

## CLINICAL STUDY

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

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## Abstract

**Objective:** To determine the feasibility and the benefits of combined resistance and interval exercise training on phenotype characteristics and skeletal muscle function in deconditioned, type 2 diabetes (T2D) patients with polyneuropathy.

**Design:** Short-term, single-arm intervention trial.

**Methods:** Eleven male T2D patients (age:  $59.1 \pm 7.5$  years; body mass index:  $32.2 \pm 4.0$  kg/m<sup>2</sup>) performed progressive resistance and interval exercise training thrice a week for 10 weeks. Besides primary diabetes outcome measures, muscle strength (MUST), maximal workload capacity ( $W_{\max}$ ), whole-body peak oxygen uptake ( $VO_{2\text{peak}}$ ) and muscle oxidative capacity (MUOX), intramyocellular lipid (IMCL) and glycogen (IMCG) storage, and systemic inflammation markers were determined before and after training. Daily exogenous insulin requirements (EIR) and historic individualized EIR were gathered and analysed.

**Results:** MUST and  $W_{\max}$  increased with 17% (90% confidence intervals 9–24%) and 14% (6–21) respectively. Furthermore, mean arterial blood pressure declined with 5.5 mmHg (–9.7 to –1.4). EIR dropped with 5.0 IU/d (–11.5 to 1.5) compared with baseline. A decline of respectively –0.7 mmol/l (–2.9 to 1.5) and –147  $\mu\text{mol/l}$  (–296 to 2) in fasting plasma glucose and non-esterified fatty acids concentrations were observed following the intervention, but these were not accompanied by changes in  $VO_{2\text{peak}}$ , MUOX, IMCL or IMCG, and blood glycolysated haemoglobin, adiponectin, tumor necrosis factor- $\alpha$  and/or cholesterol concentrations.

**Conclusion:** Short-term resistance and interval exercise training is feasible in deconditioned T2D patients with polyneuropathy and accompanied by moderate improvements in muscle function and blood pressure. Such a specific exercise regimen may provide a better framework for future exercise intervention programmes in the treatment of deconditioned T2D patients.

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## Introduction

Physical exercise has long been recognized as an effective interventional strategy in the treatment of type 2 diabetes (T2D). 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 T2D patients. However, studies assessing the effects of exercise training in long-standing, insulin-treated, T2D patients with complications are generally lacking. The latter is partly due to the many difficulties encountered when trying to define an appropriate exercise programme 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 programmes.

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 programme for long-standing, insulin-treated T2D patients. To compensate

for neuropathy-related muscle weakness and cardio-respiratory 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) represent a safe and effective type of exercise regimen that may increase maximal workload and peak whole-body oxygen uptake capacity ( $VO_{2\text{peak}}$ ) in deconditioned subjects (16, 17). Since exercise intensity and subsequent muscle fibre-type recruitment patterns during resistance exercise and HIT are likely to be similar, HIT on top of resistance exercise could represent an attractive style of exercise training in deconditioned T2D patients. By directly transferring gains in muscle strength into more functional movements, performance capacity for endurance-type activities may also show greater long-term benefits. As the population of long-term diagnosed T2D 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 deconditioned T2D 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 (MUOX) (24) and intramyocellular lipid (IMCL) (25, 26) and/or glycogen (25) content are associated with the development of skeletal muscle insulin resistance. Although insulin sensitivity has been reported to improve following resistance (27–29) as well as endurance-type (2, 30–32) exercise training, the concomitant structural changes in resting IMCL and/or glycogen content following exercise interventions in T2D 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, 40–43), 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 adipokine 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 T2D patients with polyneuropathy, it would also be appropriate to assess various markers relevant to the inflammatory state (high sensitivity C-reactive protein (hsCRP), tumor necrosis factor (TNF)- $\alpha$ , interleukin (IL)-6 and adiponectin) before and after exercise intervention.

The present study aims to define the feasibility and the clinical benefits of 10 weeks of resistance- and interval-type exercise training in long-standing, insulin-treated T2D 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.

## Methods

### Subjects

Eleven male T2D patients were selected from an outpatient clinic to participate in this hospital-based case-control intervention study. Subjects had been diagnosed with T2D for over  $12.1 \pm 7.0$  years and had been on exogenous insulin treatment for  $7.0 \pm 8.0$  years. They had no history of participating in any regular exercise programme for at least 10 years. All subjects had been on a stable regimen of diabetes medication for at least 3 months before being recruited. Patients using thiazolidinediones and/or  $\beta$ -blockers for less than 6 months, and subjects with impaired liver function (serum-aspartate amino transferase and/or  $\gamma$ -glutamyltransferase  $> 2$  times the 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 four motor (peroneal, tibial, median and ulnar) and three sensory (sural, median and ulnar) nerves using electromyography 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 3 (48). In accordance, an NCS score of 4 or higher was a prerequisite for inclusion in the study.

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 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 sulphonylurea (glimperide). Data on daily insulin requirements preceding the study were gathered by a retrospective search through the individual patient

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

<i>n</i> =11	Baseline	Change	90% CI	<i>P</i> value
Age (years)	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 (IU)	92.5±37.0	–5.0	–11.5 to 1.5	0.196
Body mass index (kg/m <sup>2</sup> )	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
<i>W</i> <sub>max</sub> RAMP test (W)	152±39	21 <sup>‡</sup>	10 to 32	0.006
VO <sub>2peak</sub> per kg BW (ml/kg per min)	24.3±1.4	0.9	–0.2 to 2.1	0.171
%pred VO <sub>2peak</sub>	79.2±15.1	3.1	–0.4 to 6.7	0.138
<i>W</i> <sub>max</sub> Steep RAMP (W)	275±62	41 <sup>‡</sup>	27 to 56	0.000
1RM strength LowerB (kg)	100.6±23.5	18.0 <sup>†</sup>	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)	0.77±0.56	0.07	–0.12 to 0.26	0.520
HbA <sub>1c</sub> (%)	7.63±0.99	–0.18	–0.54 to 0.18	0.386
FPG (mmol/l)	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)	3.4±0.4	0.1	–0.2 to 0.32	0.644
Triacylglycerol (mmol/l)	2.3±0.4	–0.2	–0.9 to 0.2	0.386
NEFA (μmol/l)	459±243	–147	–296 to 2	0.103
hsCRP (mg/l)	2.07±1.77 <sup>a</sup>	0.21	–0.38 to 0.80	0.532
Adiponectin (μg/l)	5.4±2.6	–0.1	–0.8 to 0.6	0.751
TNF-α (ng/l)	7.2±1.5	0.1	–0.7 to 0.9	0.783

Numbers are mean ± s.d. 90% CI, 90% confidence interval; T2D, type 2 diabetes; NCSs, nerve conduction 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*<sub>max</sub> RAMP test, maximum workload capacity during RAMP: linear incremental (15 or 20 W/min) cycling exercise, or Steep RAMP: cycling protocol of 25 W/10 s until exhaustion; VO<sub>2peak</sub> maximal oxygen uptake per kg bodyweight; Relative age-, height-, body weight- and sex-adjusted cardio respiratory fitness (%Pred. VO<sub>2peak</sub>) was based on the equation by Fairbairn *et al.* (49); 1RM, average 1 repetition maximum (kg) for respective two lower (LowerB) and three upper body (UpperB) exercises; HbA<sub>1c</sub>, glycosylated haemoglobin; FPG, fasting plasma glucose; hsCRP, high sensitivity C-reactive protein; TNF-α, tumor necrosis factor alpha; \*significant difference, *P*<0.05, <sup>†</sup>*P*<0.01; <sup>‡</sup>*P*<0.001, paired Student's *t*-test.

<sup>a</sup>Based on *n*=10.

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

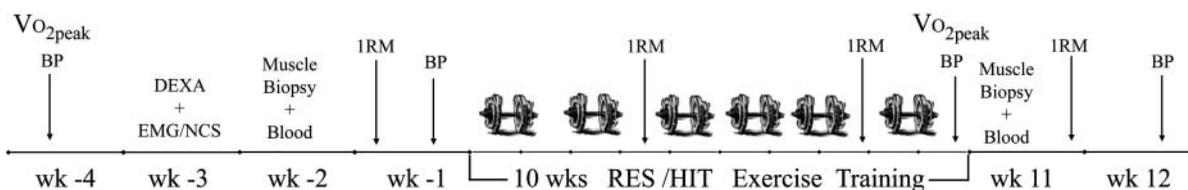
### Blood pressure recording

Before and after the 10 week exercise programme (Fig. 1), systolic and diastolic blood pressure were recorded on two separate occasions during a 15-min supine rest period

using a Dinamap 1846SX automatic blood pressure measuring device (model 8262, Critikon, Tampa, FL, USA). Each measurement was performed under standardized supine rest. Mean arterial blood pressure (MAP) was calculated from the last three stable blood pressure measurements (i.e. mean arterial pressure difference < 4 mmHg) over a 10-min period during the two 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

VO<sub>2peak</sub> and maximal workload capacity (*W*<sub>max</sub>) were measured during an incremental exhaustive exercise test until exhaustion, performed on a cycle ergometer using a linearly increasing (15 or 20 W/min) 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. VO<sub>2peak</sub>) was based on the equation by Fairbairn *et al.* (49).



**Figure 1** Overview of study protocol, including timing of the different tests before and after the 10-week resistance (RES) and high-intensity interval (HIT) exercise training programme:  $VO_{2peak}$ , exhaustive cycle ergometry test to determine maximum power output ( $W_{max}$ ) and peak oxygen uptake ( $VO_{2peak}$ , ml/kg per body weight per min); 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 two lower and three upper body exercises.

Cardiac function was monitored using a 12-lead electrocardiogram with heart rate being recorded continuously and sampled at 1 kHz through a data log device (Co2ntrol).

### Strength testing

At least 1 week before the first exercise session, subjects participated in two exercise trials to become familiarized with the exercise protocol and the equipment. Proper lifting technique was demonstrated and practiced for each of the two lower-limb exercises (leg press and leg extension) and for the three 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 week before the experimental trial, subjects' 1 repetition maximum (1RM) was determined (50). To individualize the training programme to the level of co-morbidity and subsequently maximize the progress in muscle strength, 1RM strength testing was repeated at 4 and 8 weeks after the start of the training programme after which the absolute exercise-training intensity was adjusted accordingly (Fig. 1).

### Blood sampling and analysis

Two weeks before the start of the exercise programme 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 ( $35.2 \pm 6.0$  kJ per kg body weight (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 0800 h. 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, high-density lipoprotein

(HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, non-esterified fatty acids (NEFA) and triacylglycerol concentrations were analysed with the Cobas Fara semi-automatic analyzer (Roche Diagnostics). Blood glycolysated haemoglobin ( $HbA_{1c}$ ) content was determined through HPLC (Bio-Rad Diamat). The serum concentration of adiponectin was quantified using a commercially available Human Adiponectin ELISA (#HADP-61K, Linco Research Inc., St Charles, MO, USA).  $TNF-\alpha$  concentration was analysed using a solid-phase, chemiluminescent immunometric assay (IMMULITE  $TNF-\alpha$ , DPC Biermann GmbH, Bad Nauheim, Germany). hsCRP was measured by means of immunophelometry (Cardiophase, Dade Behring GmbH). C-peptide was analysed through an electrochemiluminescent immunoassay (#03184897, Roche GmbH).

### Muscle biopsy and immunohistochemical analyses

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

For histochemical analyses, multiple serial transverse cryosections (5  $\mu$ m) 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 intramyocellular lipid (IMCL) content stained by oil red O together with immunolabelled cellular constituents, we used the protocol as previously described (51). The proportion of types I, IIa and IIx muscle fibres was determined by ATPase staining (52). To assess intramyocellular glycogen content, we used the modified periodic acid Schiff 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 (SDH) activity in the muscle cross sections using histochemical staining (54).

## Training procedures

The backbone of the exercise programme was progressive resistance training (PRT), with HIT protocol as a supplement. Four bouts of resistance-type exercise targeting the upper body were performed ( $2 \times 10$  reps of 50% of 1RM). Thereafter, resistance training was continued with horizontal leg press and leg extension ( $2 \times 10$  reps). After 5 weeks, the intensity of the PRT was progressively increased from 50 to 60% 1RM to accommodate for the generally deconditioned 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 system (55). In heart failure patients, HIT exercise has been shown to increase  $VO_{2\text{peak}}$  and  $W_{\text{max}}$ , 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  $W_{\text{max}}$  during a steep ramp test (increments of 25 W/10 s, (55)) alternated with 60 s of unloaded cycling. In total, a single training session required  $\sim 45$  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, Berelux, Valkenswaard, The Netherlands). If blood glucose was  $< 6.0$  mmol/l 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 twice following an exercise session, the diabetic nurse was consulted to adjust exogenous insulin dose.

## Statistical analysis

All data are expressed as means  $\pm$  s.d. Repeated measures ANOVA and paired Student's *t*-test were applied to compare the physical, biochemical and immunohistochemical test results before and after the 10 weeks 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 intervals (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.

## Results

### Subjects' characteristics

Subjects' characteristics are shown in Table 1. Relative baseline cardiorespiratory fitness (%pred  $VO_{2\text{peak}}$ ) was  $79.2 \pm 15.1\%$ . Mean duration of T2D was  $12.1 \pm 7.0$  years since diagnosis, and subjects had been on exogenous insulin therapy for  $7.0 \pm 8.0$  years. The average insulin doses over a 2-year period prior to the study were raised from  $64.1 \pm 38.0$  IU/d towards  $92.5 \pm 37.0$  IU/d before entering the study. This represents an average increase in insulin dose of  $+3.6 \pm 2.6$  IU/d per 3 month period. Under normal clinical conditions, the latter would have resulted in (virtual) daily exogenous insulin requirements (EIR) of  $96.3 \pm 37$  IU/d. After 10 weeks of training, the average daily dose of exogenous insulin dropped to  $87.7 \pm 37.0$  IU/d, representing a virtual improvement of  $-8.6$  IU/d (90% CI:  $-15.6$  to  $-1.5$  IU/d) from the expected trend of increasing exogenous insulin dose. All subjects finished the 10-week training programme. A total of four subjects reported mild and uncomplicated hypoglycaemia (capillary blood glucose 2.7–3.8 mmol/l) following the fourth ( $n=2$ ) and ninth ( $n=2$ ) exercise session. Only one out of the four 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 weeks of training that limited further progression of the training intensity. Compliance to the programme was good with a mean participation rate of  $83 \pm 13\%$  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 weeks of training. Body weight and waist circumferences remained constant throughout the training period. Both systolic blood pressure and 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% (6–21) or +21 W (10–32). Both maximum power output during a steep ramp test and overall muscle strength improved with 17 (9–24) and 17% (10–24) respectively over the 10-week intervention period.  $VO_{2\text{peak}}$  remained constant, with an average change of 0.9 ml/kg per BW per min ( $-0.2$  to 2.1).

### Plasma analyses

Table 1 summarizes all biochemical analyses of the blood samples. Except for small changes of  $-0.71$  mmol/l ( $-2.9$  to 1.5) in FPG and  $-147$   $\mu\text{mol/l}$  ( $-296$  to 2) in plasma NEFA concentration, no changes in blood hsCRP, TNF- $\alpha$ , C-peptide, HbA<sub>1c</sub>, lipid profile, triglyceride

**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 weeks 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 ± s.d.) in arbitrary units as measured by immunohistochemistry indicates the amount of mitochondrial enzyme activity; ORO, oil red O stain activity (mean ± s.d.) in arbitrary units as measured by immunohistochemistry as a measure of intramyocellular triglyceride concentration; PAS, periodic acid-Schiff stain activity in arbitrary units (mean ± s.d.) 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.

or adiponectin concentrations were detected following the 10-week exercise programme.

### Immunohistochemical analyses

Table 2 summarizes both mixed and fibre-type-specific IMCL and glycogen content and muscle fibre oxidative capacity before and after 10 weeks of resistance exercise. The training programme employed in the present study did not result in any significant changes in absolute IMCL, glycogen or SDH content in types I, IIa and 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 types IIa and IIx muscle fibres ( $P < 0.05$ ) both before and after 10 weeks of exercise. Muscle fibre-type distribution and muscle fibre cross-sectional area did not differ between pre-training (type I,  $47 \pm 7\%/867 \pm 3482 \mu\text{m}^2$ ; type IIa,  $41 \pm 8\%/7700 \pm 2225 \mu\text{m}^2$ ; type IIx,  $16 \pm 8\%/5250 \pm 1847 \mu\text{m}^2$ ) and post-training conditions (type I,  $45 \pm 10\%/7831 \pm 2667 \mu\text{m}^2$ ; type IIa,  $45 \pm 9\%/8400 \pm 3293 \mu\text{m}^2$ ; type IIx,  $10 \pm 3\%/4704 \pm 2040 \mu\text{m}^2$ ) after the 10-week resistance exercise programme ( $P > 0.05$ ).

### Discussion

In the present study, we show that 10 weeks of supervised resistance and HIT significantly improves muscle strength, workload capacity and blood pressure regulation in long-standing, insulin-treated T2D 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 programmes designed to prevent and/or treat chronic metabolic disease are generally not applicable in long-standing, insulin-treated T2D patients with diabetic polyneuropathy. Alternatively, resistance training has been proposed to augment functional capacity before participation in the more generic endurance exercise intervention programmes. Since the population of long-standing, insulin-treated T2D 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 T2D patients.

Following 10 weeks of moderate intensity exercise training consisting of resistance and relatively high-intensity interval exercise, we established a  $17 \pm 14$  and  $14 \pm 13\%$  increase in muscle strength and workload capacity respectively. Previous resistance-type exercise intervention studies in uncomplicated T2D patients have applied higher-intensity exercise and reported strength increases between 25 and 75% (29, 56–60). Some of these intervention studies report significant improvements in HbA<sub>1c</sub> (56, 57), and glucose area under the curve (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<sub>1c</sub> 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<sub>1c</sub>, 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-standing T2D

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-standing T2D patients with diabetic neuropathy. In accordance with other resistance-type exercise training studies in T2D (57, 60), improvements in muscle strength and workload capacity were accompanied by a moderate reduction in MAP (Table 1). Although this is an interesting finding, the underlying mechanisms cannot be deduced from our feasibility study and are likely to be multi-factorial (65). Nevertheless, the potential cardiovascular benefits for patients with long-standing T2D warrant further investigation.

Based on the experience with deconditioned heart failure patients (16), we implemented HIT as a supplement to moderate-intensity PRT. 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  $W_{\max}$  (Table 1). Despite the improvement in  $W_{\max}$ , MUOX (SDH enzyme activity) and  $VO_{2\text{peak}}$  did not increase significantly. Compared with a training study with younger and early-diagnosed T2D patients (66), it could be speculated that the implemented intensity, duration and frequency of exercise in the present study may have restricted an upregulation of myocellular oxidative capacity and  $VO_{2\text{peak}}$ . 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 4–8 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  $VO_{2\text{peak}}$  (14, 61, 64, 69). Therefore, larger-scale studies are needed to gain more insight into the muscle fibre-type-specific adaptation following resistance, interval, endurance or combined types of exercise training in different T2D subpopulations.

The present study supports the notion that intermediate exercise programmes are warranted to bring more deconditioned patients with long-standing T2D to a level at which they will be able to participate in more generic diabetes intervention programmes. 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 programmes prescribing such an exercise regimen could be of great value to increase muscle strength and functional performance in deconditioned T2D patients with polyneuropathy.

Insulin resistance and 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 apparent discrepancy might be attributed to differences in types of exercise, exercise intensity, a more prolonged intervention period, the use of cholesterol lowering agents (71, 72) or simply because of the selected T2D subpopulation. 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 FPG following the exercise intervention (Table 1). The latter was accompanied with an attenuated rise in EIR (Table 1). In the present study, we did not apply hyperinsulinemic euglycemic clamping to assess whole-body insulin sensitivity as the latter are likely to interfere with EIR. Therefore, we can only speculate whether the observed trends in reduced FPG and attenuated rise in EIR are the result of structural changes in hepatic and/or peripheral insulin sensitivity or exercise-induced improvements in  $\beta$ -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 T2D, which is generally associated with a progressive worsening of glycemic control despite increasing exogenous insulin doses (75).

Lowered peak oxygen uptake and elevated IMCL and glycogen contents have been associated with the development of insulin resistance and/or T2D (25). Despite the significant gain in functional performance, we observed no structural changes in fibre-type specific IMCL, glycogen or SDH content in types I, IIa and IIx or mixed muscle fibres in *M. vastus lateralis* (Table 2). Furthermore, fibre-type composition had not changed after 10 weeks 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 IMCL 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 expand on previous findings that the reported improvements in physical fitness, muscle strength as well as the attenuated rise in EIR are not necessarily accompanied by significant changes in muscle fibre-type characteristics, MUOX and/or muscle lipid and/or glycogen content.

Long-term adherence to resistance-type exercise training in T2D patients has proven problematic (82). Therefore, proper supervision is considered an important factor to maintain programme adherence (82). Even

though we implemented three exercise sessions per week, subjects showed excellent compliance ( $83 \pm 13\%$  attendance of the training sessions) and no dropout. The greater workload capacity and increased strength following the 10-week intervention should be sufficient to enable these patients to participate in a more generic exercise intervention programmes. As such, the applied exercise regimen might represent an effective interventional strategy to enable patients to pursue a more active, healthier lifestyle.

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 EIR in long-standing, insulin-treated T2D patients with diabetic polyneuropathy. Such a specific exercise regimen may provide a better framework for future exercise intervention programmes in the treatment of deconditioned T2D patients.

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