Effects of one-legged high-intensity interval training on insulin-mediated skeletal muscle glucose homeostasis in patients with type 2 diabetes

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Abstract

Aim: To examine the effect of high-intensity interval training (HIIT) on glucose clearance rates in skeletal muscle and explore the mechanism within the muscle.

Methods: Ten males with type 2 diabetes mellitus (T2DM) and ten matched healthy subjects performed 2 weeks of one-legged HIIT (total of eight sessions, each comprised of 10 × 1 minute ergometer bicycle exercise at >80% of maximal heart rate, interspersed with one min of rest). Insulin sensitivity was assessed by an isoglycaemic, hyperinsulinaemic clamp combined with arteriovenous leg balance technique of the trained (T) and the untrained (UT) leg and muscle biopsies of both legs.

Results: Insulin-stimulated glucose clearance in T legs was ~30% higher compared with UT legs in both groups due to increased blood flow in T vs UT legs and maintained glucose extraction. With each training session, muscle glycogen content decreased only in the training leg, and after the training, glycogen synthase and citrate synthase activities were higher in T vs UT legs. No major changes occurred in the expression of proteins in the insulin signalling cascade. Mitochondrial respiratory capacity was similar in T2DM and healthy subjects, and unchanged by HIIT.

Conclusion: HIIT improves skeletal muscle insulin sensitivity. With HIIT, the skeletal muscle of patients with T2DM becomes just as insulin sensitive as untrained muscle in healthy subjects. The mechanism includes oscillations in muscle glycogen stores and a maintained ability to extract glucose from the blood in the face of increased blood flow in the trained leg.

KEYWORDS
diabetes mellitus, glucose metabolism, leg balance, overweight

Trial registration: The study is registered at clinicaltrials.gov, ID NCT01732705.
1 | INTRODUCTION

Type 2 diabetes is a disease with rapidly increasing incidence that carries long-term complications and premature death. The key factors in the pathophysiology of type 2 diabetes are insulin resistance, insufficient insulin secretory capacity and genetic disposition combined with excess energy intake and physical inactivity. Physical training alleviates insulin resistance,1–3 may improve insulin secretory capacity4–6 and improves glycaemic control7,8 in patients with type 2 diabetes. Large clinical lifestyle studies with interventions including weight loss and increased physical activity have shown fewer hospitalizations, fewer medications and lower healthcare costs,9 but on specific cardiovascular endpoints, the results have been disappointing in large-scale studies.10,11

While there is no question about the efficacy (ie per protocol) of physical training as a treatment modality for insulin resistance, the effectiveness (ie intention to treat) is less evident. The major problem is that the patients do not exercise on a regular basis. There are many barriers to overcome, safe environments, convenience and lack of time12–15 are among the major reasons for not exercising on a regular basis. High-intensity interval training (HIIT) with short periods of intense exercise interspersed with brief periods of rest is a time-efficient exercise modality, which might surpass some of the barriers.

As with almost any kind of exercise, an acute high-intensity training bout will lower glucose concentrations in patients with type 2 diabetes.16–19 However, training for 2 weeks or more in patients with type 2 diabetes has generally used suboptimal methods for determining the effect of HIIT on insulin secretion and sensitivity, which are the key parameters in the pathophysiology of type 2 diabetes. Thus, by using measurements of interstitial glucose concentrations, HOMA or HbA1c, both marginal or no effect20,21 and significant effects22–24 of HIIT on glucose homeostasis have been reported. One study23 estimated the effect on insulin secretion and found no effect of HIIT.

One previous study25 used the glucose clamp technique combined with positron emission tomography and combined with a glucose tracer and found that six sessions of sprint training improved glucose uptake in muscle. However, muscle biopsies or arteriovenous balances across muscle were not performed, and therefore, no mechanism could be described and only one very low clamp step (plasma insulin ~90 pmol/L) was used.25 Other studies have addressed the molecular effects of high-intensity interval training in skeletal muscle,26–30 but only one study included patients with type 2 diabetes.22 In general, these studies have showed that HIIT leads to increases in proteins (activity and/or content) related to mitochondrial biogenesis, such as citrate synthase (CS), complexes in the mitochondrial respiratory chain, silent mating type information regulator 2 homolog 1 (SIRT1), peroxisome proliferator-activated receptor γ coactivator-1α (PGC1α) and mitofusin (Mfn) 2. Also, skeletal muscle GLUT4 protein and glycogen content seem to increase with HIIT, and furthermore, an increased capacity to fat oxidation (hydroxyacyl-coenzyme A dehydrogenase (HAD) activity)26 is observed after HIIT.

This experiment tests the prediction that high-intensity interval training increases skeletal muscle insulin sensitivity in patients with type 2 diabetes and that the mechanism includes exercise-induced oscillations in muscle glycogen stores. To this end, we conducted a 2-week HIIT training programme performed as one-legged training. With completion of the intervention, a 2-step hyperinsulinaemic clamp with arteriovenous catheterization of both legs was carried out. Changes in muscle glycogen and relevant proteins were analysed in muscle biopsies from the trained and the untrained leg.

2 | RESULTS

The patients with type 2 diabetes and the control group were matched in regard to age, body composition, resting metabolic rate, plasma concentration of glucoregulatory hormones and biomarkers of inflammation, and exercise capacity (Table 1). Lipid concentrations in plasma were similar, except total and LDL cholesterol, which were lower in the patients with type 2 diabetes compared with the healthy control subjects.

2.1 | Training intervention

All eight training sessions were monitored, and training was well tolerated with no dropouts. The control subjects and the patients with type 2 diabetes exercised with the same pedalling cadence (Table 2) and oxygen consumption during exercise (Figure 1). However, the control subjects were able to exercise at a significantly higher workload during all training sessions (Table 2). The relative exercise intensity (as per cent of maximal heart rate) was generally similar in the two groups, but between the exercise bouts the heart rate did not decrease to the same extent in the patients with type 2 diabetes (Figure 1), resulting in slightly higher peak and higher nadir heart rate values in the patients with type 2 diabetes compared with the control subjects (Figure 1). Glycogen use with an exercise session was reduced by ~30% and similarly in both groups (Figure 2).

In response to the training programme, one-legged maximal oxygen uptake did not change with either the trained or the untrained leg (Table 3). The maximal workload capacity with the trained leg increased by ~40% (P < 0.0001), but not with the untrained leg (data not
Thus, after the training intervention, the workload capacity was significantly higher in the trained compared with the untrained leg in both groups (Table 2). Leg muscle and fat mass did not change with the training programme, and these variables were similar between the groups and the trained and untrained leg after the training programme (Table 3).

2.2 | Glucose homeostasis

During steady state of the clamp, the concentration of glucose in plasma was maintained at the individual fasting concentrations (isoglycaemic clamp) with no statistically significant difference between the fasting state and the two clamp steps in the groups (Table 4). Whole-body glucose clearance rates were significantly higher in controls during the clamp (Figure 3). The extraction of glucose from the blood, expressed as the difference between arterial and venous plasma glucose concentrations divided by the arterial plasma glucose concentration, increased with insulin stimulation in both groups ($P < 0.0001$) and with overall higher extraction in the control group compared with the patients with type 2 diabetes ($P = 0.009$; Table 5). Glucose extraction was never significantly higher in the trained compared with the untrained leg ($P = 0.05$; Table 5), but higher in controls compared with the patients with type 2 diabetes at baseline and during clamps step I (Table 5).

Leg blood flow was not different at baseline between trained and untrained legs or between the groups. With insulin infusion, leg blood flow increased ($P < 0.05$) and it was always higher in the trained compared with the untrained leg ($P < 0.0001$) with no group differences ($P = 0.65$; Table 5). The insulin-stimulated glucose clearance of the trained leg calculated as the glucose extraction multiplied with the plasma flow was on average $\sim$30% higher in the trained compared with the untrained leg (Table 5 and Figure 3). No significant interaction was found between training and group, that is the effect of training did not differ significantly between the controls and subjects with type 2 diabetes mellitus (Figure 3).

2.3 | Substrates and metabolites

Arterial plasma concentrations of glycerol and FFA were higher in the patients with type 2 diabetes compared with controls, and the concentrations decreased markedly with increasing plasma insulin concentrations in both groups (Figure 4). The decrease in the arterial concentrations is a reflection of the decrease in release of glycerol and FFA from the legs during the clamp (Figure 5). In line with the decrease in FFA and glycerol, arterial concentrations of $\beta$-hydroxybutyrate decreased markedly with the clamp (Fig-
The uptake in the legs showed no difference between trained and untrained legs (Figure 5). However, while the leg uptake of β-hydroxybutyrate in the healthy control subjects was almost eliminated with insulin infusion, the uptake continued during hyperinsulinaemia in the patients with type 2 diabetes, albeit at diminished rates compared with baseline (Figure 5). Arterial plasma concentrations of lactate increased in both groups from baseline to hyperinsulinaemia (Figure 4), and the release of lactate during insulin infusion was higher \( (P < 0.05) \) in trained compared with untrained legs (Figure 5).

### 2.4 | Leg calorimetry and metabolism

Leg RQ always increased with increasing plasma insulin concentrations, with no difference between the groups or between the legs (Figure 6). Leg VO\(_2\) increased with insulin in T, but the increase did not reach statistical significance in UT legs (Figure 6). From baseline to maximal insulin concentrations, glucose oxidation rates increased by 4- to 5-fold, with no difference between UT and T legs in

<table>
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<tr>
<th>Cadence (rpm)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>76 ± 2</td>
<td>82 ± 2</td>
<td>79 ± 1</td>
<td>81 ± 2</td>
<td>86 ± 2</td>
<td>87 ± 2</td>
<td>86 ± 3</td>
<td>84 ± 1</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>79 ± 2</td>
<td>77 ± 2</td>
<td>79 ± 3</td>
<td>80 ± 4</td>
<td>80 ± 4</td>
<td>80 ± 5</td>
<td>82 ± 4</td>
<td>79 ± 4</td>
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<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
<td>Healthy control</td>
<td>90 ± 7(^\dagger)</td>
<td>98 ± 8(^\dagger)</td>
<td>103 ± 8(^\dagger)</td>
<td>110 ± 8(^\dagger)</td>
<td>114 ± 9(^\dagger)</td>
<td>117 ± 9(^\dagger)</td>
<td>122 ± 10(^\dagger)</td>
<td>126 ± 10(^\dagger)</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>73 ± 5</td>
<td>73 ± 7</td>
<td>75 ± 8</td>
<td>78 ± 8</td>
<td>80 ± 9</td>
<td>84 ± 9</td>
<td>88 ± 9</td>
<td>95 ± 11</td>
</tr>
</tbody>
</table>

Eight sessions of high-intensity interval training in a group of type 2 diabetics and healthy controls. Values are mean of the 10 exercise bouts in each session. Values are means ± standard error of the mean (SEM). \(^\dagger\)Different from patients with type 2 diabetes \( (P < 0.05) \).

**FIGURE 1** Ten patients with type 2 diabetes and 10 matched healthy controls performed eight high-intensity interval training sessions as ten 1-minute ergometer bicycle one-legged exercise interspersed with 1-minute resting recovery. Data are shown as pooled mean data from all exercise sessions (heart rate, panel A) or from training sessions 2 and 6 (oxygen uptake, panel B) nested in groups. A, Percentage of maximal heart rate. The decrease in heart rate during the recovery periods was greater \( (P < 0.05) \) in the healthy controls compared with the patients with type 2 diabetes. B, Oxygen uptake.

**FIGURE 2** Glycogen concentration in skeletal muscle biopsies obtained before and after one exercise session of high-intensity interval training with one leg. Data are mean ± SEM. *Significant difference between pre- and post-exercise \( (P < 0.05) \).
either group (Table 6). Oxidation of lipids was prominent at baseline, decreased with the first clamp step and was completely suppressed at maximal insulin concentrations (Table 6). Breakdown of muscle glycogen was present at baseline, but with increasing plasma insulin concentrations this switched to glycogen storage with the effect being larger \((P < 0.05)\) in T compared with UT legs; no difference in glycogenesis between healthy controls and patients with type 2 diabetes could be detected (Table 6). Finally, lipogenesis (from glucose) was absent at baseline, but the process increased substantially with insulin, in particular at maximal insulin concentrations in clamp step 2 (Table 6).

The concentration of glycogen was higher \((P < 0.05, \text{main effect})\) in T compared UT muscle (Figure 7). The small numerical increase in glycogen concentration with insulin stimulation (Figure 7) was not statistically significant, and no difference between the healthy control subjects and the patients with type 2 diabetes was detected.

### 2.5 Muscle enzyme activities and gene expressions

CS activity was significantly higher \((P < 0.05, \text{main effect})\) in T compared UT muscle (Figure 8). CS activities were not different between the groups. Activities of HAD and LDH did not change with training and were not different between the groups (Figure 8). With CS activity being a biomarker for mitochondrial mass, PGC1α was measured (Figure 8), but no difference between UT and T muscle was seen. CPT1 and CPT2 are important proteins in the transfer of long-chain fatty acids into the mitochondria, but no significant difference of their gene expression levels was observed between T and UT muscle (Figure 8).

GS activity was generally not affected by training and similar between the groups (Figure 9). However, a significant effect of training \((P < 0.05)\) was seen with maximal glucose-6-phosphate stimulation (Figure 9).
**FIGURE 3** Whole-body glucose clearance rates during a two-step, iso-osmolar, hyperinsulinaemic clamp (A). Graphical representation of leg glucose clearance rates in trained (T) and untrained (UT) legs at baseline and during the two clamp steps of hyperinsulinaemia (B). Data are shown as mean (A and B) ± SEM (A). #Significant difference between healthy controls and patients with type 2 diabetes (P < 0.05) *Different (P < 0.05) from UT leg. †Different from corresponding leg in type 2 diabetes at all insulin concentrations (P < 0.05). See text and Table 4 for details

### TABLE 5 Leg glucose clearance rates, blood flow and glucose extraction

<table>
<thead>
<tr>
<th></th>
<th>Leg glucose clearance (mL/min/kg lean leg mass)</th>
<th>Leg blood flow (mL/min)</th>
<th>Leg glucose extraction ((A-V)/A) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Healthy control</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline UT</td>
<td>0.21 ± 0.05</td>
<td>318 ± 28</td>
<td>1.4 ± 0.4†</td>
</tr>
<tr>
<td>T</td>
<td>0.24 ± 0.05†</td>
<td>276 ± 29</td>
<td>1.7 ± 0.5†</td>
</tr>
<tr>
<td>Step I UT</td>
<td>4.28 ± 0.30†</td>
<td>387 ± 46</td>
<td>23.7 ± 2.8†</td>
</tr>
<tr>
<td>T</td>
<td>5.60 ± 0.31†</td>
<td>487 ± 61†</td>
<td>25.1 ± 2.6†</td>
</tr>
<tr>
<td>Step II UT</td>
<td>8.08 ± 0.45†</td>
<td>476 ± 54</td>
<td>33.8 ± 2.3</td>
</tr>
<tr>
<td>T</td>
<td>10.40 ± 0.52†</td>
<td>625 ± 83†</td>
<td>34.9 ± 2.5</td>
</tr>
<tr>
<td><strong>Type 2 diabetes</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Baseline UT</td>
<td>0.07 ± 0.05</td>
<td>338 ± 58</td>
<td>0.4 ± 0.4</td>
</tr>
<tr>
<td>T</td>
<td>0.04 ± 0.05</td>
<td>355 ± 58</td>
<td>0.2 ± 0.3</td>
</tr>
<tr>
<td>Step I UT</td>
<td>3.04 ± 0.26†</td>
<td>418 ± 44</td>
<td>13.3 ± 1.1</td>
</tr>
<tr>
<td>T</td>
<td>4.03 ± 0.34†</td>
<td>516 ± 73†</td>
<td>14.7 ± 1.9</td>
</tr>
<tr>
<td>Step II UT</td>
<td>6.75 ± 0.54†</td>
<td>451 ± 57</td>
<td>28.1 ± 1.9</td>
</tr>
<tr>
<td>T</td>
<td>8.37 ± 0.60†</td>
<td>545 ± 69†</td>
<td>29.7 ± 2.6</td>
</tr>
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</table>

Ten patients with type 2 diabetes and ten healthy control subjects carried out eight one-legged high-intensity interval training bouts every other day, leaving a trained (T) and an untrained (UT) leg. Forty hours after the last training session, an isoglycaemic, two-step (80 and 400 mU/min/m²) hyperinsulinaemic clamp combined with leg arteriovenous (A-V) catheterization was carried out. Glucose was measured in the plasma phase of the blood, and leg glucose clearance was calculated as plasma flow (blood flow × (1 - arterial haematocrit)) × ((A-V)/A) glucose concentrations divided by kg leg muscle mass. Within T and UT legs, glucose clearance rates were always higher (P < 0.001) in healthy controls vs patients with type 2 diabetes. Leg blood flow was always similar in healthy controls and patients with type 2 diabetes and always higher in T vs UT leg during insulin infusion (P < 0.02).

*Different from UT leg in the same group (P < 0.05). Leg glucose extraction was never significantly different between UT and T legs, but higher in healthy controls vs patients with type 2 diabetes at baseline and step I.

†Different from corresponding leg in patients with type 2 diabetes (P < 0.05).

### 2.6 Mitochondrial respiration

Intrinsic respiratory capacity in permeabilized muscle fibres was not different between healthy control subjects and patients with type 2 diabetes, and it was unaffected by HIIT (Figure 10). Also, mass-specific respiratory capacity did not change with HIIT (data not shown). Respiratory control ratio (state 3/state 2) was similar in trained and untrained muscle in healthy subjects (6.9 ± 0.8 and 6.6 ± 0.9 respectively) and in T2DM (7.6 ± 0.8 and 7.6 ± 1.1 respectively).

### 2.7 Skeletal muscle protein expression

GLUT4 was 23 and 5% higher in T vs UT muscle (healthy and T2DM respectively) (main effect P = 0.08) with no group difference (Figure 11). Protein expression of complexes I, II and IV tended (main effects, P = 0.09, P = 0.055, and P = 0.09 respectively) to be lower in the patients with type 2 diabetes compared with the healthy control subjects (Figure 11). Apart from this, no other proteins changed expression with training or were differently expressed between groups.

### 3 DISCUSSION

The present study is the first to show that high-intensity interval training markedly increases insulin-mediated glucose clearance rates in leg skeletal muscle in patients with type 2 diabetes and in healthy control subjects. The training effect was of the same magnitude in the two groups, demonstrating that the effect of training on insulin-mediated glucose uptake rates is independent of insulin
The improvement in insulin sensitivity was predominantly the result of increased glucose delivery (blood flow) in the trained leg, while glucose extraction rates were maintained, even in the face of the higher glucose delivery. At baseline and during clamp step 1 (Table 5), the extraction of glucose from the blood was markedly lower in the patients with type 2 diabetes compared with the healthy controls. The reduced ability to extract glucose from the blood, at a time when glucose delivery is the same, is the physiological hallmark of insulin resistance.

The one-legged training model used here has previously been used in similar studies on the effect of endurance training and strength training. With one-legged endurance training for 10 weeks, the patients with type 2 diabetes...
diabetes improved insulin sensitivity by a factor 3 in the low physiological range of plasma insulin concentrations, and the general improvement was ~30–40% with endurance and with strength training. These effects are in accordance with the ~30% improvement in insulin sensitivity reported in the present study, where the data should be seen in the face of a very limited time spent on training (total of 1 hour and 20 minutes of active exercise) across eight training sessions in 2 weeks. The intensity of the exercise in HIIT is clearly much higher compared to endurance training, and it confirms that intensity to some extent can compensate for time.

The literature on the effect of HIIT training or a single HIIT exercise bout on glycaemic control (fasting plasma glucose and HbA1C concentrations, post-prandial glucose homeostasis, time spent in hyperglycaemia, feasibility of the intervention) in type 2 diabetes is abundant, for example. Very few studies have

**FIGURE 6** Leg oxygen uptake (A) and respiratory quotient (B) at baseline and during a two-step, hyperinsulinaemic, euglycaemic clamp in ten patients with type 2 diabetes (T2DM) and ten healthy control subjects. RQ values and VO2 in the trained (T) leg increased significantly (P < 0.05) with increasing insulin concentrations. *Significant difference between T and untrained (UT) legs (P < 0.05). Values are means ± SE.

**TABLE 6** Leg calorimetry and metabolism

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Clamp step I</th>
<th>Clamp step II</th>
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<tr>
<td></td>
<td>UT leg</td>
<td>T leg</td>
<td>UT leg</td>
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<tr>
<td>Glucose oxidation</td>
<td></td>
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<tr>
<td>Healthy control</td>
<td>1.32 ± 0.35</td>
<td>1.03 ± 0.42</td>
<td>2.60 ± 0.25</td>
</tr>
<tr>
<td>T2DM</td>
<td>1.03 ± 0.27</td>
<td>0.89 ± 0.50</td>
<td>2.84 ± 0.52</td>
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<tr>
<td>Lipid oxidation</td>
<td></td>
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<tr>
<td>Healthy control</td>
<td>0.44 ± 0.14</td>
<td>0.45 ± 0.18</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>T2DM</td>
<td>0.40 ± 0.23</td>
<td>0.50 ± 0.31</td>
<td>0.16 ± 0.06</td>
</tr>
<tr>
<td>Glycogenesis</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Healthy control</td>
<td>−1.32 ± 0.39</td>
<td>−1.06 ± 0.47</td>
<td>2.04 ± 0.50</td>
</tr>
<tr>
<td>T2DM</td>
<td>−1.13 ± 0.25</td>
<td>−1.04 ± 0.49</td>
<td>1.55 ± 0.25</td>
</tr>
<tr>
<td>Lipogenesis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy control</td>
<td>0.02 ± 0.01</td>
<td>0.04 ± 0.03</td>
<td>0.11 ± 0.07</td>
</tr>
<tr>
<td>T2DM</td>
<td>0.01 ± 0.01</td>
<td>0.05 ± 0.04</td>
<td>0.15 ± 0.10</td>
</tr>
</tbody>
</table>

Ten patients with type 2 diabetes and ten healthy control subjects performed high-intensity interval training with one leg every other day for two weeks (eight training sessions). See methods for calculations. Values are means ± SEM. Unit: mg·min⁻¹·kg muscle⁻¹.* and †Difference between untrained (UT) and trained (T) legs, P < 0.05 and P < 0.1 respectively.

**FIGURE 7** Concentration of glycogen in muscle biopsies obtained before the initiation of the clamp (baseline) and at the end of clamp step II (insulin) in the trained (T) and in the untrained (UT) leg of 10 healthy (healthy) control subjects and 10 patients with type 2 diabetes (T2DM) after 2 weeks of high-intensity interval training. Data are mean ± SE. *T > UT, main effect (P < 0.05)
mechanistically explored the effects of HIIT on insulin action in patients with type 2 diabetes, and the present study extends the previous findings by demonstrating the effect of HIIT directly in skeletal muscle (metabolically speaking the leg consists predominantly of skeletal muscle) and provides further mechanistic observations.

### 3.1 Physiological and molecular mechanisms

Training-induced increase in insulin-mediated glucose uptake in skeletal muscle is brought about by several mechanisms that may act in concert. Delivery of glucose and insulin to the muscle is important and will be facilitated by an increase in insulin-mediated bulk leg blood flow and enhanced insulin-mediated capillary recruitment. An increased capillary density, and thereby reduced diffusion distance, would also facilitate an enhanced insulin action, but the training period in the present study is probably too short to have induced noticeable angiogenesis. Transport of glucose from the capillaries into the muscle cell is a passive transport, dependent on the concentration gradient and the presence of glucose transporter 4 protein (GLUT4). Here, we did not find a significant increase (main effect, $P = 0.08$; Figure 11) in total GLUT4 content. This is in contrast to the significant increases found in other studies using similar training modalities and duration of training or different modalities and longer duration. We have no good explanation for the lack of significant increase in GLUT4 in the present study. We have used the same method and antibodies previously, and the quality of the blots was good (Figure 11). Trustworthy training-induced increases in

**FIGURE 8** Citrate synthase (A), hydroxyacyl-coenzyme A dehydrogenase (B), and lactate dehydrogenase (C) enzyme activities and gene expression of peroxisome proliferation-activated receptor gamma, coactivator 1 alpha (PGC1α) (D), carnitine palmitoyltransferase 1 (CPT1) (E) and 2 (CPT2) (F) in skeletal muscle in the trained (T) and the untrained (UT) leg in 10 healthy subjects and 10 patients with type 2 diabetes (T2DM) who performed one-legged high-intensity interval training for 2 weeks. Data are mean ± SE, except for mRNA data which are geometric mean ± SE. $^*T > UT$, main effect ($P < 0.05$)

**FIGURE 9** Glycogen synthase (GS) activity in trained (T) and untrained (UT) muscle. GS was measured with three different concentrations of glucose-6-phosphate in the medium. Data are mean ± SE. $^*T > UT$, $P < 0.05$

**FIGURE 10** Mitochondrial respiratory capacity in permeabilized skeletal muscle fibres in trained (T) and untrained (UT) leg from healthy, control subjects and from patients with type 2 diabetes (T2DM). Respiration was stimulated with glutamate (G) and malate (M) without ADP (state 2), which is equivalent to LEAK respiration, and subsequently with the addition of ADP (state 3) and finally with dual electron input in complex I + II with the addition of succinate (S). See text for details of the protocol. Data are means ± SE.
GLUT4 protein are usually about 20%-30%, but this may not always come out statistically significant. Once inside the muscle cell, glucose flux must occur in the metabolic processes, glycolysis, oxidation and glycogenesis. In other training studies, the role of abundance, activities and site-specific phosphorylation of key proteins (isoforms of AMPK, Akt, AS160, TBC1D4) in these processes have been demonstrated. In the present study, GS activity (Figure 9) and protein content (Figure 11) were measured and a training-induced increase in activity (but not content)
was demonstrated in both groups, in line with previous findings in similar groups.3,38 The stimulus for the increase in GS activity is most likely the fluctuations in muscle glycogen content (Figure 2) associated with each training bout, while the limited number of exercise sessions was obviously not sufficient for an increase in GS protein content. Along the same line, we would predict that a change in muscle fibre type composition would not take place within the relatively short intervention period, and therefore, fibre types were not measured. The training-induced increase in GS activity is reflected in the results from the indirect calorimetric calculations across the legs, in which the rate of glycogenesis during insulin stimulation was higher in the trained leg (Table 6). The negative rate glycogenesis at baseline (Table 6) reflects glycogenolysis. Numerically, the rate of glycogenesis (Table 6) and GS activity (Figure 9) was higher in the healthy control subjects compared with T2DM patients, but the difference never reached statistical significance.

3.2 | Indirect calorimetry and mitochondrial respiration

Calculated values of glucose oxidation rates (Table 6) increased with insulin in UT and T legs as expected. For the T leg, this is in accordance with the measured rates of leg oxygen uptake, while the increase in oxygen uptake in the UT leg was moderate (Figure 6). The increase in oxygen uptake from baseline to maximal insulin was of the same magnitude and of similar absolute numbers as previously found in a similar study, where the intervention was one-legged endurance training for 10 weeks.2 The rate of non-oxidative glycolysis is reflected by the lactate A–V balance showing increasing release of lactate with insulin and more in T compared with UT legs (Figure 5). The higher release of lactate in T leg reflects a higher glucose uptake rate (and glycolytic rate), as shown previously2,32, and the additional formation of lactate in T legs could also reflect a training-induced increased LDH activity,43 which was, however, not detected in the present study (Figure 8). The intrinsic mitochondrial respiratory capacity does not increase with high-intensity training24,45 (Figure 10), which leaves the option that pyruvate formed by glycolysis give rise to release of lactate and alanine (the latter not measured in the present study). However, in absolute terms, mass-specific mitochondrial respiratory capacity (ie not corrected for mitochondrial content) may increase with a training-induced mitochondrial biogenesis. The plasticity of mitochondria has been known since the mid-sixties,46 and an increase in biomarkers of mitochondrial content31 in response to high-intensity training in human muscle has also been shown.12,45 In the present study, we found only small increases in some of these biomarkers (Figures 8 and 11), but if the mitochondrial biogenesis had been of substantial magnitude, the higher lactate release from the trained legs may not have been seen. The finding that CS activity and protein biomarkers of mitochondrial biogenesis were not markedly increased is supported by the lack of increase in mRNA levels of particularly PGC1α, but also CPT1 and CPT2 (Figure 8).

Of note, we found similar mitochondrial respiratory capacities (normalized to CS) in the healthy control subjects and the patients with type 2 diabetes (Figure 10). The patients with type 2 diabetes were clearly insulin-resistant (Figures 3 and 12; Table 5), supporting the notion that there is a dissociation between insulin resistance and mitochondrial respiratory function as previously found in skeletal muscle,47–50 adipose tissue51 and hepatic tissue.52

3.3 | Contribution of leg muscles to insulin-mediated glucose uptake rates

The measurements of whole-body and leg glucose uptake rates (Tables 3 and 5; Figure 3) allow for an estimation of the relative contribution of leg muscles to the whole-body glucose uptake. Leg muscle mass is very close to leg lean mass (determined by DXA scan), since the amount of connective tissue and other non-fat tissue is limited but whole-body muscle mass is far from whole-body lean mass. However, MR studies have provided a value of 35% of body weight as muscle mass43 and by using these values it can be calculated that ~65% of the whole-body muscle glucose uptake during maximal insulin is accounted for by the leg muscles. This percentage was similar for T2DM and the healthy control subjects. Further, if both legs had been trained, the contribution would obviously have been higher, ~74%.
3.4 | Leg balances of substrates and metabolites

The arterial concentrations and leg balances of substrates and metabolites (Figures 4 and 5) were as expected. Thus, glycerol and FFA arterial plasma concentrations were higher in T2DM compared with healthy controls, and the concentrations decreased (markedly for FFA) with increasing insulin concentrations, reflecting the decrease in the release from legs (Figure 5) and insulin-mediated inhibition of lipolysis. While the leg FFA release was numerically lower in the healthy controls, the difference did not reach statistical significance ($P < 0.1$). Ketone bodies, represented by the measurements of β-hydroxybutyrate (Figures 4 and 5), decreased markedly in the artery, which is expected with a hyperinsulinaemic suppression of whole-body lipolysis and leg lipid oxidation rates (Table 6). Interestingly, the uptake of β-hydroxybutyrate in the leg muscles was significantly higher in T2DM compared with the healthy controls (Figure 5) as seen previously. The higher uptake was not due to a higher availability because the arterial concentrations were identical in the two groups (Figure 4). Thus, a higher uptake would be due to a higher A-V extraction, but a significant difference between the groups could not be detected. The fate of the β-hydroxybutyrate taken up by the leg muscles is unclear. At baseline, during fasting plasma insulin concentrations, muscle oxidation rates are close to zero and only with markedly increased arterial concentrations of β-hydroxybutyrate can oxidation be detected (by isotope infusion experiments). Nevertheless, it has previously been proposed that 3%–5% of oxygen consumption can be ascribed to ketone body oxidation at baseline and in the present study, this was set to 4% and 1% (during the clamp) in the calorimetric calculations. The plasma concentration of β-hydroxybutyrate was in the present study measured by spectrophotometry, and the data are similar to those found with other methods (gas chromatography, mass spectrometry). Nevertheless, the ketone balance data should be interpreted with caution because the lower technical limit of the analysis is 0.03 mmol/L.

3.5 | Training parameters

The participants’ individual exercise capacity was carefully recorded before the training intervention was started, and during the training sessions, relevant exercise parameters were noted. All sessions were supervised, and compliance was 100%. There is no doubt that all participants were dedicated to the training, which is testified by the recorded pedalling cadence, workload (Table 2), heart rate and oxygen uptake (Figure 1). The initial exercise capacity was similar between the two groups (Tables 1 and 4), but during training, the workload was higher in the healthy controls (Table 2). However, the oxygen consumption during the training sessions (Figure 1) was similar. Thus, the exercise efficiency was higher in the healthy controls compared with the patients (ie same oxygen consumption but higher workload). Perhaps, the slightly lower successive minimum and maximum per cent maximal heart rate in the healthy controls (Figure 1) was a contributing factor to this. The differences in heart rate may be the result of cardiac autonomic neuropathy in the patients with type 2 diabetes which has previously been shown to decrease cardiac output at a given workload, but this issue was not further investigated in the present study.

As a result of the training, maximal workload capacity increased in both groups even though we were unable to demonstrate an increase in one-legged $\hat{V}O_2$max (Table 4). This is not surprising because a one-legged maximal bicycle exercise test is not easy to perform, and a levelling off of oxygen uptake is very hard to obtain. Furthermore, the exercise test was carried out the day after the clamp experiment (Figure 13) at which blood was sampled and haematocrit was indeed significantly decreased at the end of the clamp experiment (data not shown), and this will have a negative impact on the $\hat{V}O_2$max test. Nevertheless, the increased blood lactate concentration measured at maximal exercise effort with the trained leg (increase from 6.8 ± 0.9 (pre-training) to 9.7 ± 1.1 (post-training) mmol/L [healthy subjects, $P < 0.05$] and from 6.8 ± 0.7 (pre-training) to 8.1 ± 0.8 (post-training) mM [patients with type 2 diabetes, $P < 0.05$]) also documents the improved exercise capacity of the trained leg muscles. This is further supported by the slightly higher (~10%-13%) CS activity in the trained leg (Figure 8). Other studies, using similar training regimen and duration, have found significant increases in CS and other mitochondrial enzyme activities, but these studies were performed in young, healthy males.

3.6 | Perspectives and potential implementation of HIIT

The data presented here document the positive effect of HIIT as a training modality on glucose homeostasis in patients with (and without) type 2 diabetes. Even though it is difficult to compare different training modalities (HIIT, strength and endurance training), it seems clear that the effect of HIIT is in the same order of magnitude as other training regimens. It is probably not essential to use one specific training method, rather it only seems to be a matter of motivating the patients with type 2 diabetes to perform some form of regular exercise. The important issue is that the energy expenditure and the intensity are sufficiently high. For endurance training, a relative intensity of ~60%-70% of $\hat{V}O_2$max is appropriate minimum 3 days a week.
FIGURE 13 Study design. Two test
days before the high-intensity interval
training period of 2 weeks with exercise
every other day. Experimental day at day
17, approximately 40 hours after the last
training bout. OGTT, oral glucose tolerance
test; DXA, whole-body dual-energy X-ray
absorptiometry; ECG, electrocardiogram;
VO₂ max, maximal oxygen uptake; HIT1-
8, high-intensity interval training sessions
1-8

The frequency is determined by the fact that the effect of a
single exercise bout is short-lived, lasting 3 but not
5 days, and the effect of regular endurance training is
decaying after 5 days of de-training. For strength training
and HIIT, such de-training studies have not been performed
in patients with type 2 diabetes, but it would probably be
fair to say that the insulin sensitizing effect would have the
same “half-life.”

It is currently debated whether HIIT is a training
modality that is useful in daily clinical practice. HIIT
has been reported to be safe, even in selected high-risk
populations with cardiovascular disease, but many HIIT
protocols for clinical populations with elevated cardiovas-
cular risk have been modified to be less strenuous and
some authors advocate to be cautious when prescribing
HIIT to patients with cardiometabolic disease. A recent
large-scale study concluded that HIIT feasibility in heart
failure patients remains unsolved, while a pilot trial in
obese, middle-aged subjects (mostly women) concluded
that HIIT may be appropriate and attainable for this
population.

3.7 | Strength and limitations

An advantage of the one-legged training model and the
combination with arteriovenous catheterization is that day-
to-day variations in the measurements are eliminated, as
well as the influence of changes in diet. Furthermore, the
hormonal milieu is the same for the trained and the
untrained leg. A disadvantage is the potential co-training
effect on the non-training leg, which may not be truly
untrained at the time of the experiment. However, this
would only underestimate the effect of training. The aim of
the present study was to explore possible effects of and
mechanisms associated with HIIT. Consequently, no clini-
cal endpoints were selected and no long-term effects can
be reported. Finally, a limitation could be that the study is
performed exclusively with healthy male subjects and male
patients.

3.8 | Summary

Eight sessions of one-legged HIIT improved insulin-mediated
glucose uptake and clearance rates in the trained leg
compared with the untrained leg in patients with type 2
diabetes as well as a matched healthy control group. The
mechanism for this effect includes an enhanced insulin-me-
diated delivery of glucose to the skeletal muscle, preserva-
tion of the ability to extract glucose from the blood,
tendency to increased GLUT4 content and increased GS
activity. The latter likely stimulated by frequent oscillations
in muscle glycogen stores. Content of proteins in the insu-
lin signalling cascade did not change, while biomarkers for
mitochondrial mass increased. We conclude that HIIT train-
ing is as effective as other training modalities for improv-
ing skeletal muscle insulin sensitivity.

4 | METHODS

4.1 | Study population

Ten Caucasian males with type 2 diabetes mellitus were
enrolled in the study together with an age, body mass
index (BMI) and maximal oxygen uptake (VO₂ max)
matched control group with normal glycosylated haemoglo-
bin values (HbA₁c) and normal oral glucose tolerance test
(Figure 12). Type 2 diabetes mellitus was defined as HbA₁c
≥6.5 mmol (47.5 mmol/mol). All participants were catego-
rized as sedentary according to the International Physical
Activity Questionnaire (IPAQ). Exclusion criteria included
weight instability (> ±2 kg/3 months), insulin treatment, a
history of cardiovascular or cerebrovascular disease or
signs of ischaemia during a cardiac stress test. Participant
characteristics are given in Table 1. Pharmacological treat-
ment (Table 1) was predominantly glucose-lowering agents
(in the patients), antihypertensive drugs and statins. Since
statins have been described to decrease maximal mitochon-
drial respiration, these drugs were paused for one week
before the experiment, while the others were paused on the
day of the experiment. The study was approved by the Danish National Committee on Health Research Ethics (H-4-2011-137) and conducted according to the Helsinki Declaration. Participants were given oral and written information and provided written informed consent prior to the study.

### 4.2 | Exercise intervention

A total of eight supervised training sessions every other day were conducted as one-legged bicycle interval exercise. The participants were randomly allocated into training with the right or the left leg. Ten intervals of 1 minute were interspersed with 1-minute recovery periods of rest, making the total time commitment 22 minutes including a 2-minute warm-up. The workload of the first training session was set at 70% of maximal one-legged workload and gradually increased to obtain a heart rate above 80% of max during the last two intervals of each session. Workload was applied at the beginning of each interval lasting for 1 minute and switched off at rest periods for 1 minute. Heart rate was monitored throughout the training sessions, and on two training sessions (numbers 2 and 6), oxygen uptake was measured continuously during the sessions (Jaeger Oxycon Pro; VIASYS Healthcare, Hoechberg, Germany). The non-training leg was resting on a chair next to the bicycle.

### 4.3 | Experimental protocol

An overview of the study design is shown in Figure 13. Applied methods for maximal oxygen uptake tests, whole-body dual-energy X-ray absorptiometry (DXA) scan and resting energy expenditure have been described previously. Measurements of maximal oxygen uptake (VO2max) were carried out using two-legged as well as one-legged ergometer cycle exercise.

On the experimental day, participants returned to the laboratory 40 hours after the last training session. Both femoral veins and a brachial artery were catheterized for measuring the arteriovenous balance for substrates and metabolites. An antecubital vein was catheterized to infuse insulin and glucose. A two-step, isoglycaemic, hyperinsulinaemic clamp with insulin infusion at 80 and 400 mU/min/m2 was performed as previously described. Plasma glucose was maintained at isoglycaemia by arterial blood analyses (ABL 700, Radiometer, Bronshoj, Denmark) every 5 minutes and subsequent adjustment of glucose infusion rate.

Arterial and venous blood samples were drawn, and femoral artery blood flow was measured by Ultrasound Doppler (Acuson S2000, Siemens Healthcare, Ballerup, Denmark) two times before initiation of the clamp (baseline) and three times within the last 30 minutes of each clamp step (I and II). Intra-arterial blood pressure was recorded throughout the clamp.

### 4.4 | Blood and muscle biopsy sampling

Micro biopsies were obtained before and immediately after the last training session to measure glycogen consumption in the thigh muscle (vastus lateralis). Percutaneous needle biopsies using Bergström technique (modified for suction) from vastus lateralis were obtained from trained and untrained leg before and immediately after the last clamp step. Visible non-muscle material was immediately removed, and one part of the muscle was directly frozen in liquid nitrogen and stored at −80°C.

### 4.5 | Analyses in skeletal muscle and blood

Mitochondrial respiration was performed in permeabilized muscle fibres as described previously with the exception that the buffer in the present study contained catalase (280 U/mL) and that chamber oxygenation was kept above 300 nmol/mL. The protocol measures O2 flux during state 2 (using glutamate and malate for complex I respiration (GM2)), state 3 (addition of ADP (GM3)) and addition of succinate for respiration with dual electron input to complex I + II (GMS3). The respiratory rates are reported as normalized for citrate synthase (CS) enzyme activity (ie reported as intrinsic capacity) and corrected for residual oxygen consumption by addition of antimycin A. Quality control of the muscle preparation was performed by adding cytochrome c (10 µmol/L) after GM3, and if the respiratory rates increased by >10%, the data were discharged.

The frozen muscle biopsies were freeze-dried and further cleansed from blood, adipose and connective tissue under a stereomicroscope, and the muscle powder was used in the analyses. Western blots were performed as described using freeze-dried dissected muscle tissue. The membranes were probed with antibodies against protein kinase B (Akt) (Cell Signaling Technology, Akt (pan) 4691, Leiden, The Netherlands), glucose transport isofrom 4 (GLUT4) (Fisher Scientific, PA1-1065 Invitrogen, Wal-tham, MA, USA), glycogen synthase (GS) (Cell Signaling Technology, 3893), hydroxycyl-CoA dehydrogenase (HAD) (Abcam, HADA ab54477, Cambridge, UK), mito-fusin-2 (Mfn2) (Cell Signaling Technology, 11925), phosphatidylinositol-3-kinase (PI3Kinase) (EMD Millipore, ABS234 Merck KGaA, Darmstadt, Germany), voltage-de-pendent anion channel protein 1 (VDAC) (Cell Signaling Technology, 4661) or the five complexes in the
mitochondrial oxidative phosphorylation system (Mitoprofile Total OXPHOS Human WB Antibody Cocktail, Abcam, ab110411). Quantification was done by the Image Quant Tl software (GE Healthcare Europe GmbH, Brondby, Denmark) where intensities of specific bands were normalized to the entire UV intensity of the corresponding sample representing its total protein content on the membrane (except the membranes with the COM I-V antibody cocktail). Furthermore, a pool of all samples was included on each gel/membrane to calibrate specific signals in order to compare across membranes. Therefore, each sample has been measured relative to its own UV intensity and then the mean of the intensity of the specific band of the three pooled samples on the membrane.

All GS activity measurements were performed in 96-well microtitre plates as previously described. The GS activity was determined in the presence of 1.67 mmol/L UDP glucose. The GS activity was measured at three levels of glucose-6-phosphate (0.02, 0.167 and 8 mmol/L) and is given as nmol/min/mg dw muscle weight. Gene expression of peroxisome proliferation-activated receptor gamma, coactivator 1 alpha (PPARGC1A) and carnitine palmitoyltransferase 1 and 2 (CPT1 and CPT2) was performed by reverse transcription polymerase chain reaction (RT-PCR). RT was performed with 500 ng total RNA with poly-dT primer in 20 µL with the OmniScript RT Kit (Qiagen, Valencia, CA, USA) according to the user manual. We amplified 0.5 µL cDNA in single reactions with the Quantitect SYBR Green Master Mix (Qiagen) and 100 nmol/L of each primer. The Stratagene MX3000p was used to calculate mRNA expression levels, where we have applied the 2−ΔΔCq method to determine the relative abundance of each mRNA. We used the NormFinder algorithm to calculate the most stable reference gene and found the ribosomal protein lateral stalk subunit P0 (RPLP0) to be most useful among RPLP0, GAPDH and peptidylprolyl isomerase A (PPIA). The used primers are listed in Table S1. For each of the primer set, we measured the mRNA level relative to the mean of the control group and the mean of the reference gene from all groups.

Plasma concentrations of substrates and metabolites (glucose, free fatty acids (FFA), glycerol β-hydroxybutyrate, cholesterol, triglyceride, hsCRP, orosomucoid) were measured by spectrophotometry (Cobas 6000 c 501, Roche, Glostrup, Denmark). HbA1c was analysed on a DCA Vantage Analyser (Siemens Healthcare Diagnostics, Tarrytown, NY, USA). Concentrations of hormones and cytokines in plasma were analysed by ELISA technique: insulin (Dako A/S, Glostrup, Denmark), C-peptide, glucagon (80-CPTHU-E01 and 48 GLUHU-E01 respectively; Alpco Diagnostics, Salem, NH, USA), cortisol (DE1887, Demeditec Diagnostics, Kiel, Germany), growth hormone (59121 IBL Intl., Hamburg, Germany), IL-6 and TNFα (HS600B and HSTA00D respectively; R&D Systems, Oxon, UK).

4.6 | Indirect calorimetry

Leg muscle metabolism was determined by indirect calorimetry. Blood gasses (O2 and CO2) in arterial and femoral venous blood samples were analysed (ABL 700, Radiometer, Bronshoj, Denmark) immediately after sampling. Calculations of rates of glucose oxidation, lipid oxidation and lipid synthesis were done from measurements of oxygen consumption (VO2) and carbon dioxide production (VCO2) rates and calculated respiratory quotient (RQ) values (VCO2/VO2). RQ values were corrected for estimates of protein and β-hydroxybutyrate oxidation rates and used in all calculations of oxidation and synthesis rates. Thus, protein oxidation was set to be 10% of oxygen consumption at baseline and 4% and 0% during clamp steps I and II respectively. CO2 production was calculated from a known RQ value of 0.87 for branched-chain amino acids. Ketone body (β-hydroxybutyrate and acetoacetate) oxidation has been reported to account for 5% and 3%, and in the present data set assumed to be 4% at baseline and 1% during clamp steps I and II. CO2 production from was calculated from a known RQ value of 0.89 for β-hydroxybutyrate. When corrected RQ values were >1.0, glucose oxidation and lipid synthesis were assumed to be the only net metabolic processes using O2 and producing CO2. To calculate lipogenesis, we assumed that the triglycerides formed were tripalmitate, tristearate and trioleate in the ratio 3:2:1 respectively. Synthesis of this average lipid from 1 g of glucose requires 25.8 mL of O2 and produces 239.6 mL of CO2. Glycogenesis was estimated as [glucose uptake rates – (glucose oxidation + lipogenesis + lactate release) (in glucose equivalents)], and negative values indicate glycogen breakdown.

4.7 | Calculations

All leg balance data and leg metabolism data are expressed relative to the leg lean mass. This was derived from the DXA scanning where leg lean mass was calculated as total leg mass – (fat mass + bone mineral content). Leg lean mass is almost equivalent to leg muscle mass.

4.8 | Statistical analysis

Heart rate data from all training sessions were pooled for each subject and then nested in the two groups. Minimum and maximum values from each interval were compared.
Single measurement comparisons were made using paired or unpaired Student's t test. Non-parametric statistics were applied for outcomes that did not meet the requirement of normality or with unequal variance. Two-way analyses for repeated measures were applied for comparison of repeated measurement (eg leg balance data during the clamp). All data are shown as mean ± standard error of mean (SEM). P-values <0.05 were considered significant.

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CONFLICTS OF INTEREST

The authors declare that there is no duality of interest associated with this manuscript.

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