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Statins induce biochemical changes in the Achilles tendon after chronic treatment

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ABSTRACT

Statins have been widely prescribed as lipid-lowering drugs and are associated with tendon rupture. Therefore, this study aimed to evaluate the possible biochemical changes in the Achilles tendon of rats after chronic treatment with statins. Dosages of statins were calculated using allometric scaling with reference to the 80 mg/day and 20 mg/day, doses recommended for humans. The rats were divided into the following groups: treated with simvastatin (S-20 and S-80), treated with atorvastatin (A-20 and A-80), and the control group that received no treatment (C). Measurements of low-density lipoprotein (LDL) in the plasma were performed. The levels of non-collagenous proteins, glycosaminoglycans (GAGs) and hydroxyproline were quantified. Western blotting for collagen I was performed, and the presence of metalloproteinases (MMPs)-2 and -9 was investigated through zymography. The concentration of non-collagenous proteins in S-20 was less than the C group. There was a significant increase in pro-MMP-2 activity in A-80 group and in active MMP-2 in S-20 group compared to the C group. A significant increase in latent MMP-9 activity was observed in both the A-80 and S-20 groups when compared to C group. In the A-20 group, there was a lower amount of collagen I in relation to C group. In addition, a higher concentration of hydroxyproline was found in the S-20 group than the C group. The analysis of GAGs showed a significant increase in the A-20 group when compared to C group. The treatment induced remarkable alterations in the Achilles tendon and the response of the tissue seems to depend of the used statin dosage. The presence of MMP-2 and MMP-9 is evidence of the degradation and remodeling processes in the extracellular matrix of the tendons. Our results show that statins induce imbalance of extracellular matrix components and possibly induce microdamage in tendons.

1. Introduction

Statins are a group of drugs prescribed for the treatment of hyperlipidemia, and they act by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, an enzyme involved in cholesterol production. HMG-CoA reductase catalyzes the conversion of HMG-CoA to mevalonate, and therefore serves as a therapeutic target for the action of statins (Campo and Carvalho, 2007). Consequently, HMG-CoA reductase inhibitors induce a reduction of LDL in the plasma, a reduction of intracellular cholesterol and an increase of the LDL receptors (Maron et al., 2000).

Statins are widely prescribed medications with various clinical benefits, including the prevention of myocardial infarction and the formation of atherosclerotic plaques (O'Sullivan, 2007). Additionally, many studies have shown that the clinical benefits associated with statins are either independent of or indirectly dependent on a reduction in LDL-cholesterol (Campo and Carvalho, 2007). These pleiotropic effects, have been well-studied in an effort to identify additional potential uses for these drugs in the treatment of other pathologies, such as (Liao and Laufs, 2005) hypertension, (Yang et al., 2005), Alzheimer's disease (Campo and Carvalho, 2007), sepsis (Giusti-Paiva et al., 2004) and osteoporosis (Jadhav and Jain, 2006).

Despite being quite effective medicines, statins have some adverse effects, including constipation, headaches, sleep disturbances, and other more serious effects, such as hepatotoxicity and musculoskeletal complications (Hoffman et al., 2012; O'Sullivan, 2007). More recently, cases of tendinitis and tendon ruptures have been associated with the use of statins (Marie and Noblet, 2009). These complications have been observed in several tendons, including the distal biceps (Savvidou and Moreno, 2012), the patellar (Beri et al., 2009), the quadriceps (Nesselroade and Nickels, 2010; Rubin et al., 2011) and the Achilles tendons (Beri et al., 2009; Carmont

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et al., 2009; Chazerain et al., 2001), the latter of which are commonly injured (Marie et al., 2008).

Simvastatin and atorvastatin are some of the most widely used statins in the treatment of hypercholesterolemia, and they are highly efficacious and tolerable (Maron et al., 2000). However, they can also result in tendinopathies (Marie et al., 2008). Tendinopathies seem to be a rare adverse effect of statins, but it is also thought that many of these types of adverse effects are left unreported to pharmacovigilance centers (Chazerain et al., 2001).

Recent studies have shown that statins are responsible for inhibiting the secretion of metalloproteinases (MMPs) in lung fibroblasts (Kamio et al., 2010) and endothelial cells (Izidoro-Toledo et al., 2011). Furthermore, they reduce the expression of collagen I in smooth muscle (Schaafsma et al., 2011). Of note, tendons are formed primarily by collagen I, and MMPs play an important role in maintaining and remodeling the extracellular matrix in tendons (Kannus, 2000).

In addition to lowering cholesterol and inhibiting MMPs (Izidoro-Toledo et al., 2011), statin use has also been shown to promote apoptosis in fibroblasts (Yokota et al., 2008), further suggesting an association between statin use and tendon rupture. Together, these factors can weaken the integrity of the tendon tissue and thus predispose the tendon to rupture.

Statins are widely prescribed drugs, and investigating the biochemical changes in tendons caused by statins may help to understand the causes of tendinitis and tendon rupture associated with the use of those drugs. Because there are no studies that address the effects of statins on the components of the extracellular matrix in tendons, this study was designed to investigate the effects of chronic oral statin treatment on the Achilles tendon in rats.

2. Methods

2.1. Experimental groups

Animal care was in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes and was consistent with the ethical principles of animal experimentation adopted by the Brazilian College of Animal Experimentation (COBEA), was approved by the Ethics Committee on Animal Experiments of State University of Campinas, SP, Brazil and filed under no. 2473-1.

We used 50 male Wistar rats, young adults, weighing on average 300 g. The rats were housed two per cage in a 12 h light:dark cycle at 23 °C, with free access to standard rat chow and water.

The animals were divided into five experimental groups: rats treated with a daily dose equivalent to 20 mg of atorvastatin (pharmanostra, Rio de Janeiro, Brazil) (A-20) or simvastatin (Galena, Campinas, Brazil) (S-20), rats treated with a daily dose equivalent to 80 mg of atorvastatin (A-80) or simvastatin (S-80) and the control group that received no treatment (C). To dilute the statins we used an aqueous solution of 0.5% carboxymethylcellulose. The treatment lasted for 2 months and a gavage was used to administer the solutions (Camerino et al., 2011).

We accounted for the metabolic rate of the animals and used allometric scaling to calculate the necessary drug dosage (Pachaly and Brito, 2001). Thus, it was necessary to use an animal model in which the pharmacokinetic and pharmacodynamics parameters of the drug of interest were known. In this study, we used as animal model the human and animal target Wistar rats. After two months of treatment, the animals were euthanized with isoflurane (Isofurine – Cristália, Itapira, Brazil) and the tendons were collected for biochemical analysis.

2.2. LDL-cholesterol determination

For the determination of LDL-cholesterol in the plasma of the rats an enzymatic assay was used. Through this assay it is possible to measure LDL-cholesterol directly (Kit LDL-C Plus-Roche).

2.3. Extraction procedures

The calcaneal tendon ($n = 5$) was cut in small pieces and treated with 50 volumes of 4 M guanidine hydrochloride (GuHCl) containing 20 mM EDTA, 1 mM PMSF in 50 mM sodium acetate buffer, pH 5.8, for 24 h at 4 °C with mild stirring (Heinergard and Sommarin, 1987). Afterwards, the material was centrifuged (13,000 × *g*, 25 min, 4 °C) and the supernatant was used for non-collagenous protein dosage and Western blotting for collagen I.

2.4. Quantification of proteins

Samples of the extracts of each experimental group were used. Non-collagenous proteins (NCPs) were quantified according to the Bradford method (1976), using bovine serum albumin as standard. The absorbance was measured at 595 nm.

2.5. Hydroxyproline quantification

After washing in PBS (phosphate-buffered saline—5 mM phosphate buffer, 0.15 M NaCl and 50 mM EDTA), fragments from the tendons were immersed into acetone for 48 h and then into chloroform:ethanol (2:1) for 48 h. Fragments were weighed and hydrolyzed in 6 N HCl (1 mL for each 10 mg of tissue) for 16 h at 110 °C. The hydrolysate was neutralized with 6 N NaOH, and 20 μL of each sample was treated with chloramine T solution, as described by Stegemann and Stalder (1967) and Jorge et al. (2008), with some modifications. The absorbance was measured at 550 nm in a spectrophotometer. Hydroxyproline concentrations from 0.2 to 6 μg/mL were used for a standard curve.

2.6. Agarose gel electrophoresis

The fragments of the tendons were dehydrated, and sulfated glycosaminoglycans were released from proteoglycans by digestion with a papain solution (Merck, Darmstadt, Germany) (40 mg/g of dry tissue) containing 100 mM sodium phosphate buffer, pH 6.5, 40 mM EDTA, and 80 mM β-mercaptoethanol (Harab and Morão, 1989). The reaction was stopped by the addition of 4 mM iodoacetic acid for 1 h. The sulfated GAGs were precipitated in ethanol and separated by agarose gel electrophoresis (0.6%) in 0.05 M propylenediamine according to Dietrich and Dietrich (1976).

2.7. Quantification of sulfated GAGs

The samples digested by papain solution were used to quantify the GAGs of the tendons of the different experimental groups. The quantification was determined using the dimethylmethylene blue method (Farndale et al., 1986) using chondroitin sulfate as the standard. The absorbance was measured at 540 nm using an Asys Expert Plus Microplate Reader (Biochrom, Holliston, MA, USA).

2.8. Western blotting for collagen I

For collagen I detection, were precipitated 10 μg of total protein from the guanidine extract, using a solution containing 1 M sodium acetate buffer pH 7.4 (100 mL) and 9 volumes of ethanol (1350 mL) for 24 h at 4 °C. After three washes (150 mL acetate buffer 1 M sodium pH 7.4 and 1350 mL of ethanol), the precipitate obtained was dried at 37 °C and resuspended in reducing sample buffer (0.5 M Tris–HCl pH 6.8, 26% glycerol, 20% SDS, 0.1% Bromophenol Blue). Proteins tendon subjected to electrophoresis on SDS-polyacrylamide (6%) were transferred to nitrocellulose membrane, as described by Towbin et al. (1979).

The membranes were transferred to the device Snap i.d. (Millipore, Billerica, USA). They were blocked with BlØk-CH reagent for 15 s and then the membranes were incubated with primary antibody (C2456 – Sigma–Aldrich for collagen I) at a dilution of 1:500 for 10 min. The membranes were washed three times in TBS. Incubation with secondary antibody (A8786 – anti-mouse Sigma–Aldrich and A2306 – anti-rabbit Sigma–Aldrich) was performed for 10 min and washed again. The revelation was performed with DAB (dimethylaminobenzaldehyde). The band densitometry was made using the Scion Image software Alpha 4.0.3.2 (Scion Corporation).

2.9. Zymography

Metalloproteinase analyses were made according to Marqueti et al. (2006). The samples were incubated in extraction buffer (Tris–HCl 50 mM pH 7.4, NaCl 0.2 M, Triton X-100 0.1%, $CaCl_2$ 10 mM and protease inhibitor 100 μL/10 mL) at 4 °C for 24 h. For MMP-2 and MMP-9 analysis were applied 20 μg of proteins in each lane of sodium dodecyl sulfate (SDS) – 10% polyacrylamide gels prepared with 2 mg/mL gelatin. Then, the gel was incubated in incubation buffer (Tris–HCl 50 mM pH 8.4, 5 mM de $CaCl_2$ e 1 μM de $ZnCl_2$) overnight at 37 °C. Gels were stained with Coomassie Brilliant Blue R-250 and bleached (methanol solution 30% and 10% acetic acid in water). Finally, the gel was placed in shrinking solution (30% methanol and 3% glycerol). The band densitometry was made using the Scion Image software Alpha 4.0.3.2 (Scion Corporation).

2.10. Statistical analysis

All results were expressed as mean ± standard deviation. The results were analyzed by analysis of variance (ANOVA-one-way) followed by Tukey test. The level of significance was $p < 0.05$. The analysis was carried out in GraphPad Prism 3.0 program. It was used $n = 5$ for each technique.

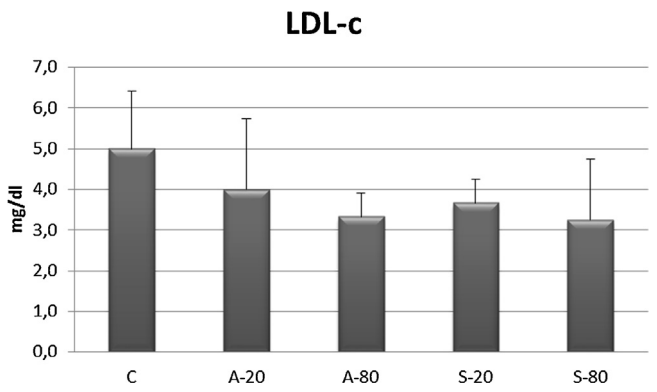

Fig. 1. Plasma levels of LDL-cholesterol (mg/dL) in the different groups. There are no significant differences between the groups, but all groups treated with statins had a tendency toward reduction of LDL-cholesterol. (A) atorvastatin; (C) control; (S) simvastatin.

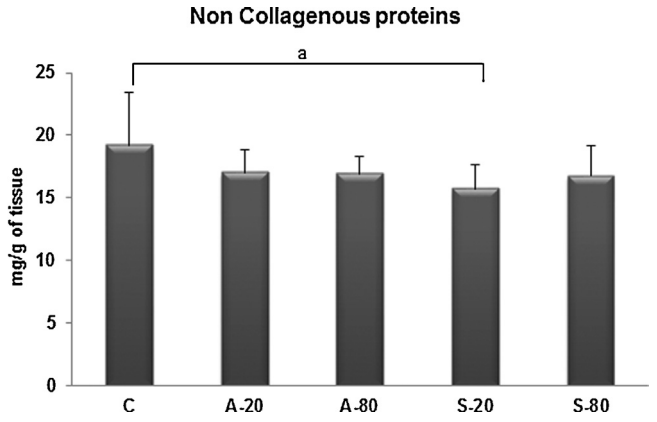

Fig. 2. Concentration of non-collagenous proteins (mg/g wet tissue) in the different groups. The S-20 group had smaller amounts of non-collagenous proteins compared to the C group. (a) $p < 0.05$ relative to C. (A) atorvastatin; (C) control; (S) simvastatin.

3. Results

Biochemical analyses were performed on Achilles tendon extracts and LDL was determined in the plasma of the rats after two months of treatment with atorvastatin or simvastatin.

3.1. LDL-cholesterol determination

LDL-cholesterol dosage was made to evaluate the biological effect of the statins (Fig. 1). Although we do not find significant differences between the groups, it was observed a reduction of LDL cholesterol in plasma of rats treated with statins when compared to the control group.

3.2. Quantification of proteins

The quantification of non-collagenous proteins allowed obtaining the concentration of total non-collagenous proteins in the extracts of the tendons. There was a significant reduction in non-collagenous proteins in the S-20 group relative to the C group (Fig. 2).

3.3. Hydroxyproline quantification

The quantification of hydroxyproline (Fig. 3), which indirectly measures the total collagen content in the tissue (mg/g dry weight),

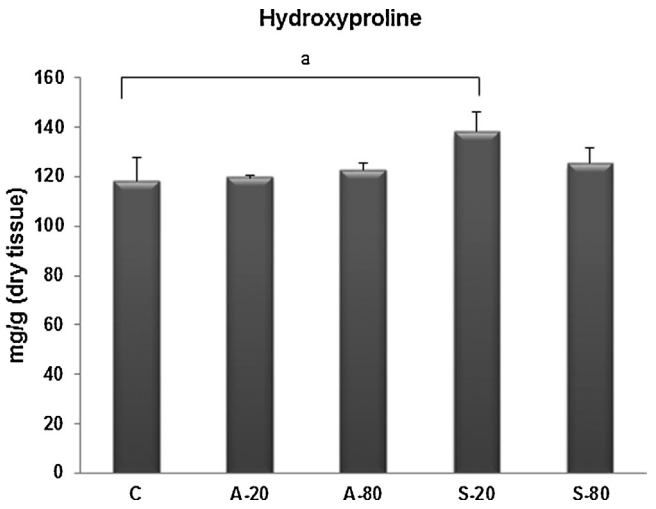

Fig. 3. Concentration of hydroxyproline (mg/g dry tissue) in the different groups. A high concentration was detected in group S-20 compared to the C group. (a) $p < 0.05$ relative to C. (A) atorvastatin; (C) control; (S) simvastatin.

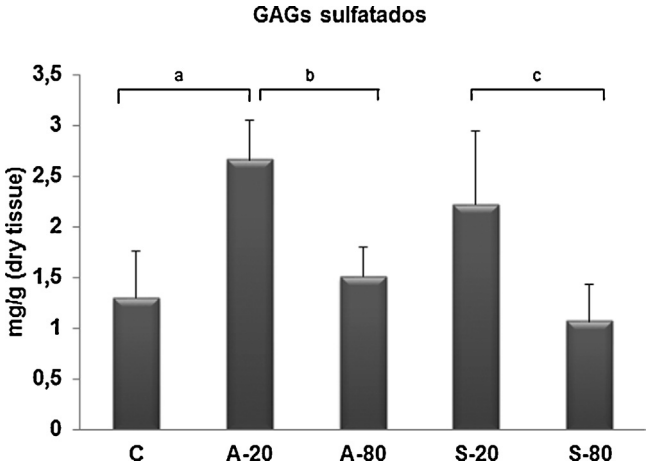

Fig. 4. Concentration of sulfated glycosaminoglycans (mg/g dry tissue) in the different groups. In group A-20, there are higher concentrations of GAGs compared to the C group. A smaller amount of GAGs was observed in groups that received statins equivalent to 80 mg/day. (a) $p < 0.05$ relative to C; (b) $p < 0.05$ between the A-20 and the A-80 groups; (c) $p < 0.05$ between the S-20 and S-80 groups. (A) atorvastatin; (C) control; (S) simvastatin.

revealed a significant increase in the S-20 group compared to the C group.

3.4. Quantification of sulfated GAGs

The total amount of GAGs in the Achilles tendon was measured (Fig. 4) and there was a significant increase in GAGs in the A-20 group compared to the C group. Statistical analysis also revealed differences between the A-20 group and the A-80 group and between the S-20 group and the S-80 group; there were lower levels of GAGs in the groups that received dosage equivalent to 80 mg/day relative to those that received dosage equivalent to 20 mg/day.

3.5. Agarose gel electrophoresis

The individual GAGs were analyzed using agarose gels and showed the presence of dermatan sulfate in all groups (Fig. 5). Densitometric analysis of the bands showed no significant differences (data not shown).

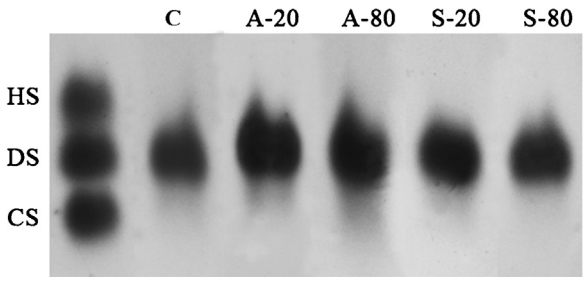

Fig. 5. Agarose gel electrophoresis. HS (heparan sulfate), DS (dermatan sulfate), and CS (chondroitin sulfate) standards are shown on the left. No difference was detected between the groups after 2 months of treatment. (A) atorvastatin; (C) control; (S) simvastatin.

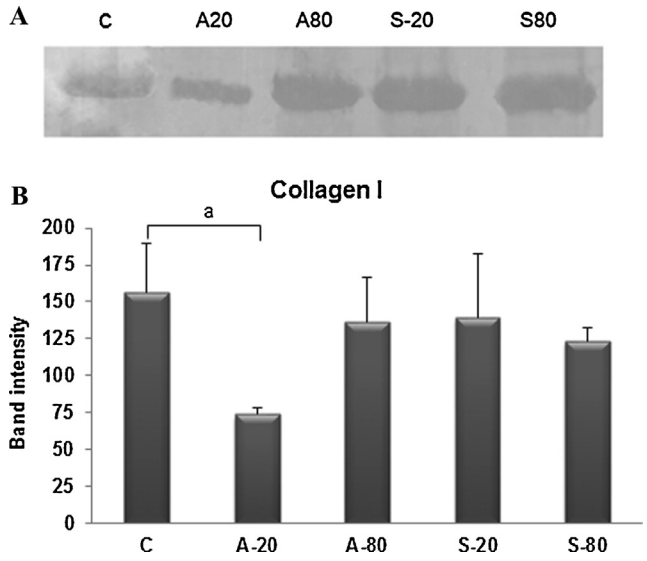

Fig. 6. (A) Western blotting for collagen I. Collagen I is found in all of the groups. (B) Densitometry of the bands corresponding to collagen type I of the groups. (a) $p < 0.05$ relative to (C); group A-20 had less type I collagen compared to the C group. When compared to the C group, all of the groups treated with statins had a tendency toward reduction of type I collagen. (A) atorvastatin; (C) control; (S) simvastatin.

3.6. Western blotting for collagen I

Western blotting for collagen I (Fig. 6A) allowed analyzing specifically the collagen I, the major component of the tendon. According to the results observed, the A-20 group had a smaller band than other groups; densitometric analysis of the bands (Fig. 6B) revealed a statistically significant reduction of collagen I in the A-20 group compared to the C group.

3.7. Zymography

The isoforms of the enzymes MMP-2 and MMP-9 were analyzed by zymography. The presence of latent MMP-9 and active MMP-9 was detected in all of the groups except for the C group (Fig. 7A). Densitometric analysis of the bands (Fig. 7B) showed that groups A-80 and S-20 had significantly more latent MMP-9 relative to the C group. The A-20 group showed a significant increase in the activity of the active MMP-9 compared to the C group. Although no significant differences were found among other groups, it is noteworthy that there was an increase in the activities of active MMP-9 in all of the groups treated with statins.

Both active MMP-2 and pro-MMP-2 were observed in all of the groups (Fig. 7). The densitometric analysis of the bands (Fig. 7C) showed a significant increase in activity of pro-MMP-2 in the A-80 group and active MMP-2 in the S-20 group relative to the C group.

4. Discussion

Statins belong to a class of drugs prescribed for the treatment of dyslipidemia. These drugs are effective and relatively safe with few adverse effects (O'Sullivan, 2007). However, there have been reports of tendon complications because of statin therapy (Beri et al., 2009; Carmont et al., 2009; Marie et al., 2008; Nesselroade and Nickels, 2010; Pullatt et al., 2007). This is the first report in the literature showing the effect of the different doses of statins in the tendon of rats. Therefore, in this study, we analyzed the possible biochemical changes in the Achilles tendon of rats after chronic treatment with simvastatin or atorvastatin.

In this work, allometric scalling was used to calculate de appropriate dosage of drug to administer to the rats to mimic the dosage administered in man (Pachaly and Brito, 2001). In humans, pharmacokinetic and pharmacodynamic parameters of statins are well established. The dosages of 20 mg/day and 80 mg/day were determined because they correspond to the minimum and maximum dosages, respectively, employed in human therapy (Goodman and Gilman, 2006).

Statins are the most effective drugs for reducing LDL-cholesterol levels (Maron et al., 2000). In this study the determination of LDL-cholesterol was made to evaluate the biological effect of the statins. Although we do not find significant differences in the determination of LDL-cholesterol, trend toward a reduction of this lipoprotein in all of the groups treated with statins when compared to the control group was observed. Hyperlipidemia was not induced in the rats used in this study, and therefore, it was expected that lowering LDL-cholesterol levels would not be so pronounced, considering that the ratio of LDL-cholesterol in the plasma of normal rats is low (Sanchez-Muniz and Bastida, 2008).

Extracellular matrix of the tendons contain various non-collagenous proteins and their function are related with stabilizing collagen fibers, considering that these proteins seem to have a property to bind to other macromolecules or in cell surface (Kannus, 2000; Partington and Wood, 1963). After treatment with statins, there was a significant reduction in the levels of non-collagenous proteins in the tendons of the S-20 group, thus indicating a protein degradation or reduced protein synthesis in tenocytes. In fact, there is an increase in protein degradation associated with tendon irregularities (Karousou et al., 2008; Vieira et al., 2012). Non-collagenous proteins were reduced in inflamed tendons (Vieira et al., 2012). Probably the degradation of the non-collagenous proteins may be increased due to treatment with simvastatin.

MMPs are enzymes that participate in the maintenance of tissue homeostasis of various organs (Malemud, 2006). The presence of MMP-2 and MMP-9 are signs of degradation and matrix remodeling, and if their activities are not properly regulated, the integrity of the tissue can be affected, which makes the tendon more susceptible to injury (Karousou et al., 2008). It has been reported that various statins inhibit the expression of MMPs, an effect mediated by a reduction in the intermediates of the HMG-CoA reductase pathway, such as the isoprenoid farnesyl pyrophosphate and geranylgeranyl pyrophosphate, These intermediates are important in the post-translational modification of proteins such as Rho, which is present in signaling pathway for the secretion of MMPs (Barter et al., 2010; Kamio et al., 2010; Turner et al., 2005). Nevertheless, in our study, we found an increase of pro-MMP-2 and active MMP-2 in the A-80 and S-20 groups, respectively. These groups also showed a significant increase in latent MMP-9. *In vitro* experiments conducted by Lee et al. (2012) demonstrated that atorvastatin and simvastatin can increase the expression of MMP-9 in macrophages, thereby increasing the phosphorylation of MAP kinases that are involved in the signaling pathway mediated by lipopolysaccharides. Thus, depending on the cell signaling pathway and tissues

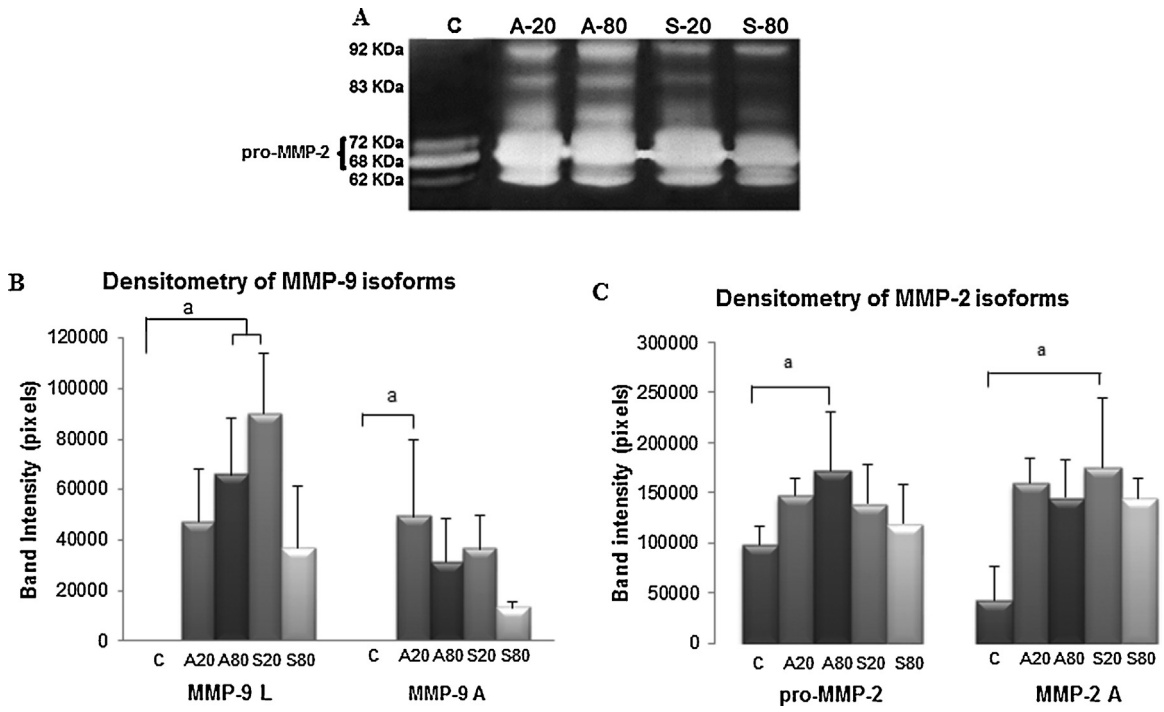

Fig. 7. (A) Zymography for analysis of MMP-2 and MMP-9. Observe the presence of latent MMP-9 (92 kDa) and active MMP-9 (83 kDa) in the A-20, A-80, S-20 and S-80 groups. All groups studied show pro-MMP-2 (72 and 68 kDa) and active MMP-2 (62 kDa). (B) Densitometry of the bands corresponding to the MMP-9 isoforms. The activity of the latent MMP-9 is elevated in the A-80 and S-20 groups; a higher value was observed for the active MMP-9 in the A-20 group compared to the C group. (C) Densitometry of the bands corresponding to the MMP-2 isoforms; pro-MMP-2 is more active in the A-80 group when compared to the C group and active MMP-2 shows increased activity in the S-20 group. (a) $p < 0.05$ relative to C. (A) atorvastatin; (C) control; (S) simvastatin.

involved, these drugs appear to exert different effects on the activity of MMPs.

The active isoform of MMP-9 is generally involved in inflammatory processes and tendon rupture. In these cases, there is an increase in the expression of MMP-9 (Jones et al., 2006). The A-20 group showed a pronounced increase of this enzyme in comparison to the C group. Although we did not find significant differences in the remaining groups that received statins, both latent MMP-9 and active MMP-9 were present. An excess of MMPs proteolytic activities can lead to progressive tendon degeneration, weakening of the tendon extracellular matrix, and a consequent reduction in the biomechanical properties of the tendon (Karousou et al., 2008). Thus, based on the profile of MMPs found in our study, an inflammatory process may have been induced by statin treatment that detrimentally affected the integrity of the tendon.

Hydroxyproline is a major component of the collagen (Neuman and Logan, 1950). Our results showed an increase in hydroxyproline in the S-20 group, which is consistent with the analyses of total collagen content in patellar tendinopathy, in which greater amounts of hydroxyproline in lesion regions have been observed (Samiric et al., 2009). The highest concentration of hydroxyproline was found in the S-20 group and may be a result of an increase in collagen fragments resulting from the increased activity of MMPs-2 and -9 (Vieira et al., 2012). Furthermore, if statins caused injuries to the tendons analyzed in this study, it was expected that tenocytes would synthesize more collagen to restore the normal composition of the tendons. In the collagen I analysis, the S-20 group showed a reduction of this component. Probably, tendon cells may have synthesized other types of collagen in greater amounts than collagen I, in response to tendon damage (Thomopoulos et al., 2002). Thus, this may be an explanation for the higher content of hydroxyproline and the reduction of collagen I founded in the S-20 group.

Although only the A-20 group showed a significant reduction in collagen I, trend toward a reduction in collagen I in the remaining groups that received statins was observed. Morphological analysis of atherosclerotic plaques performed by Cherpachenko et al. (2009) showed a decrease in collagen fibers in the plaques of patients who used statins for the treatment of hyperlipidemia. In another study, it was found that simvastatin inhibited the synthesis of collagen I in smooth muscle cells, an effect that is closely related to the reduction of intermediate isoprenoids in the cholesterol synthesis pathway (Schaafsma et al., 2011). Therefore, our results suggest that atorvastatin was mainly responsible for drastically inhibiting the synthesis of type I collagen in tendon. Type I collagen is the major extracellular matrix component of tendons; it forms highly oriented fibrils that confer the tensile strength of this tissue (Gelse et al., 2003). Therefore, any imbalance between collagen synthesis and degradation may weaken the tissue and thereby predispose the tendon to microdamage and ruptures (Riley et al., 2002).

As mentioned previously, in the A-20 group, MMP-9 showed augmented activity; therefore, the reduction of collagen type I found in the same group may be due to the proteolytic activity of this enzyme. It is important to note that collagen I is one of the substrates of the MMP-9 and that this enzyme degrades fragments of collagen after tendon rupture (Chakraborti et al., 2003; Riley et al., 2002).

There are no reports that statins may directly affect the synthesis of GAGs. However, in our study, we observed a greater amount of sulfated glycosaminoglycans in the A-20 group relative to the C group. It is possible that the increased MMPs found in the A-20 group caused degradation of the collagen, thereby inducing the tenocytes to synthesize more glycosaminoglycans to assist in the repair process. A biochemical analysis of patellar tendons in chronic degenerative processes showed an increase in the synthesis and deposition of GAGs compared to normal tendons (Samiric et al., 2009). Thus, our results suggest that treatment with atorvastatin may be involved in chronic disorders of tendons. Until now there is no explanation for the significant differences in content of

GAGs found between the A-20 group and the A-80 group and also between the S-20 group and the S-80 group.

When administered orally, statins undergo intensive first-pass metabolism in the liver, and a small proportion of the administered dose is accumulated in extra-hepatic tissues (Blum, 1994; Corsini et al., 1999). Despite having a low systemic bioavailability (Schachter, 2004), our results suggest that these drugs were active in the tendons and that the chronic use of the statins may cause detrimental changes to the extracellular matrix of the tendon.

The findings of this study suggest that there is a possible reverse effect when we analyzed the dose equivalent to 80 mg. Possibly larger doses caused tendon injuries in the initial period of the treatment (first few weeks), while lower doses caused lesions late onset, or after a longer period of statin administration (2 months). This explains the results obtained for the groups treated with a dose equivalent to 20 mg. Furthermore, statins have pleiotropic effects which are widely studied (Farmer, 2000; Liao and Laufs, 2005). It has been shown that statins can promote recovery from a tissue that has suffered an injury, for example, skin (Rego et al., 2007; Toker et al., 2009) and bone (Skoglund and Aspenberg, 2007; Wang et al., 2007). So, it is possible that initially, the administration of statins caused an injury in the normal tendon. Then, after that initial lesion, the same drug, administered chronically may have acted recovering this tendon. This may be a hypothesis to explain the possible reversed results founded in the groups treated with dosage equivalent to 80 mg. If analyzes were performed in periods exceeding 2 months of treatment, it is possible that the reverse effects also would be observed at a dose equivalent to 20 mg which takes longer time to act.

Although apparently there is a reverse effect on the dose equivalent to 80 mg, it is important to note that initially both doses caused changes in the extracellular matrix of tendons. The presence of active MMP-9 in all of the groups treated with statins is a strong evidence of tendon injury. Furthermore, a tendon that suffers an injury never completely regains the biomechanical properties it had prior to injury (James et al., 2008).

5. Conclusions

Finally, our results clearly suggest that statins cause remarkable changes to the extracellular matrix of the Achilles tendon. The response of the tendon to the treatment is different depending on the dose and type of statin used. The presence of MMP-2 and MMP-9 suggest that the degradation and remodeling of the extracellular matrix were triggered. Our results provide evidence indicating that statins promote an imbalance between the synthesis and degradation of several molecules, particularly collagen I, and possibly induce microdamage in the tendons. Therefore, it is necessary to monitor all patients using statins, especially those who have tendon complications or those that have potential risk of developing tendinopathy.

Conflict of interest statement

The authors state no conflict of interest.

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