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Effects of high-intensity interval training (HIIT) on skeletal muscle atrophy, function, and myokine profile in diabetic myopathy

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ABSTRACT

Purpose: Diabetes is a metabolic disorder characterized by chronic hyperglycemia due to insulin deficiency and/or loss of its action. Diabetic myopathy causes functional limitations in diabetic patients. The beneficial effects of high-intensity interval training (HIIT) are widely reported. We have hypothesized that HIIT application would prevent the development of diabetic myopathy.

Methods: Male, Wistar albino rats (10 W) were randomly divided into four groups (1)Control(C), (2)Diabetes (DM), (3)Training(HIIT), and (4)Diabetes + Training(DM + HIIT). Streptozotocin(60 mg/kg) was injected for the induction of diabetes. The maximum exercise capacity(MEC) of animals was determined by an incremental load test. HIIT protocol (4 min 85–95 % MEC, 2 min 40–50 % MEC, 6 cycles, 5 days/week) was applied for 8 weeks. In the end, functional parameters, atrophy, and resistance to fatigue in soleus and EDL muscles were evaluated. IL-6, FNDC5, and myonectin levels were measured in EDL, soleus, and serum.

Results: We observed atrophy, fatigue sensitivity, and proinflammatory alterations (IL-6 increase) in the EDL samples due to diabetic myopathy which were not observed in the soleus samples. HIIT application prevented the aforementioned detrimental alterations. Both force-frequency response and parallelly the twitch amplitude increased significantly in the DM + HIIT group. Half relaxation time (DT_{50}) increased in both exercising and sedentary diabetics. FNDC5 was significantly higher in the exercising animals in soleus samples. Myonectin was significantly higher in the soleus muscle only in the DM + HIIT group.

Conclusion: Current findings show that diabetic myopathy develops earlier in glycolytic-fast-twitch fibers(EDL) than in oxidative-slow-twitch fibers(soleus). Furthermore, HIIT application prevents atrophy in skeletal muscle, increases resistance to fatigue, and has an anti-inflammatory effect.

New findings: The current study analyzes the myokine profile and skeletal muscle function under the effect of diabetes HIIT-type exercise. We also measured maximal exercise capacity and tailored the exercise program individually according to the result. Diabetic myopathy is an important complication of diabetes yet still, it is not understood completely.

Our results show that HIIT-type training would be beneficial in diabetic myopathy but further investigation is needed to understand the whole molecular mechanism.

1. Introduction

Diabetes mellitus (DM) is a metabolic disease that is characterized by chronic hyperglycemia due to impaired insulin secretion and sensitivity and it damages various tissues *(cardiovascular system, retina, kidneys, nervous system, etc.)* over time [43]. Diabetic myopathy is characterized

Abbreviations: ANOVA, Analysis of Variance; BW, Body Weight; CT, Contraction Time; DM, *Diabetes mellitus*; DT_{50}, Decay Time 50; DW, Dry Weight; EDL, *Extansor digitorum longus*; FHL, *Flexor hallicus longus*; FNDC5, Fibronectin Type 3 domain containing protein 5; HF, High frequency; HIIT, High-intensity interval ttraining; IL-6, Interleukin-6; LF, Low frequency; MEC, Maximal exercise capacity; Ps, Power stroke; STZ, Streptozotocin; TNF-α, Tumor necrosis factor α; WC%, Water content (%); WW, Wet weight; Vo_{2max}, Maximal oxygen consumption; $+dp/dt_{max}$, Maximum contraction and relaxation rates.

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by muscular atrophy *(decreased muscle mass)*, muscle weakness *(decreased muscle contractility)*, and increased muscle fatigue [2,10]. Since skeletal muscle is considered the primary organ in locomotion and glucose metabolism, it has a key role in the prognosis of diabetes and its complications [5,26]. Oxidative fiber-dominant muscles are more affected by atrophy from immobility, microgravity, and denervation, while glycolytic fiber-dominants are more vulnerable to diabetes, cancer cachexia, chronic heart failure, and aging [56].

Exercise has many beneficial effects such as reducing the risk of cardiovascular, pulmonary, and metabolic diseases, as well as cancer, anxiety, and depression. Previous studies have shown that diabetic atrophy can be prevented with chronic exercise [49]. Although its beneficial effects, determining the right training protocol is crucial. In the last decades, a new form of exercise has been introduced which is called "High-intensity interval training (HIIT)". HIIT basically consists of active-resting periods between successive high-intensity exercise sessions. Although different HIIT protocols have been defined, it is generally maintained for 1–4 min at an intensity corresponding to 80–90 % of Vo_{2max} as the high-intensity period, while the intensity is reduced to 50–60 % of Vo_{2max} in the active rest period [8]. There are various studies that report HIIT could improve various physiological parameters *(especially cardiovascular and metabolic fitness)* better than any other conventional exercise program [35,44,9,19]. Although there are lots of positive data, an underlying mechanism of this difference is needed to be revealed.

Myokines are defined as cytokines and other peptides that are produced, expressed, and released by muscle fibers and exert either autocrine, paracrine, or endocrine effects [52]. Exercise *(or simply skeletal muscle contraction)* affects the secretion profile of myokines that's why they are also called "exercise factors". Myokines mediate communication between skeletal muscle and other organs, such as the brain, adipose tissue, liver, and pancreas, as well as within the muscle itself *(autocrine effect)* [52]. Interleukin-6 (IL-6) is the first defined myokine which has both proinflammatory and anti-inflammatory effects. IL-6 secretion is affected by the duration and intensity of skeletal muscle contraction [14,15,16,33]. In acute exercise serum, IL-6 concentration increases and it exerts an anti-inflammatory effect via suppressing tumor necrosis factor-α (TNF-α) [14,42]. There are some studies that report the insulin-mimetic effect of IL-6. It increases glucose uptake and fatty acid oxidation via altering the AMPK and PI3K-Akt pathways [48]. Irisin which is synthesized by the degradation of FNDC5, is defined as an important mediator of the beneficial effects of exercise [31,46]. Irisin increases thermogenesis and total energy expenditure whereas it has a crucial role in exercise-induced cardioprotection [57]. Furthermore, irisin has a role in skeletal muscle hypertrophy, myogenic differentiation, and activation of satellite cells [31,47]. Myonectin is a fairly novel myokine that possesses insulin-mimetic effects [38]. Myonectin increases muscle mass via stimulating protein synthesis while suppressing catabolism [6,51].

In the current study, we aimed to examine the effects of HIIT on the Streptozotocin-induced diabetic myopathy model. We hypothesize that HIIT application would prevent the detrimental effect of diabetes on skeletal muscles. Furthermore, we have planned to examine the exercise-induced alterations in the myokine profile that is crucial in muscle-organ crosstalk. Finally, we have planned to examine two different skeletal muscles that have different dominant fiber types *(oxidative and glycolytic)*. We expect fiber type dominancy would affect the magnitude of the detrimental effects of diabetes and response to exercise at the same time.

2. Methods

2.1. Animals and ethical approval

Animal experiments were conducted in the laboratory of Ankara University, Faculty of Medicine, Department of Physiology. Animals were handled under the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996) [12]. Ankara University, Animal Experiments Local Ethics Committee (AU HADYEK) approved the current methodology (2021–8-47).

The minimum sample size was calculated considering that the one-way ANOVA would be used in statistical analysis. When the parameters were determined as follows: the power of the test (1-β) = 0.8, the effect size (d) = 0.5, and the Type-I error (α) = 0.05; the minimum sample size was calculated as n = 48. Calculations were made with G*power (ver. 3.1.9.6) software. According to previous experience, we expected at least 20 % data loss *(mortality caused by diabetes, animals excluded from the study, low-quality data, etc.)*. Therefore, we increased the sample size to n = 60.

Male, 10 weeks old (weighing 250–300 g), Wistar albino *(Rattus norvegicus)* rats (n = 60) were obtained from Ankara University Experimental Animals Breeding and Research Laboratory. Animals were housed in a 12-hour light/dark cycle at a constant temperature (22–24 ℃) and humidity (50–55 %) and had access to *ad libitum* chow (Korkutelim Inc. Antalya, Turkey) and tap water (Table 1). Rats were adapted to our laboratory for one week before the start of the experiments.

Then, animals were randomly divided into four groups which were:

(1) Control (C) (n = 15)
(2) Diabetes (DM) (n = 15)
(3) Training (HIIT) (n = 15)
(4) Diabetes + Training (DM + HIIT) (n = 15)

The flow chart of the experimental procedures was given in Fig. 1.

2.2. Induction of experimental type I diabetes

Animals were fasted overnight (12–16 h) before the injection. Streptozotocin (STZ) (Sigma Aldrich, SO130, Missouri, USA) dissolved in 0.1 M Citrate Buffer (pH: 4.5) was injected in 50 mg/kg dose *i.p.* to induce experimental Type I diabetes. We monitored blood glucose levels on the 1st, 3rd, and 7th days following the injection, and rats with all three measurements above 300 mg/dl (16.7 mmol/l) were diagnosed as diabetic if not excluded from the experiment. We made a small incision at the tip of the tail for measurements and used a standard glucometer (OnCall Plus Acon Labs. Inc. USA). Blood glucose and body weight were monitored regularly throughout the study.

2.3. Incremental load test and training program

We used a rodent-specific treadmill manufactured by Ankara University, Faculty of Engineering. Animals were gradually adapted to the treadmill for 1 week to minimize the adverse effects of stress.

The maximal exercise capacity (MEC) of each animal was determined by the incremental load test [58,21]. Briefly, the animal started the test at 25° inclination and 8 m/sec speed. After 20 min of warm-up,

Table 1
Nutrient composition of the standard rat chow.

Composition of the chow	
Carbohydrate	48.80 %
Raw protein	23.50 %
Raw Cellulose	6.50 %
Raw Ash	4.91 %
Raw fat	3.00 %
Lysine	1.30 %
Phosphorus	0.74 %
Calcium	0.62 %
Methionine	0.43 %
Sodium	0.04 %
Moisture	10.16 %

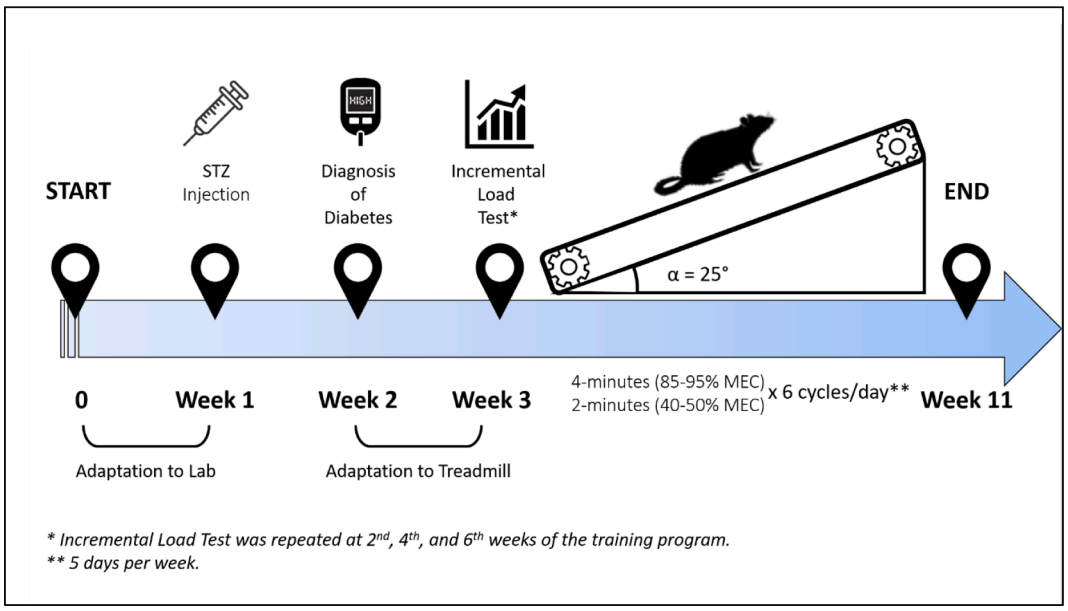


Fig. 1. Flow chart of experimental procedures and the training program.

the speed was increased by 2 m/sec every two minutes. The speed at which the animal could not continue despite the electrical and mechanical stimulation was considered the MEC value of the animal. The intensity of the training protocol was determined individually for all exercising animals according to their MEC values. High-intensity interval training (HIIT) consists of repeating cycles. In the current protocol, one cycle has 4 min of high-intensity (85–95 % MEC) and 2 min of active-resting (40–50 % MEC) periods. We applied 6 cycles in one session. HIIT sessions were scheduled on weekdays in the morning (between 9:00–12:00). The training protocol was continued for 8 weeks.

The slope of the treadmill was kept constant (25°) throughout the training, and the exercise intensity was adjusted by changing the speed of the treadmill. The incremental load test was repeated at the training program's 2nd, 4th, and 6th weeks. If there was a change in the animal's MEC, the running speed was adjusted to ensure the targeted intensity.

We also calculated the workload of the training programs which were planned according to the MEC values of the animals. We used the following formula:

$$\text{Workload (Watt)} = \text{Force (N)} \times \text{Distance Travelled (m)} \times \cos(\alpha).$$

2.4. Termination of experiments

Animals that had been fasted overnight (12–16 h) were sacrificed 24 h after the last exercise session. Fasting blood glucose levels of the animals were measured from the tail vein. Then, animals were anesthetized by *i.p.* injection of sodium thiopental (50 mg/kg). The depth of anesthesia was monitored by checking the finger-pinch response. After cessation of reflex, we rapidly opened the chest cavity and entered to right ventricle with a heparinized syringe (21G). We drained ≈8–10 ml of blood and transferred it to a gold-capped Vacutainer® tube *(Clot activator and serum separating gel)*. Blood samples were centrifuged at 5000 g, +4 °C for 10 min. The supernatant was kept, and the pellet was discharged. The obtained serum was aliquoted and stored at −80 °C for further analysis.

The *soleus* and *extensor digitorum longus* (EDL) muscles of the animals were removed bilaterally. All samples were weighed. The length and radius of the muscles were measured with a digital caliper. We used the right pairs of muscles for isolated skeletal muscle function. After functional measurements, the muscle samples were desiccated with a humidity scale (AXIS AGS100) and dry weights were measured, humidity percentage was calculated.

The left pairs of the muscles were cut into two pieces, first one was put into buffered formalin solution (10 %) for histological analysis whereas the other part was snap-frozen in liquid nitrogen and stored at −80 °C for biochemical analysis.

2.5. Isolated skeletal muscle function

2.5.1. Preparation

We used an isolated organ bath system (Commat LTD. IOBS99 Ankara, Turkey) which is specific for skeletal muscle. Briefly, the bath consists of two glass chambers which are the reservoir and the organ chamber. The organ chamber is a double-layered glass reservoir, which has a detachable glass stand inside for skeletal muscle attachment. The valves inside the organ chamber let us replenish the content of the chamber when needed. First, the reservoir of the bath was filled Krebs-Henseleit buffer (mM: 118 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.2 KH, 2 PO_4, 25 $NaHCO_3$, 2.5 $CaCl_2$, and 11 Glucose; pH: 7.2–7.4) at 28 °C. The buffer was continuously gassed with carbogen (95 % O_2 and 5 % CO_2) throughout the experiment. The organ chamber was filled with buffer and the muscles were mounted vertically *(soleus and EDL samples were mounted to two separate systems simultaneously)* to the glass stand between two platinum electrodes (not touching the muscle). The muscles were stimulated with the electrical field stimulation method by using a specific stimulator (Biopac Systems Inc. CA, USA). The voltage was 60 Volts and the pulse width was 0.5 ms for all stimuli. The optimal voltage and buffer temperature were predetermined in our preliminary experiments (data not shown).

The optimal length of the muscle prior to the contraction was determined by trying various preloads (1, 1.5, 2, or 2.5 g). The preload at which the muscle made the maximum contraction was determined as its optimal length. The initial tension of the muscle was set to the specified value for subsequent analyses. The whole procedure was summarized in Fig. 2.

Contraction data were converted from g to N/cm^2 using the following formula [50]:

$$\text{Force (N)}/\text{cm}^2 = [\text{force (N)} \times \text{skeletal muscle density (1.06 g/cm}^3) \times \text{muscle length (cm)}] / \text{muscle weight (g)}.$$

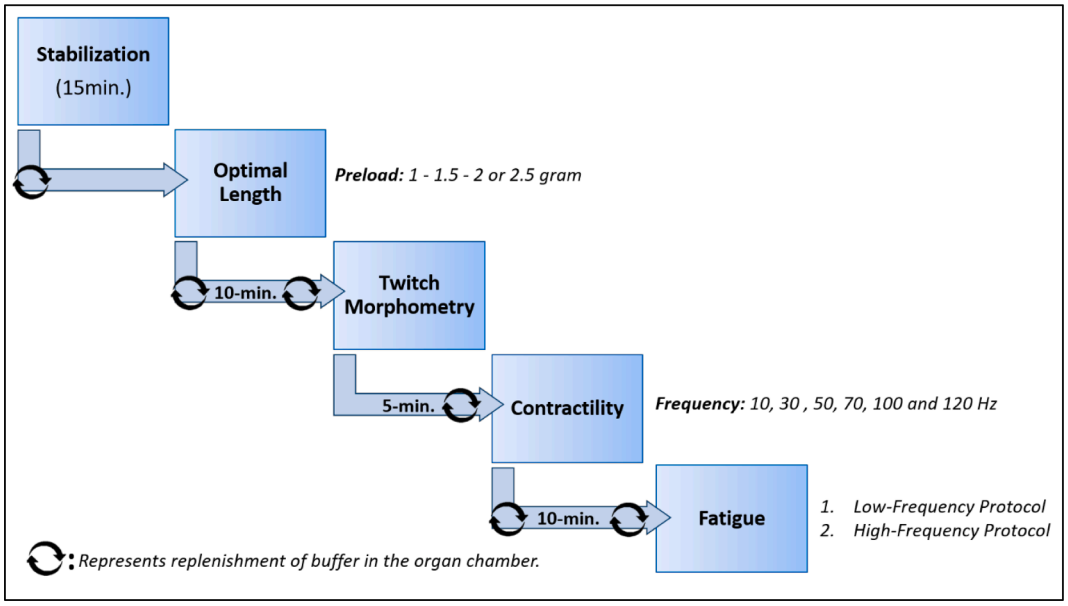


Fig. 2. Functional measurement steps after mounting the muscles to the isolated organ bath.

2.5.2. Twitch Morphometry

We evaluated the amplitude (Ps), contraction time (CT), half-relaxation time (DT_{50}), maximum contraction, and relaxation rates ($\pm dP/dt_{max}$). All parameters were calculated by averaging the data of five twitches.

2.5.3. Contractility

The muscles were stimulated at six consecutive frequencies (10, 30, 50, 70, 100, and 120 Hz, respectively) for five seconds. The contractile responses were recorded and the force-frequency graph was plotted.

2.5.4. Fatigue

The fatigue protocols are shown in Fig. 3. We applied two different fatigue protocols, which were low-frequency and high-frequency fatigue protocols [40].

The pattern was the same in both protocols. Train time was 8 s whereas the train duration was 30 s. 6 cycles were applied. We recorded twitches before and after the protocol (T_{PRE} and T_{POST}). The fatigue index was calculated according to the following formula [13]:

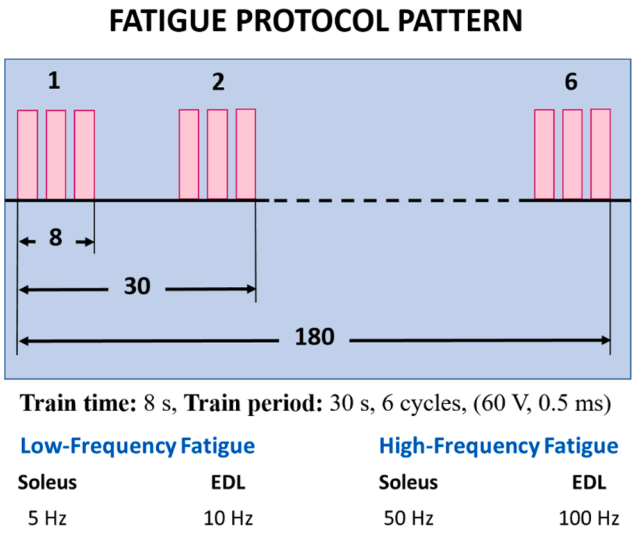


Fig. 3. Application and details of the fatigue protocols.

Fatigue index (%) = Amplitude of T_{POST} (g) / Amplitude of T_{PRE} (g) × 100.

2.6. Biochemical analyzes

2.6.1. Serum insulin concentration

Fasting serum insulin levels of animals were measured with ELISA (Elabscience, Cat No: E-EL-R3034). Assay was completed according to the manufacturer's instructions.

2.6.2. Myokine profile analysis

Soleus and EDL muscle samples were homogenized according to the same protocol. Briefly, the sample was weighed and put into a mortar filled with liquid nitrogen, and it was crushed into small pieces with a pestle. The tissue debris was transferred to a glass tube and homogenization buffer was added at a ratio of 1:9 *(w/v)*. Ice-cold phosphate-buffered saline (PBS; 0.02 mol/L, pH 7.0–7.2) was used as the homogenization buffer. Tissue was further degraded with a special homogenizer (Glas Col 099 K54 Cole Palmer). Then, homogenates were centrifuged at 5000*g*, +4 °C, for 15 min, and the supernatant was stored. Total protein concentration was measured using the Bradford Assay.

Interleukin-6 (IL-6), Fibronectin type III domain-containing protein 5 (FNDC5), and Myonectin concentrations were measured with ELISA (Elabscience Cat. No: E-EL-R0015, Elabscience Cat. No: E-EL-R1104, and MyBioSource Cat. No: MBS1600363, respectively). The measurements were made both in muscle homogenates and serum samples. Myokine concentrations in muscle homogenates were normalized by the total protein concentration of the homogenate. Assays were completed according to the manufacturer's instructions.

2.7. Histological evaluation

For fiber diameter analysis, the muscle samples (soleus and EDL) were fixed in neutral buffered 10 % formalin solution for two days. Before dehydration by graduated alcohol series, samples were washed under tap water. Afterward, they were cleared in Xylol and incubated in liquid paraffin for 4 h at 60 °C then embedded into blocks. Then we cut the block into 5 μm thick sections with a rotary microtome (Leica RM 2125). The sections were stained with Hematoxylin-Eosin and captured

with an AxioCam MRc5 camera at 40× magnification under a light microscope (Zeiss Axio Scope.A1, Oberkochen, Germany). The shortest diameter of muscle fiber was measured and recorded by Axiovision Rel 4.8 software. At least ten muscle fiber cross-sections were evaluated and averaged on each image.

2.8. Statistical analysis

GraphPad Prism (GraphPad Prism for Windows v5 2007) was used for statistical analysis. First, the test of normality (Shapiro-Wilk) was applied to all data sets. We decided to prefer parametric tests, due to the distribution and nature of the data. One-way ANOVA was selected as the multiple comparison test. Tukey test was selected as the Post-Hoc test. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Metabolic parameters

Terminal body weight, fasting blood glucose, and insulin levels of the animals were given in Fig. 4.

Terminal body weight showed a significant difference between groups ($F_{(3,50)} = 33.54$; $p < 0.0001$). Body weights of the diabetic animals (DM and DM + HIIT) were significantly lower compared to control animals ($p < 0.0001$ for each). Exercise did not cause any significant alteration in the body weight of both diabetic and non-diabetic animals ($p > 0.05$).

Fasting blood glucose levels differ between the groups ($F_{(3,49)} = 7.958$; $p < 0.0001$). Fasting blood glucose levels of both diabetic groups (DM and DM + HIIT) were significantly higher compared to the control animals ($p = 0.001$ and $p = 0.0328$, respectively). Training did not alter the fasting blood glucose levels both in diabetic and non-diabetic animals ($p > 0.05$). The blood glucose levels that we measured weekly during animal experiments were not measured after fasting and they were always above 300 mg/dl (data not shown).

There was a significant difference among groups in fasting serum insulin levels ($F_{(3,32)} = 46.48$; $p < 0.0001$). Insulin levels were approximately 40-fold lower in diabetic animals compared to control ($p < 0.0001$ for each). Training did not affect insulin levels both in diabetic and non-diabetic animals ($p > 0.05$).

3.2. Atrophy parameters

Wet and dry weights were weighed for soleus and EDL muscles. After drying dry weights were also measured and the water content (WC) of the samples was calculated. Both wet and dry weights were normalized to the terminal body weight (Table 2).

The dry weight (DW/BW) of the soleus samples did not differ among groups ($F_{(3,41)} = 1.254$; $p = 0.3026$). Wet weight (WW/BW), and water

Table 2
Wet weight (WW), dry weight (DW), and water content (WC) for soleus and EDL muscles. All weights were normalized to terminal body weight (BW) and multiplied by 10^3 for ease of notation (n = C: 12, DM: 12, HIIT: 12, DM + HIIT: 15) (*: $p < 0.05$ vs. Control) (x̂±SD).

	SOLEUS		
	WW / BW × 10^3	**DW / BW × 10^3**	**WC (%)**
Control	0.67 ± 0.12	0.15 ± 0.03	77.85 ± 2.38
HIIT	0.77 ± 0.17	0.17 ± 0.02	78.29 ± 3.39
DM	0.82 ± 0.13	0.15 ± 0.02	80.88 ± 3.45
DM + HIIT	0.87 ± 0.12*	0.16 ± 0.02	81.62 ± 1.94*
	EDL		
	WW / BW × 10^3	**DW / BW × 10^3**	**WC (%)**
Control	0.57 ± 0.06	0.13 ± 0.02	77.90 ± 1.32
HIIT	0.64 ± 0.07	0.14 ± 0.01*	77.66 ± 2,78
DM	0.57 ± 0.05	0.11 ± 0.01*	80.91 ± 1,41*
DM + HIIT	0.58 ± 0.06	0.12 ± 0.01	79.13 ± 1.33

*: $p < 0.05$ vs. Control.

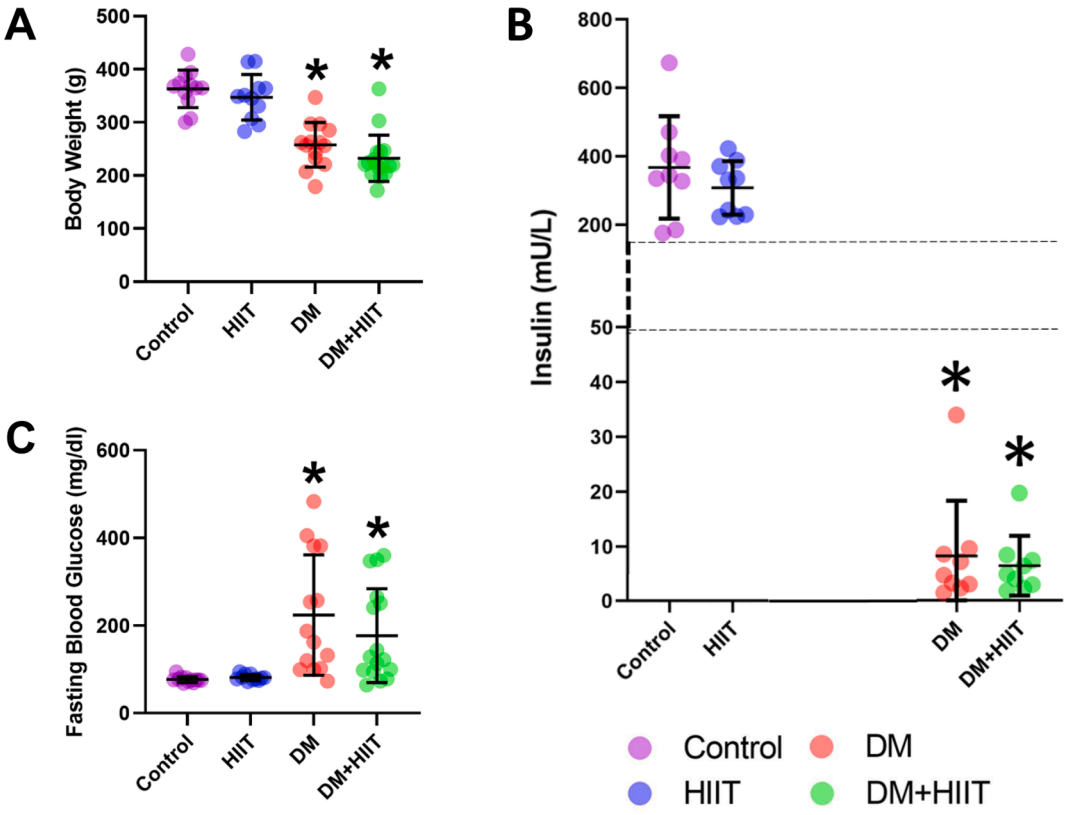


Fig. 4. Metabolic parameters of the animals. **(A)** Body weight **(B)** Fasting serum insulin level **(C)** Fasting blood glucose (*: $p < 0.05$ vs. Control).

content (WC %) of soleus samples showed a significant difference between the groups ($F_{(3,42)} = 4.975$; $p = 0.0048$ and $F_{(3,46)} = 5.556$; $p = 0.0024$, respectively), and the values in the DM + HIIT group were significantly higher compared to the control for both parameters ($p = 0.0028$ and $p = 0.0075$, respectively). We did not observe any other significant difference among other groups for WW/BW and WC % ($p > 0.05$).

In EDL muscle, WW/BW ratio did not differ among the groups ($F_{(3,43)} = 3.472$; $p > 0.05$), whereas DW/BW and % WC was differed ($F_{(3,39)} = 11.44$; $p < 0.0001$ and $F_{(3,43)} = 7.528$; $p = 0.0004$ respectively). The dry weight of the EDL samples in the HIIT group was significantly higher compared to the control ($p = 0.0438$) while it was significantly lower in the DM group compared to the control ($p = 0.0226$). WC % was increased in the DM group compared to controls ($p = 0.0016$).

We also measured fiber diameter in histological slides which is a better approach for determining atrophy (Fig. 5).

Fiber diameters did not differ among groups in soleus samples ($F_{(3,22)} = 1.625$; $p = 0.2124$). In EDL samples, there was a marked difference between groups ($F_{(3,24)} = 22.80$; $p < 0.0001$). There was a significant increase in the HIIT group compared to the control ($p = 0.0021$) whereas diameter was markedly decreased in the DM + HIIT group compared to controls ($p = 0.0080$).

3 Maximum Exercise Capacity (MEC).

The MEC values were measured at 0, 2, 4, and 6 weeks only in the exercise groups (HIIT and DM + HIIT) (Fig. 6).

The MEC value shows a time-dependent increase in both groups ($F_{(3,53)} = 15.43$; $p < 0.0001$). There was a divergence trend between the MEC values of diabetic and non-diabetic animals after the fourth week. However, it did not reach significance ($F_{(1,18)} = 1.729$; $p = 0.2051$). The workload values were differed between non-diabetic and diabetic animals ($F_{(3,34)} = 43.38$; $p < 0.0001$).

3.3. Isolated skeletal muscle function

3.3.1. Twitch morphometry

The twitch amplitude (Power stroke = Ps) for soleus and EDL samples, were given in Fig. 7. Time to peak (contraction time = CT) and half relaxation time (decay time$_{50}$ = DT$_{50}$) for soleus and EDL samples were given in Fig. 8. Maximum contraction and relaxation rates for soleus and EDL samples were given in Fig. 9.

Twitch amplitudes in soleus and EDL muscles differ among groups ($F_{(3,24)} = 12.05$; $p < 0.0001$ and $F_{(3,25)} = 4.277$; $p = 0.0144$, respectively). In the soleus samples, amplitudes were significantly higher in the DM + HIIT group compared to the control ($p < 0.0001$) and also to the DM group ($p = 0.0204$). In the EDL samples, amplitudes in the DM + HIIT group were markedly higher compared to the control ($p = 0.0399$).

Time to peak values differed among groups in soleus samples ($F_{(3,21)} = 5.021$; $p = 0.0088$) while there was no significant difference among groups in EDL samples ($F_{(3,22)} = 2.450$; $p = 0.0905$). In soleus samples, contraction times in the HIIT group were markedly lower compared to the control ($p = 0.0257$) and there was no significant difference among other groups ($p > 0.05$).

Half relaxation time values differed among groups both in soleus ($F_{(3,25)} = 25.17$; $p < 0.0001$) and EDL ($F_{(3,26)} = 5.096$; $p = 0.0066$) samples. In soleus samples, there was a significant increase in both diabetic groups (DM and DM + HIIT) compared to the control ($p = 0.0003$ and $p < 0.0001$ respectively). In EDL samples, there was a

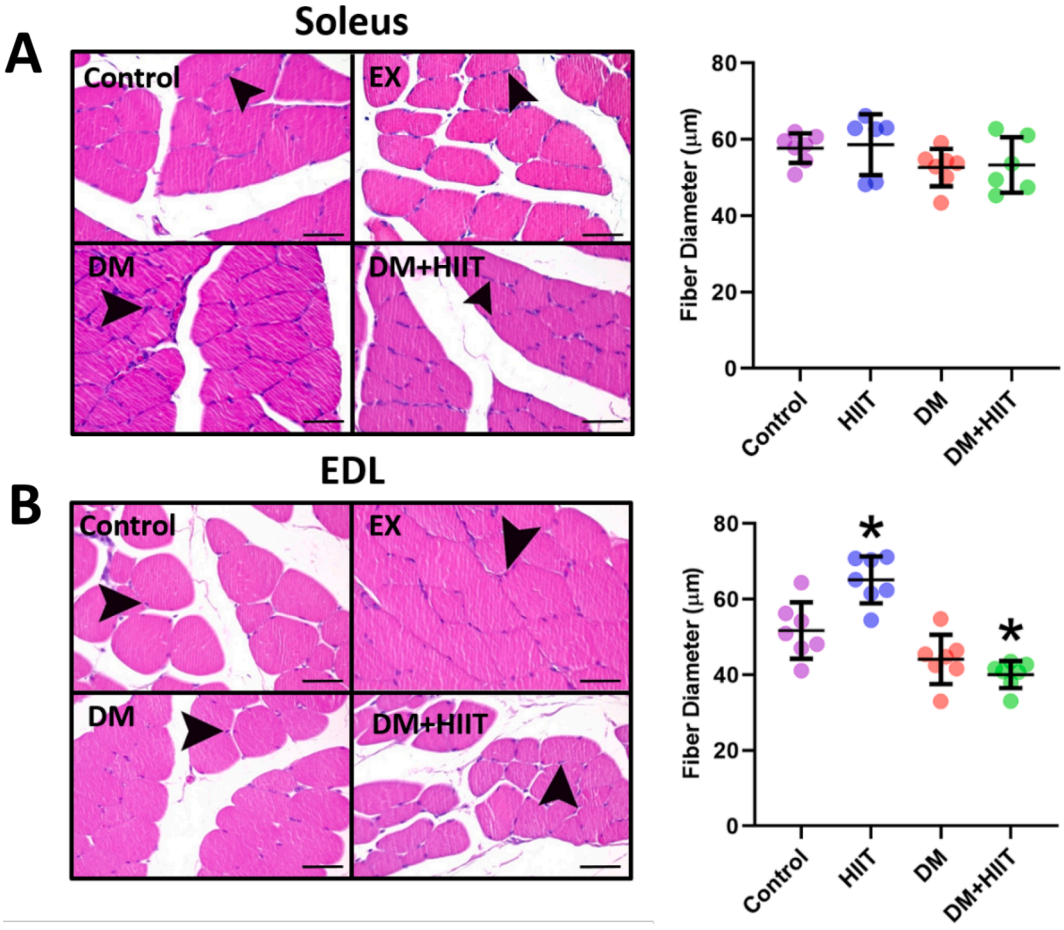


Fig. 5. Fiber diameters **(A)** Skeletal muscle cross-sectional slides and fiber diameter graph in soleus samples **(B)** Skeletal muscle cross-sectional slides and fiber diameter graph in soleus samples in EDL samples. **Dye:** Hematoxylin-Eosin, **Arrowhead**: Nuclei of muscle fibers, **Scale bar** = 50 µm. (*: $p < 0.05$ vs. Control).

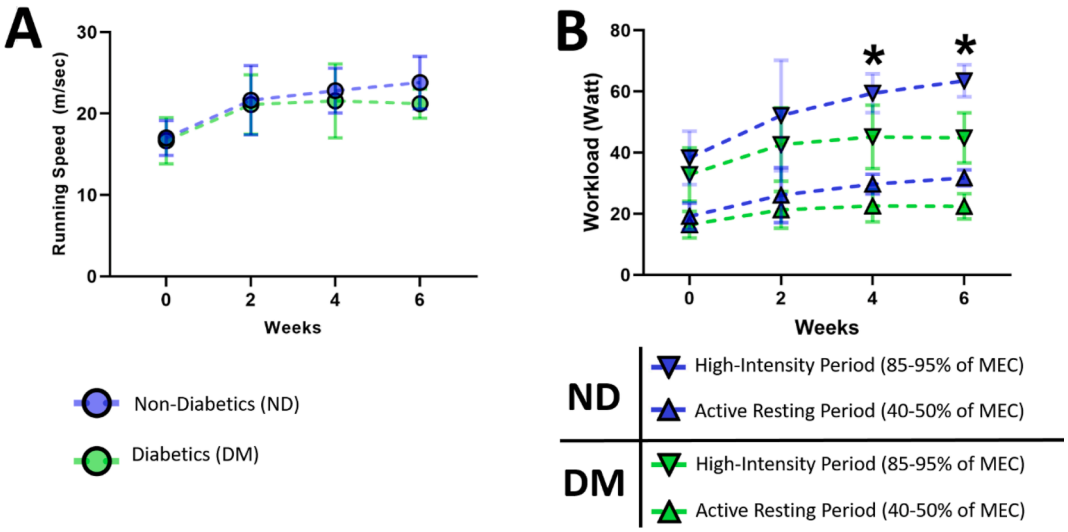


Fig. 6. Incremental test results and exercise program **(A)** MEC values measured in diabetic and non-diabetic exercise groups *(represented as maximal running speed)* **(B)** Workload values of the training program applied to non-diabetic and diabetic animals (*: $p < 0.05$ vs. Diabetics) (n = Non-diabetics: 7, diabetics: 12).

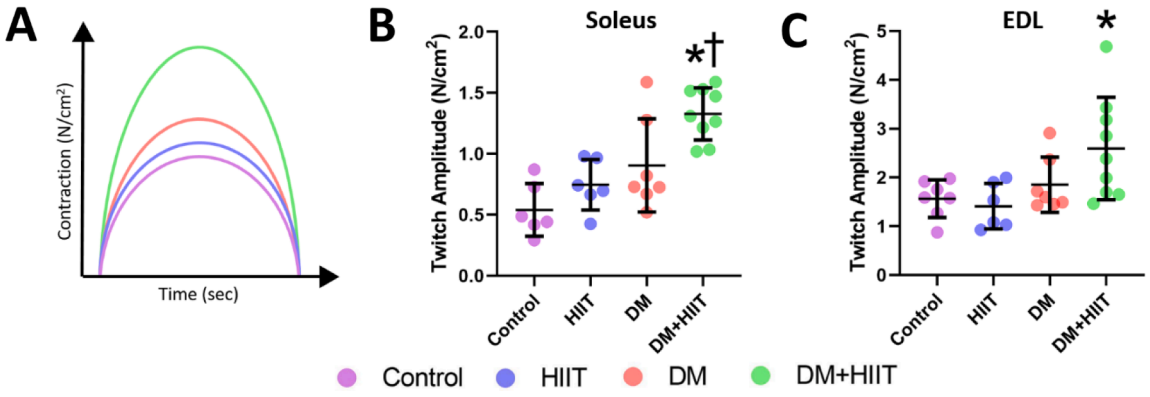


Fig. 7. The power stroke (Ps) of the twitch samples. **(A)** Representative twitches **(B)** Twitch amplitudes recorded in soleus samples **(C)** Twitch amplitudes recorded in EDL samples. (*: $p < 0.05$ vs. Control; †: $p < 0.05$ vs. DM).

marked increase only in the DM group compared to the control ($p = 0.0080$), and it was normalized by training (in the DM + HIIT group).

While the maximum contraction rate of the soleus samples differs among groups ($F_{(3,25)} = 5.610$; $p = 0.0044$), the maximum relaxation rate did not ($F_{(3,25)} = 1.746$; $p = 0.1832$). There was a significant difference in maximum contraction rate only between the DM + HIIT group and the control group ($p = 0.0035$).

Both the maximum contraction and relaxation rates in the EDL muscle samples were different between groups ($F_{(3,26)} = 4.777$; $p = 0.0088$ and $F_{(3,27)} = 5.382$; $p = 0.0049$, respectively). There was a significant difference between the DM + HIIT group and the control group both in the maximum contraction ($p = 0.0370$) and relaxation rate ($p = 0.0352$). Additionally, there was a significant difference between DM and DM + HIIT groups only in relaxation rate data ($p = 0.0410$).

3.3.2. Contractility

The contractile response to the given stimuli at various frequencies (10, 30, 50, 70, 100, and 120 Hz) was measured both in the soleus and EDL muscles (Fig. 10).

The contractility curves recorded in soleus and EDL muscles showed significant differences between the groups ($F_{(3,23)} = 5.736$; $p = 0.0044$ and $F_{(3,25)} = 5.198$; $p = 0.0063$, respectively). The DM + HIIT curve dramatically diverges from others for both muscles. The difference between DM + HIIT and the control group was significant for the soleus muscle for all time points. For the EDL muscle, there was a similar trend but significance was observed at some time points (30, 50, 70, and 100 Hz).

There was no significant difference among other curves, for any frequency both in the soleus and in EDL muscles ($p > 0.05$).

3.3.3. Fatigue

High-frequency (HF) and low-frequency (LF) fatigue indices were calculated separately for soleus and EDL muscles (Fig. 11).

Both LF and HF fatigue indices did not differ among groups for soleus samples ($F_{(3,23)} = 1.755$; $p = 0.1839$ and $F_{(3,20)} = 1.836$; $p = 0.1732$, respectively). In the EDL samples, both indices (LF and HF) markedly differed between groups ($F_{(3,26)} = 7.492$; $p = 0.0009$ and $F_{(3,24)} = 3.292$; $p = 0.0378$, respectively). While the LF fatigue index was significantly decreased in the DM group compared to the control in EDL samples ($p = 0.0202$), training normalized the index in diabetic animals. Additionally, there was a significant difference between DM and DM + HIIT groups ($p = 0.0223$). We observed a similar pattern in the HF fatigue index calculated in EDL samples. Diabetes markedly decreased the index ($p = 0.0342$) and training normalized that effect in diabetic animals. But there was not a significant difference between DM and DM + HIIT groups ($p > 0.05$).

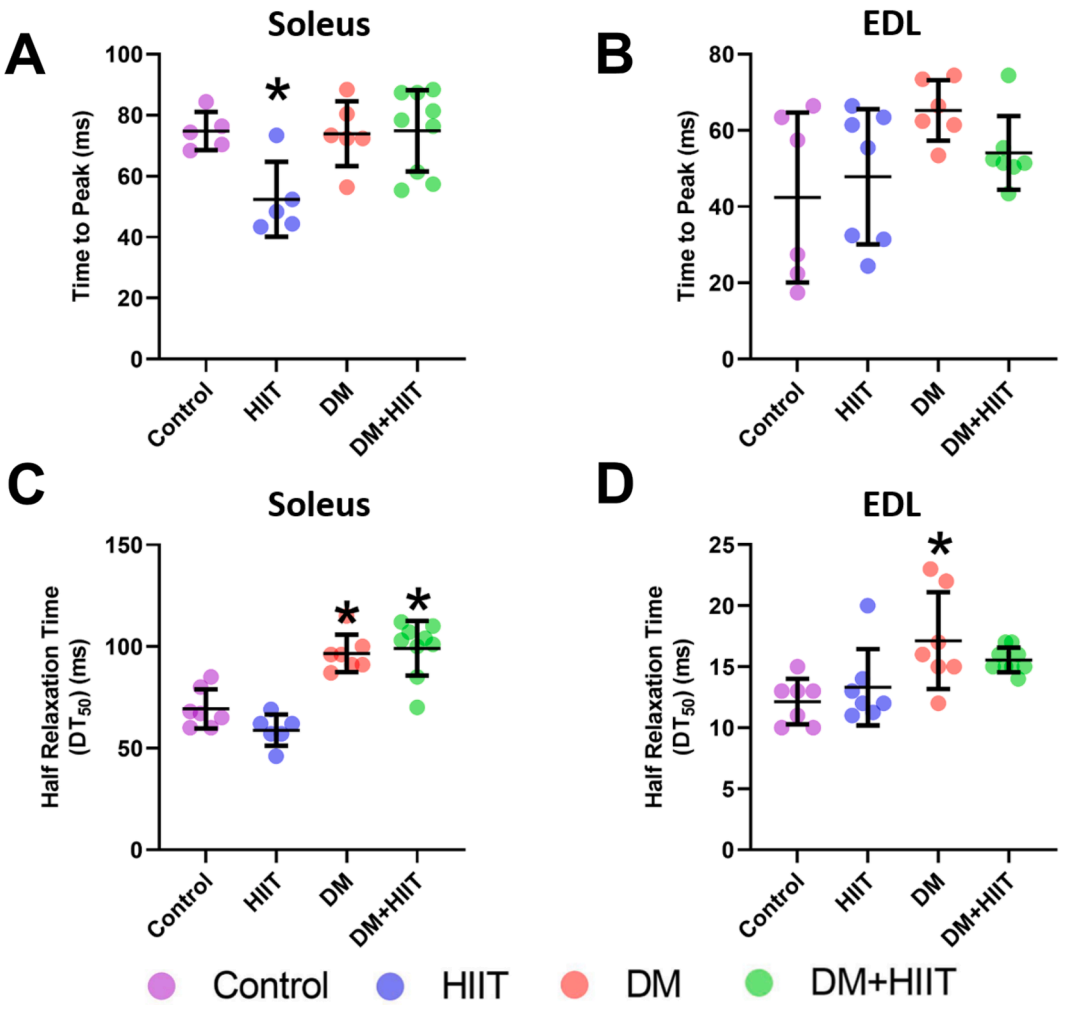


Fig. 8. Contraction and relaxation times. **(A)** Time to peak (contraction time = CT) in soleus samples **(B)** Time to peak in EDL samples **(C)** Soleus muscle half relaxation time (DT_{50}) **(D)** EDL muscle half relaxation time (DT_{50}) (*: $p < 0.05$ vs. Control).

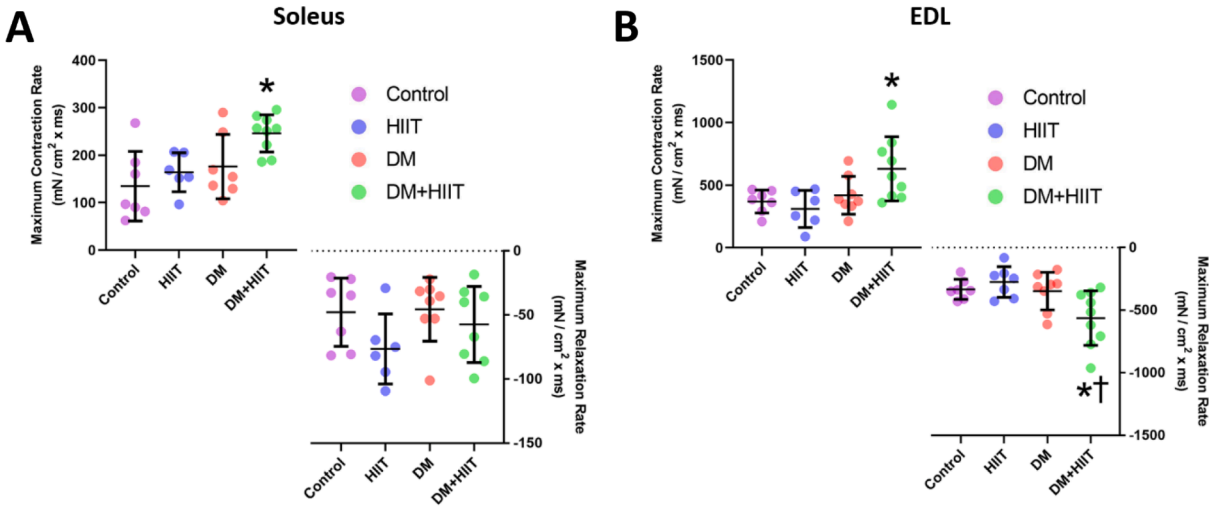


Fig. 9. Maximum contraction and relaxation rates **(A)** Maximum contraction and relaxation rates for the soleus samples **(B)** Maximum contraction and relaxation rates for the EDL samples (*: $p < 0.05$ vs. Control; †: $p < 0.05$ vs. DM).

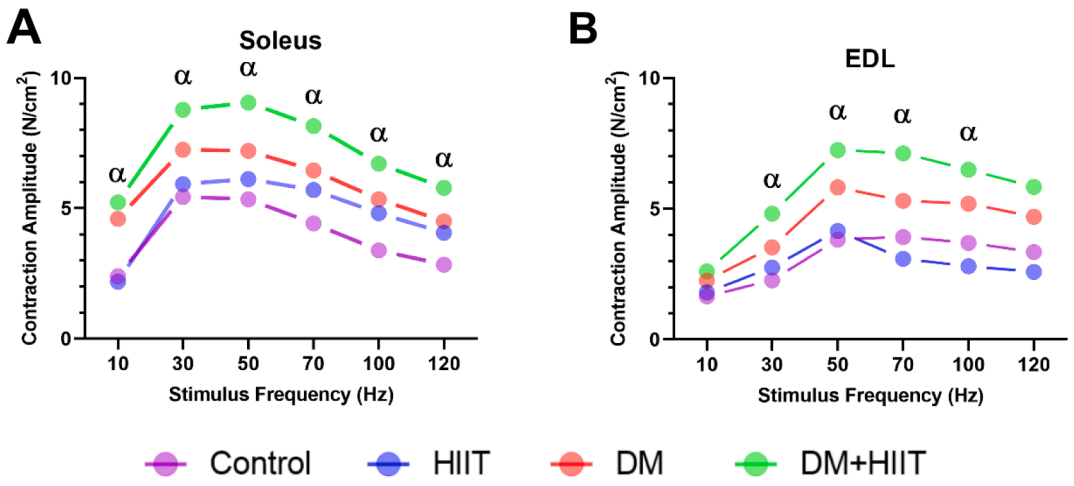


Fig. 10. Contractility graphs consist of the contraction amplitude and frequency of the stimuli (10, 30, 50, 70, 100, and 120 Hz). **(A)** Contractility graph of the soleus muscles **(B)** Contractility graph of the EDL muscles (α: $p < 0.05$C vs. DM + HIIT).

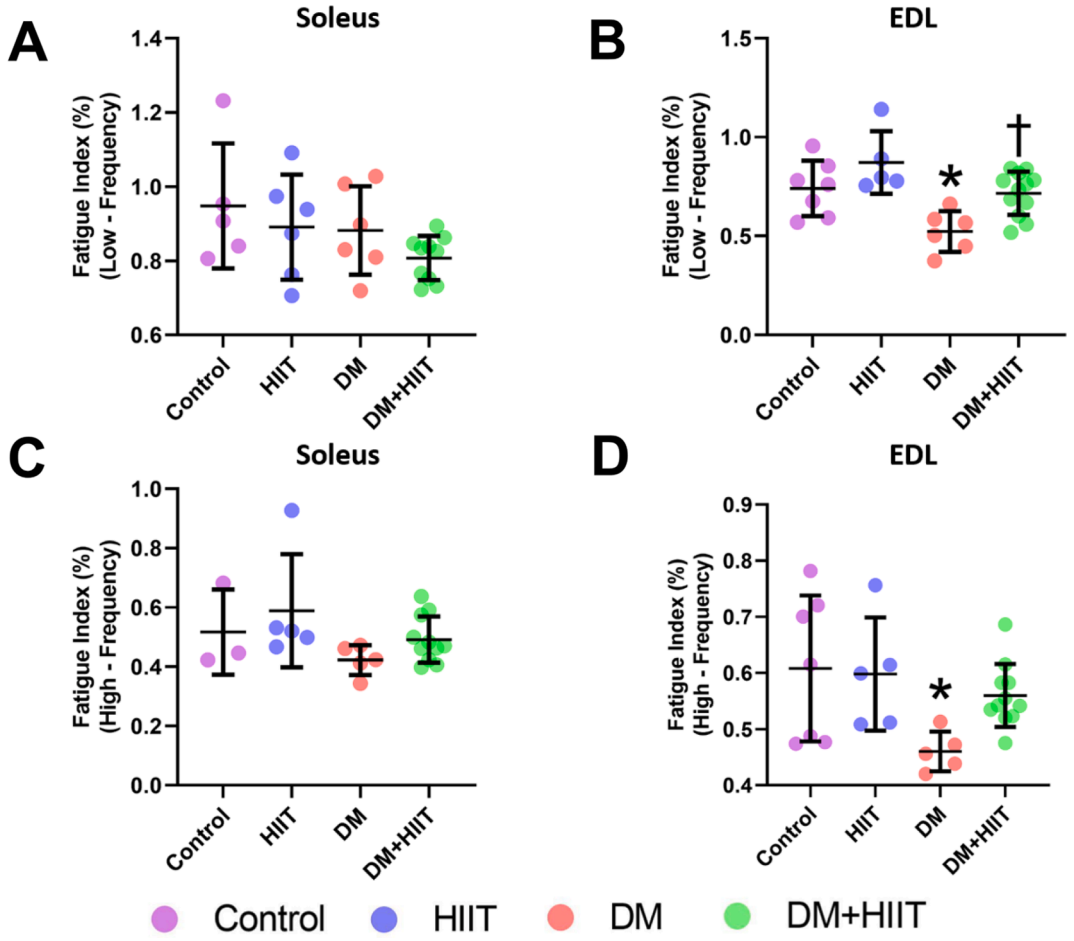


Fig. 11. Fatigue indices (A) Low frequency (LF) fatigue index for soleus muscle (B) Low frequency (LF) fatigue index for EDL muscle (C) High frequency (HF) fatigue index for soleus muscle (D) High frequency (HF) for EDL muscle (*: $p < 0.05$ vs. Control; †: $p < 0.05$ vs. DM).

3.4. Myokines

3.4.1. Interleukin-6 (IL-6)

IL-6 concentrations were measured in soleus and EDL homogenates and serum samples. (Fig. 12).

In soleus homogenates, we did not observe a significant difference between groups ($F_{(3,24)} = 0.2619$; $p = 0.8521$). On the other hand, IL-6 levels differed among groups in EDL homogenates and in serum samples ($F_{(3,21)} = 9.568$; $p = 0.0003$ and $F_{(3,21)} = 6.547$; $p = 0.0027$, respectively). We observed a similar pattern both for EDL homogenates and serum in pairwise comparisons. IL-6 levels were significantly increased in DM groups compared to controls ($p = 0.0015$ and $p = 0.0043$, respectively) and training normalized these effects (C vs. DM-HIIT, $p > 0.05$ for each). Besides, there was a significant difference between DM

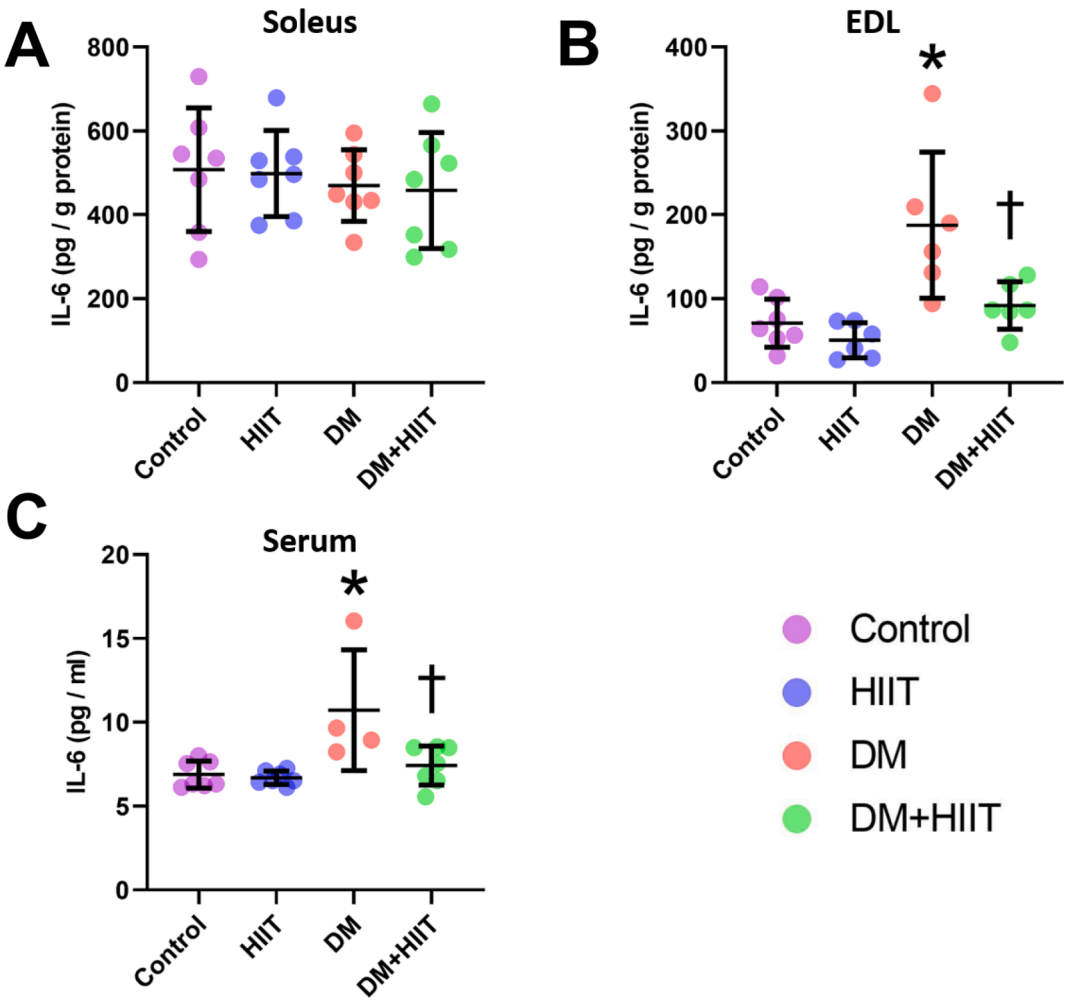


Fig. 12. Interleukin-6 (IL-6) concentrations **(A)** IL-6 concentrations in soleus homogenates **(B)** IL-6 concentrations in EDL homogenates **(C)** IL-6 concentrations in serum samples (*: $p < 0.05$ vs. Control; †: $p < 0.05$ vs. DM).

and DM + HIIT groups both in EDL homogenates ($p = 0.0123$) and in serum samples ($p = 0.0149$).

3.4.2. *Fibronectin type III domain-containing protein 5 (FNDC5)*

FNDC5 concentrations were measured in soleus and EDL muscle homogenates and in serum samples (Fig. 13).

We did not observe a significant difference among groups both in EDL homogenates and in serum samples ($F_{(3,24)} = 0.04217$; $p = 0.9882$ and $F_{(3,24)} = 2.003$; $p = 0.1404$, respectively). On the other hand, FNDC5 levels differed among groups in soleus homogenates ($F_{(3,23)} = 12.82$; $p < 0.0001$). There was a significant training-induced FNDC5 increase that was prominent both in diabetic and non-diabetic animals ($p = 0.0195$ and $p = 0.0397$, respectively). Additionally, there was a marked difference between the DM and DM + HIIT groups ($p = 0.0001$).

3.4.3. *Myonectin*

Myonectin concentrations were measured in soleus and EDL homogenates and in serum samples. (Fig. 14).

We did not observe a significant difference among groups both in EDL homogenates and in serum samples ($F_{(3,23)} = 1.611$; $p = 0.2141$ and $F_{(3,24)} = 2.842$; $p = 0.0591$, respectively). However, there was a marked difference in soleus muscle homogenates ($F_{(3,23)} = 3.235$; $p = 0.0409$). Myonectin levels were significantly higher in the DM + HIIT group compared to the control group ($p = 0.0241$), whereas no significant difference was found among other groups ($p > 0.05$).

4. Discussion

According to the current results, ten weeks of diabetes (Type I) is not long enough for the complete manifestation of all components (atrophy, weakness, and fatigue) of diabetic myopathy. Nevertheless, in glycolytic fiber-dominant EDL muscle, we were able to observe the initial symptoms of diabetic myopathy. In the EDL muscle samples, there were significant pro-inflammatory alterations and decreased fatigue resistance whereas there were no significant alterations in the oxidative fiber-dominant soleus muscles. Moreover, HIIT application alleviated the aforementioned detrimental effects of diabetes on EDL muscle.

Fasting blood glucose levels of diabetic animals were significantly high compared to non-diabetics and terminal body weights were markedly low. Additionally, we measured the fasting serum insulin levels. There was an approximately 20-fold difference between diabetic and non-diabetic animals and the insulin levels of diabetic animals were similar. These results are consistent with the literature and prove that we established a homogenous and efficient diabetes model [7,24,61]. Although exercise has a widely demonstrated beneficial effect on glycemic control in individuals have Type II diabetes [53,29], it did not alter the fasting blood glucose and insulin levels in diabetic animals. Since we applied STZ to induce Type I diabetes, the damage done to the pancreatic β-cells was irreversible [36]. Thus, HIIT application after irreversible damage was not able to increase insulin levels and reduce blood glucose in diabetic animals. Exercise increases energy expenditure and stimulates weight loss in general however in the current study,

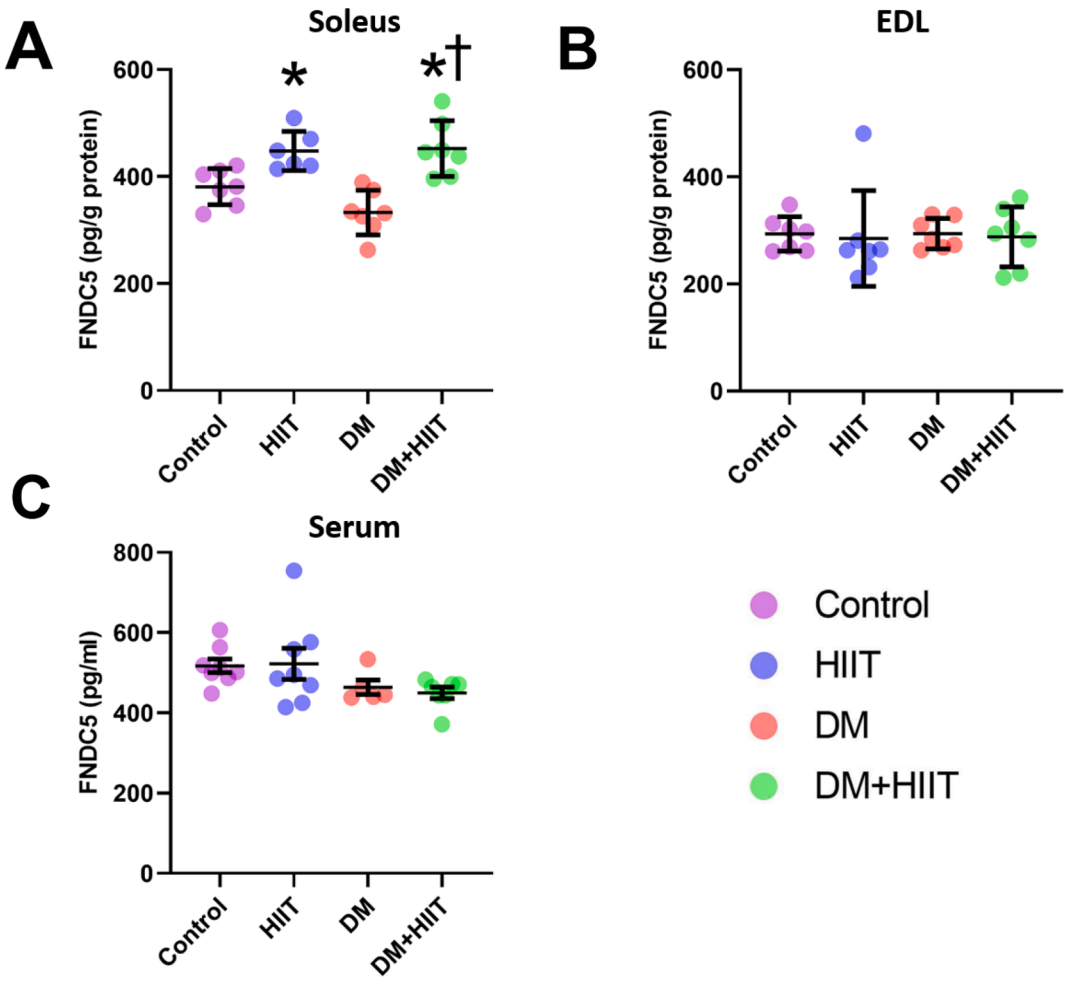


Fig. 13. Fibronectin type III domain-containing protein 5 (FNDC5) concentrations **(A)** FNDC5 concentrations in soleus homogenates **(B)** FNDC5 concentrations in EDL homogenates **(C)** FNDC5 concentrations in serum samples (*: $p < 0.05$ vs. Control; †: $p < 0.05$ vs. DM).

exercise did not alter the terminal body weights of animals. There are some parallel studies in the literature [59,32] and we also demonstrated similar results in our previous study [7]. It may be related to an increase in muscular mass parallel to fat loss. Parallelly, we observed hypertrophy in EDL muscles with HIIT application in healthy animals. Measurement of body composition would be a valuable approach to understanding of this phenomenon. We could not measure body composition in the current study which was one of the limitations of our study.

We measured the maximal exercise capacity of every animal with the incremental load test before training and repeated the test in the 2nd, 4th, and 6th weeks. We hypothesized that diabetes may reduce the maximal exercise capacity of the animal but there was not a significant difference in running speed between diabetic and non-diabetic animals. On the other hand, the discrepancy between diabetic and non-diabetics showed an increasing trend after the 4th week so we can say the difference may become significant if the test could be repeated in the 8th or 10th week of the training. Although there is a correlation between maximal exercise capacity and maximal oxygen consumption (V_{O2max}) [25], the MEC value is an indirect measurement and it is not as definitive as V_{O2max}. If we could have measured the V_{O2max} values of animals throughout training with a metabolic treadmill, the effect of diabetes on exercise capacity would be more distinct. Technical limitations in our lab precluded V_{O2max} measurement which was also an important limitation of the current study. Yet still, measuring individual MEC values and tailoring the training program according to the desired percentage of MEC is a better approach than applying the same protocol to all animals. We think this approach was one of the strong sides of our design. We also calculated the workload of the training program which was planned according to the MEC values of the animals. According to the results, the workload of the non-diabetic animals was significantly high compared to the diabetic animals. The difference between two groups were originated from the body weight difference of the animals. Even though we equalized the MEC values by altering the running speed, diabetic animals could achieve an exercise program in lower workload.

One of the cardinal features of diabetic myopathy is the loss of muscular mass (muscular atrophy). It was demonstrated both in human patients [2,3] and in animal models [60]. In the current study, we desiccated the muscle samples before weighing them to eliminate the effect of tissue edema and normalized this value to the terminal body weight of the animal. According to the results, diabetes caused atrophy in EDL samples but not in soleus. Fiber diameter was also measured in histological slides to evaluate atrophic alterations. Since there was a decreasing trend in the EDL samples of diabetic groups (DM and DM + HIIT) it only reached significance in the DM + HIIT group. Exercise reduced the atrophic alterations seen in diabetic EDL muscles, but weighing and histological data were not consistent. On the other hand, HIIT application induced hypertrophy in EDL samples but not in soleus samples of non-diabetic animals. This effect was prominent both in weighing and fiber diameter results. According to current results, we can conclude that muscles with different fiber types are differentially affected by diabetes and exercise. EDL is more susceptible to diabetes-induced atrophy. Similarly, Armstrong et al. demonstrated that glycolytic fiber loss was more prominent in diabetes [4]. Pepato et al. made a

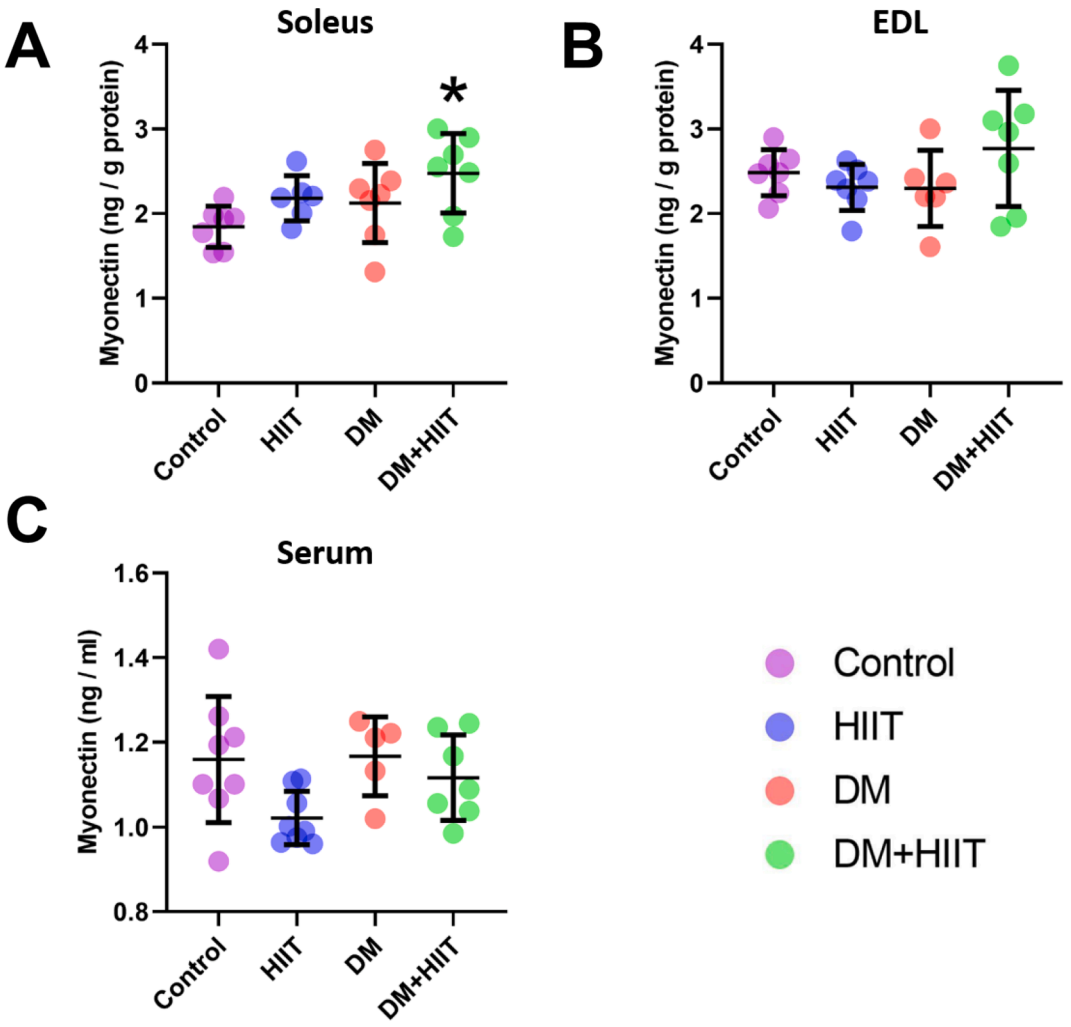


Fig. 14. Myonectin concentrations **(A)** Myonectin concentrations in soleus homogenates **(B)** Myonectin concentrations in EDL homogenates **(C)** Myonectin concentrations in serum samples (*: $p < 0.05$ vs. Control; †: $p < 0.05$ vs. DM).

longitudinal diabetes study and showed prominent glycolytic fiber loss while there was almost no loss in oxidative fibers [41]. Kelleher et al. reported that glycolytic fiber loss was twofold high in diabetes compared to oxidative fibers [28]. Our results were consistent with these findings.

We measured contractility and evaluated the twitch morphology to observe muscle strength. According to current results, diabetes did not cause a contractile impairment in soleus and EDL samples, making us think that diabetic myopathy was not completely established in the current diabetes duration. On the contrary, some studies demonstrated diabetes-induced contractile impairment in similar diabetes durations [11,40,55]. In the current situation, our data is insufficient to explain the source of the discrepancy. Further analysis like intracellular calcium measurements is needed to shed light on this matter. Twitch morphology is consistent with the contractility data. Twitch amplitude and maximal contraction/relaxation rates did not differ between diabetic and non-diabetic animals. Diabetes decreases the removal rate of calcium from the cytosol and impairs the relaxation in muscles [54]. Relaxation time which is an important indicator of relaxation problems is elongated in diabetic animals for both muscle samples. Moreover, the HIIT application normalized the elongated relaxation time in EDL samples but did not significantly affect soleus samples.

IL-6 levels in diabetic serum and EDL muscle samples were significantly high compared to control animals. The dramatic increase in IL-6 levels can be interpreted as the pro-inflammatory effect of diabetes and the muscles were differentially affected by this systemic inflammation. Moreover, exercise application restored the increased IL-6 levels to control values. Shamsi et al. demonstrated a diabetes-induced IL-6 increase in the *flexor hallucis longus* (FHL) muscle and they also observed a restoration in IL-6 levels following exercise application to diabetic animals [34]. Kazemi et al. also used an STZ-induced diabetes model and they measured the various inflammatory cytokines (IL-6, TNF- α, and IL-1β). They reported a significant increase in IL-6 levels following STZ-induced diabetes. According to their data, this pro-inflammatory deterioration was restored by exercise application [27]. Pattamaprapanont et al. preferred a high-fat feeding model and low-dose STZ injection model and applied endurance exercise to animals for 8 weeks. They could not observe a significant alteration in IL-6 levels in soleus muscle samples [39]. Our results were consistent with the literature and make us think that diabetic medium initiated pro-inflammatory changes in the EDL muscle which was not evident in the soleus muscle. However, IL-6 has a unique cytokine that can have bidirectional effects *(both pro and anti-inflammatory)* [18,23]. The bidirectional effect of IL-6 is regulated by the general cytokine profile that is co-secreted with IL-6. Even though we demonstrated a significant IL-6 increase in diabetic animals, evaluation of the other components of the inflammatory cascade is needed to prove the inflammatory effect of the diabetic medium. Furthermore, a comparative analysis of the IL-6 receptor intensity in different muscles would also be a valuable approach. We could not make the aforementioned analyzes which were one of the limitations of the current study.

Neither diabetes nor exercise made a marked alteration in FNDC5

levels in serum and EDL samples. On the other hand, there was a prominent increase in soleus samples both in diabetic and non-diabetic animals. Nadimi et al. reported no alteration both in irisin and FNDC5 levels following STZ-induced diabetes [37]. Amri et al. measured irisin levels irisin levels in the nicotinamide-STZ combination model. They could not observe any diabetes-induced alteration in serum irisin levels whereas 10 weeks of HIIT application made a significant increase in serum irisin levels [1]. On the contrary, Fouda et al. reported a diabetes-induced decrease in serum irisin levels that was restored with moderate-intensity swimming exercise [17]. Hashemvarzi et al. also observed a diabetes-induced serum FNDC5 and irisin decrease [20]. In the current study, we evaluated FNDC5 levels which is the precursor of irisin and we could not find a diabetes-induced FNDC5 decrease both in serum and muscle samples. Taken together with other results, normal FNDC5 levels make us think that all features of diabetic myopathy were not completely established. Various studies in the literature report the exercise-induced irisin increase [22,45,30]. We observed a marked increase in soleus samples that was consistent with the literature. On the other hand, there was no FNDC5 response in EDL samples. Muscles that have different fiber types and anatomical positions were differentially affected by the same training regimen. It can be concluded that our training program stimulates oxidative fiber-dominant soleus muscles more than glycolytic fiber-dominant EDL muscles.

Finally, we evaluated the myonectin levels in serum EDL and soleus samples. Serum myonectin levels did not differ among groups. On the other hand, there was a parallel increase in DM + HIIT groups of soleus and EDL samples. Since myonectin is a considerably novel molecule studies measuring myonectin levels are scarce. Rahmati-Ahmadabad et al. used a STZ-induced diabetes model and evaluated myonectin gene expression in the soleus muscle. Diabetes markedly reduced myonectin gene expression in soleus muscle samples and both HIIT and moderate-intensity chronic exercise application increased the myonectin gene expression [45]. Zhang et al. made a well-attended study with type-II diabetic participants and demonstrated that diabetes caused a marked decrease in the serum myonectin levels of the patients. They also speculated that there might be a correlation between myonectin levels and the intensity of diabetic nephropathy [62]. In the current study, there was a similar pattern between myonectin levels and contractile parameters (both of which were increased in DM + HIIT groups). This analogy and the given potential of this myokine in the literature show that further studies concerning myonectin and exercise would be valuable.

5. Conclusion

In conclusion, HIIT may become a valuable training program for diabetic patients against diabetic myopathy. In current study, we could not observe the complete manifestation of all features of diabetic myopathy. Therefore, preferring longer diabetes durations or higher STZ doses would give better results. Analyzing the inflammatory cytokines like TNF-α, IL-1β, IL-1Ra, and IL-10 is crucial for understanding the pivotal role of IL-6 in muscle-organ crosstalk. Additionally, Leptin is a key hormone in metabolism and body weight control. Furthermore, it has also regulatory functions in skeletal muscle metabolism. Measuring leptin levels would give valuable insights about general and tissue level metabolic alterations during diabetes and exercise. Unfortunately, leptin levels could not be measured in these animals which was one of the limitations of the current study. Last but not least, observation of intracellular calcium alteration is needed to reveal the underlying mechanisms of contraction.

Finally, skeletal muscle is not an isolated organ in the human body. It should be considered a motor unit with its corresponding motor nerve. Besides, diabetic medium affects the nerves together with the muscles. Therefore, additional analysis of motor nerve function is crucial.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Data Availability Statement.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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