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Original article

Investigation of the relationship between plasma ghrelin levels and muscle atrophy in experimental diabetic rats

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Abstract

In this study, the relationship between plasma ghrelin levels and muscle atrophy was examined in an experimental diabetic rat model.

56 male Wistar albino rats, aged 8-10 weeks, were used in the study. The rats were divided into 8 groupsD1: one-week diabetes, C1: one-week control, D2: three-week diabetes, C2: three-week control, D3: six-week diabetes, C3: six-week control, D4: eight-week diabetes, C4: eight-week control. To induce diabetes, rats were injected with a single intraperitoneal dose of 45 mg/kg streptozotocin. At the end of the experiments, body weights and fasting blood sugar levels were measured. mTOR and myostatin levels of gastrocnemius muscle and plasma ghrelin levels were measured by ELISA method. Gastrocnemius muscle weight, cross-sectional area and histopathological images were examined.

It was observed that the gastrocnemius weights of the D2, D3, D4 groups decreased significantly compared to their controls ($p \leq 0.01$). Muscle cross-sectional area decreased significantly in groups D3 and D4 compared to controls ($p \leq 0.01$). Muscle mTOR levels were found to be significantly lower in all diabetic groups compared to controls ($p \leq 0.01$). Although muscle myostatin levels were higher in the diabetic groups, this increase was only significant in the D4 group. Plasma ghrelin levels were significantly lower in all diabetic groups compared to controls ($p \leq 0.01$). A positive correlation was determined between plasma ghrelin levels and the final weights, muscle cross-sectional area, gastrocnemius weights and mTOR levels of the rats.

Time-dependent muscle atrophy developed in diabetic rats and there was a relationship between muscle atrophy and plasma ghrelin level. We suggest that ghrelin plays a role in diabetes-induced muscle atrophy as well as cachexia and sarcopenia.

Keywords: atrophy, diabetes, ghrelin, mTOR, muscle

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Introduction

Muscle atrophy is a pathological condition resulting from an imbalance in contractile protein synthesis and degradation, triggered by long-term inactivity, old age, and chronic diseases such as diabetes mellitus (Cohen et al. 2015). Muscle atrophy can reduce the capacity to perform daily living activities and quality of life, and subsequently increase mortality (Zinna and Yarasheski 2003).

Diabetes mellitus (DM) is a metabolic disorder characterized by insulin deficiency or absence. Although it affects more people every day, it is predicted to affect more than 700 million people in 2045 (IDF 2019). Although diabetes has many harmful effects on tissues and organs, it is a fact that chronic uncontrolled hyperglycemia causes loss of structure and function in tissues and organs (Gumieniezek et al. 2001). Since muscle tissue uses glucose dependent on insulin, muscle tissue is one of the organs most affected by disorders in glucose metabolism. This condition is called diabetic muscle atrophy (diabetic myopathy), which is characterized by a decrease in skeletal muscle size and strength. An increase in the number of glycolytic fibers in the skeletal muscle, muscle atrophy, and decreases in capillary density are common in both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) (Gumieniezek et al. 2001, D'souza et al. 2013).

Diabetic myopathy is an often-overlooked complication, despite being a significant contributor to the worsening of the diabetic condition. Ghrelin is a 28 amino acid peptide produced primarily by the oxy gland of the stomach. Ghrelin is a gastric hormone that circulates in acylated (AG) and non-acylated (UnAG) forms. Recent studies have shown that both forms of ghrelin have protective effects against muscle atrophy that develops under different conditions (Cappellari and Barazzoni 2019, Wu et al. 2020). Although some studies have demonstrated beneficial effects of pharmacological doses of ghrelin for cachexia and sarcopenia, a recent study reported that ghrelin deletion reduced body weight but had no effect on muscle function in aging mice (Cappellari and Barazzoni 2019).

Therefore, the role of ghrelin in muscle atrophy remains unclear. Although ghrelin has protective effects against muscle atrophy, the knowledge about plasma ghrelin values during diabetic muscle atrophy is not sufficient. In this study, the extent to which muscle loss developed in rats induced with streptozotocin (STZ) diabetes and the existence of a relationship between plasma ghrelin level and muscle atrophy were investigated.

Materials and Methods

Ethics committee approval was received for the study from Dicle University animal experiments local ethics committee, dated 07/02/2019, protocol number 15670.

Animals and experimental design

56 male Wistar Albino rats, 8-10 weeks old and weighing 350-400 grams, were used. The weights of all animals were measured before the experiment. All animals were fed with standard pellet feed and tap water with free access to water and food during the experiment under optimum conditions (temperature; 25±2C0, relative humidity; 40-60%, light cycle 12/12 hours light/dark and appropriate ventilation).

A total of 8 groups were created, 4 experimental and 4 control, with 7 rats in each group. For the experimental diabetes model, streptozotocin (catalog no:572201, Merck, Germany) dissolved in phosphate-citrate buffer with a pH value of 4.5 was administered to rats that had been fasted the night before. A single dose of 45 mg/kg streptozotocin was injected intraperitoneally to the diabetic groups 15 minutes after nicotinamide administration. Physiological saline placebo was applied to the control groups. After 48 hours, fasting blood sugar was measured from the blood sample taken from the tail veins of the rats with a glucometer (Plusmedfast test, Tyson BioresearchInc., Taiwan). Those whose fasting blood sugar level was above 14 mmol (250 mg/dl) were considered diabetic and included in the study.

A single dose of placebo (physiological saline) was administered to the control groups at the beginning of the experiment into the abdominal cavity. Control Group 1 (C1): was sacrificed after 1 week, Control Group 2 (C2): 3 weeks, Control Group 3 (C3): 6 weeks and Control Group 4 (C4): 8 weeks. Diabetic Experimental Group (D1); Rats were sacrificed 1 week after inducing diabetes with STZ, Diabetic Experiment Group (D2): 3 weeks, Diabetic Experiment Group (D3): 6 weeks, Diabetic Experiment Group (D4): 8 weeks.

The weights and fasting blood sugar of all rats were measured from the tail vein once a week. At the end of the specified weeks, all groups were sacrificed under ketamine-xylasin anesthesia following a 12-hour fast. Fasting blood glucose levels were determined with blood taken from their tails. Live weights were determined. All animals were then sacrificed by cardiac puncture and blood samples were taken. Blood samples were centrifuged at 3700 rpm for 15 min at 4°C. Supernatants were taken into 2 ml Eppendorfs. The gastrocnemius muscle of the right leg was removed and weighed with a precision scale. The gastrocnemius

Table 1. Weight changes of rat experimental groups.

	Before Experiment (g) mean±SD	After Experiment (g) mean ±SD	Difference (%)
C1	384.8±41.9	385.0±41.0	+0.0
D1	378.2±60.4	367.5±57.3*	-2.8
C2	450.7±21.6	445.4±21.9	-1.1
D2	397.8±24.4	331.4±28.3*	-16.6
C3	408.7±34.4	411.8±29.6	+0.7
D3	402.8±44.8	323.1±44.3*	-19.7
C4	433.7±24.0	442.1±30.4	+1.9
D4	401.1±42.4	281.2±36.0*	-29.8

C – Control group, D – Diabetic group, * $p<0.01$ compared to the control group

muscle was fixed in 10% formaldehyde for histopathological evaluation.

Biochemical analysis and histopathology

Muscles removed from the freezer were homogenized to determine muscle mTOR and myostatin levels. At the beginning of the homogenization process, the weights of the muscle tissues were determined on a precision scale. 0.5 g muscle was placed in glass tubes and kept cold. Phosphate buffer solution was added to the muscle tissues at a ratio of 10 times their weight. Glass tubes filled with muscle and buffer fluid were placed in ice-filled plastic containers and homogenized for 60 seconds at 16 000 rpm. Then, the resulting homogenate was centrifuged in a centrifuge tube at 4700 rpm for 20 minutes at 40°C. After centrifugation, the supernatant parts of the samples were taken into Eppendorf tubes. mTOR and myostatin were determined with the supernatants in Eppendorf tubes. It was measured spectrophotometrically using the "SunRed Ghrelin ELISA" kit for the determination of plasma ghrelin, the "SunRed mTOR ELISA kit" for the determination of gastrocnemius muscle mTOR, and the "SunRed Myostatin ELISA kit" for the determination of gastrocnemius muscle myostatin.

Muscle tissue samples were placed in 10% neutral buffered formalin solution and subjected to routine paraffin tissue monitoring. 4-6 µm thick sections were stained with Hematoxylin-eosin and visualized. The cross-sectional area of the muscle was measured under a microscope using hematoxylin and eosin staining.

While performing histopathological scoring, muscle thickness, muscle core degeneration, cell infiltration and apoptosis parameters were evaluated. For the evaluated parameters, measurements were made from 10 different areas per section in each group. For muscle core degeneration, the number of degenerated nuclei was divided by the total number of nuclei and the average of the measurements was taken. Other parameters were calculated using a similar method. Measurements were made for muscle thickness, muscle core degeneration, cell infiltration, and apoptosis parameters with a scaning:

0: no change, 1: mild, 2: moderate, 3: high, 4: very intense

Statistical analysis

The results obtained were analyzed using the SPSS (version 24) package program. Measurement data were tested for normal distribution and homogeneity with the Shapiro-Wilk test. In the analysis of the data, independent student t test was used for normally distributed data and Mann-Whitney U test was used for non-normally distributed data. Spearman's correlation analysis was used to evaluate the relationships of the parameters with each other. The results were shown as arithmatic mean ± standard deviation. $p<0.05$ was considered significant.

Results

The weight of rats pre- and post-experiments was shown in Table 1. Weight of rats in diabetic groups were significantly changed before and after experiment ($p\leq0.01$). Since diabetes cause weight loss, the weight loss in diabetic groups was expected.

The difference in first and final blood sugar was significant in diabetic groups ($p\leq0.01$). The diabetes was not treated in diabetic groups, therefore high level of fasting blood sugar was observed in final blood measurements in diabetic groups (Table 2).

There was no significant difference in the gastrocnemius muscle weights of the diabetic groups compa-

Table 2. Fasting blood sugar of experimental rats.

	First Blood Sugar (mg/dl) ±SD	Final Blood Sugar (mg/dl)±SD	Significance
C1	187.43±38.47	187.14±44.25	
D1	319.00±78.40	344.86±86.96	p≤0.01
C2	104.57±6.63	116.86±7.47	
D2	307.71±44.24	385.57±78.30	p≤0.01
C3	116.00±7.85	103.86±4.63	
D3	311.17±66.96	425.83±57.17	p≤0.01
C4	106.57±6.40	129.57±14.34	
D4	273.67±64.74	497.00±60.46	p≤0.01

C – Control group, D – Diabetic group

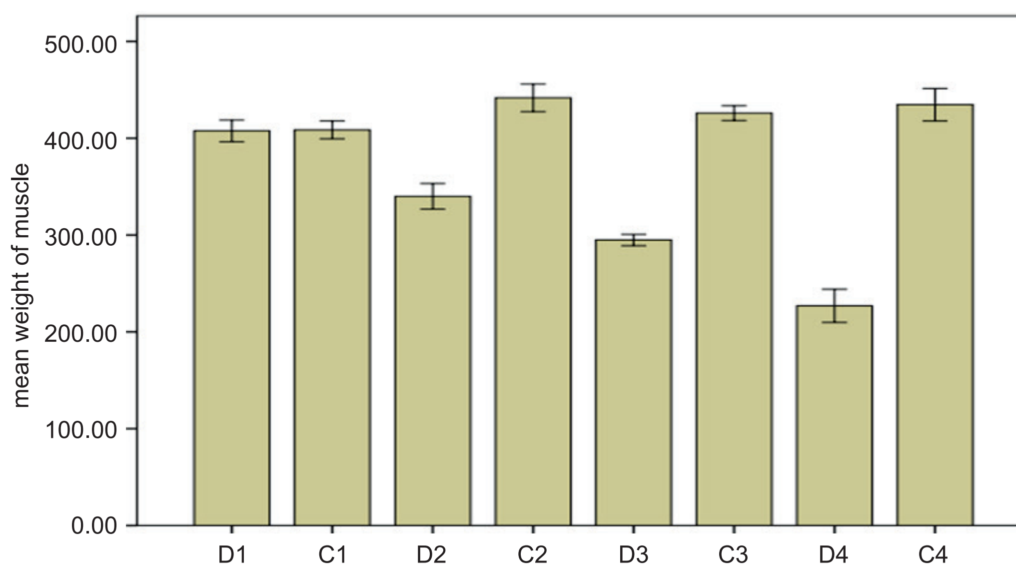

Fig. 1. Gastrocnemius weight of the rat groups

red to the control group for the same week (p=0.898) while a significant difference was found in the other diabetic groups compared to the their corresponding control groups for the same week of D2, D3, D4 (p≤0.01). One week of diabates didn't cause any muscle weight change, however after 3rd week, gastrocnemius muscle weight was decreased in diabetic groups (Fig.1).

There was no significant difference in muscle cross-sectional area in the D1 and D2 groups compared to their control group. (p=0.559, p=0.269). A significant difference was found in muscle cross-sectional area in D3 and D4 compared to their controls (p ≤0.01). similar to weight loss, cross area of muscles was decreased in D3 and D4. İn D2 groups, cross area was non-significantly decreased. Muscular atrophy was increased following prolonged duration of diabetes (Fig. 2).

The mTOR level of D1 was nonsignificantly lower than that of the C1 group (p=0.126). mTOR levels in D2, D3 and D4 groups were significantly lower compared to C2, C3 and C4 groups (p≤0.01). Since mTOR is an enzyme whose expression decreases in muscle atrophy and muscle atrophy was observed in our diabetic groups, the results support the literature.

Myostatin level of D1, D2 and D3 was non-significantly lower than their corresponding control groups (p=0.274, p=0.062 and p=0.564, respectively). Myostatin level of D4 was significantly higher (p≤0.01) than in level of C4 group. Myostatin is an active enzyme in muscle atrophy and its expression is expected to increase. However, since there was no intense atrophy in the first weeks, an increase was observed as time progressed in this study.

Plasma levels of Ghrelin was significantly lower in all diabetic groups compared to their corresponding control groups (p≤0.01). Since ghrelin is a hormone that increases during fasting and the blood sugar of the diabetic groups in this study was high, it is an expected result that ghrelin was low (Table 3).

There was no significant correlation between plasma ghrelin level and the initial weights and muscle myostatin levels of the rats (p>0.01). A significant positive correlation was determined between plasma

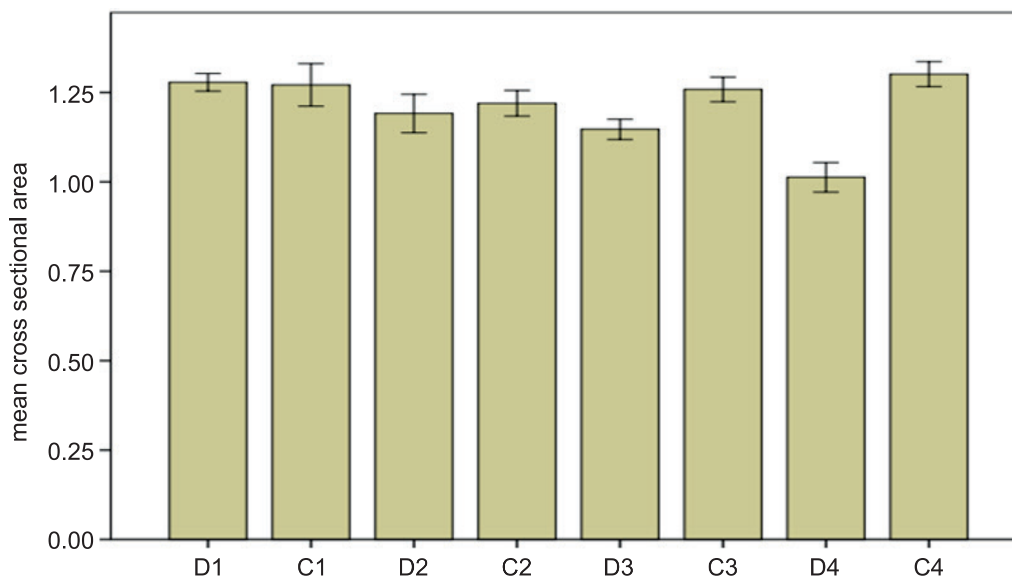

Fig. 2. Gastrocnemius muscle cross-sectional area of the rat groups

Table 3. mTOR, myostatin and ghrelin values of experimental groups.

	mTOR (pg/ml)	P	Miyostatin (pg/ml)	P	Ghrelin (pg/ml)	p
C1	90.14±13.61	p=0.126	53.85±6.03	p=0.274	1858.24±53.95	p≤0.01
D1	75.8571±17.77		49.00±9.96		1280.01±169.22*	
C2	91.71±11.64	p≤0.01	56.71±7.20	p=0.062	1773.42±37.71	p≤0.01
D2	70.42±9.14		52.00±3.00		1276.06±132.43*	
C3	84.57±11.81	p≤0.01	53.57±9.60	p=0.564	1755.29±76.47	p≤0.01
D3	61.42±13.17		57.28±6.15		1150.40±355.55*	
C4	88.28±17.55	p≤0.01	52.57±9.55	p≤0.01	1807.76±53.33	p≤0.01
D4	40.85±4.77		62.14±5.01*		1037.44±136.18*	

C – Control group, D – Diabetic group

Table 4. Correlation of ghrelin and other parameters.

	Ghrelin	
	R	P
Weight (before experiment)	0.209	0.121
Weight (after experiment)	0.594*	0.000
Fasting Blood Sugar (final)	-0.693*	0.000
Muscle Cross-sectional area	0.579*	0.000
Weight of muscle	0.660*	0.000
mTOR	0.524*	0.000
Myostatin	-0.187	0.169

ghrelin level and the final weights of the rats, muscle cross-sectional area, gastrocnemius muscle weight and muscle mTOR levels (p≤0.01). A significant negative correlation was found between plasma ghrelin value and fasting blood sugar of rats (p≤0.01) (Table 4).

A significant difference was found between the groups in terms of muscle thickness, muscle core degeneration, cell infiltration and apoptosis parameters (p≤0.001) (Table 5).

Hematoxylin-eosin stained muscle sections were shown in Fig.3. Normal muscle histology was observed in control groups with regular muscle bundles and fibroblast cells. In diabetes group, hyperplasic muscle cells, leukocyte infiltration, degenerating

Table 5. Histopathological scoring results of the groups.

	Muscle thickness	Degeneration	Infiltration	Apoptosis	
C1	4 (3-4)	1 (0-1)	0 (0-1)	1 (0-1)	p≤0.001
D1	3 (2-4)	2 (0-3)	1 (0-2)	0 (0-2)	
C2	3 (2-4)	1 (0-1)	1 (0-2)	3 (2-4)	p≤0.001
D2	3 (1-3)	2 (1-4)	2 (0-3)	2 (1-3)	
C3	3 (2-4)	0 (0-3)	1 (0-1)	1 (0-2)	p≤0.001
D3	1 (0-3)	3 (2-4)	3 (2-4)	1 (0-3)	
C4	3 (2-4)	1 (0-2)	1 (0-1)	1 (0-2)	p≤0.001
D4	0 (0-2)	3 (3-4)	4 (3-4)	4 (3-4)	

Data was shown as median (minimum-maximum)

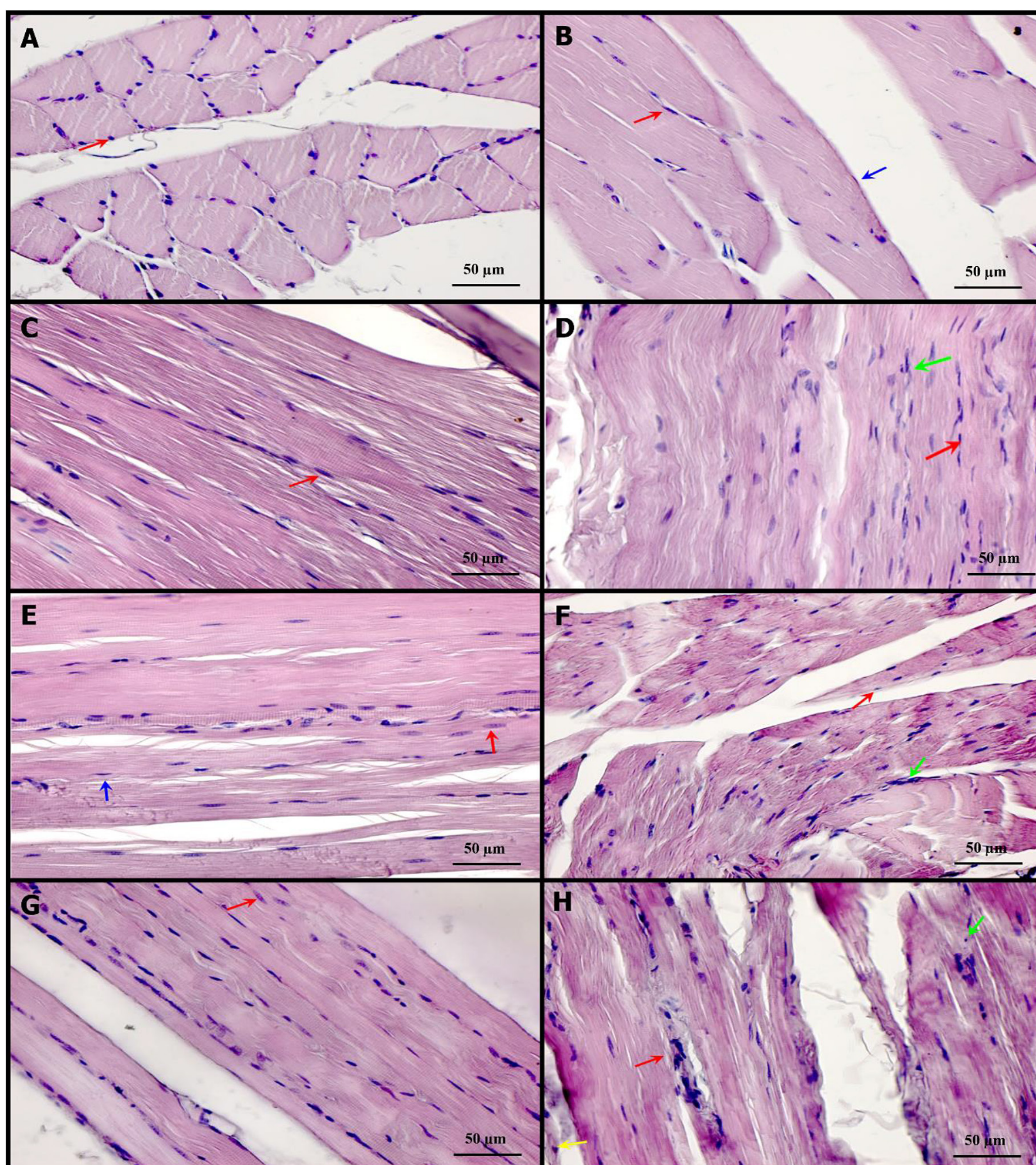

Fig. 3. Hematoxylin-eosin staining of rat skeletal muscles. A) C1: regular muscle bundles, muscle cells (red arrow); B) D1: normal muscle histology, fibroblast cells (blue arrow); C) C2: normal muscle appearance, muscle cells (red arrow); D) D2: hyperplasic muscle cells (red arrow) and leukocyte infiltration (green arrow); E) C3: normal muscle histology, muscle cells (red arrow) and fibroblast cells (blue arrow); F) D3: muscle degeneration and apoptosis in cells (red arrow) and increased leukocyte infiltration (green arrow); G) C4: regular muscle appearance (red arrow); H) D4: Increased degeneration in the nuclei of muscle cells (red arrow), increased leukocyte infiltration (green arrow) and thinning of muscle fibers (yellow arrow). x20

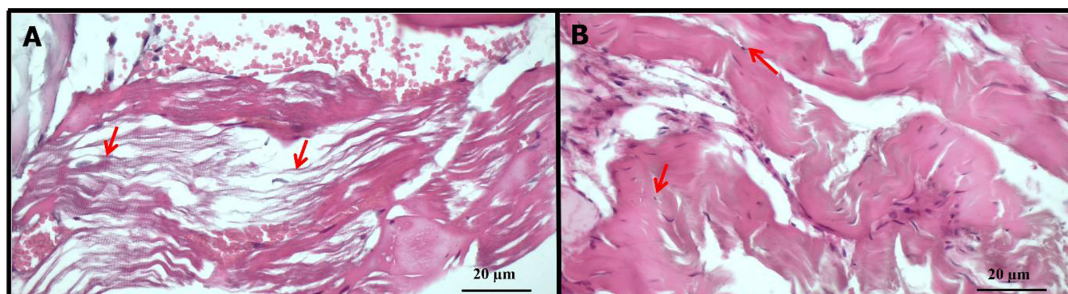

Fig. 4. High magnification of muscular sections of the rat diabetes group. A) Thinning of muscular fibers (arrows); B) Degenerating muscle cells (arrows). x40

muscle cell nuclei, and thinning of muscle fibers were observed.

A higher magnification of muscle tissue belonging to diabetes group show in Fig. 4. Thinning of muscle fibers and degenerated muscle nuclei were evident in diabetes groups.

Discussion

Diabetes mellitus is associated with various macro and microvascular complications due to high blood sugar concentration, resulting in increased morbidity burden and decreased life expectancy. Therefore, glucose homeostasis is very important for long-term maintenance of health. Skeletal muscles function as a basic organ in glucose homeastasis. In healthy people, nearly 70-90% of plasma glucose is taken up and used by skeletal muscles (Evans et al. 2019). Skeletal muscle plays an important role in cellular glucose uptake, retention of amino acid reserves in protein form, and fatty acid oxidation (Argilés et al. 2016).

Diabetes causes total body weight loss as a result of tissue and muscle loss due to insufficient use of glucose in the body. Diabetes mellitus can cause skeletal muscle damage and atrophy with the direct effects of high glucose and low insulin (Lambertucci et al. 2012). This clinical condition is called diabetic myopathy. Uncontrolled diabetes is known to negatively affect skeletal muscle growth and regeneration. (Nguyen et al. 2011). Serious weight loss in the STZ-induced diabetes model has also been shown in previous studies (Ramesh and Pugalendi 2009, Kavishankar and Lakshmidevi 2014). The reason for weight loss is the decrease in protein synthesis in all tissues as a result of decreased ATP production due to insulin deficiency or absolute absence of insulin (Kavishankar and Lakshmidevi 2014). Insufficient use of glucose in the body leads to protein and fat breakdown. This increase in protein catabolism also occurs in structural proteins, which leads to a decrease in total body weight and muscle weight (Ramesh and Pugalendi 2009). In this study, weight loss was observed within the first week after diabetes was induced. As the time living with diabetes increased, statistical weight losses occurred. Among the experimental groups, the weight loss was the lowest in D1, while the weight loss was the highest in D4.

Skeletal muscle fibers generally consist of fast-twitch glycolytic (type 2) and slow-twitch oxidative (type 1) muscle fibers. The soleus muscle consists mainly of type 1 fibers, while the gastrocnemius muscle consists of type 2 fibers. This ratio of muscle fiber types is susceptible to various diseases and can change. It has been reported that type 2 fibers are mostly affected by atrophy, especially in conditions of sarcopenia, cachexia and starvation-related muscle loss (Talbot and Maves 2016). This study used the gastrocnemius muscle to investigate the effect of diabetes on muscle atrophy. Many studies have reported that muscle mass decreases in diabetes (Doustar et al 2007, Ramesh and Pugalendi 2009, Kavishankar and Lakshmidevi 2014, Ato et al. 2019). In this study, a decrease in gastrocnemius muscle weight was observed in diabetic rats. While there was no decrease in the D1 group; a significant decrease was detected in the diabetic groups D2, D3 and D4. Additionally, it was determined that the decrease in muscle mass and the resulting muscle atrophy increased over time in diabetic rats.

It has been reported that the cross-sectional area of muscle fibers decreases in muscle atrophy due to DM (Doustar et al 2007, Ato et al. 2019). Similar to other studies, the cross-sectional area of the gastrocnemius muscle was observed to decrease in all diabetic groups compared to controls, except D1. The cross-sectional area of muscle fibers was found to be significantly lower in the D3 and D4 groups compared to the controls.

In diabetic myopathy, cellular degeneration, inflammation, decrease in muscle fiber length and diameter, and vacuolization of the sarcoplasm are observed in the muscle (Doustar et al 2007). In the histopathological examination of the muscles, all control groups had normal histological appearance. Histological images were found normal in the D1 and D2 groups; This shows that there is no atrophy in this group. In groups D3 and D4,

a decrease in protein density was observed in some muscle bundles, degeneration and apoptosis in the nuclei located in the periphery, and an increase in mononuclear cell infiltration in the connective tissue areas between the muscle bundles. In the D4 group, an increase in connective tissue cells in the fibrous area and thinning of some muscle fibers towards the periphery were observed.

One of the main pathways controlling protein translation and muscle growth is the insulin-IGF1/Akt/mTORC1 signaling pathway (Wood et al. 2021). Studies have shown that insulin deficiency or resistance in diabetes reduces the specific activity of Akt kinase by 34% compared to controls. This impairment in the PI3K-Akt pathway plays a role in decreasing both insulin-mediated glucose uptake and protein synthesis in rodents and patients with DM (Perry et al. 2016). The primary regulator of protein synthesis in skeletal muscle is the activation of mammalian target of rapamycin (mTOR), which is activated by insulin or insulin-like growth factor-1 (IGF1) and Akt via mechanical stimuli (Watson and Baar 2014). It has been reported that mTOR activity decreases in diabetes (Su et al. 2015, Perry et al. 2016, Wood et al. 2021). In this study, mTOR activity was measured by ELISA method. Similar to other studies, mTOR activity in the gastrocnemius muscles of diabetic groups was found to decrease more than in controls. Only in the DD-1 group, this decrease was not significant due to the short duration of diabetes. Considering that mTOR is the main pathway of protein synthesis; it appears that this decrease in protein synthesis in diabetes increases the severity of atrophy.

Myostatin (MSTN), a member of the TGFβ family, is secreted predominantly by skeletal muscle and functions as a negative regulator of muscle growth. In muscle cell cultures, myostatin has been reported to upregulate atrophy-associated ubiquitin ligases; specifically, this regulation was found to be FOXO dependent. In fact, myostatin treatment blocks the IGF1-PI3K-Akt pathway and activates FOXO1, allowing increased expression of atrogin-1. Myostatin is a negative regulator of myoblast proliferation and differentiation. Normally, it functions to regulate muscle hypertrophy, but it is involved in the induction of muscle loss in diseases that cause muscle wasting and cachexia (Elkina et al. 2011). Myostatin expression varies in diabetic rats. There are studies showing that in diabetic rats induced by STZ, the expression of both myostatin and myostatin protein precursors increases in skeletal muscle (Coleman et al. 2016). Increased MSTN expression indicates that it contributes to muscle atrophy in insulin deficiency. In this study, myostatin levels of the gastrocnemius muscle were measured. Myostatin levels were not significantly higher in the D1 and D2 groups. It was found to be significantly higher in groups D3 and D4. Myostatin levels paralleled the muscle atrophy seen in diabetic groups.

Ghrelin, known as the hunger hormone, stimulates growth hormone secretion, stimulates food intake and promotes fat through the growth hormone secretagogue receptor (GHS-R). Since ghrelin is a potent GH secretagogue that regulates muscle growth and differentiation through the GH-insulin-like growth factor (IGF) axis, pharmacological dosing of ghrelin has recently emerged as an interesting candidate for the treatment of sarcopenia and cachexia (Ali and Garcia 2014). A study showed that overexpression of ghrelin in skeletal muscle protects against chemical-induced muscle atrophy (Garcia et al. 2008). Again, many studies have reported that ghrelin application alleviates the severity of muscle atrophy in muscle atrophy caused by fasting, cisplatin, doxorubicin, burns and sarcopenia (Porporato et al. 2013).

Conflicting results have been obtained in studies conducted in diabetic humans and rats regarding plasma ghrelin levels. In some studies, ghrelin levels were found to be significantly lower in obese individuals, type 2 diabetic individuals, and individuals with high insulin resistance (Pöykkö et al. 2003, Pulkkinen 2010). Elsawy et al. (2016) showed that ghrelin decreased in the STZ-induced diabetes model. There are studies showing that plasma ghrelin in type 1 diabetic patients is significantly lower than in the control group (Prodam et al. 2014). In another study in which different types of diabetes models were studied, plasma ghrelin in patients was found to be higher in maturity-onset diabetes type (MODY) than in patients with T1DM and T2DM, but lower than in the control group (Nowak et al. 2015). This study suggested that circulating ghrelin concentration may depend, at least to some extent, on the etiology of diabetes. In other studies, plasma ghrelin levels in rats with type 1 diabetes were found to be higher than in controls (Shankar et al. 2020). We think that the observed change in ghrelin concentration may be due to differences in glycemic control levels.

In this study, plasma ghrelin levels were found to be lower in diabetic groups than in healthy control groups. A decrease in ghrelin concentrations was detected as the duration of diabetes increased. As shown in previous studies, we can say that this develops due to chronic inflammation and insulin resistance (Pöykkö et al. 2003, Nowak et al. 2015).

A positive correlation was determined between plasma ghrelin level and final weight of rats, gastrocnemius muscle weight, cross-sectional area of muscle fiber, and muscle mTOR level. These results show us that there is a significant positive relationship between

plasma ghrelin level and muscle atrophy. A recent study shows that the protective effect of ghrelin on starvation and denervation-induced muscle atrophy is mediated by mTOR and Akt signaling (Porporato et al. 2013).

Decreased ghrelin levels in our diabetic groups affect muscle atrophy through the mTOR pathway, which is also supported by the positive relationship between them. This study showed that ghrelin also plays a role in the protection of diabetic muscle. In the future, larger experimental studies investigating treatment methods with ghrelin in diabetic muscle are needed.

Conclusion

In addition to being a force-generating mechanism, skeletal muscle is an essential organ for glucose storage and metabolism. Therefore, maintaining or improving skeletal muscle mass is an effective way to manage blood sugar. For this reason, diabetic muscle atrophy has attracted more attention recently and many studies have been conducted on this subject.

Muscle mTOR and myostatin levels, muscle weight, muscle fiber size and histopathological images of diabetic groups show us that muscle atrophy develops. It was found that there was a positive relationship between atrophy data and plasma ghrelin level. The idea of ghrelin as a treatment option for atrophy was recently supported by our study. With this study, we can conclude that ghrelin plays a role in diabetes-induced muscle atrophy, as well as cachexia and sarcopenia. Using ghrelin as a treatment option in the treatment of atrophy may be promising for the future.

Acknowledgements

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