



## **Alterations in Serum Myostatin Levels via Swimming Exercise in High Fructose Mediated Metabolic Syndrome Model**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. Author MEK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors CA and MFG managed the analyses of the study. Authors ZA and FT managed the literature searches. All authors read and approved the final manuscript.*

### **Article Information**

DOI: 10.9734/JPRI/2021/v33i1531285

#### Editor(s):

(1) Dr. Prabakaran Nagarajan, The Ohio State University, USA.

#### Reviewers:

(1) Sylwester Kowalik, University of Life Sciences in Lublin, Poland.

(2) Cláudia Rucco Penteadó Detregiachi, University of Marília (UNIMAR), Brazil.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/66293>

**Original Research Article**

**Received 05 January 2021**

**Accepted 10 March 2021**

**Published 23 March 2021**

### **ABSTRACT**

**Aims:** Metabolic syndrome is a table of abdominal diseases characterized by insulin resistance, obesity, dyslipidemia, and hypertension, and it is a serious risk factor for the development of cardiovascular diseases. The purpose of this study was to understand how metabolic syndrome causes a change in myostatin levels, and to understand if swimming exercise have protective and therapeutic effects in fructose mediated metabolic syndrome model by regulating serum myostatin level.

**Methodology:** A total of 21 Sprague Dawley male rats were used in the study. Metabolic syndrome was induced by adding 30% fructose into drinking water for 5 weeks The rats (6-8 weeks old, weighing 200-250 g) were randomly divided into 3 groups as 7 rats in each cage. Swimming exercises were applied three days a week for six weeks. On exercise days, rats were exercised for 20 minutes in a day. Blood was collected from all animals in aprotinin tubes to be used in the necessary analysis with decapitation. Serum samples obtained after centrifugation were kept at -80

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°C until the assay were performed. In serum samples, myostatin was measured by ELISA method.  
**Results:** There was a statistically significant difference resulting from G2, which had the highest value in glucose and triglyceride levels between groups. G2 had the lowest HDL levels. Myostatin levels were significantly higher in G2 compared to other groups.  
**Conclusion:** Moderate swimming exercises are protective and therapeutic in preventing chronic diseases such as diabetes, obesity and metabolic syndrome. In line with these results, it can be said that swimming exercise is a therapeutic practice that regulates impaired fasting serum glucose, increased TG levels and decreased HDL levels and downregulates serum myostatin levels.

*Keywords: Metabolic syndrome; swimming exercise; myostatin (MSTN); lipid profile; myokine.*

## 1. INTRODUCTION

Metabolic syndrome is a table of abdominal diseases characterized by insulin resistance, obesity, atherogenic dyslipidemia, hypertension and hypercoagulability, and it is a serious risk factor for the development of chronic diseases such as obesity and type II diabetes [1]. It is accepted that lifestyle disorder, lack of physical activity, imbalanced and excessive nutrition cause metabolic syndrome by making insulin resistance obvious [2]. One of the main causes of increased metabolic syndrome is fructose in the diet. Fructose is found naturally in many fruits and honey, and in industry as sweeteners [3]. Depending on the dose and duration of use of the high fructose diet, the characteristic features of metabolic syndrome such as glucose intolerance, insulin resistance, dyslipidemia, and fatty liver develop in experimental animals [4]. Exercise is an effective method to improve insulin sensitivity [5]. Because, with exercise, glucose transport to insulin sensitive tissues is increased and this situation is especially observed in bones and muscles [6].

Myokine is the general name given to cytokines / peptides that are secreted in response to the contraction of skeletal muscles and have autocrine, paracrine and endocrine effects [7]. Myokines can undertake important tasks such as energy metabolism and angiogenesis [8]. Known to inhibit muscle hypertrophy, myostatin performs this function by limiting the physiological functions of muscle in many species, including humans [9]. Mice with suppressed myostatin expression are larger than other mice and show a marked increase in skeletal muscle mass. The main characteristic of myostatin deficient mice is very low body fat ratio [10]. Braga and friends (2013), demonstrated a significant increase in lipid metabolism and energy expenditure in their study of mice with myostatin deficiency [11].

In the light of this information, the purpose of this study was to understand how metabolic syndrome causes a change in myostatin level, and to understand if the swimming exercise have protective and therapeutic effects in fructose mediated metabolic syndrome model by regulating serum myostatin level.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Design

A total of 21 Sprague Dawley male rats were used in the study. Metabolic syndrome was induced by adding 30% fructose into drinking water for 5 weeks [12] and weight controls were made at the end of each week. Control group was fed with standard diet and normal tap water. To confirm the induction of metabolic syndrome, fasting blood glucose, triglyceride and HDL levels were measured with an auto analyzer in serum samples. Metabolic syndrome induction was confirmed by determining the presence of 3 of the NCEP-ATP III diagnostic criteria (high fasting blood glucose 'x=170,23±10,33 mg/dL', high triglyceride'x=168,53±34,34 mg/dL' and low HDL 'x=28,78±7,25' mg/dL levels) in groups fed with fructose. The rats (6-8 weeks old, weighing 200-250 g) were randomly divided into 3 groups as 7 rats in each cage after feeding for 5 weeks with 30% fructose diet.

Group 1 (G1): Control group (rats without metabolic syndrome)

Group 2 (G2): Metabolic syndrome group (without exercise application)

Group 3 (G3): Metabolic syndrome group (with exercise application)

### 2.2 Exercise Protocol

Swimming exercises were applied three days a week for six weeks. On exercise days, rats were exercised for 20 minutes a day and between

09:00 and 10:00 in the morning. A 30 cm deep and 184 cm wide water tank was used for swimming exercises. During the exercises, the rats were allowed to swim with their own body weight without any weight attached.

### 2.3 Sample Collection

At the end of the studies, blood was collected from all animals in aprotinin tubes between 09:00 and 10:00 in the morning. Serum samples obtained after centrifugation were kept at -80°C until the assay were performed. In serum samples, myostatin was measured by ELISA method and expressed as pg/ml. However, the determinations of glucose (GL), triglyceride (TG), high density lipoprotein (HDL) in serum samples were studied in the Firat University Animal Hospital Laboratory in an auto analyzer.

### 2.4 Statistical Evaluations

Mann-Whitney U and Kruskal-Wallis tests were applied to the obtained data. These tests were carried out with SPSS Statistics V22.0 software (IBM, NY, USA).

## 3. RESULTS

There was a statistically significant difference resulting from G2, which has the highest value in glucose levels between groups ( $p < 0.05$ ). G2 was the lowest in HDL levels, and this level was statistically significant compared to the other groups ( $p < 0.05$ ). At TG levels, G2 was at the highest level, this level was statistically significant compared to G1 and G3 ( $p < 0.05$ ). Myostatin levels were significantly higher in G2 compared to other groups ( $p < 0.05$ ) Table 1.

## 4. DISCUSSION

As a result of the findings obtained after the study, we determined that metabolic syndrome caused by high fructose diet leads impairment in glucose ( $x = 190,42 \pm 10,14$  mg/dL) TG ( $x = 176,28 \pm 51,23$  mg/dL) and HDL ( $x = 26,85 \pm 5,20$  mg/dL) levels and increased myostatin levels in circulation Table 1. We have shown that the swimming exercises we applied against these impairments have therapeutic potential in the metabolic syndrome model. It has been previously reported that high myostatin level in the circulatory system may be associated with metabolic syndrome [13]. In a study involving metabolically unhealthy obese young adults, it was shown that myostatin levels were statistically significantly higher than normal weight individuals [14]. Contrary to both our study and most studies in the literature, there is a study showing that myostatin levels decrease in patients with diabetes and metabolic syndrome [15]. In experimental animal models, myostatin has been shown to play a role in diabetic muscle atrophy, accordingly, increased myostatin expressions are associated with decreased muscle mass and fiber cross-sectional area [16]. In a study investigating the relationship between plasma and muscle myostatin expression levels, it has been shown that myostatin levels are increased in type 2 diabetes. In the same study, a statistically significant relationship was reported between high triglyceride levels, one of the components of metabolic syndrome, and high myostatin levels [17], these results support our study. In addition to these studies, it has been shown that regular exercise reduces myostatin levels in both healthy and overweight and obese individuals [18].

Table 1. Alterations in GL, HDL, TG and Myostatin level

Parameter	Group	N	Mean	St. Dev.	X <sup>2</sup>	U	p
GL (mg/dL)	G1	7	142,57	11,99	11,59	4,00	0,003
	G2	7	190,42	10,14			
	G3	7	151,14	33,85			
HDL (mg/dL)	G1	7	35,71	2,21	14,28	3,00	0,001
	G2	7	26,85	5,20			
	G3	7	39,14	3,43			
TG (mg/dL)	G1	7	70,42	20,27	14,18	0,00	0,001
	G2	7	176,28	51,23			
	G3	7	140,42	28,73			
Myostatin (pg/ml)	G1	7	485,85	256,95	8,63	8,00	0,013
	G2	7	721,31	130,52			
	G3	7	518,27	94,17			

An exercise-mediated decrease in muscle-derived myostatin plasma levels was reported in a study which diabetic rats were exercised [19]. In a study investigating myostatin mRNA expressions in diabetic rats, it was reported that the myostatin mRNA expressions of diabetic rats which swimming exercise applied were increased compared to non-diabetic and non-exercised rats, and it was emphasized that this may be due to the role of myostatin in energy balance [20]. Besides being an important regulator of myostatin in muscle growth and development [21]. It is known that it is effective in energy metabolism, making myostatin a good therapeutic agent. In studies conducted with myostatin inhibitors on this subject, it has been reported that insulin sensitivity and inhibition of myostatin in diabetic patients can significantly reduce diabetes [19,5,22,23]. In the presented study, it was found that swimming exercise is a therapeutic practice that prevents impaired fasting serum glucose, increased TG levels and decreased HDL levels and downregulates increased serum myostatin levels.

## 5. CONCLUSION

This study, which emphasizes the therapeutic ability of exercise, is capable of contributing to the literature in terms of examining the myostatin levels that differ in metabolic syndrome. The results of this study are useful in understanding the changes in the serum myostatin level caused by a high fructose diet. Moderate swimming exercises are protective and therapeutic in preventing chronic diseases such as diabetes, obesity and metabolic syndrome.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been approved by the Firat University Local Ethics committee (Protocol no:2019/93).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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*Peer-review history:*  
The peer review history for this paper can be accessed here:  
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