Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (4): 473-479, 2024
DOI: 10.9775/kvfd.2024.31671

Research Article

Effects of Statins on Skeletal Muscle Contractile Properties in Rats

Cengiz ÜNSAL [1 (*)] Ece KOÇ YILDIRIM [1] Ayşe Nur AKKOÇ [2] Hümeyra ÜNSAL [1]

[1] Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Physiology, TR-09016 Aydın - TÜRKİYE
[2] Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Pathology, TR-09016 Aydın - TÜRKİYE

[(*)] **Corresponding author:** Cengiz ÜNSAL
Phone: +90 256 220 6000-6120
Cellular phone: +90 539 248 1550
E-mail: cunsal@adu.edu.tr

How to cite this article?
Ünsal C, Koç Yıldırım E, Akkoç AN, Ünsal H: Effects of statins on skeletal muscle contractile properties in rats. *Kafkas Univ Vet Fak Derg,* 30 (4): 473-479, 2024.
DOI: 10.9775/kvfd.2024.31671

Article ID: KVFD-2024-31671
Received: 25.01.2024
Accepted: 13.05.2024
Published Online: 11.06.2024

Abstract

The aim of this study was to investigate the short-term effects of statins on rat skeletal muscle with the use of electrophysiological methods. A total of 28 adult male Wistar albino rats were given atorvastatin, rosuvastatin, and pravastatin (40 mg/kg each) intragastrically for four weeks. At the end of the study, the gastrocnemius muscle was isolated from the surrounding tissues under deep anaesthesia. It was tied with silk thread in the Achilles tendon area and then connected to an isometric force transducer. The contractile properties of the gastrocnemius muscle were assessed. Serum levels of total cholesterol and creatine kinase (CK) were measured. The muscle tissue samples were stained with hematoxylin eosin and histopathologically evaluated under light microscope. The findings revealed that cholesterol levels were significantly lower in all groups except the control group, whereas CK levels were higher in the statin groups than the control group. All three statins decreased the contractile strength and caused fatigue quickly in the gastrocnemius muscle. Histopathological changes in gastrocnemius muscle were more related to early moderate necrosis in rats that were received statins. This study demonstrates that all three statins decrease the ability of skeletal muscle to generate force and its resistance to fatigue in rats.

Keywords: Creatine kinase, Myopathy, Rat, Skeletal muscle contractility, Statin

Introduction

Cardiovascular diseases have remained one of the most important health problems for all over the world. The total number of cases increased from 271 million in 1990 to 523 million in 2019, while the number of related deaths increased from 12.1 million to 18.6 million [1]. Statins significantly reduce the incidence of coronary heart disease, being the most effective hypolipidemic compounds that reduce mortality in patients with cardiovascular diseases [2-4]. A study that was conducted in the US from 2003-2012 reported that approximately 71% of adults diagnosed with cardiovascular diseases, 63% of those diagnosed with diabetes and 54% of patients with hypercholesterolemia were taking cholesterol-lowering medications [5].

Statins are very well tolerated by most patients. However, 10-12% of patients may develop muscle-related side effects [6]. Other side effects of statins include new-onset type 2 diabetes, neurological and neurocognitive effects, hepatotoxicity, renal toxicity, and the adverse effects on gastrointestinal, urogenital, and reproductive systems [7,8]. In a survey study conducted in France, muscle symptoms such as muscle pain, tenderness or weakness were reported in 10% of patients taking statins for the treatment of hypercholesterolemia and one third of these patients had to discontinue the treatment. The most prescribed statins are low dose rosuvastatin and atorvastatin. Soreness was the most described symptom and many patients reported stiffness during exercise, cramps, weakness, or loss of strength. About 38% of patients reported that their symptoms prevented even moderate exertion during daily activities, while 42% of patients experienced significant interference with daily activities [9].

Lovastatin, pravastatin, and simvastatin are grouped as type 1 statins, while fluvastatin, cerivastatin, atorvastatin, and rosuvastatin are grouped as type 2 statins [10]. The side effects of statins may vary depending on the metabolic profile of statins. Moreover, other medications taken in combination may interfere with the mechanisms responsible for the metabolism or clearance of statins, leading to increased statin exposure and a significantly increased risk of myotoxicity [11].

This study aimed to investigate the possible short-term effects of three statins (atorvastatin, rosuvastatin, and pravastatin) from two different groups mentioned above,

widely used in clinical practice, on the skeletal muscles of rats with the use of electrophysiological methods.

Material and Methods

Ethical Statement

This study was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Approval no: 64583101/2018/054).

Animals

A total of 28 male Wistar albino rats weighing ~350 g were used. The rats were housed in a research room with a 12/12 light/dark cycle, a temperature of 22±2°C, and 50-70% humidity. The rats were randomly divided into four groups (n=7/group) as atorvastatin, rosuvastatin, pravastatin, and control groups. Commercially available statins were purchased, grounded into powder, dissolved in saline, and administered via the intragastric gavage at a dosage of 40 mg/kg per day for four weeks. The control group received equal volume saline for the same period. The animals were weighed weekly throughout the experiment.

Muscle Preparation

At the end of the experiment, the rats were anesthetized with a combination of ketamine HCl (60 mg/kg) and xylazine HCl (10 mg/kg) and placed on a warm pad to help regulate their body temperature when they are under deep anaesthesia. The rats were then positioned on their right side and their tail and right hind leg were immobilized with flaster. The left hind leg was shaved, the skin was cut (between the lumbar vertebrae and the achilles tendon) and separated from the underlying tissues by using blunt dissection. The gastrocnemius muscle was isolated from the surrounding tissues and ligated with a silk thread at the achilles tendon area. The achilles tendon was then connected to an isometric force transducer (BSL, SS12LA, BIOPAC®, USA) [12]. After this, the stimulator (BSLTSMA, BIOPAC®, USA) was set to deliver one stimulus at a time (0.5 ms and 0.5 V). The stimulus was increased until the twitch amplitude remained constant. The maximum voltage was determined to be 3 V. The optimal length was determined by gradually stretching the muscle by 1 mm after each stimulus until the twitch amplitude was stabilized. This length was used for all subsequent measurements. The following parameters were determined from the recordings: single twitch force (g), time to peak twitch (ms), half relaxation time (ms), force-frequency relationship (muscle responses obtained by nerve stimulation at frequencies of 10, 20, 40, 60, 80, and 100 Hz for 0.5 ms and a total of 200 ms), maximum isometric tetanic contraction (the frequency with the strongest contraction was determined from 10, 20, 40, 60, 80 and 100 Hz frequencies), and muscle fatigue protocol (50 Hz frequency, 0.5 ms stimulation duration, and 300 ms). This procedure was repeated every second for five minutes, and the values for fatigue initial force (g) and fatigue duration (s) were calculated based on these data [13]. The muscle was allowed to rest for 5 min following the muscle fatigue protocol. The single-twitch protocol was performed again to determine the value of the amplitude of the recovery period (g).

Biochemical and Histopathological Analyses

After evaluating the contractility properties of the muscles, the animals were euthanized following intracardiac blood collection. Using commercial kits, the total cholesterol (Abbot® 7D62-21, USA) and creatine kinase (CK) levels (Abbot® 7D63, USA) were measured in the serum. The muscle tissue samples were stained with the hematoxylin and eosin and evaluated under a light microscope.

Statistical Analysis

The data were analyzed using IBM SPSS Statistics for Windows, version 19.0 (IBM Co., Armonk, NY, USA). Mean and standard error of the mean were presented. The distribution of the obtained data was evaluated using the Shapiro-Wilk test. Changes in body weight were analyzed by repeated measures ANOVA, while other variables were analyzed by one-way ANOVA followed by Duncan's post hoc test.

Results

As shown in *Fig. 1*, the body weights of all groups were normally distributed and there was no significant difference among groups at the beginning of the experiment (P=0.437, F=0.931). The differences among the groups remained statistically insignificant throughout the experiment while the body weights of the animals were seen to increase by time (P=0.026, F=4.141). Cholesterol levels were significantly lower in the rats that received statins than those of the controls. In addition, atorvastatin led to further reduction in cholesterol levels compared

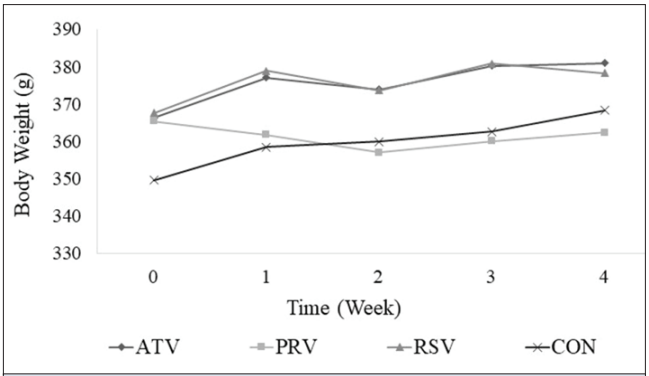

Fig 1. Body weight changes of the rats (g). The body weights of the animals were seen to increase by time (P=0.026).

Table 1. *Cholesterol and creatine kinase levels (mean ± SD)*

Parameters	CON	ATV	RSV	PRV
CHL (mg/dL)	116.06±3.61[a]	47.96±2.44[b]	75.23±4.81[c]	68.16±1.89[c]
CK (U/L)	626.50±6.02[a]	1489.25±163.66[c]	998.00±43.62[b]	707.75±54.31[ab]

Values in the same row *with* different *superscript* letters *differed significantly (P<0.001).*
CON: Control group; ATV: Atorvastatin group; RSV: Rosuvastatin group; PRV: Pravastatin group; CHL: Cholesterol; CK: Creatine kinase

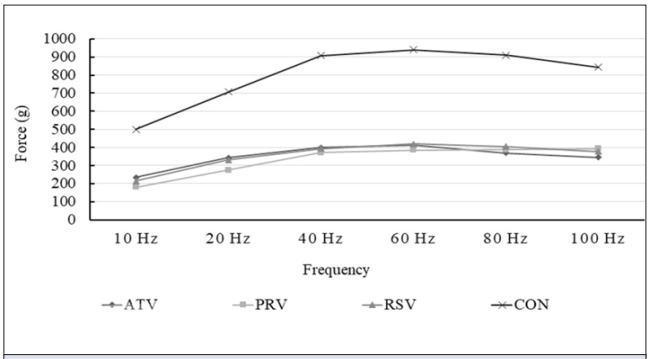

Fig 2. Values of the force (g)-frequency (Hz) of the gastrocnemius muscle. The control group differs significantly from the other groups at all measurement points (P<0.001).

Table 2. *Contractile properties of gastrocnemius muscle (mean ± SD)*

Parameters	CON	ATV	RSV	PRV	P Value
TPT (msec)	47.50±1.68[a]	36.57±3.41[b]	40.17±3.12[ab]	40.75±2.13[ab]	P<0.05
HRT (msec)	34.00±1.90	31.14±2.03	29.67±2.58	30.25±1.79	NS
STF (g)	535.96±50.99[a]	194.90±14.00[b]	181.17±22.63[b]	157.89±26.35[b]	P<0.0001
MITC (g)	998.67±77.79[a]	438.94±52.58[b]	437.21±42.95[b]	396.97±67.54[b]	P<0.0001
FD (s)	53.28±5.66[a]	16.21±1.12[b]	17.88±3.66[b]	14.48±3.25[b]	P<0.0001
FIF (g)	815.53±76.55[a]	309.78±60.86[b]	358.73±38.90[b]	354.31±65.80[b]	P<0.0001
ARP (g)	198.69±32.85[a]	127.64±13.72[ab]	81.24±24.63[b]	70.03±12.09[b]	P<0.01

Values in the same row with different superscript letters differed significantly. CON: Control group;; ATV: Atorvastatin group; RSV: Rosuvastatin group; PRV: Pravastatin group. TPT: Time to peak twitch; HRT: Half relaxation time; STF: Single twitch force; MITC: Maximum isometric tetanic contraction; FD: Fatigue duration; FIF: Fatigue initial force; ARP: Amplitude of the recovery period.

to rosuvastatin and pravastatin (P<0.001, F=36.634). CK levels in the rats, received statins (especially atorvastatin and rosuvastatin), were higher than the controls (P<0.001, F=19.210) *(Table 1)*.

Single twitch force of the rats that were received statins decreased compared to the controls (P<0.0001, F=30.128). The difference regarding time to peak twitch was significant between the control and atorvastatin groups (P<0.05, F=3.302), whereas the difference among groups in respect of half relaxation time was not significant (P=0.446, F=0.919). The statin administration caused a reduction (P<0.001) in force at all frequency ranges that were used in the force-frequency evaluation *(Fig. 2)*. The initial force and duration of fatigue were also reduced in the statin-received rats compared with the controls (P<0.0001, F=14.215, F=23.654 respectively). Single twitch force, which is the recovery time value taken after fatigue, was found to decrease in statin-received rats. The difference was significant in the rosuvastatin, and pravastatin groups compared to the control group (P<0.01, F=6.164), whereas the difference between the atorvastatin group and other groups was not significant *(Table 2)*.

The transverse and longitudinal sections of the muscle tissue, stained with the hematoxylin and eosin, were analyzed under the light microscope *(Fig. 3, Fig. 4)*. Necrosis was objectively scored as minimal (1)-up to %10 individual fibers involved in the whole area; mild (2)-up to 20% of fibers in the area; moderate (3)-up to 50% of fibers in the area; or severe (4)-greater than 50% of fibers in the area [14]. Degenerative necrotic changes in the muscles were mild in the rats, treated with atorvastatin and rosuvastatin, and moderate in the rats treated with

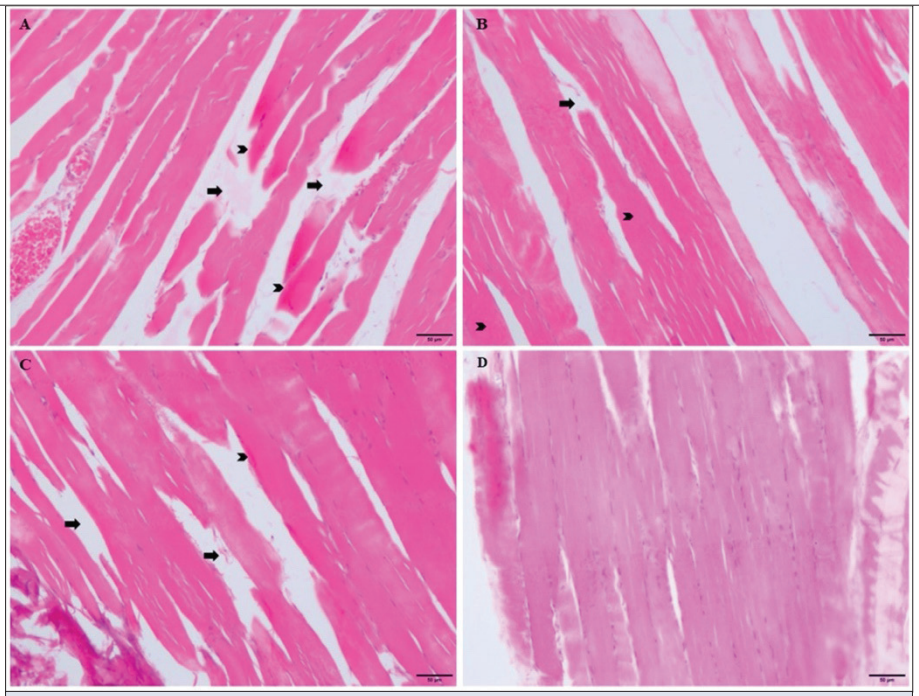

Fig 3. A-D: Cross-section of the gastrocnemius muscle. Atorvastatin, rosuvastatin, pravastatin, and control group, respectively. *Thick arrow:* myofibrillar rupture; *arrowhead:* hyper-eosinophilic fibres. H&E staining, scale bar: 50 µm

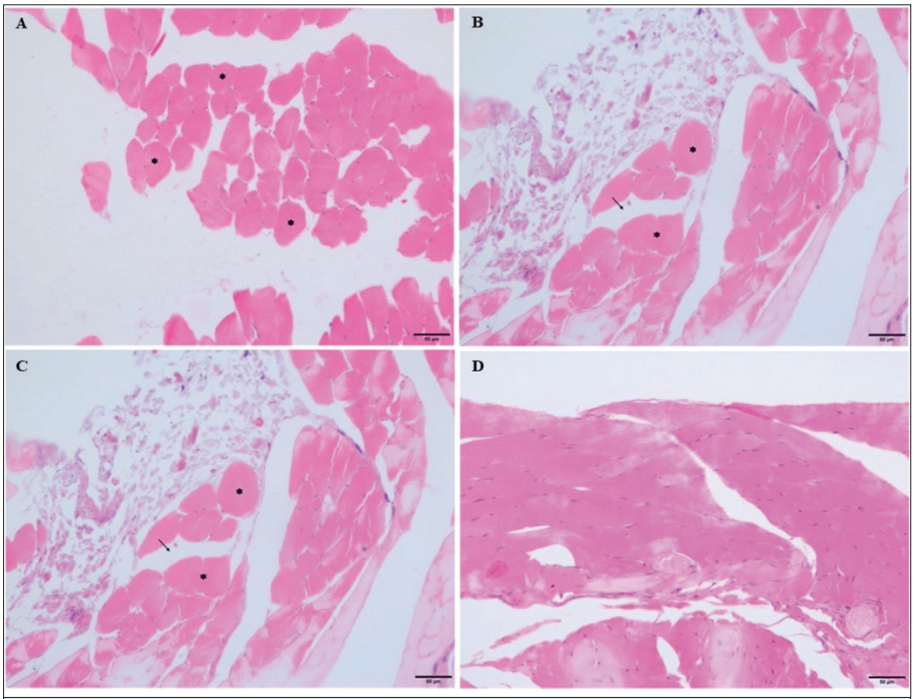

Fig 4. A-D: Longitudinal section of the gastrocnemius muscle. Atorvastatin, rosuvastatin, pravastatin, and control group, respectively. *: coagulation-like appearance, swelling; *thin arrow:* oedema between muscle bundles. H&E staining, scale bar: 50 µm

pravastatin. A marked disorganization of the muscle bundles, myofibrillar rupture, hypereosinophilic staining, coagulation-like appearance, fiber breakdown, and myofibrillar swelling and disintegration were observed in the longitudinal sections of the muscles in the statin-treated rats. In the rosuvastatin group, there was loss of transverse striation in some areas of the muscle. Additionally, there was dilation caused by edema between muscle bundles. Perivascular cell infiltration was seen in some muscle sections of the atorvastatin-received rats. Hypereosinophilic staining, coagulation-like appearance, fiber breakdown, and myofibrillar swelling were more

prominent in the pravastatin-received rats. Cross sectional evaluation revealed that the angular appearance of the muscle bundles disappeared, became rounded and separated. Hypereosinophilic staining and a coagulated appearance were observed in some of the transverse sections in the atorvastatin-received rats. In addition, degeneration in muscle bundles was prominent in the pravastatin-received rats.

Discussion

Long-term low dose of statin does not have a negative effect on body weight [15] which is consistent with this study. The increase in body weight by time in this study is due to the ongoing development of the rats. In rats, all three statins caused a decrease in cholesterol levels compared to the control group. It has been reported that different statins either do not lower cholesterol levels [16,17] or cause a weak reduction [15] in normo-cholesterolemic rats. However, the selected statins in this study led to significant reductions in cholesterol levels. The differences between studies may be due to the fact that the cholesterol-lowering effects of statins vary between species and depend on the dosage. Pecoraro et al.[18] stated that statins reduced total cholesterol in different species and the reduction was greater in animals that were fed with a high cholesterol diet. It was found that lipophilic statins (cerivastatin, simvastatin, fluvastatin, atorvastatin, lovastatin, and pitavastatin) had significantly greater cytotoxic effects in cell culture than hydrophilic statins (pravastatin and rosuvastatin) in a concentration-dependent manner [19]. Previous studies in rats have emphasized that a particularly high dose of statins is required to induce muscle necrosis [14,20]. Degenerative changes in the muscles have been reported to occur after a certain amount of time, such as 43 days, after administering simvastatin at a dose of 60 mg/kg/day in rats [20]. It has been suggested that myopathy may be initiated as early as the first day of statin administration and the necrosis may develop as early as 5 days after administration [21]. However, the data obtained in this study revealed that there was no evidence of statin-induced muscle severe necrosis. In our study, changes in gastrocnemius muscle were more related to early mild to moderate necrosis. We found a more pronounced degeneration in rats that received pravastatin. In contrast to the findings in our study, Bergman et al.[22] did not observe significant changes in the muscles of the mice that received pravastatin. El-Ganainy et al.[23] observed mild to severe necrosis in 60% of rats received atorvastatin (100 mg/kg/day), and mild necrosis in only 10% of rats, received rosuvastatin (80 mg/kg/day), after three weeks of statin administration. The changes in CK levels were one of the findings that reflects the severity of muscle degeneration. The known mechanism for the release of CK is the damage in the muscle tissue or changes in the permeability of the myocyte membrane. The rats, received atorvastatin, had the highest CK levels, followed by those received rosuvastatin when compared to the control rats. The CK levels of the control rats were above the normal levels. The reason for this may be that the blood samples were collected immediately after the in situ muscle protocol. This could be the same effect through which extended and intense training can harm muscle tissue and lead to a rise in serum CK levels which can be caused by both metabolic and mechanical factors [24]. It was reported that CK values doubled in rats, received 100 mg/kg/day of atorvastatin compared to the control rats [25]. CK levels increased in rats treated with high dose rosuvastatin. Interestingly, the increase in CK was started after the first 10 days of treatment and returned to normal levels by day 16 [14]. The CK values of the rats in the pravastatin group were similar to those in the control group, although slightly elevated. However, pravastatin group also displayed more prominence histopathologic findings of muscle degeneration. It is worth noting that some patients with statin-induced muscle symptoms had normal serum CK levels, despite the presence of marked weakness and histopathologic myopathy findings [26]. Reijneveld et al.[27] found that CK levels were similar to the control group after administration of pravastatin to young rats. However, elevated plasma CK levels can be used as a biomarker of tissue histopathology and severity of muscle toxicity [28]. According to the aforementioned studies, it can be stated that there is an increase in CK levels depending on the dose of statin.

Simvastatin [20], rosuvastatin [14,29], and atorvastatin [25,30] have been shown to cause muscle necrosis in rats. However, the doses used in the studies mentioned above are higher than those used in this study. In addition, the severity of the lesions varies depending on the statin dose administered. The muscles contain a significant proportion of type IIB fibers [14,20,25], and in these muscles, type IIB fibers become necrotic first [14]. Each of the three sections that make up the gastrocnemius has a different fibre composition. The red part has a high proportion of type I (slow twitch) fibers and low oxidative capacity fibers are absent, and therefore this muscle part has a high degree of fatigue resistance. The mixed part, adjacent to the red part and separated from it by a fibrous septum, contains a low proportion of type I fibers and a high proportion of IIB fibers. The largest white part contains only IIB fibers [31]. Therefore, there might be a correlation between the metabolic nature of muscle fibers and their susceptibility to statin-induced necrosis and these changes can be observed in fibers without concomitant changes in contractile elements, the endoplasmic reticulum or other subcellular components, suggesting that different parts of the muscle may be affected [20].

All three statins decreased the contractile strength of the gastrocnemius muscles and caused them to fatigue quickly. It was also determined that the time to twitch peak was shortened in the atorvastatin group compared with the control group. The administration of atorvastatin in rats results in a decrease in exercise capacity and impairment of the mitochondrial respiratory chain complexes in skeletal muscle. The correlation between exercise capacity and muscular oxidative capacity suggests that atorvastatin-induced mitochondrial dysfunction is responsible for the reduction in exercise capacity [32]. It is expected that some aspect of the intracellular Ca^{2+} transient, such as the peak amplitude and/or duration of the Ca^{2+} transient, will correlate with the contractile response during an isometric twitch [33]. Statins have been found to affect calcium channels that are crucial for muscle contraction and other fundamental functions [34]. The disruption of calcium homeostasis by statins is thought to be due to impairment of mitochondrial respiration or disturbance of intracellular calcium signaling [35,36]. We suggest that the decrease in the time to peak caused by the administration of atorvastatin may be due to a disturbance in the homeostasis of calcium.

Within the statin groups, the amplitude of the recovery period was closest to the control in atorvastatin group. However, amplitudes were significantly decreased in other statin groups compared to the control group. Sustained physical exercise reduces the ability to produce voluntary force. After this period, a rapid recovery of strength occurs due to the restoration of central fatigue (usually within 2 min) and aspects of peripheral fatigue related to excitation-contraction coupling and muscle reperfusion (usually within 3 to 5 min). Muscle function may not fully recover for several hours due to prolonged impairment in intracellular Ca^{2+} release or sensitivity [37,38]. The muscle phosphocreatinine metabolic recovery rate is an index of muscle oxidative capacity in vivo. A longer recovery time indicates impaired oxidative phosphorylation and/or mitochondrial ATP synthesis. The exposure to statins leads to a significant increase in metabolic recovery time after exertion. This suggests that statins may impair mitochondrial oxidative function [32,39,40]. The reduction in exercise endurance in mice by atorvastatin has been linked to muscle mitochondrial dysfunction as a result of ubiquinone deficiency. Pravastatin does not have a similar effect both on ubiquinone levels and exercise endurance in mice. The difference in membrane permeability of statins is thought to be the reason for their effect on muscle ubiquinone levels [41].

In conclusion, this study demonstrates that all three statins decrease the ability of skeletal muscle to generate force and its resistance to fatigue in rats. While the severity of degeneration and impairments in the contractile properties of the muscle are similar, there are differences in recovery period and time to peak twitch, suggesting that there may be other potential mechanisms besides common ones. This study attempted to examine the effects of statins by mechanical assessment of the muscle. For further studies, it will be important to conduct comprehensive analyses and approaches to explain the underlying mechanisms and to consider the time factor.

Declarations

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author (C. Ünsal) upon reasonable request.

Financial Support: This research was supported by Research Fund the Aydın Adnan Menderes University. Project number: VTF-19014.

Ethical Statement

This study was approved by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (Approval no: 64583101/2018/054).

Conflict of Interest: The authors state no conflict of interest.

Author Contributions: CÜ designed this study, collected the data set and revised the manuscript; EKY, ANA, HÜ collected the data set and made contributions to results and discussion.

References

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, Barengo NC, Beaton AZ, Benjamin EJ, Benziger CP, Bonny A, Brauer M, Brodmann M, Cahill TJ, Carapetis J, Catapano AL, Chugh SS, Cooper LT, Coresh J, Criqui M, DeCleene N, Eagle KA, Emmons-Bell S, Feigin VL, Fernandez-Sola J, Fowkes G, Gakidou E, Grundy SM, He FJ, Howard G, Hu F, Inker L, Karthikeyan G, Kassebaum N, Koroshetz W, Lavie C, Lloyd-Jones D, Lu HS, Mirijello A, Temesgen AM, Mokdad A, Moran AE, Muntner P, Narula J, Neal B, Ntsekhe M, Moraes de Oliveira G, Otto C, Owolabi M, Pratt M, Rajagopalan S, Reitsma M, Ribeiro ALP, Rigotti N, Rodgers A, Sable C, Shakil S, Sliwa-Hahnle K, Stark B, Sundstrom J, Timpel P, Tleyjeh IM, Valgimigli M, Vos T, Whelton PK, Yacoub M, Zuhlke L, Murray C, Fuster V, GBD-NHLBI-JACC Global Burden of Cardiovascular Diseases Writing Group: Global burden of cardiovascular diseases and risk factors, 1990-2019: Update from the GBD 2019 study. *J Am Coll Cardiol,* 76 (25): 2982-3021, 2020. DOI: 10.1016/j.jacc.2020.11.010

2. Schwalm JD, Walli-Attaei M, Yusuf S: New approaches needed to improve prevention of cardiovascular disease. *JAMA Netw Open,* 6 (1):e2251162, 2023. DOI: 10.1001/jamanetworkopen.2022.51162

3. Rasmussen P, Yandrapalli S, Aronow W: Statin therapy for primary cardiovascular prevention in adults older than 75 years. *Kardiol Pol,* 79 (1): 18-24, 2021. DOI: 10.33963/KP.15743

4. Nanna MG, Abdullah A, Mortensen MB, Navar AM: Primary prevention statin therapy in older adults. *Curr Opin Cardiol,* 38 (1): 11-20, 2023. DOI: 10.1097/HCO.0000000000001003

5. Gu Q, Paulose-Ram R, Burt VL, Kit BK: Prescription cholesterol-lowering medication use in adults aged 40 and over: United States, 2003-2012. *NCHS Data Brief* (177): 1-8, 2014.

6. Abd TT, Jacobson TA: Statin-induced myopathy: A review and update. *Expert Opin Drug Saf,* 10 (3): 373-387, 2011. DOI: 10.1517/14740338.2011.540568

7. Patel KK, Sehgal VS, Kashfi K: Molecular targets of statins and their potential side effects: Not all the glitter is gold. *Eur J Pharmacol,* 922:174906, 2022. DOI: 10.1016/j.ejphar.2022.174906

8. Wu Q, Fang L, Zhu Y, Zheng L: Reviews on statin-associated side effects. **In,** Liu D (Ed): Statins - From Lipid-lowering Benefits to Pleiotropic Effects. *IntechOpen*, DOI: 10.5772/intechopen.1000845, 2023.

9. Rosenbaum D, Dallongeville J, Sabouret P, Bruckert E: Discontinuation of statin therapy due to muscular side effects: A survey in real life. *Nutr Metab Cardiovasc Dis,* 23 (9): 871-875, 2013. DOI: 10.1016/j.numecd.2012.04.012

10. Murphy C, Deplazes E, Cranfield CG, Garcia A: The role of structure and biophysical properties in the pleiotropic effects of statins. *Int J Mol Sci,* 21 (22), 2020. DOI: 10.3390/ijms21228745

11. Catapano AL: Statin-induced myotoxicity: Pharmacokinetic differences among statins and the risk of rhabdomyolysis, with particular reference to pitavastatin. *Curr Vasc Pharmacol,* 10 (2): 257-267, 2012.

12. Koc Yildirim E, Dedeoglu Z, Kaya M, Uner AG: The effect of swimming training on adrenomedullin levels, oxidative stress variables, and gastrocnemius muscle contractile properties in hypertensive rats. *Clin Exp Hypertens,* 43 (2): 131-137, 2021. DOI: 10.1080/10641963.2020.1825726

13. MacIntosh BR, Esau SP, Holash RJ, Fletcher JR: Procedures for rat in situ skeletal muscle contractile properties. *J Vis Exp,* 56:e3167, 2011. DOI: 10.3791/3167

14. Westwood FR, Scott RC, Marsden AM, Bigley A, Randall K: Rosuvastatin: characterization of induced myopathy in the rat. *Toxicol Pathol,* 36 (2): 345-352, 2008. DOI: 10.1177/0192623307311412

15. Pierno S, Didonna MP, Cippone V, De Luca A, Pisoni M, Frigeri A, Nicchia GP, Svelto M, Chiesa G, Sirtori C, Scanziani E, Rizzo C, De Vito D, Conte Camerino D: Effects of chronic treatment with statins and fenofibrate on rat skeletal muscle: A biochemical, histological and electrophysiological study. *Br J Pharmacol,* 149 (7): 909-919, 2006. DOI: 10.1038/sj.bjp.0706917

16. Rossoni G, Manfredi B, Civelli M, Berti F, Razzetti R: Combined simvastatin-manidipine protect against ischemia-reperfusion injury in isolated hearts from normocholesterolemic rats. *Eur J Pharmacol,* 587 (1-3): 224-230, 2008. DOI: 10.1016/j.ejphar.2008.03.026

17. Krause BR, Newton RS: Lipid-lowering activity of atorvastatin and lovastatin in rodent species: Triglyceride-lowering in rats correlates with efficacy in LDL animal models. *Atherosclerosis,* 117 (2): 237-244, 1995. DOI: 10.1016/0021-9150(95)05576-i

18. Pecoraro V, Moja L, Dall'Olmo L, Cappellini G, Garattini S: Most appropriate animal models to study the efficacy of statins: A systematic review. *Eur J Clin Invest,* 44 (9): 848-871, 2014. DOI: 10.1111/eci.12304

19. Kobayashi M, Chisaki I, Narumi K, Hidaka K, Kagawa T, Itagaki S, Hirano T, Iseki K: Association between risk of myopathy and cholesterol-lowering effect: A comparison of all statins. *Life Sci,* 82 (17-18): 969-975, 2008. DOI: 10.1016/j.lfs.2008.02.019

20. Westwood FR, Bigley A, Randall K, Marsden AM, Scott RC: Statin-induced muscle necrosis in the rat: Distribution, development, and fibre selectivity. *Toxicol Pathol,* 33 (2): 246-257, 2005. DOI: 10.1080/01926230590908213

21. Sidaway J, Wang Y, Marsden AM, Orton TC, Westwood FR, Azuma CT, Scott RC: Statin-induced myopathy in the rat: Relationship between systemic exposure, muscle exposure and myopathy. *Xenobiotica,* 39 (1): 90-98, 2009. DOI: 10.1080/00498250802585539

22. Bergman M, Salman H, Djaldetti M, Alexandrova S, Punsky I, Bessler H: Ultrastructure of mouse striated muscle fibers following pravastatin administration. *J Muscle Res Cell Motil,* 24 (7): 417-420, 2003. DOI: 10.1023/a:1027367022415

23. El-Ganainy SO, El-Mallah A, Abdallah D, Khattab MM, Mohy El-Din MM, El-Khatib AS: Rosuvastatin safety: An experimental study of myotoxic effects and mitochondrial alterations in rats. *Toxicol Lett,* 265, 23-29, 2017. DOI: 10.1016/j.toxlet.2016.10.017

24. Brancaccio P, Maffulli N, Limongelli FM: Creatine kinase monitoring in sport medicine. *Br Med Bull,* 81-82 (1): 209-230, 2007. DOI: 10.1093/bmb/ldm014

25. El-Ganainy SO, El-Mallah A, Abdallah D, Khattab MM, Mohy El-Din MM, El-Khatib AS: Elucidation of the mechanism of atorvastatin-induced myopathy in a rat model. *Toxicology,* 359-360, 29-38, 2016. DOI: 10.1016/j.tox.2016.06.015

26. Phillips PS, Haas RH, Bannykh S, Hathaway S, Gray NL, Kimura BJ, Vladutiu GD, England JD, Scripps Mercy Clinical Research C: Statin-associated myopathy with normal creatine kinase levels. *Ann Intern Med,* 137 (7): 581-585, 2002. DOI: 10.7326/0003-4819-137-7-200210010-00009

27. Reijneveld JC, Koot RW, Bredman JJ, Joles JA, Bar PR: Differential effects of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors on the development of myopathy in young rats. *Pediatr Res,* 39 (6): 1028-1035, 1996. DOI: 10.1203/00006450-199606000-00016

28. Schaefer WH, Lawrence JW, Loughlin AF, Stoffregen DA, Mixson LA, Dean DC, Raab CE, Yu NX, Lankas GR, Frederick CB: Evaluation of ubiquinone concentration and mitochondrial function relative to cerivastatin-induced skeletal myopathy in rats. *Toxicol Appl Pharmacol,* 194 (1): 10-23, 2004. DOI: 10.1016/j.taap.2003.08.013

29. Chogtu B, Ommurugan B, Thomson SR, Kalthur SG: Effect of vitamin D analogue on rosuvastatin-induced myopathy in wistar rats. *Sci World J,* 2020:4704825, 2020. DOI: 10.1155/2020/4704825

30. Hassani KM: Investigation of the effect of atorvastatin on skeletal muscles in male rats and the involved mechanisms. *Arch Razi Inst,* 77 (1): 285-291, 2022. DOI: 10.22092/ARI.2021.356683.1895

31. Santocildes G, Merino M, Fabiani F, Pages T, Marotta M, Viscor G, Torrella JR: Histomorphological and functional contralateral symmetry in the gastrocnemius muscles of the laboratory rat. *J Anat,* 241 (3): 692-701, 2022. DOI: 10.1111/joa.13674

32. Bouitbir J, Charles AL, Rasseneur L, Dufour S, Piquard F, Geny B, Zoll J: Atorvastatin treatment reduces exercise capacities in rats: Involvement of mitochondrial impairments and oxidative stress. *J Appl Physiol,* 111 (5): 1477-1483, 2011. DOI: 10.1152/japplphysiol.00107.2011

33. Sun YB, Lou F, Edman KA: The relationship between the intracellular Ca^{2+} transient and the isometric twitch force in frog muscle fibres. *Exp Physiol,* 81 (5): 711-724, 1996. DOI: 10.1113/expphysiol.1996.sp003971

34. Seto SW, Au AL, Lam TY, Chim SS, Lee SM, Wan S, Tjiu DC, Shigemura N, Yim AP, Chan SW, Tsui SK, Leung GP, Kwan YW: Modulation by simvastatin of iberiotoxin-sensitive, Ca^{2+}-activated K^{+} channels of porcine coronary artery smooth muscle cells. *Br J Pharmacol,* 151 (7): 987-997, 2007. DOI: 10.1038/sj.bjp.0707327

35. Mohaupt MG, Karas RH, Babiychuk EB, Sanchez-Freire V, Monastyrskaya K, Iyer L, Hoppeler H, Breil F, Draeger A: Association between statin-associated myopathy and skeletal muscle damage. *CMAJ,* 181 (1-2): E11-18, 2009. DOI: 10.1503/cmaj.081785

36. Mollazadeh H, Tavana E, Fanni G, Bo S, Banach M, Pirro M, von Haehling S, Jamialahmadi T, Sahebkar A: Effects of statins on mitochondrial pathways. *J Cachexia Sarcopenia Muscle,* 12 (2): 237-251, 2021. DOI: 10.1002/jcsm.12654

37. Ce E, Longo S, Limonta E, Coratella G, Rampichini S, Esposito F: Peripheral fatigue: new mechanistic insights from recent technologies. *Eur J Appl Physiol,* 120 (1): 17-39, 2020. DOI: 10.1007/s00421-019-04264-w

38. Carroll TJ, Taylor JL, Gandevia SC: Recovery of central and peripheral neuromuscular fatigue after exercise. *J Appl Physiol (1985),* 122 (5): 1068-1076, 2017. DOI: 10.1152/japplphysiol.00775.2016

39. Bonfim MR, Oliveira AS, do Amaral SL, Monteiro HL: Treatment of dyslipidemia with statins and physical exercises: Recent findings of skeletal muscle responses. *Arq Bras Cardiol,* 104 (4): 324-331, 2015. DOI: 10.5935/abc.20150005

40. Garrett TJ, Puchowicz MA, Park EA, Dong Q, Farage G, Childress R, Guingab J, Simpson CL, Sen S, Brogdon EC, Buchanan LM, Raghow R, Elam MB: Effect of statin treatment on metabolites, lipids and prostanoids in patients with statin associated muscle symptoms (SAMS). *PLoS One,* 18 (12):e0294498, 2023. DOI: 10.1371/journal.pone.0294498

41. Muraki A, Miyashita K, Mitsuishi M, Tamaki M, Tanaka K, Itoh H: Coenzyme Q10 reverses mitochondrial dysfunction in atorvastatin-treated mice and increases exercise endurance. *J Appl Physiol,* 113 (3): 479-486, 2012. DOI: 10.1152/japplphysiol.01362.2011