidities and patient motivation as important factors determining long-term program adherence.

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Chapter 8 General discussion and future research

Regular exercise has been recommended for diabetes patients for many years, and was identified along with diet and insulin as one of the three components of good diabetes therapy as early as 1939 [1]. From a physiological perspective, structured exercise interventions have at least equal therapeutic strength as currently applied pharmaceutical solutions aimed to improve glycemic control [2-4] and cardiovascular risk profile [5 6]. The aim of this thesis was to gain more insight in the clinical health benefits of exercise therapy in patients with Type 2 diabetes. In this final chapter, the results and conclusions of the previous chapters are integrated and the clinical implications of the work presented in this thesis are discussed. Furthermore, therapeutic guidelines for tailored exercise interventions in Type 2 diabetes patients are proposed and suggestions for future research are provided.

8.1 IMPAIRED GLYCEMIC CONTROL IN TYPE 2 DIABETES

To investigate the potential benefits of exercise on either diurnal and/or nocturnal hyperglycemia, it is important to have more detailed information on daily blood glucose profiles. Therefore, in **chapter 2**, we investigated 24 h interstitial glucose profiles in Type 2 diabetes patients on oral blood glucose lowering medication and compared this to healthy, normoglycemic controls under the same strictly standardized, but otherwise free-living conditions. In the healthy control group, hyperglycemia was hardly present, while in the Type 2 diabetes patients hyperglycemia was experienced for as much as 13±2 h per day. A strong correlation was observed between the prevalence of hyperglycemia and HbA_{1c} content in the Type 2 diabetes patients, but even patients with apparent acceptable glycemic control (HbA_{1c} ≤7.0) were still experiencing hyperglycemia for 11±0.9 h throughout the day. Although the latter is an interesting finding, the results described in **chapter 2** should be interpreted cautiously, especially since recent CGMS investigations indicate that glycated haemoglobin levels (HbA_{1c}) reflect both postprandial and fasting hyperglycemia [7]. Moreover, their relative contributions appear to depend both on the level of overall glycemic control [7] and on the stage of the disease [8]. Therefore, future CGMS studies should not only standardize diet and physical activity levels, but preferably also quantify

pancreatic β-cell status [9] in order to classify a patients' disease stage. For now, continuous glucose monitoring (CGMS), in addition to HbA_{1c}, represents a useful tool to monitor more accurately the amount of glycemic instability in different glucose intolerant populations. Furthermore, CGMS provides us with an excellent investigational tool that can be used for future research to more directly evaluate alternative therapeutic strategies to reduce hyperglycemic blood glucose excursions.

8.2 ACUTE VERSUS MORE LONG-TERM EFFECTS OF EXERCISE THERAPY ON GLYCEMIC CONTROL

Using the same CGMS methodology, we investigated the acute effects of moderate intensity, resistance type exercise on glycemic control in long-standing, insulin-treated Type 2 diabetes patients **(chapter 3)**. Although these Type 2 diabetes patients were receiving oral blood glucose lowering therapy in combination with exogenous insulin, they still showed 7.6±1.4 h of hyperglycemia during the 24 h baseline conditions **(chapter 3)**. Importantly, the next day following an acute bout of resistance type of exercise, hyperglycemia was reduced by almost 40 percent. No direct significant effect on mean 24 glucose values or glycemic instability (CONGA*n*) was detected, which implies that more long-term application of resistance type exercise may be needed to achieve such improvements [10]. In accordance, in **chapter 5 and 6** we reported attenuated exogenous insulin needs, while only after 5 months significant improvements in glycosylated hemoglobin levels were observed **(chapter 6)**. Obviously, non-exercising control groups and more frequent clinical assessments would be required to quantify the size and time-path of the insulin sensitizing and blood glucose lowering effects of different types of exercise interventions. The current gold standard to monitor such changes in insulin sensitivity and glucose disposal capacity is the hyperinsulinemic euglycemic clamp technique [11 12]. However, the methodological difficulties that arise from the application of a hyperinsulinemic euglycemic clamp in insulin-treated Type 2 diabetes patients would severely complicate this type of clinical research. Alternatively, the minimal-invasive CGMS measurements are more practical, do not interfere with medication schemes and still provide us with clinically relevant information on improvements in glycemic control throughout the exercise intervention period. In accordance, the combined results from **chapter 3, 5 and 6** indicate that acutely observed improvements in glycemic control through the application of CGMS may predict or reflect the medium-to-long-term clinical effectiveness of a certain type of exercise regimen aimed at improving glucose homeostasis. Therefore, instead of HbA_{1c} monitoring, the CGMS approach may enable us to more directly define the exercise parameters that modulate the capacity of exercise to improve glycemic control. Given the current lack of specific exercise guidelines [13-15], future research should be aimed at defining the characteristics that constitute the most effective population-based exercise regimen that maximizes the therapeutic value of exercise in the prevention and/or treatment of Type 2 diabetes.

8.3 EXERCISE AS AN ANTI-HYPERTENSIVE AGENT

Besides improving glycemic control a recent meta-analysis showed that structured exercise intervention studies in non-insulin dependent Type 2 diabetes patients reduces systolic blood pressure with -4.16 mmHg (95% CI -9.46 to 1.14) [16]. In accordance, similar reductions in blood pressure were observed following short **(chapter 5)**, medium **(chapter 6)** and long-term **(chapter 7)** exercise therapy. The limited follow-up period and lack of a non-exercising control group in our exercise intervention studies prevent us to exclude other factors that may have modulated mean arterial blood pressure. Furthermore, our experimental design does not allow us to estimate the amount of cardiovascular risk reduction through improvements in diabetes outcome control **(chapter 7)**. However, the relative and absolute reductions in mean arterial blood pressure are clinically relevant and are similar to the effects of add-on blood pressure lowering therapy using a combination of an ACE-inhibitor and thiazide diuretic [17]. As such, the simultaneous improvements in glycemic and blood pressure control, presented in **chapter 4, 5 and 6**, extend on findings from the Steno-2 study [18] and the ongoing Look AHEAD trial [19 20] by showing that a tailored exercise intervention in combination with pharmaceutical measures is safe, feasible and effective in long-term insulin-treated Type 2 diabetes patient with a high cardiovascular risk profile. Furthermore, such an approach may also improve long-term cardiovascular outcome in this specific subgroup of Type 2 diabetes patients that has generally been excluded from lifestyle intervention studies. Although both resistance- and endurance-type exercise seem to reduce mean arterial blood pressure to a similar extent in Type 2 diabetes populations [3], further research is needed to explore their separate contribution and way of action in different insulin resistant populations.

8.4 DOES EXERCISE THERAPY IMPROVE LIPID METABOLISM?

Although blood lipid profiles in Type 2 diabetes populations have been shown to improve following long-term exercise interventions with [19 21 22] or without dietary restriction [23 24], we observed no clinically relevant changes in fasting blood lipid profiles following either short **(chapter 5)**, medium- **(chapter 6)** or long-term **(chapter 7)** exercise interventions. The latter extends on recent findings [25] and may be related to the lack of a simultaneously diet-induced weight loss [26], a compensatory decline in daily physical activity level [27] or the fact that in our Type 2 diabetes population baseline Total-cholesterol, LDL-cholesterol and triglycerides levels were already 15-35% lower in comparison with aforementioned 'exercise-only' intervention studies [23 24]. Nevertheless, in accordance with earlier reports (for references see [28]), body composition analyses using DEXA and MRI **(chapter 6)** revealed that, despite an unaltered body weight, 5 months of combined endurance and resistance type of exercise training is able to induce regional changes in fat and lean muscle mass in obese Type 2 diabetes patients. Opposed to very low calorie diet-induced loss of fat mass [29], the reduction in truncal fat mass described in **chapter 6** occurs independent of clinically important

changes in fasting blood lipid profile in our population of obese long-term insulin-treated Type 2 diabetes patients. Although we did not measure postprandial lipemia, several lines of research [30-32] indicate that our exercise intervention has been of sufficient volume and intensity to modulate post-prandial lipid handling. The latter may explain the loss of truncal fat mass, as well as the observed trend in lower post-absorptive non-esterified fatty acid (NEFA) levels **(chapter 5 and 6)**. Future studies should aim to unravel the mechanisms of action and modulating effects of different modes of exercise training on post-absorptive and post-prandial lipid handling in Type 2 diabetes patients [33]. Ideally, dietary intake and hormonal responses should be monitored to differentiate between the impact of isocaloric exercise bouts of different volumes and intensities on both post-prandial glycemica and lipidemia.

8.5 DECONDITIONING IN DIABETES ASSESSED THROUGH IN VIVO AND EX VIVO DETECTION TOOLS

Most patients with Type 2 diabetes have a significantly lower oxidative capacity than age-matched controls [34-36]. Whether this lower oxygen uptake capacity is attributed to a low habitual physical activity level, reduced mitochondrial content or an intrinsic mitochondrial defect is a topic of intense debate [37-46]. However, recent experimental evidence indicates that mitochondrial respiration is not abnormal when normalized for mitochondrial content [43 46], which implies that low habitual physical activity level or cardiovascular dysfunction may explain the generally deconditioned state in the Type 2 diabetes patient. This also raises the question whether an increased physical activity level following the implementation of an exercise intervention program could reverse the deconditioned state of long-term Type 2 diabetes patients with a high cardiovascular risk profile. Before trying to answer the latter question, we compared a non-invasive *in vivo* method using ^{31}P nuclear magnetic resonance spectroscopy (MRS) to assess mitochondrial function with the outcome of other markers of muscle oxidative capacity. Despite our small sample size, the result in **chapter 3** showed overall good correlations between *in vivo* and *ex vivo* measurements of mitochondrial function in long-standing, insulin-treated Type 2 diabetes patients, which are qualitatively and quantitatively consistent with previous results measured in healthy subjects. It was concluded that ^{31}P MRS recovery kinetics is an appropriate tool to investigate skeletal muscle oxidative capacity in response to a well-structured exercise intervention in a deconditioned insulin resistant population. Most other studies on the role of mitochondrial dysfunction in insulin resistant states have applied ^{31}P MRS transfer experiments to measure ATP synthesis-flux in resting skeletal muscle [38 39 44]. However, as recently shown by Szendroedi et al. [44] the lower basal or insulin-stimulated ATP synthesis rates in insulin resistant subjects are almost fully explained by the higher lipid availability and lower insulin sensitivity in these subjects. Furthermore, the more fundamental question still remains to be answered to what extent

resting ATP synthesis-flux truly reflects mitochondrial respiratory capacity during exercise conditions. For now, post-exercise PCr recovery analysis seems a more suitable ^{31}P MRS technique to monitor mitochondrial respiratory capacity in response to therapeutic exercise interventions in insulin resistant populations. However, since hemo-dynamic factors in muscle have a direct influence on PCr recovery kinetics [47], future ^{31}P MRS experiments in diabetes patients should simultaneously monitor skeletal muscle blood flow [48] and differentiate between findings observed in the presence and absence of impaired cardiovascular dynamics [49].

8.6 CARDIOVASCULAR DECONDITIONING MAY ATTENUATE THE RESPONSE TO EXERCISE

In respectively **chapter 5 and 6** we reported on the results following 2.5 and 5 months of resistance and interval type endurance exercise. Neither *in vitro* (SDH enzyme activity, **chapter 5**) or *in vivo* whole-body oxidative capacity ($\dot{V}O_{2peak}$, **chapters 5 and 6**) and ^{31}P MRS recovery kinetics **(chapter 6)** improved significantly, despite significant improvements in muscle strength and maximal exercise capacity. These results may appear somewhat in contrast with the overall improvement in mitochondrial content, skeletal muscle oxidative capacity, insulin sensitivity and glycemic control following a 4 months combined weight loss and daily moderate-intensity exercise intervention in a relatively young population of early-diagnosed Type 2 diabetes patients [50]. However, other medium-term endurance type of exercise interventions in more advanced and older Type 2 diabetes patients report no clinically significant improvements in whole-body oxidative capacity [10 51-53]. So far, no comparative exercise intervention studies in early- versus more-advanced stage Type 2 diabetes have been performed. A complicating factor when studying these Type 2 diabetes subpopulations is that apparent differences in maximum workload capacity will lead to important differences in absolute exercise intensity and energy expenditure. The latter is likely to produce a different physiological training stimulus. Although the lack of improvement in mitochondrial function parameters in **chapters 5 and 6** may indicate that diabetes related co-morbidities attenuate the adaptive response in mitochondrial oxidative capacity to endurance exercise training, a lower energy expenditure or training load may explain this as well. As such, it would seem logical to further increase exercise intensity, duration and/or –frequency. However, the associated co-morbidity [54-57], and deconditioned state of both skeletal muscle [58] and the cardiovascular apparatus [57 59 60] limits the intensity and volume of endurance exercise training in long-standing, insulin-treated Type 2 diabetes patients. In accordance, the results from **chapter 5 and 6** suggest that intermediate programs are needed to bring the patient to a level at which they are able to participate in more generic diabetes intervention programs. Such intermediate programs should implement short, relatively high-intensity, exercise bouts applied in an intermittent fashion with the intention to increase muscle strength and functional performance. These so-called short 'in-and-out' exercises do

not produce feelings of dyspnoea or discomfort and have been proven safe and effective in cardiac patients [61 62]. The overall improvements in muscle strength, blood pressure and glycemic control indicate that from a clinical perspective such intermediate programs in long-term, Type 2 diabetes patients with a high cardiovascular risk profile are feasible and improve general health and fitness.

8.7 ENERGY EXPENDITURE DETERMINES THERAPEUTIC STRENGTH OF EXERCISE

Both patients and health care providers often tend to believe that a brisk walking program produces lower cardiovascular benefits than medical fitness. In contrast to this believe, **chapter 7** shows that through the prescription of either brisk walking or medical fitness similar exercise intensities and training responses can be achieved in relatively deconditioned Type 2 diabetes patients. Although this may seem obvious, it is important to stress that the brisk walking program that we applied in **chapter 7**, not only consisted of walking exercises. In addition, also resistance type exercise was applied aimed at improving overall muscle strength. Therefore, our outdoor brisk walking program can be considered a total body workout, enabling a comparable physiological training stimulus in comparison with indoor-based medical fitness. Based on average heart rate and indirect calorimetry measurements performed during either brisk walking [63] or circuit resistance training in the elderly [64], estimated energy expenditure in both exercise interventions ranged between 0.23-0.33 kJ. $kg^{-1}min^{-1}$. When prescribing exercise as a therapy for an individual diabetes patient, it is important to estimate total energy expenditure that can be achieved through the recommended type of exercise. Several studies have shown that the energy equivalent of an endurance exercise bout appears to represent the major determinant of the exercise-induced changes in glucose homeostasis [5 65 66]. Therefore, a lesser exercise intensity should be compensated for by an increase in exercise duration. Nonetheless, given our 60% drop out throughout the 12-month intervention period in **chapter 7**, we can only speculate on whether higher attendance rates would have resulted in a greater improvement in the training response. Future exercise intervention studies in Type 2 diabetes patients should aim to monitor an individual's total weekly or monthly energy expenditure and relate this to an individual clinical progress throughout the follow-up period. In accordance, a post-hoc analysis of a daily walking exercise program indicated that long-term metabolic improvements in Type 2 diabetes patients requires a minimum weekly energy expenditure of 4.2 MJ [5]. In both our long-term exercise intervention groups this would have required a participation rate of 83% or an average of 2.5 exercise sessions per week. Since no reliable information on habitual physical activity levels was gathered in our long-term exercise study **(chapter 7)**, it is not possible to obtain a reliable estimate of average weekly energy expenditure in an individual patient. Nevertheless, our exercise intervention study indicates that the prescription of

moderate intensity exercise may be sufficient to improve long-term glycemic control in subjects with a baseline HbA_{1c} >7.5%.

8.8 WHAT IS THE ROLE OF RESISTANCE-TYPE EXERCISE TRAINING?

In terms of metabolic adaptation, apparent differences exist between endurance and resistance type exercise training. Besides the consecutive effects of each bout of exercise on insulin sensitivity [67 68], resistance type exercise training has been associated with a substantial gain in skeletal muscle mass. The latter also improves whole-body glucose disposal capacity [69]. Some studies report even greater benefits of resistance as opposed to endurance type exercise training on glycemic control and insulin sensitivity in long-term Type 2 diabetes patients [10]. However, recent evidence indicates that both types of exercise interventions have similar therapeutic strength in uncomplicated Type 2 diabetes patients [25]. Its combined application is probably more effective, especially in patients with HbA_{1c} levels less than 7.5% [25]. As reported in **chapter 6**, the application of combined resistance and interval exercise not only improved glycemic control and blood pressure, but also increased muscle mass in Type 2 diabetes patients. As such, our results indicate that despite a deconditioned state, resistance type exercise training can be effective in attenuating the loss of muscle mass, thereby improving muscle strength and functional capacity. The latter enables a healthier, more active lifestyle and, as such, may motivate patients to continue their exercise regime. Although the study scale prevents us to generalize our findings, it may be worth mentioning that after finishing the 5-month exercise intervention study, 9 out of the 11 patients voluntarily decided to continue their tailored exercise intervention.

Another vastly expanding diabetes subgroup that might benefit from resistance-type exercise training is formed by the elderly (>70 yrs) patients with Type 2 diabetes. Aging is associated with the loss of skeletal muscle mass, and represents one of the main factors responsible for the increase in Type 2 diabetes incidence at an advancing age [70]. The loss of muscle mass is proportionally related to the reduction in blood glucose disposal capacity and the decline in muscle strength [71]. The latter prevents many elderly diabetes patients to participate in lifestyle intervention programs. Although even low-impact endurance-type exercise has been reported to improve glycemic control in elderly patients with Type 2 diabetes [72 73], it has been suggested that the insulin sensitizing response to exercise is attenuated with an advancing age [74 75]. Since Type 2 diabetes patients show an accelerated decline in muscle mass and strength with aging [76], it would be preferred to focus more on increasing skeletal muscle mass and strength when designing exercise intervention programs for elderly diabetes patients [77 78]. Effective exercise intervention programs should include resistance type exercise, with exercise volume and -intensity being progressively increased towards 3 sets of 8-10 repetitions with intensities ranging from 50-80% of 1RM for 7-10 exercises and/or muscle groups [69 79-81]. Dietary co-interventions might further improve the benefits of resistance type exercise training in elderly patients [82 83].

8.9 PRESCRIBING PROGRESSIVE RESISTANCE-TYPE EXERCISE TRAINING

It has been established that progressively intensified resistance training over a period of 6 wk (3*30 min exercise per wk) upregulates the activity of key proteins in the insulin signaling cascade, resulting in enhanced insulin-stimulated blood glucose disposal in muscle [84]. Moreover, resistance type exercise training has been shown to reduce blood HbA_{1c} levels with -0.4 to -0.8 % in patients with Type 2 diabetes [3]. In accordance, it has been advised to implement resistance type exercise training with exercise-volume and -intensity being progressively increased towards 3 sets of 8-10 repetitions performed at 70-80% of 1RM per muscle group [13]. When applying resistance type exercise, strength improvements also tend to follow a linear dose-response relationship, at least in healthy subjects [85 86]. If the therapeutic aim of resistance type exercise is to stimulate muscle glycogen storage depletion and net muscle mass gain, both exercise intensity and volume should be kept high [67 87]. However, studies assessing the impact of exercise intensity in resistance type exercise interventions in patients with Type 2 diabetes remain lacking. In accordance with the methodology described in **chapter 3**, resistance-training studies using CGMS may be helpful to establish optimal dose-response relationships in different subpopulations or even individual patients with Type 2 diabetes.

8.10 PREFERRED FREQUENCY AND TIMING OF EXERCISE

The enhanced insulin sensitivity following an acute bout of exercise has been reported to persist for a period ranging from 2 [88], 4-6 [89], 12-16 [90 91], 24 [52 92] to up to 48 h following the cessation of exercise [88]. As such, the benefits of exercise on glycemic control can largely be attributed to the cumulative effects of each successive bout of endurance or resistance type exercise, rather than the structural adaptive response to prolonged exercise training [52]. In fact, long-term training effects on glycemic control may be lost entirely 6-14 days after cessation of training [93 94]. Therefore, it is preferred to perform exercise everyday. The American College of Physicians (ACP) guidelines prescribe an exercise frequency of at least 3 times per week with no more than 2 consecutive days without physical activity [95]. The ADA [15] now recognizes that these guidelines need to be considered a minimal therapeutic dose and, therefore, represent a less than optimal therapy.

Furthermore, current guidelines on exercise prescription as therapeutic strategy [15 96] do not consider an optimal timing of a daily exercise routine. In this respect, it is interesting to note that in Type 2 diabetes patients hyperglycemic episodes during the day are most predominant in the morning in the post-prandial state [97 98]. This so-called *'dawn-phenomenon'* [99] seems to be related to the diurnal variation in endogenous glucose production [100]. Since moderate-intensity exercise suppresses endogenous glucose production, it might be advantageous to schedule moderate intensity endurance exercise sessions in the morning, preferably in the post-prandial state. More research is warranted to assess the impact of timing of exercise and nutrition on daily hyperglycemia in patients with Type 2 diabetes [66 101].

8.11 PREVENTION OF OVERLOAD INJURIES IN EXERCISE TRAINING

In accordance with the experience from the studies presented in this thesis, many patients with Type 2 diabetes experience some musculoskeletal [54-57] and/or cardiorespiratory deconditioning [59]. Despite the relatively low impact of both exercise programs applied in **chapter 7**, the rate of withdrawal and percentage of patients that were unable to adhere to 70% of the exercise sessions was high. Almost 50 percent of the withdrawals related to orthopedic overload injuries, equally distributed between the 2 study arms. Although the progressive nature of our 2 exercise interventions was adjusted for the deconditioned status of our subjects, the musculoskeletal overuse injuries might be caused by obesity- and diabetes-related subclinical osteoarthritis [102 103] on top of neuropathy related peripheral muscle weakness [56]. In future endurance type of exercise interventions, certain overuse injuries might be prevented through adaptations in biomechanical loading on feet and lower extremities [104-107], as well as through the application of resistance type exercise aimed at strengthening myotendinous structures. The latter concept is supported by resistance type of exercise studies that reported long-term program adherence between 68% [108] and 72% [109], without concomitant musculoskeletal overuse injuries. Nevertheless, more long-term tailored exercise interventions studies are needed to test the usefulness of a differentiated approach.

8.12 PREVENTION OF DROP OUT IN THERAPEUTIC EXERCISE INTERVENTION PROGRAMS

As reported in **chapter 7**, motivational factors and time-constraints appeared the next most important reason for drop out. Although long-term program adherence may vary between as much as 10 and 80% [24 108 110 111], the high drop out rate reported in **chapter 7** indicates that future exercise interventions might benefit from psychological strategies such as motivational interviewing [112] or booster sessions [113-115]. Furthermore, restricting the travel time towards a training facility [116] and providing the patient with feedback on physical activity levels [117] may improve long-term adherence as well. Although aforementioned approaches are likely to reduce program drop out throughout the course of a supervised exercise program, scientific studies are warranted that combine aforementioned approaches.

8.13 LESSONS MAY BE LEARNED FROM CARDIAC REHABILITATION

Despite the growing body of evidence showing the health benefits of exercise in Type 2 diabetes, a recently published large scale U.S. survey shows that the majority of patients with diabetes do not engage in regular physical activity. [118]. For now, there is no reason to believe that this is any different in the rest of the world.Although the advice from the physician has been shown a strong predictor of attempts to change lifestyle habits [119], health professionals may not take the time nor provide enough specific information to help patients successfully change their physical activity be-

haviour. [120] Apparently, most physicians and diabetes nurses around the world find it difficult to prescribe structured exercise routines for individual patients. The high costs, lack of reimbursement, low compliance, and/or absence of proper infrastructure may indeed work as barriers [116]. However, not so long ago, cardiac patients and their doctors experienced similar problems [121].Nevertheless, exercise-based cardiac rehabilitation programs have been shown effective and feasible [122], if available evidence-based guidelines are supported by a motivated and knowledgeable staff and applied in a patient-tailored way [123].

8.14 SAFETY CONSIDERATIONS BEFORE INITIATING EXERCISE THERAPY

Before exposing patients with Type 2 diabetes to more vigorous exercise programs, the ADA and U.S. Preventive Services Task Force recommend exercise testing for silent myocardial ischaemia (SMI) if 10-years' cardiovascular risk exceeds 10% [13 124]. Cardiac dysfunction [125] and SMI are estimated to be present between 6-22% [126] of the Type 2 diabetes patients, with cardiac autonomic dysfunction, disease duration, and male gender being the best predictors for SMI [126]. Moreover, poor physical fitness [127], scintigraphy abnormalities [128], diabetic retinopathy [128] and an advancing age > 60 y [129] in combination with the traditional cardiac risk factors also represent good predictors for the likelihood of a cardiac event. In **chapter 7** a total of 4 out of 96 candidates were excluded from the long-term exercise intervention based on an abnormal stress-ECG. In the remaining 92 subjects no cardiovascular event were reported during the 12 months follow-up period. On itself, these numbers raise some questions on cost-effectiveness of a stress-ECG as a screening tool for SMI. Therefore, as an alternative screening tool, the UKPDS Risk Engine v2.0 (available free of charge at www.dtu.ox.ac.uk) may be of help to calculate an individual patient's risk for coronary heart disease [130]. Although arbitrary, the UKPDS Risk Engine indicates that ECG stress testing in Type 2 diabetes is useful in most patients with >2 cardiovascular risk factors, in middle-aged patients with a diabetes duration >5 years, as well in elderly patients >70 years. Although a stress-ECG is not the most sensitive diagnostic tool to detect SMI [131] and predict coronary events [132], other research indicates that it is still the most cost effective tool when trying to minimize the risk of a coronary event [133]. In case SMI is expected, more sensitive diagnostic tests such as myocardial perfusion scintigraphy [134], electron beam computerized tomography [135] and/or coronary angiography [136] should be considered before more vigorous exercise is prescribed. Even in the absence of SMI, a stress test will detect chronotropic incompetence [137] as well as exercise-related hypertension and provide more objective information on the individual fitness level [59]. Ideally, this information should be used to further tailor an exercise program for the individual patient with Type 2 diabetes [138].

8.15 FUTURE RESEARCH

Based on a thorough review of the literature, a more differentiated approach for exercise therapy in Type 2 diabetes has recently been proposed [138]. However, before more differentiated exercise prescription guidelines can be used as clinical treatment guidelines, its medium to long-term efficacy should first be evaluated in more large-scale randomized controlled clinical trials. By definition, most randomized clinical trials completely disregard a patient's free choice or preference for a specific type of exercise. In fact, potentially interested patients may feel excluded, while others may find it difficult to adhere to a non-preferred type of exercise intervention. Therefore, to simulate a more realistic type of health care environment, randomized clinical trials should be performed in which subjects can choose from different exercise programs. Moreover, for each type of exercise intervention control groups should perceive a similar amount of supervision and guidance. Such an approach is likely to result in higher adherence rates and will provide us with more definitive answers on how to implement exercise therapy more effectively in the chain of diabetes health care.

On top of these clinical and methodological issues, recent studies now indicate that genetic factors [139 140] should be considered as well to determine which subgroup Type 2 diabetes patients is likely to benefit the most from tailor made exercise interventions. However, to unravel these genetic influences, well-defined exercise intervention studies with a high compliance rate are warranted. Although such mechanistic studies often do not represent a realistic clinical approach, these studies should provide more insight into the pathomechanics of exercise in Type 2 diabetes patients with a different genetic and/or co-morbidity profile. The combined approach of mechanistic and clinical implementation studies is expected to lead towards more specific and evidence-based exercise prescription guidelines that optimize long-term therapeutic outcome at an affordable socio-economic cost price.Given the size and expanding nature of the Type 2 diabetes pandemic, the field of *Exercise Science & Sports Medicine* has the scientific, socio-economic and medical ethical obligation to contribute to such studies and move the field of diabetes care into action.

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List of abbreviations

^{1}H	single proton hydrogen
1RM	1 repetition maximum
^{31}P	phosporus-31
ACEI	angiotensin converting enzyme inhibitor
ACP	American College of Physicians
ADA	American Diabetes Association
ACSM	American College of Sports Medicine
ADP	adenosine diphosphate
AGE	advanced glycation end-products
AMP	adenosine monophoshate
AMPK	AMP-activated protein kinase
ANOVA	analysis of variance
ARB	angiotensinogen II receptor blocker
ATP	adenosine triphosphate
AU	arbitrary units
BMI	body mass index
BrW	brisk walking
BW	body weight
°C	degree celsius
CGMS	continuous subcutaneous glucose monitoring
CI	confidence interval
CoA	co-enzyme A
CONGA	continuous overall net glycemic action
CV	coefficient of variation
CVD	cardiovascular disease
DAG	diacylglycerol
DEXA	dual X-ray absorptiometry
DBP	diastolic blood pressure
DOMS	delayed onset muscle soreness
EASD	European Association for the Study of Diabetes

EDTA	ethyleen-diamine-tetra-acetate
EMG	electromyography
En%	energy percent
ELISA	Enzyme-Linked ImmunoSorbent Assay
EUDIP	European Union diabetes indicators
FFM	fat free mass
FM	fat mass
FPG	fasting plasma glucose
g	gram
g	gravitation force
GLUT4	glucose transporter-4
h	hour
HbA_{1c}	glycosylated hemoglobin type A1C
HDL-C	high density lipoprotein cholesterol
HF	heart frequency
HIT	high intensity interval training
HOMA(-IR)	homeostasis model assessment insulin resistance index
hsCRP	high-sensitivity C-reactive protein
Hz	hertz
IDF	International Diabetes Federation
IGT	impaired glucose tolerance
IHL	intrahepatic lipids
IL-6	interleukin-6
IMCL	intramyocellular triacylglycerol
iNOS	inducible nitric oxide synthase
ITT	intention to treat
I.U.	international units
J	joule
kD	kilo dalton
kg	kilogram
L	liter
LDL	low density lipoprotein cholesterol
LLMM	leg lean muscle mass
LM	lean mass
M.	*musculus*
MAP	mean arterial blood pressure
MCP-1	monocyte chemoattractant protein-1
MDP	mean diastolic blood pressure
MF	medical fitness
min	minute
MJ	megajoules

mM	millimolair
mmHg	millimeter mercury
MR(I)	magnetic resonance (imaging)
MRS	magnetic resonance spectroscopy
MSP	mean systolic bloodpressure
NEFA	non-esterified fatty acids
NIRS	near-infrared spectroscopy
NMR	nuclear magnetic resonance
NCS	nerve conduction study
NS	not significant
OGIS	oral glucose insulin sensitivity
OGLA	oral blood glucose lowering agents
OGTT	oral glucose tolerance test
ORO	oil-red-O
P	probability value
P_i	inorganic phosphate
PAS	periodic acid-Schiff
PCr	phosphocreatine
PDE	phosphodiesters
PP	post prandial
PPAR-α	peroxisome proliferators activated receptor α
PRT	progressive resistance training
Q_{max}	maximal aerobic capacity in muscle
QoL	quality of life
RAGE	receptor for AGE
RAS	renin-angiotensin system
RES	resistance exercise
RAND-36	36-Item Short Form Health Survey developed by RAND Corporation
RBP-4	retinol binding protein-4
ROS	reactive oxygen species
s	second
SBP	systolic blood pressure
SD	standard deviation
SDH	succinate dehydrogenase
SEM	standard error of the mean
SNS	sympathetic nervous system
SOCS	suppression of cytokine signalling
SU	sulfonylurea
T2D	Type 2 diabetes
TBLM	total body lean mass
T-CHOL	total cholesterol

TNF-α	tumour necrosis factor α
TRIG	triacylglycerol
UV	ultraviolet
$\dot{V}O_2$	oxygen uptake
$\dot{V}O_{2peak}$	peak oxygen uptake
V_{PCr}	initial PCr recovery rate
W	watt
WC	waist circumference
WHO	World Health Organisation
wk	week
W_{max}	maximal workload capacity

Summary/Samenvatting

SUMMARY

Exercise therapy is considered an important cornerstone in the prevention and treatment of Type 2 diabetes. For practical, economical as well as medical reasons, its clinical application is still underutilised. This doctoral thesis describes a variety of studies that investigate the feasibility and efficacy of both short-, medium- and long-term exercise interventions in different subpopulations Type 2 diabetes patients. Novel research methodologies are introduced to provide more insight into the clinical benefits of exercise intervention in the diabetes state.

In **chapter 2**, continuous subcutaneous glucose monitoring (CGMS) was used to investigate 24 h blood glucose profiles in Type 2 diabetes patients on oral blood glucose lowering medication. In the healthy control group, hyperglycemia was only present during 2±1% throughout the day, while in the Type 2 diabetes patients hyperglycemia was experienced for as much as 55±7% of the time (13±2 h / 24 h) while using the same standardized diet. Breakfast-related hyperglycemia contributed most to the total amount of hyperglycemia and postprandial glycemic instability. In the diabetes patients, blood HbA_{1c} contents correlated well with the duration of hyperglycemia and the postprandial glucose responses. However, our CGMS measurements show that standard measures for glycemic control underestimate the amount of hyperglycemia prevalent during real-life conditions in Type 2 diabetes. Given the macro- and microvascular damage caused by postprandial hyperglycemia, CGMS provides an excellent tool to evaluate alternative therapeutic strategies aimed at reducing hyperglycemic blood glucose excursions.

Chapter 3 describes a study in which we applied an *in vivo* method using ^{31}P nuclear magnetic resonance spectroscopy (MRS) to assess mitochondrial function and compared the outcome with *in vitro* markers of muscle oxidative capacity in a group of long-term insulin-treated Type 2 diabetes patients. Several ^{31}P MRS parameters of mitochondrial respiratory function showed moderate to good correlations with the percentage of type-I fibres and type-I fibre-specific succinate dehydronase (SDH)-activity (Pearson's R between 0.70-0.75) as a marker for oxidative capacity in skeletal muscle tissue. *In vivo* and *in vitro* parameters of local mitochondrial respiration also

correlated well with whole body fitness level ($\dot{V}O_{2peak}$) in these patients (Pearson's R between 0.65-0.90). These good correlations between *in vivo* and *in vitro* measurements of mitochondrial respiratory function in long-term, insulin-treated Type 2 diabetes subjects, justify the use of ^{31}P MRS as a means to assess mitochondrial function in relation to Type 2 diabetes.

In **chapter 4** we applied CGMS to evaluate whether a single bout of combined resistance and high-intensity interval exercise improves 24 h glycemic control in patients with long-standing, insulin-treated, Type 2 diabetes under free-living conditions. The prevalence of hyperglycemic blood glucose excursions was reduced by 39% over a 24 h period (equivalent to 3 h) following an acute bout of 45 min moderate-intensity exercise. Average glucose concentrations 24 h before and after the exercise bout did not differ significantly. Mean glucose concentrations and the prevalence of hyperglycemic periods correlated well with baseline blood HbA_{1c} content (Pearson's R=0.69). However, in accordance with the results presented in **chapter 2** these standard measures for glycemic control underestimate glycemic instability in insulin-treated Type 2 diabetes patients. Nevertheless, our results indicate that on top of intense pharmaceutical blood glucose lowering therapies, an acute bout of exercise effectively reduces the prevalence of hyperglycemia over a 24 h period under free-living conditions in these deconditioned, long-standing Type 2 diabetes patients.

In **chapter 5**, we assessed the feasibility and short-term benefits of a specifically designed exercise program for the category long-standing Type 2 diabetes patients on exogenous insulin treatment. After 10 wks of progressive resistance and high intensity interval training muscle strength and maximum workload capacity increased substantially. Furthermore, mean arterial blood pressure declined and the gradual rise in exogenous insulin requirements was attenuated. These benefits were not accompanied by changes in oxidative capacity, intramyocellular lipid or glycogen content, blood HbA_{1c} content, blood adiponectin, TNF-α and/or cholesterol concentrations. Short-term resistance and interval exercise training is feasible and may provide a better framework for future exercise intervention programs in the treatment of long-standing, deconditioned, Type 2 diabetes patients.

Subsequently in **chapter 6**, we report on the medium-term health benefits of this combined progressive interval and resistance type of exercise training. All subjects completed the study and showed on average a high compliance (>83%) to the prescribed exercise sessions. After 5 months, combined exercise training had significantly reduced blood HbA_{1c} contents with 0.4% and showed a tendency for lower fasting plasma glucose concentrations. Compared to baseline this was associated with an attenuated rise in exogenous insulin requirements, increased exercise performance capacity, increased leg lean muscle mass and reduced truncal fat mass. Cardiovascular risk was reduced as mean arterial pressure decreased with 7.7 mmHg. The combined exercise intervention did not result in changes in either ^{1}H MRS derived IMCL content, ^{31}P MRS derived parameters reflecting *in vivo* muscle oxidative

capacity, or whole body fitness levels ($\dot{V}O_{2peak}$). Given the overall health benefits, it is concluded that long-term, insulin-treated Type 2 diabetes patients should be stimulated to participate in specifically designed exercise intervention programs that combine low-impact resistance and high-intensity interval type exercise training.

Despite the growing body of scientific evidence on the health benefits of exercise, most meta-analyses report a lack of studies that have tried to assess the long-term efficacy of exercise prescription in Type 2 diabetes. In **chapter 7** we assessed the long-term clinical health benefits of randomized prescription of 12 months of supervised group-based brisk-walking versus a more individualized medical fitness program as addendum to primary diabetes care. After 12 months, only 40% of the 92 participants were still actively participating in the structured exercise program consisting of 3 times a week 60 min brisk walking or medical fitness. Besides motivational problems, 50% of the dropout was attributed to overuse injuries. Mean arterial blood pressure declined with 7.9 mmHg, while glycemic control, fasting lipid profile, physical fitness, body weight and quality of life (RAND36) remained unchanged in both groups. Post-hoc analyses indicated that patients with HbA_{1c}>7.5% prior to intervention significantly reduced blood HbA_{1c} content with 0.9%, with no differences between groups. Overall, metabolic and cardiovascular changes did not differ between brisk walking and medical fitness. As such, our long-term exercise intervention study shows that group-based brisk walking represents a low-cost but equally effective interventional strategy to improve cardiovascular risk profile and glycemic control when compared to the implementation of more individualized medical fitness.

In **chapter 8** the results and conclusions of the previous chapters are integrated and the clinical implications of the work presented in this thesis are further discussed. Therapeutic guidelines for tailored exercise interventions in Type 2 diabetes patients are proposed and suggestions for future research are provided.

The overall conclusion of the work presented in this dissertation is that in future research a combined approach of mechanistic and clinical implementation studies is expected to lead towards more specific and evidence-based exercise prescription guidelines that optimize long-term therapeutic outcome at an affordable socio-economic cost price. Given the size and expanding nature of the Type 2 diabetes pandemic, the field of *Exercise Science & Sports Medicine* has the scientific, socio-economic and medical ethical obligation to contribute to such studies and move the field of diabetes care into action.

SAMENVATTING

Type 2 diabetes, in de volksmond ook wel ouderdomssuiker genoemd, werd van oudsher beschouwd als een strikt medisch probleem. Echter, de sterke toename van Type 2 diabetespatiënten in onze Westerse samenleving zal binnen afzienbare tijd leiden tot een forse maatschappelijke ziektelast die op zijn beurt weer zal leiden tot aanzienlijke logistieke en financiële problemen in de gezondheidszorg. Vrijwel iedereen kent inmiddels wel iemand met ouderdomssuiker, maar wellicht nog verontrustender is het feit dat deze stofwisselingsziekte op steeds jongere leeftijd, en inmiddels ook bij jonge kinderen, wordt vastgesteld.

In **hoofdstuk 1** wordt een uitgebreid overzicht van de literatuur beschreven. Hieruit blijkt onder andere dat regelmatige lichaamsbeweging reeds van oudsher beschreven is als een belangrijke hoeksteen in de preventie en behandeling van Type 2 diabetes. Om uiteenlopende redenen wordt 'Bewegen-op-Recept' heden ten dage in de klinische praktijk nog maar in zeer beperkte mate als therapeuticum ingezet. Dit proefschrift beschrijft een aantal wetenschappelijke studies waarin de haalbaarheid en effectiviteit van zowel korte-, midden-, als lange-termijn beweeginterventies in verschillende subpopulaties Type 2 diabetespatiënten wordt onderzocht. Tevens worden een aantal nieuwe methoden geïntroduceerd waarmee we nog beter de klinische voordelen van inspanningsinterventies in Type 2 diabetes kunnen onderzoeken.

Om een beter inzicht in de glucosehuishouding van Type 2 diabetespatiënten te verkrijgen werd in **hoofdstuk 2** onder gestandaardiseerde voeding- en leefomstandigheden middels een microdialyse techniek (zgn. continue glucose monitoring of CGMS) continue de 24 uurs glucosewaarden in het onderhuids weefsel gemeten. Ter controle werden deze metingen ook verricht in een normaal glucose tolerante controle groep met dezelfde lichaamsstelling. In de gezonde controle groep blijken hoge glucose pieken slechts ongeveer 2% van de dag op te treden. Ondanks het gebruik van orale bloedglucose verlagende middelen blijken Type 2 diabetespatiënten onder dezelfde leef- en dieetomstandigheden gedurende 55±7% (oftewel 13±2 uur) van de dag zogenaamde hyperglykemische episoden door te maken. De hyperglykemie na het ontbijt blijkt het sterkst bij te dragen aan de ontregeling van de bloedglucose spiegel. In de diabetespatiënten bleek de mate van hyperglykemie goed te correleren met bloed HbA_{1c} gehalte. Echter, onze CGMS metingen tonen tegelijkertijd aan dat deze standaard maat voor bloedsuikerregulatie de ontregeling in de glucosehuishouding aanzienlijk onderschat. Deze glucoseontregeling vormt een belangrijke factor in het ontstaan van vaatwandschade in zowel de kleine als grote bloedvaten. Deze vaatwandbeschadigingen leiden op de lange termijn tot een scala aan diabetes gerelateerde complicaties. Deze studie laat zien dat continue glucose monitoring een zeer geschikte meettechniek is om alternatieve bloedglucose verlagende therapieën gericht op het voorkómen van lange-termijn complicaties op directere wijze te evalueren.

In **hoofdstuk 3** wordt een studie beschreven waarin, aan de hand van fosfor-31 magnetische resonantie spectroscopie (^{31}P MRS), de *in vivo* functie van de mitochondriën binnenin de spiercel wordt vergeleken met de oxidatieve *in vitro* kenmerken van de skeletspier bij een groep insuline behandelde patiënten met lange-termijn Type 2 diabetes. Uit deze studie blijkt dat een aantal dynamische ^{31}P MRS parameters redelijk tot goed (Pearson's correlatie coëfficiënt van 0.70-0.75) correleren met het percentage oxidatieve type I vezels en spiervezelspecifieke succinaat dehydrogenase (SDH) activiteit als maat voor oxidatieve capaciteit in de type I spiervezels. Tevens bleken in deze patiënten bovenstaande *in vivo* en *in vitro* parameters van de lokale mitochondriële ademhalingsketen goed te correleren (Pearson's R 0.65-0.90) met het zuurstofopname vermogen ($\dot{V}O_{2peak}$) gemeten op heel lichaamsniveau. Dergelijke goede correlaties tussen *in vivo* en *in vitro* metingen in insuline behandelde patiënten met lange-termijn Type 2 diabetes rechtvaardigen het gebruik van ^{31}P MRS ter evaluatie van de mitochondriële respiratie in relatie tot Type 2 diabetes.

In **hoofdstuk 4** wordt opnieuw de CGMS-methodiek toegepast om onder normale dagelijkse omstandigheden bij insuline behandelde Type 2 diabetespatiënten het effect van een eenmalige kracht- en intervaltraining op de 24 uurs bloedglucoseregulatie te meten. Uit de metingen blijkt dat de hyperglykemische episoden over een periode van 24 uur na training met 39% (oftewel 3 uur) worden verminderd. De gemiddelde 24 uurs glucoseconcentratie bleef daarentegen onveranderd. Zowel de prevalentie van hyperglykemie als de gemiddelde hyperglykemie bleek opnieuw goed te correleren met het HbA_{1c} niveau (Pearson's R 0.69). Echter, in overeenstemming met de resultaten gepresenteerd in **hoofdstuk 2** blijken deze standaard maatstaven voor glykemische controle ook in insuline behandelde Type 2 diabetespatiënten de glykemische instabiliteit te onderschatten. Desalniettemin geven onze resultaten aan dat acute inspanning als aanvulling op intensieve farmaceutische behandeling de prevalentie van de 24-uurs glucosehuishouding direct kan verbeteren in deze subgroep gedeconditioneerde Type 2 diabetespatiënten.

Vervolgens wordt in **hoofdstuk 5** de haalbaarheid en korte termijn effecten van een specifiek ontwikkeld trainingsprogramma voor deze groep met insuline behandelde Type 2 diabetespatiënten nader onderzocht. Na 10 weken progressieve kracht- en intervaltraining wordt een sterke toename in spierkracht en maximaal inspanningsvermogen gezien.Verder neemt de gemiddelde arteriële bloeddruk af en is er een verminderde toename in de exogene insuline behoefte. Deze gunstige veranderingen blijken echter niet samen te gaan met een verandering in zuurstofopname vermogen, de hoeveelheid opgeslagen intramyocellulaire lipiden (IMCL), opgeslagen spierglycogeen, bloed HbA_{1c} gehalte, bloed adiponectine, TNF- α, en/of cholesterol concentraties. Op basis van deze positieve bevindingen kan worden geconcludeerd dat gestructureerde beweegprogramma's bestaande uit zowel kracht- als intervaltraining haalbaar zijn en kunnen dienen als raamwerk voor toekomstige beweeginterventies in gedeconditioneerde patiënten met Type 2 diabetes. Aansluitend

worden in **hoofdstuk 6**, de middenlange termijn gezondheidseffecten van dit gecombineerd interval- en krachttrainingsprogramma gerapporteerd. Ondanks de lage fysieke belastbaarheid is er geen uitval van proefpersonen en blijkt men in staat om gemiddeld 83% van alle trainingsbijeenkomsten bij te wonen. Na 5 maanden blijkt deze gecombineerde beweeginterventie het bloed HbA_{1c} gehalte met gemiddeld 0,4 procentpunt te laten dalen terwijl ook de nuchtere glucosespiegel neigt te dalen. Ten opzichte van de uitgangswaarden zien we tegelijkertijd een verminderde toename in de exogene insuline behoefte, een sterke toename van het inspanningsvermogen, een toename in beenspiermassa en een significante afname van de vetmassa in de romp. Gezien een gemiddelde bloeddrukafname van 7,7 mmHg, kan men spreken van een gunstiger cardiovasculair risicoprofiel. Bovengenoemd beweegprogramma resulteerde uiteindelijk niet in veranderingen in IMCL concentratie (gemeten met proton (^{1}H)-MRS) of *in vivo* (met ^{31}P MRS) bepaalde mitochondriële respiratie of op de fiets gemeten zuurstofopname vermogen ($\dot{V}O_{2peak}$) tijdens maximale inspanning. Op basis van de gunstige gezondheidseffecten wordt geconcludeerd dat langetermijn, insuline-behandelde Type 2 diabetespatiënten zouden moeten worden gestimuleerd om te participeren in dergelijke specifiek ontworpen beweeginterventieprogramma's. Deze programma's zouden moeten bestaan uit relatief laag-intensieve krachttrainingsvormen en hoog-intensieve intervaltraining.

Ondanks al het wetenschappelijk bewijs omtrent de gezondheidsbevorderende effecten van gestructureerde beweegprogramma's, rapporteren de meeste meta-analyses een gebrek aan lange-termijn studies die de lange termijn effecten van 'Bewegen-op-Recept' bij Type 2 diabetes hebben onderzocht. In **hoofdstuk 7** zijn binnen een eerstelijns setting derhalve op gerandomiseerde wijze de lange-termijn gezondheidseffecten van 12 maanden gesuperviseerde groepstrainingen bestaande uit sportief wandelen vergeleken met een meer geïndividualiseerd medisch fitnessprogramma als uitbreiding van standaard diabeteszorg. Beide programma's bestonden uit 3 x per week 60 min sportief wandelen of medische fitness. Na 12 maanden namen nog slechts 40% van de 92 deelnemers actief deel aan beide beweegprogramma's. Naast gebrek aan motivatie, blijkt 50% van de uitval te worden veroorzaakt door overbelastingsklachten van het bewegingsapparaat. Op groepsniveau zagen we desondanks een gemiddelde arteriële bloeddruk daling van 7.9 mmHg, terwijl de glucosehuishouding, het nuchter lipidenprofiel, fysieke fitheid, lichaamsgewicht en kwaliteit van leven (gemeten middels de RAND36 vragenlijst) in beide interventiegroepen onveranderd bleven. Een post-hoc analyse van de onderzoeksgegevens geeft aanwijzingen dat, onafhankelijk van het type beweeginterventieprogramma, diabetespatiënten met een uitgangs-HbA_{1c} boven de 7.5% na 1 jaar een significante HbA_{1c} daling van gemiddeld 0.9 procentpunt boeken.

Globaal gesproken blijken ten opzichte van de uitgangswaarden de metabole en cardiovasculaire aanpassingen gelijkwaardig tussen de sportief wandel- en medische fitness groep. Derhalve laat deze lange-termijn beweeginterventie-studie zien dat

binnen de eerstelijns diabeteszorg een sportief wandelprogramma goedkoper, maar even effectief als een medisch fitness programma is in het verbeteren van het cardiovasculaire risicoprofiel.

In **hoofdstuk 8** worden de resultaten en conclusies van voorgaande hoofdstukken geïntegreerd en worden de verdere klinische implicaties van de onderzoeken uit dit proefschrift bediscussieerd. Vervolgens worden een aantal therapeutische richtlijnen voor 'Bewegen-op-Recept' in patiënten met Type 2 diabetes voorgesteld en suggesties gedaan voor verder onderzoek op dit terrein.

De algehele conclusie van deze dissertatie luidt dat toekomstige beweeginterventie-studies in Type 2 diabetes vooral zouden moeten bestaan uit een combinatie van mechanistisch en klinisch toegepast onderzoek. Het is de verwachting dat dergelijk translationeel onderzoek zal leiden tot meer specifieke en wetenschappelijk onderbouwde therapeutische richtlijnen om de lange termijn effecten van beweeginterventieprogramma's in Type 2 diabetespatiënten verder te optimaliseren tegen een maatschappelijke aanvaardbare kostprijs. Gezien de grootte en te verwachtte toename van het aantal Type 2 diabetespatiënten in onze maatschappij, heeft het veld van de Bewegingswetenschappen en Sportgeneeskunde de wetenschappelijke, sociaaleconomische en medisch-ethische verplichting om een belangrijke bijdrage te leveren aan dergelijke onderzoek om daadwerkelijk het hele werkveld rondom de diabeteszorg in beweging te brengen.

Epiloog

Nu ik ben aangekomen bij het meest gelezen, maar ongetwijfeld minst geciteerde deel van dit proefschrift, rest mij de uitdaging om in mooi proza terug te blikken op de 4 jaargetijden van mijn promotietraject. Enkele clichés hierbij zijn helaas onvermijdelijk maar het moet toch echt gezegd, dit proefschrift was zonder de vele 'vaste krachten' en 'seizoensarbeiders' nooit en te nimmer in deze vorm tot stand gekomen.

In tegenstelling tot wat wellicht een ieder zou verwachten begint mijn promotietraject in het winterseizoen.Van oudsher kenmerkt dit traject zich voornamelijk door het feit dat buiten nog niet in de tuin, akker of boomgaard kan worden gewerkt en er veel tijd is om na te denken over waarom bepaalde oogsten zijn mislukt en na te pluizen welke wetenschappelijk vruchten er in de voorafgaande seizoenen door anderen inmiddels zijn geplukt.

In de voorafgaande herfst ben ik namelijk de inspanningsfysioloog (annex pianist) Hans Keizer tegen het lijf gelopen. Geïnspireerd door zijn pionierswerk (en een voor mij zeer herkenbare manier van associatief denken) ben ik geënthousiasmeerd om met hem als dagelijks begeleider en Goof Schep als opleider sportgeneeskunde een klinisch inspanningsfysiologisch promotieonderzoek onder supervisie van prof. dr. Harm Kuipers op te starten in het Máxima Medisch Centrum (destijds St. Joseph Ziekenhuis) te Veldhoven. Deze mogelijkheid doet zich voor dankzij een VWS subsidie ter stimulering van sportgeneeskundig onderzoek. Ik weet het niet helemaal zeker maar ik denk toch dat ik de Vereniging voor Sportgeneeskunde en collega Maarten Koornneef van VWS moet bedanken voor hun lobbywerk in deze.

Aangezien eerdere onbezoldigde wetenschappelijk uitstapjes op het terrein van de voetbiomechanica en dynamische drukmetingen door externe krachten worden gefrustreerd (Craig Nevin, one day you will be understood!), is dit voor mij een uitgelezen mogelijkheid om als sportarts i.o. mijn wetenschappelijke interesses en ambities om te zetten in een doctors titel. Ook al vielen de eerste resultaten van onze vibratie-

trainings-experimenten bij diabetes en COPD patiënten (waarvoor mijn oprechte dank aan Martin Huizing, Gerrit van Kranenburg, de 2 Guido's, Ronald Erdtsieck en Stijn Mol) erg tegen, ik leer elke dag bij en bij eenieder is er het volste vertrouwen dat de onderzoekscultuur van het ouwe St. Joep een ideale setting is om klinisch onderzoek te doen. Eerder is namelijk gebleken dat mede door een uitstekend functionerende medische ethische toetsingscommissie (waarvoor dank aan alle leden!), zelfs invasief en translationeel inspanningsfysiologisch trainingsonderzoek tot de mogelijkheden behoort in dit perifere ziekenhuis.

Tijdens het smeden van nieuwe plannen met een wel heel erg multidisciplinair begeleidingsteam blijken plotseling de financiële middelen toch te beperkt om grote aantallen diabetespatiënten binnen het gefuseerde MMC te gaan trainen. Terwijl Gérard Koot nog ontzettend zijn best doet om alle plannen binnen de ziekenhuisbegroting te laten passen, ontstaan er 2 clubleidingen met duidelijk andere visies over de juiste opstelling van het elftal. Ik zit er midden tussenin en probeer tevergeefs het compromis te zoeken. Het is uiteindelijk toch mijn 1e promotor Harm Kuipers die knopen doorhakt en duidelijk de prioriteiten bij de wetenschap legt. Achteraf bezien de juiste keuze en het is dan toch mede dankzij jouw bestuurlijke ervaring, Harm, dat er meer structuur en rust is gekomen. Toch blijft er een nare nasmaak kleven aan dit winterseizoen. Beste Goof, het was voor jou ongetwijfeld een teleurstelling om middenin het wedstrijdseizoen te moeten merken dat duursporters en middenafstandsatleten uiteenlopende ambities kunnen hebben. Het respect voor je wilskracht en doorzettingsvermogen is er bij mij nog steeds en ik hoop dat het oogstseizoen van de post-chemo trainingen en die onwillige liesslagaders voor jou spoedig mag aanbreken. Zonder de immer in Access calculerende collega Hoogeveen (Thx Adwin!), en een bezielend secretariaat onder leiding van Jolande, alsmede de coöperatieve opstelling van de afdeling Cardiologie en SMA Cardiosport was mijn patiëntgebonden onderzoek nooit uitvoerbaar gebleken. Bedankt dus om in alle vrijheid een stukje innovatieve sportgeneeskunde in een perifeer ziekenhuis te kunnen bedrijven.

Op mijn eigen atletiekbaantje vriest het ondertussen nog steeds en tempoloopjes zitten er nog steeds niet in. Inmiddels vinden er wel verkennende schaatstochtjes op natuurijs plaats naar het Diagnostisch Centrum Eindhoven (Luc Harms, nog welgemeend bedankt voor je coöperatieve opstelling in deze) en de Technische Universiteit Eindhoven waar aan het eind van een onverlichte tunnel een enorme witte magneet maar nauwelijks patiënten weet aan te trekken.

Afgeleid door de ruimhartige ondersteuning vanuit HTI, lijkt de Inspectra™ in eerste instantie licht in de duisternis te brengen. Echter, het NIRS-apparaat zakt weldra door het ijs wanneer de immer kritische en energieke Joyce Kramer samen met Carola van Pul het magische licht vakkundig ontmaskert. Beste Pieter, Carola en Chris,

het was ontzettend fijn om een tijdje in jullie klinische werkplaats te mogen kijken en als relatief software-analfabeet van jullie expertise gebruik te mogen maken. Jullie meerwaarde als klinisch fysici voor een ziekenhuis staat wat mij betreft buiten kijf!

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Tegelijkertijd met een grote voorjaarsschoonmaak wordt er in het MMC hard gewerkt om onder hoge tijdsdruk een onmogelijke groep diabetespatiënten samen te stellen. Dat lukt wonderwel nog ook en hiervoor wil ik Louis, Harm, Hannie, Ronald, Gerrie en alle anderen die hierbij betrokken waren, expliciet bedanken. Carla van Mensvoort en de afdeling diëtetiek bedank ik voor de verwerking van de vele voedingslijsten en de prettige manier van samenwerking. Ondanks alle bezuinigingen weet zelfs de afdeling fysiotherapie dankzij Machteld Jongmans en Paul Chatrou toch nog 0.1 fte en trainingsruimte vrij te plannen. Ook Ad Smets, als onvermoeibare klinisch neurofysioloog, blijkt plotsklaps ook een enthousiasmerende docent voor promovendus en BW-student. Ad, bedankt voor jouw onvoorwaardelijk steun en bijdrage aan het objectiveren van diabetische neuropathie. Maar ook zonder het creatieve boekhouden van Jef Pluymen en de expertise van de diverse MMC-laboranten waren de EMG- en DEXA-metingen niet mogelijk gebleken.

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Echter, op het moment dat het voorjaarszonnetje echt lijkt door te breken treedt mijn oorspronkelijk co-promotor tamelijk onverwachts terug als dagelijks begeleider. Hans, we konden al goed met elkaar overweg, maar ik ben blij dat onze leermeester-gezel relatie tot een evenwaardige vriendschap is uitgegroeid en hoop dat in de toekomst jouw idealen door je wetenschappelijke nakomelingen zullen kunnen worden gerealiseerd. Bedankt voor je geloof en blind vertrouwen in mij en de kansen die je me hebt gegeven.
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Curriculum vitae and publications

CURRICULUM VITAE

Name:	Stephan Florent Eugenie PRAET
Date of birth:	01-03-1971
Place of birth:	Hulst, the Netherlands
Current Position:	Sports & Exercise Physician Pro*sano* Sports & Exercise Medicine, ZorgSaam Hospitals, Terneuzen, The Netherlands
E-mail:	Stephan.Praet@BW.unimaas.nl / SPraet@sportsmedicine.nl
Key words:	sports & exercise medicine, Type 2 diabetes, clinical foot biomechanics
Hobbies:	running, cycling (road-race and ATB), wine & dine, travelling

HIGHER EDUCATION

1989 – 1994
Master of Science, Department of Human Movement Sciences,
Vrije University Amsterdam, The Netherlands
Major: Functional Anatomy
Minor: Exercise Physiology
Psychology of Human movement

1992 – 1993
Undergraduate Program Exercise and Sports Sciences, Penn State University, USA

1994 – 1997
Master's Program, School of Medicine,
Vrije University Amsterdam, The Netherlands

1997 – 1999
Clinical Training Period, School of Medicine,
Vrije University Amsterdam, The Netherlands
(Medical Degree: October 29th , 1999)

2000 – 2004
Advanced Clinical Training in Sports Medicine
Orthopaedic Surgery / Cardiology,
Máxima Medical Center, Veldhoven, the Netherlands
Registered as sports physician December 1st, 2004

2003 – 2007
PhD candidate, NUTRIM research institute, Department of Movement Sciences,
Faculty of Health, Medicine and Life Sciences, Maastricht University,
The Netherlands.
Topic: "*Exercise therapy in Type 2 Diabetes*",
Thesis defense December 14th, 2007.
Supervisors: prof. H. Kuipers, MD PhD
prof. C.D.A. Stehouwer, MD PhD
Co-supervisor: L.J.C. van Loon, PhD

RESEARCH EXPERIENCE ABROAD

1992 – 1993
Internship at the Center for Locomotion Studies (CELOS), Penn State University, State College, U.S.A., supervised by prof. P.R. Cavanagh.

1998
Medical Elective student at Sports Science Institute of South Africa, University of Cape Town, South Africa,
supervised by prof. T.D. Noakes / prof. C. Vaughan,
Topics 1) *'Posterior impingement syndrome in fast cricket bowlers'.*
2) *'Right vs. left foot pressure dynamics in running'.*

STUDY GRANTS:

1992 – 1993
Grant-in-Aid from the Athletic Department, Penn State University, USA

1998 – 1999
Study Grant from Foundation of the Vrijevrouwe of Renswoude, The Hague, NL

1998 – 1999
Study Grant Schuurman Schimmel-van Outeren Foundation, Aerdenhout, NL

CAREER DESCRIPTION AND PROFESSIONAL VISION

As a result of his combined training in Human Movement Sciences, Sports Medicine and Clinical Exercise Physiology, Stephan Praet has been involved in the development of exercise testing and training programs for COPD, heart failure and post-chemotherapy cancer patients.

During his specialisation program in Sports Medicine he got inspired by the pioneering work from dr. Hans Keizer, which resulted in a PhD program on Exercise therapy in Type 2 Diabetes at the NUTRIM Research institute, Maastricht University/ Academic Hospital Maastricht, supervised by Prof. dr. Harm Kuipers, Prof. dr. Coen Stehouwer and dr. Luc van Loon. During the past 4 years he has been involved in research projects on the clinical application of exercise therapy in Type 2 diabetes in both primary and secondary health care settings with over 100 Type 2 diabetes patients involved.

Through transfer of knowledge from Sports Medicine and Exercise Science his clinical research is aimed at improving the clinical care of Type 2 diabetes patients. Besides the current focus on exercise physiology he also has a special interest in clinical foot biomechanics, including the diabetic foot.

Currently he is a member of the scientific reviewing board of PLoS Medicine, the European Journal of Sports Science and the Clinical Journal of Sports Medicine and advisor of the Sports and Exercise Theme Group of the Dutch Diabetes Association (DVN).

Through his basic and applied research and as a member of several professional (Dutch Sports Medicine Assocation, American College of Sports Medicine, South African Sports Med Assoc, FIMS and European Association for the Study of Diabetes) and patients' (Dutch Diabetes Association) organizations he tries to draw further attention towards the clinical application of exercise as a strategy to improve the metabolic control and quality of life in patients with diabetes and other chronic diseases.

Dr. Stephan Praet is a regular invited speaker on topics like the health benefits of exercise intervention in Type 2 diabetes, clinical foot biomechanics and gait analysis through dynamic foot pressure measurements.

PUBLICATIONS

Praet S.F.E., Jonkers R.A.M., Schep G., Stehouwer C.D.A., Kuipers H., Keizer H.A., van Loon L.J.C.: 'Long-standing, insulin-treated type 2 diabetes patients with complications respond well to a resistance type exercise program', accepted Eur J Endocrinology.

Praet S.F.E. and van Loon L.J.C.: 'Optimizing the therapeutic benefits of exercise in type 2 diabetes', J Appl Physiol 2007; 103(4):1113-20.

De Feyter H.M., **Praet S.F.E.**, van den Broek, N.M.A., Kuipers H., Stehouwer, C.D.A. Nicolay K., Prompers J.J., van Loon L.J.C.: 'Exercise training improves glycemic control in long-standing, insulin treated type 2 diabetes patients', Diabetes Care, 2007:30(10):2511-3.

Manders R.J., **Praet S.F.E.**, Vikstrom M.H, Saris W.H. and van Loon L.J.: 'Protein hydrolysate co-ingestion does not modulate 24 h glycemic control in long-standing type 2 diabetes patients', Eur J Clin Nutr, epub ahead of print August 22nd, 2007.

Praet S.F.E, Feyter H.M., Jonkers R.A., Nicolay K, Pul C.V., Kuipers H, van Loon L.J.C. and Prompers J.J. (31)P: 'MR spectroscopy and in vitro markers of oxidative capacity in type 2 diabetes patients', Magn Reson Mater Phy. 2006 19;321-31.

Manders R.J., **Praet S.F.E.**, Meex R.C., Koopman R, de Roos A.L., Wagenmakers A.J., et al.: 'Protein hydrolysate/leucine co-ingestion reduces the prevalence of hyperglycemia in type 2 diabetic patients', Diabetes Care. 2006;29:2721-2.

Praet S.F.E., Manders R.J.F., Meex R.C.R., Lieverse A.G., Stehouwer C.D.A., Kuipers H., Keizer H.A., van Loon L.J.C.: 'Glycemic instability is an underestimated problem in type 2 diabetes', Clin. Sci. 2006;111:119-26.

Praet S.F.E., Manders R.J., Lieverse A.G., Kuipers H., Stehouwer C.D., Keizer H.A., et al.: 'Influence of Acute Exercise on Hyperglycemia in Insulin-Treated Type 2 Diabetes', Med Sci Sports Exerc. 2006;38:2037-44.

Praet S.F.E., Louwerens J.W.: 'The influence of shoe design on plantar pressures in neuropathic feet', Diabetes Care. 2003;26:441-5.

Toussaint H.M., Commissaris D.A., Van Dieeën J.H., Reijnen J.S., **Praet S.F.E.**, Beek P.J.: 'Controlling the Ground Reaction Force During Lifting', J. Mot Behav. 1995;27:225-34.

PEER REVIEWED SCIENTIFIC ABSTRACTS

Praet S.F.E., van Rooij L.S.J., et al.: 'Long-term brisk walking vs medical fitness in type 2 diabetes', 43rd Annual meeting of the European Association for the Study of Diabetes, Sept. 17-21, 2007, Amsterdam, The Netherlands.

Praet S.F.E., Bronts H.M. and van Loon L.J.C.: 'Proper shoe design alone may prevent diabetic foot injuries', 5th International Symposium on the Diabetic Foot, May 9-12, 2007, Noordwijkerhout, The Netherlands

Praet S.F.E., de Feyter H.M., et al.: 'Response of insulin-treated long-term Type 2 diabetes subjects to a 5-months exercise training programme', 42nd Annual meeting of the European Association for the study of Diabetes, Sept. 14-17 2006, Copenhagen-Malmoe, Denmark/Sweden.

Praet S.F.E., Stehouwer C.D.A., et al.: 'Long-standing, insulin-treated type 2 diabetes patients with complications respond well to resistance training', Copenhagen Muscle Research Centre (CMRC) International symposium: 'Exercise, insulin sensitivity and diabetes – what is new?', Sept. 13, 2006, Copenhagen, Denmark.

Praet S.F.E., van Rooij L., Enneking Th and van Loon L.J.C.: 'Metabolic effects in type 2 diabetes following Medical Fitness vs Brisk Walking', 6 months follow-up form the Dutch ELDiaS study, DESA 2006, Diabetes Exercise and Sports Association Congress, Sept. 7-9, 2006, Papendal the Netherlands.

Praet S.F.E. and van Loon L.J.C.: 'Plantar Pressure Profiles of Diabetes Patients Outside and Inside a walking shoe.' Bejing International Diabetes Foot & Related Diseases Forum, Bejing, Oct 9-12, 2005, Bejing, China.

Praet S.F.E., Manders R.J., et al.: 'Effect of Exercise on 24hr glucose profile in insulin treated type 2 Diabetes patients', 11th Biennial South African Sports Medicine Association Congress, Cape Town, Sept 28-Oct. 1st 2005.

Praet S.F.E., Schep G., et al.: 'The use of NIRS (InSpectra™) in the diagnosis of sports-related iliac artery flow limitations', IBEC 2003, 13th International Biochemistry of Exercise Conference, Maastricht, 13-16 July, 2003.

Praet S.F.E.: 'Radius of Gyration as a possible parameter for tuning Prosthetic feet', ECSS2001, Köln, 24-28 July 2001.

Praet S.F.E.: 'Sports Pedorthics in the Netherlands: current concepts from a historical perspective.' IVO Kongres, Friedrichshafen, 15-17 September 2000.

Praet S.F.E. and Louwerens J.W.L.: 'Shoe design and plantar pressures in neuropathic feet.' EFAS 2000 3rd congress Stockholm, June, 15-17, 2000 and NOV 2001 (year congress Dutch Orthopaedic Association), Groningen, May 17-18, 2001.

Praet S.F.E., van Blomberg M., et al.: 'Anti-polymeric antibodies, rheumatic complaints and silicone leakage of breast implants'. EULAR 99, Glasgow, June 6-11, 1999.

Praet S.F.E., Wilssens J-P., et al.: 'Right vs. left foot pressure dynamics in running', ISB-99, International Society of Biomechanics Congress, Calgary, August 8-13, 1999.

Praet S.F.E. and Cavanagh P.R.: 'The role of plantar cutaneous mechanoreceptors in human balance reflexes', 2nd World Congress in Biomechanics, Amsterdam, July 10-15, 1994.

POPULAR SCIENTIFIC PUBLICATIONS

Praet S.F.E., 'Wat is het beste beweegadvies bij Type 2 diabetes?', DiabeteSpecialist 6, 23 juni 2007, 11-13.