

## Effects of exercise intensity on *soleus* muscle myostatin and follistatin levels of hyperglycaemic rats

### Efectos de la intensidad del ejercicio sobre la miostatina y follistatina del músculo sóleo de ratas hiperglicémicas

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**Abstract.** Background: Hyperglycaemia induces dysregulations in skeletal muscle mass and function. Myostatin (*Mstn*) and follistatin (*Fs*) are two key regulators of muscle mass, which are known to be dysregulated in people with hyperglycaemia. Exercise is frequently prescribed to counteract these changes; however, the influence of exercise intensity is unknown. The purpose of this study was to compare two training programs, moderate-intensity constant (MICT) and high-intensity interval training (HIIT), on *soleus* mRNA levels of *Mstn* and *Fs* in an animal model of hyperglycaemia. Material and Methods: 36 male Wistar rats, were divided into control (n=18) and hyperglycaemic (HG, n=18; induced by a single intraperitoneal dose of Streptozotocin) groups. Subsequently, these groups were randomly subdivided into control untrained, control+moderate-intensity constant training (MICT), control+high-intensity interval training (HIIT), HG untrained, HG+MICT, and HG+HIIT (n=6 each subgroup). Training programs were performed for 8 weeks, with a frequency of 5 sessions per week. The total distance covered per session in MICT and HIIT was equal. 48 hours following the last training session, rats were anesthetized and *soleus* muscles were excised. Results: HIIT reduced and increased significantly the *Mstn* and *Fs* mRNA levels respectively, irrespective of hyperglycaemia ( $p < 0.05$ ). When *Mstn:F*s ratio was analysed, only HIIT induced a significant increase in hyperglycaemic rats ( $p < 0.05$ ). Conclusion: HIIT over MICT, changed the *Mstn* and *Fs* *soleus* mRNA levels, irrespective of hyperglycaemia. This could indicate that the regulation of these genes is exercise intensity-dependent, whereas hyperglycaemia seems to not blunt this response.

**Keywords:** Endurance training, High-intensity interval training, Myokines, Blood glucose, Insulin.

**Resumen.** Antecedentes: La hiperglicemia induce alteraciones en la masa y fuerza del músculo esquelético. La miostatina (*Mstn*) y follistatina (*Fs*) son reguladores de masa muscular, los cuales son alterados por hiperglicemia. El ejercicio es utilizado para neutralizar estos cambios; sin embargo, la influencia de la intensidad del mismo no está aclarada. Este estudio comparó dos programas de ejercicio, intensidad moderada y constante (MICT) e interválico de alta intensidad (HIIT), sobre los niveles de ARN mensajero (ARNm) muscular de *Mstn* y *Fs* en ratas hiperglicémicas. Material y Métodos: 36 ratas Wistar fueron divididas en controles (n=18) y con hiperglicemia (HG, n=18; inducida con Streptozotocina intraperitoneal). Además, estos grupos fueron subdivididos aleatoriamente en: control no-entrenado, control+entrenamiento moderado constante (MICT), control+entrenamiento interválico de alta intensidad (HIIT), HG no-entrenados, HG+MICT y HG+HIIT (cada subgrupo n=6). El entrenamiento duró 8 semanas, con 5 sesiones por semana. La distancia total recorrida por sesión en cada programa de entrenamiento fue igual. 48 horas posterior a la última sesión, las ratas fueron anestesiadas y los músculos sóleos fueron extraídos. Resultados: El entrenamiento HIIT redujo e incrementó significativamente los niveles de *Mstn* y *Fs* respectivamente, independiente de la presencia de hiperglicemia ( $p < 0.05$ ). Además, la razón *Mstn:F*s se incrementó significativamente sólo en el grupo de ratas hiperglicémicas entrenadas con HIIT ( $p < 0.05$ ). Conclusión: El entrenamiento HIIT, no MICT, cambió los niveles de ARNm de *Mstn* y *Fs* en el músculo sóleo, independiente de la presencia de hiperglicemia. Esto sugiere que la regulación de estos genes es dependiente de la intensidad del ejercicio, en donde la hiperglicemia parece no aminorar esta respuesta.

**Palabras claves:** Entrenamiento aeróbico, entrenamiento interválico de alta intensidad, mioquinas, glicemia, insulina.

## Introduction

In general, diabetes is defined as a condition where the body's blood glucose levels are higher than normal (hyperglycaemia) resulting from the body's inability to use or store blood sugar for energy (ADA, 2021). Risk factors to develop diabetes are increasing age, obesity, lack of physical activity, and subjects with prediabetes, who have higher blood glucose levels, but are not high enough to be classified as diabetic (Maruthur

et al., 2016; Seuring, Archangelidi, & Suhrcke, 2015).

Among the detrimental effects of hyperglycaemia, muscle atrophy can be initially seen in middle age (Baltadjiev & Baltadjiev, 2011), and becomes more substantial with older age (Fox et al., 2015; K. S. Kim et al., 2014), where a 50% higher sarcopenia prevalence has been described in older adults with diabetes, along with a higher risk to develop sarcopenia in subjects with diabetes (Chung, Moon, & Chang, 2021). This muscle loss leads to decreased strength, functional capacity, and ultimately increased mortality (G. Q. Chen, Mou, Yang, Wang, & Zhao, 2011; Fox et al., 2015). Considering these changes, the understanding of the molecular mechanisms of muscle mass control has been

a particular research area of interest in the context of hyperglycaemia. As such, several factors have been studied to understand muscle atrophy during hyperglycaemia. For instance, it has been described that hyperglycaemia induces dysregulations of transcription factors, such as *FoxO*, which derives in increases of ubiquitin-proteasome protein degradation and autophagy in skeletal muscle, promoting atrophy (O'Neill et al., 2019). In particular, myostatin (*MSTN*) and follistatin (*FS*) are key genes regulating muscle atrophy and growth respectively (Covington et al., 2016). This can be explained as follows: *MSTN* is a powerful negative regulator of muscle size, which in the presence of *FS*, the former is unable to bind to its receptor, inhibiting its action (Elliott, Renshaw, Getting, & Mackenzie, 2012). As an example of the previous, in castrated rats (a procedure known to induce muscle atrophy), ~2.5-fold increases of *Mstn* mRNA were detected in gastrocnemius muscle (Marine, Fabrizzi, Nonaka, Garcia de Oliveira Duarte, & de Oliveira Leal, 2018).

To counteract these consequences, lifestyle changes including the adoption and maintenance of physical activity are cornerstones to prevent and/or delay the incidence of hyperglycaemia (Chatterjee, Khunti, & Davies, 2017). Specifically, aerobic exercise training is associated with beneficial effects on glycaemic profiles in patients with hyperglycaemia (Grace, Chan, Giallauria, Graham, & Smart, 2017), which include decreased glycated haemoglobin (HbA1c) levels (Grace et al., 2017; Umpierre et al., 2011) and improved insulin sensitivity (Way, Hackett, Baker, & Johnson, 2016). Moreover, regular physical activity can reduce the risk of diabetes in people even with impaired glucose tolerance (Aune, Norat, Leitzmann, Tonstad, & Vatten, 2015). Considering the benefits associated with regular physical activity, recent efforts have been focused on comparing different types of exercise to find the most efficient type in terms of metabolic benefits in populations with metabolic dysfunctions (Lobato-Huerta, Moneda-Rovira, Martínez-Tovilla, & Meléndez-Aguilar, 2021). In that context, high-intensity interval training (HIIT) is an option that may encourage participation in physical activities and, thus, reduce the risk of chronic diseases (Gibala, Little, Macdonald, & Hawley, 2012). This exercise modality includes a training program based on brief intermittent bursts of intense exercise interspersed with periods of rest or low-intensity exercise (Dávila-Grisales, Mazuera-Quiceno, Carreño-Herrera, & Henao-Corrales, 2021; Gibala et

al., 2012; Karstoft & Pedersen, 2016).

To support the implementation of HIIT programs, exercise with higher intensities may provide similar or greater benefits than moderate-intensity exercise for metabolic health (Liubaoerjijin, Terada, Fletcher, & Boule, 2016), cardiovascular disease risk factors, and all-cause mortality reduction (Cassidy, Thoma, Houghton, & Trenell, 2017). In that regard, previous reviews have shown controversial results when assessing the effects of HIIT and moderate-intensity continuous training (MICT) on the HbA1c concentrations in individuals with diabetes (Liubaoerjijin et al., 2016). However, important limitations were observed in these reviews, which include lack of consideration for the differences in individual patient circumstances (Bertram, Brixius, & Brinkmann, 2016), and different approaches used for interval and continuous training for high-intensity exercise (Liubaoerjijin et al., 2016). To study the interaction of hyperglycaemia and the effects of exercise, preclinical models have been developed to understand the underlying mechanisms by which exercise might promote metabolic benefits, where the pharmacological induction of hyperglycaemia or insulin resistance through diet are one of the most frequently used (King, 2012; Thu, Kim, & Han, 2017). In that context, a recent study has shown that HIIT can induce *Mstn* and *Fs* mRNA changes in soleus and extensor digitorum longus muscles of lean rats after eight weeks of training (Roostaei, Pirani, & Rashidlamir, 2020). However, the effects of different exercise intensities on mRNA levels of *Mstn* and *Fs* in a context of metabolic dysfunction remain under-researched and, to the extent of our knowledge, have not been previously reported. Therefore, the present study aimed to compare two training programs, HIIT and MICT, on the skeletal muscle mRNA levels of *Mstn* and *Fs* in an animal model of hyperglycaemia.

## Material and methods

### Animals

The experiments described herein were carried out according to the guidelines laid down by the Consolidated version of the Animals (Scientific Procedures) Act 1986. Specific pathogen-free male Wistar rats (age – 45 days), born and maintained at the animal facilities of the Department of Anatomy and Physiology, Research Institute of the University of Baqiyatallah, were used for this study. Rats were housed under controlled environmental conditions (20–22°C, 12 h–12 h light-dark cycle) and were fed with standard

rodent chow (ALTRONIN-R, A. Rieper Spa, Vandoies, BZ, Italy) and tap water *ad libitum*. Animals were maintained according to the European Union guidelines for the care and use of laboratory animals. Thirty-six animals were randomly assigned to six different groups as follows: Control group (CON, n =6), MICT group (n =6), HIIT group (n =6), Hyperglycaemic group (HG, n =6), HG+MICT (n =6), and HG+HIIT group (n =6).

### Hyperglycaemia induction

Hyperglycaemia was induced by a single intravenous injection of Nicotinic Amide-Streptozotocin (STZ) (Alfa Aesar – Ward Hill, MA, USA) (40 mg/kg) diluted in 0.1M citrate buffer, pH 4.5, after 12 h of fasting. Control rats were injected with equivalent amounts of citrate buffer. One week after STZ administration, hyperglycaemia was confirmed by measuring blood glucose levels of samples extracted from the tail vein with Accu-Chek glucose strips (Roche, Mannheim, Germany). If blood glucose levels were higher than 13.9 mmol/L, those rats were considered hyperglycaemic.

### Treadmill Exercise

All rats were familiarized with a rodent treadmill (Columbus Instruments, Columbus, OH, USA) before randomization (10 m/min for 10 min x 4). Exercise protocols were developed based on the American Physiological Society's resource on exercise in animals, and from previous studies (Smith et al., 2007). The MICT and HIIT groups trained 5 days/week for 8 weeks. The total distance between the groups was matched between training programs (K. H. Kim, Kim, Park, & Kim, 2019). Detailed MICT and HIIT protocols are described in Table 1. Forty-eight hours after the last exercise bout, animals were anaesthetized with pentobarbital (90 mg/kg body weight). Following anaesthesia, soleus muscles collected and rapidly frozen in liquid nitrogen and stored at -80°C.

Table 1.  
Exercise Protocols for HIIT and MICT rats at weeks 1–8 of training.

		Exercise speed (m/min)	Exercise time (min)	High- intensity bouts (n)	Active rest speed (m/min)	Time of active rest (min)	Total distance (m)
Week-1	HIIT	16	2	5	10	1	200
	MICT	10	20	-	-	-	
Week-2	HIIT	20	2	5	10	1	240
	MICT	12	20	-	-	-	
Week-3	HIIT	22	2	5	11	1	264
	MICT	12	22	-	-	-	
Week-4	HIIT	26	2	5	12	1	308
	MICT	14	22	-	-	-	
Week-5	HIIT	28	2	5	14	1	336
	MICT	14	24	-	-	-	
Week-6	HIIT	32	2	5	16	1	384
	MICT	16	24	-	-	-	
Week-7	HIIT	34	2	5	19	1	416
	MICT	16	26	-	-	-	
Week-8	HIIT	38	2	5	22	1	468
	MICT	18	26	-	-	-	

HIIT: High-Intensity Interval Training, MICT: Moderate-Intensity Continuous Training

### Exercise Testing

Functional capacity testing consisted of an initial 5 min warm-up at 5m/min on the treadmill. Rats were then subjected to interval exercise at an initial speed of 8 m/min followed by 3 m/min increases every 3 min until exhaustion. Exhaustion was determined when the animal refused to run even after electric stimulation or was unable to coordinate steps (Gomes et al., 2016). Maximum running speeds were recorded and total distance calculated. Exercise capacity was measured as time, in minutes, to reach exhaustion, and distance covered during this test in meters.

### Weight and Biochemical assays

After the completion of the exercise training, body weight was measured. Fasting blood glucose levels were obtained by a venous blood sample extracted from the tail vein, and fasting insulin levels were measured using a mouse insulin ELISA kit (Thermo).

### Total RNA extraction and real-time quantitative polymerase chain reaction (RT-qPCR)

Total RNA was isolated from rat soleus muscle with a BioRobot EZ1 (Qiagen, Germany) and an EZ1 RNA Universal Tissue Kit (Qiagen, Germany), according to the manufacturer's instructions. RNA quality and quantity were measured with a ND-1000 camera (NanoDrop Technologies, Wilmington, Delaware, USA). Then, total RNA samples were stored at -80°C.

For the real-time polymerase chain reactions (RT-qPCR) analyses the TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems, Waltham, Massachusetts, USA) and primers designed specifically to target the *Mstn* and *Fs* gene (The primer sequences used were as follows: for *MSTN*, 52 -CAA ACA GCCTGA ATC CAA CTT AG-32 (forward) and 52 -CCGTGA GGGGGT AGC GAC AG-32 (reverse); for *FS*, 52 -GGC GTA CTGCTT GAA GTG AA-32 (forward) and 52 -GGG AAG CTG TAGTCC TGG TC-32 (reverse)) (TaqMan Gene Expression Assay, Applied Biosystems, Waltham, Massachusetts, USA), were used following manufacturer instructions, using an ABI 7500 thermocycler (Applied Biosystems, Waltham, Massachusetts, USA). Next, we added total RNA (1 µg) to each well, where a No Template Control (NTC) was also included, which had water instead of RNA (Sigma-Aldrich, Saint Louis, Missouri, USA). PCR was performed according to the protocol recommended by Applied Biosystems, which was as follows: an initial denaturation of 10 min at 95°C, followed by 40 cycles of 95°C for 15 sec and 60°C for 30 sec (X. Wang et al., 2019). The test samples and control

were assayed in duplicate, and the non-template control was assayed in triplicate. Results were expressed using the delta-delta Ct method, correcting values using the housekeeper gene *18S* (Pryor, Montani, & Adair, 2010).

### Statistical analysis

Statistical analyses were performed using GraphPad Prism software Version 8.0. Data were expressed as mean  $\pm$  SD. To investigate the effects of hyperglycaemia (Control vs Hyperglycaemic) and the different training regimes (untrained vs MICT vs HIIT), two-way ANOVA followed by Sidak's and Tukey's post hoc tests were used. For differences to be considered as statistically significant,  $p$  values had to be less than 0.05.

## Results

### Weight and Biochemistry

As expected, hyperglycaemia decreased body weight in untrained rats ( $226 \pm 4.6$  vs  $181 \pm 5.9$  g). Overall, exercise increased body weight in all groups, however, hyperglycaemic rats after HIIT were slightly heavier than their counterparts that performed MICT ( $241 \pm 5.7$  vs  $251 \pm 5.9$ ; Figure 1A). In terms of fasting glucose (untrained:  $11.2 \pm 0.9$  vs MICT:  $6.4 \pm 0.9$  vs HIIT:  $5.6 \pm 1.0$  mmol/L) and insulin (untrained:  $25.4 \pm 1.1$  vs MICT:  $16.1 \pm 1.0$  vs HIIT:  $14.3 \pm 0.8$  ng/mL), untrained hyperglycaemic rats showed higher levels compared to trained rats (Figure 1B-C;  $p < 0.05$ ). Interestingly, after HIIT, control and hyperglycaemic rats exhibited lower insulin levels in comparison with their counterparts who performed MICT ( $p < 0.05$ ).

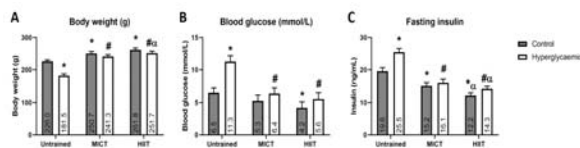


Figure 1. Animal characteristics. Body weight (A), fasting glucose (B), and insulin (C) at termination. Data are presented as mean  $\pm$  SD and the number of animals per group is 6. \*:  $p < 0.05$  vs. Control untrained; #:  $p < 0.05$  vs. hyperglycaemic untrained; a:  $p < 0.05$  vs. respective MICT group by Two-way ANOVA with Tukey's and Sidak's post-hoc tests.

### Muscle and functional changes

Soleus weight was affected by hyperglycaemia and exercise. Hyperglycaemic untrained rats exhibited lighter soleus muscles compared to controls ( $92.4 \pm 2.9$  vs  $72.9 \pm 3.7$  mg;  $p < 0.05$ ). Notably, all trained groups exhibited bigger soleus muscles, irrespective of the presence of hyperglycaemia. Moreover, rats after HIIT exhibited slighter heavier soleus than their respective MICT groups (Figure 2A-B;  $p < 0.05$ ).

In a functional context, hyperglycaemia had a small effect on the maximal running capacity, where hyperglycaemic untrained rats ran  $\sim 30$  m less than their control counterparts ( $p < 0.05$ ). As expected, exercise increased significantly distance and time to exhaustion irrespective of the presence of hyperglycaemia. Interestingly, HIIT had a higher effect compared to MICT among hyperglycaemic rats (MICT:  $356.9 \pm 21.1$  vs HIIT:  $380.4 \pm 11.3$  m;  $p < 0.05$ , Figure 2C-D).

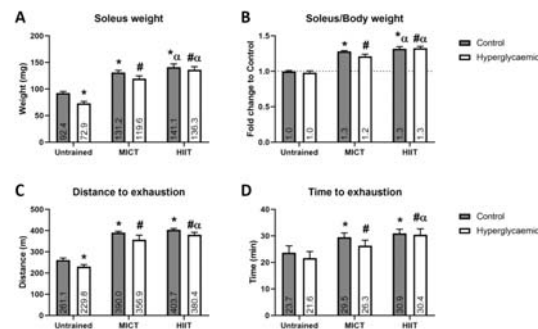


Figure 2. Soleus weight and exercise adaptation. Soleus weight (A), Soleus to body weight ratio (B), distance (C), time to exhaustion (D), and maximal running capacity test. Data are presented as mean  $\pm$  SD and the number of animals per group is 6. \*:  $p < 0.05$  vs. Control untrained; #:  $p < 0.05$  vs. hyperglycaemic untrained; a:  $p < 0.05$  vs. respective MICT group by Two-way ANOVA with Tukey's and Sidak's post-hoc tests.

### Muscle myostatin and follistatin mRNA

In terms of *Mstn* and *Fs* soleus mRNA levels, hyperglycaemia induced by pancreatic damage did not show an effect ( $p > 0.05$ ); interestingly, only HIIT decreased and increased its levels respectively, irrespective of hyperglycaemia induction (Figure 3A-B;  $p < 0.05$ ). When calculating the ratio between these two genes, only HIIT exhibited a significant effect among hyperglycaemic rats (MICT:  $4.3 \pm 3.2$  vs HIIT:  $7.5 \pm 5.9$ ;  $p < 0.05$ , Figure 3C).

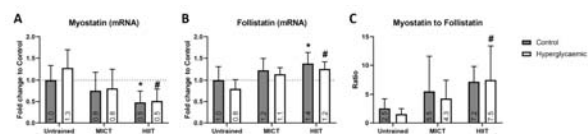


Figure 3. Data are presented as mean  $\pm$  SD and the number of animals per group is 6. \*:  $p < 0.05$  vs. Control untrained; #:  $p < 0.05$  vs. hyperglycaemic untrained by Two-way ANOVA with Tukey's and Sidak's post-hoc tests.

## Discussion

The present study aimed to compare two training programs, MICT and HIIT, on soleus mRNA levels of *Mstn* and *Fs* in an animal model of hyperglycaemia. Here, we found that HIIT over MICT, changed the mRNA levels of soleus *Mstn* and *Fs* in control and hyperglycaemic rats, whereas its ratio changed only after HIIT in the latter.

As expected, hyperglycaemia induction resulted in typical changes in the animals' phenotype, such as



decreased body weight and increases in fasting blood glucose levels. As previously reported, exercise decreased blood glucose concentrations, suggesting that exercise was properly prescribed (Loganathan et al., 2007; H. Wang et al., 2015). It is interesting how the hyperglycaemic rats exhibit slightly higher levels of fasting insulin compared to their lean counterparts. This could be explained by the previously described lower insulin clearance observed in similarly STZ-treated rats, where a higher insulin area under the curve was observed after subcutaneous insulin injections (Qinna & Badwan, 2015).

The animals' maximal running capacity (distance and time to exhaustion) was lower in hyperglycaemic rats, whereas MICT and HIIT induced a significant increase in this parameter. Interestingly, HIIT induced higher adaptations compared to MICT, specifically in hyperglycaemic rats. This could indicate that exercise intensity is a particularly relevant characteristic in a hyperglycaemic context (Grace et al., 2017). The underlying physiological mechanisms of HIIT are a prolific scientific area that has resulted in the development of several hypotheses. In that context, it is well established that exercise enhanced insulin sensitivity via improvements in insulin and AMP-activated protein kinase (AMPK) mediated signalling pathways (Yaspelkis et al., 2007). Consecutively, increases in GLUT4 vesicle formation and translocation from the cytoplasm to the cell membrane promote an increase in glucose uptake, along with the activation of calcium-dependent kinases derived from the increase in  $Ca^{+2}$  availability in skeletal muscle during contraction (SyLOW, Kleinert, Richter, & Jensen, 2017). As a result of these changes, reduction in fasting glucose levels after long-term HIIT programs have been reported (Madsen, Thorup, Overgaard, & Jeppesen, 2015); however, others have reported no major changes in this outcome (Cassidy et al., 2016; Stensvold et al., 2010). This could indicate that systemic insulin sensitivity not only depends on skeletal muscle glucose uptake, given that this response in the liver is particularly relevant as well. Therefore, future studies should explore the effects of MICT and HIIT on the liver function and structure of hyperglycaemic rats and expand the results presented here.

After 8 weeks, *Mstn* mRNA levels were significantly reduced only after HIIT in control and hyperglycaemic rats. These results are in line with the findings of Bagheri et al. (Bagheri et al., 2019), and Chen et al. (Y. Chen, Cao, Ye, & Zhu, 2009), who showed that exercise reduced

*Mstn* expression. This suggests that *Mstn* is likely to be more sensitive to exercise intensity in skeletal muscle; however, mechanistic studies are encouraged in this field. However, in our study hyperglycaemia did not induce changes in *Mstn*, nevertheless, since we did not measure its corresponding protein level, we cannot rule out potential effects.

Similarly compared to *Mstn*, *Fs* mRNA levels changed specifically after HIIT in the soleus muscles of control and hyperglycaemic rats. In support of these results, Willis et al. (Willis et al., 2019), described increases in *Fs* after intense exercise. However, Hansen et al. (Hansen et al., 2016) have reported impaired *FS* secretion in people with diabetes, suggesting that hyperglycaemia dysregulates its production. However, in our study hyperglycaemia did not affect *Fs* mRNA levels, which could suggest that its effects are particularly in a post-transcriptional fashion. Significant differences of mRNA *Fs* levels between training groups may also be due to greater muscle stimulation and greater secretion of *FS* (Willis et al., 2019). HIIT, with an increased muscle contraction and greater use of the anaerobic energy pathway, could have stimulated muscle growth signalling (e.g. mTOR and IGF-1 dependent pathways) (Cassidy et al., 2017; Galaviz-Berelleza et al., 2021). Moreover, HIIT especially if including long intervals appears as the most effective strategy to increase *FS* concentration (He et al., 2018). Therefore, like *Mstn*, *Fs* seems to be dependent on the intensity of exercise. In addition, *Mstn:F<sub>s</sub>* ratios were significantly increased only after HIIT in hyperglycaemic rats. This ratio seems to be relevant since several studies suggest that *Fs* can also bind to *Mstn*, preventing the interaction with its receptor, antagonising its activity (Abe et al., 2009; Camporez et al., 2016).

As limitations, our study only considered post-training measurements, which does not allow us to detect the sizes of the effect of our interventions, along with the absence of food consumption levels, which could be a confounder factor. Also, we only performed mRNA measurements of the mediators of interest, therefore, further studies are needed to investigate its protein changes with different exercise regimes, along with complementary mediators, such as IL-6, IL-15, and irisin, given their function in exercise-mediated metabolic benefits (Raschke & Eckel, 2013). Moreover, only soleus muscles were considered, and future studies should include muscles with different morphology and composition (e.g. fast-twitch muscles such as: extensor digitorum longus muscle).

## Conclusions

In conclusion, according to our results, HIIT had a greater effect on *Mstn* and *Fs* mRNA levels of soleus muscle of hyperglycaemic rats, along with higher improvements in their aerobic capacity. Therefore, exercise intensity appears to be a regulator of the transcription of these mediators. Mechanistic studies in this field are warranted to corroborate and expand the results presented here.

## Conflict of interest

The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

## Funding sources

This work was supported by the personal expenses of SA. SM-H is supported by the National Agency of Research and Development (ANID) through an Early Career Research Grant (FONDECYT de iniciación en investigación), code 11200391.

## Contributions

Involved in the study concept and design (SA, EA, SM, FF); acquisition of data (SA, FF, HA); analysis and interpretation of data (SA, SM, FF); drafting of the manuscript (FF, SM, JB); critical revision of the manuscript for important intellectual content (SA, EA, SM, HA, JB); statistical analysis (SM, FF), and final approval for publication (SA, EA, SM, HA, FF, JB).

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