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Elucidation of the Mechanism of Atorvastatin-Induced Myopathy in a Rat Model

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Abbreviations : LDL, low-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; CoQ10, coenzyme Q10; LDH, lactate dehydrogenase enzyme

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Abstract

Myopathy is among the well documented and the most disturbing adverse effects of statins. The underlying mechanism is still unknown. Mitochondrial dysfunction related to coenzyme Q10 decline is one of the proposed theories. The present study aimed to investigate the mechanism of atorvastatin-induced myopathy in rats. In addition, the mechanism of the coenzyme Q10 protection was investigated with special focus of mitochondrial alterations. Sprague-Dawely rats were treated orally either with atorvastatin (100 mg/kg) or atorvastatin and coenzyme Q10 (100 mg/kg). Myopathy was assessed by measuring serum creatine kinase (CK) and myoglobin levels together with examination of necrosis in type IIB fiber muscles. Mitochondrial dysfunction was evaluated by measuring muscle lactate/pyruvate ratio, ATP level, pAkt as well as mitochondrial ultrastructure examination. Atorvastatin treatment resulted in a rise in both CK (2X) and myoglobin (6X) level with graded degrees of muscle necrosis. Biochemical determinations showed prominent increase in lactate/pyruvate ratio and a decline in both ATP (>80%) and pAkt (>50%) levels. Ultrastructure examination showed mitochondrial swelling with disrupted organelle membrane. Co-treatment with coenzyme Q10 induced reduction in muscle necrosis as well as in CK and myoglobin levels. In addition, coenzyme Q10 improved all mitochondrial dysfunction parameters including mitochondrial swelling and disruption. These results presented a model for atorvastatin-induced myopathy in rats and proved that mitochondrial dysfunction is the main contributor in statin-myopathy pathophysiology.

Keywords: atorvastatin; myopathy; mitochondrial dysfunction; coenzyme Q10

Introduction

Lowering plasma cholesterol level especially low-density lipoprotein (LDL) has widely been related to reduction in cardiovascular-related mortality and morbidity. Statins represent the first line treatment for atherosclerotic disorders related to hypercholesterolemia. They competitively inhibit 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, the rate limiting step in cholesterol biosynthesis (Sirtori 2014). Despite their different physicochemical properties, all statins are capable to decrease LDL-C with different potencies (Grundy et al. 2004) The use of high dose of statins is sometimes needed especially in high risk patients to achieve therapeutic goals on all atherogenic parameters. Doubling of statins dose had shown a 5% to 7% decrease in LDL-C levels (Nicholls et al. 2010), however with increased myopathic incidence and drug discontinuation (Silva et al. 2007).

Generally, statins have a good safety profiles except for muscle toxicity and increase in liver enzymes. Myopathy is the most significant and well documented side effect described with statin use (Hu et al. 2012). While clinical studies reported myopathy incidence as 0.1% (Rallidis et al. 2012) , latest estimates indicate that up to 29% of statin users complain from musculoskeletal side effects (Stroes et al. 2015). Myopathy may comprise myositis, myalgia with or without CK increase. The most severe form of myopathy is rhabdomyolysis characterized by muscle destruction and myoglobin release (Armitage 2007).

The exact mechanism of statin-induced myopathy has not been clearly elucidated. Different theories have been suggested including altered membrane fluidity and excitability (Pierno et al. 1995), impaired calcium homeostasis (Liantonio et al. 2007), induction of apoptosis (Dirks and Jones 2006) , activation of ubiquitin-proteasome pathway (Hanai et al. 2007) and

mitochondrial dysfunction (Kaufmann et al. 2006). The main suggested theories relay on inhibiting mevalonate downstream products particularly; cholesterol, prenylated proteins and ubiquinone. It was suggested that reduced serum cholesterol may lead to altered muscle membrane fluidity and excitability. Prenylated proteins are thought to be essential in many signaling pathway. Dysprenylation of small GTPases causes vacuolation of myofibers , degeneration and swelling of organelles and eventually apoptosis (Abd and Jacobson 2011).

Mitochondrial dysfunction is one of the most compelling theories to elucidate statin myopathy. Mitochondrial dysfunction could be related to decrease in coenzyme Q10 (CoQ10) or ubiquinone. Coenzyme Q10 is a powerful antioxidant and an essential cofactor in the electron transport chain. Through inhibiting the mevalonate pathway, statins could reduce CoQ10 level which in turn disrupts cellular respiration, producing muscle related effects (Tomaszewski et al. 2011). Studies have shown that statins decrease CoQ10 levels in serum (Folkers et al. 1990; Willis et al. 1990) and skeletal muscle (Nakahara et al. 1998; Päivä et al. 2005). A recent study had shown a decrease in mitochondrial oxidative phosphorylation capacity accompanied by a decrease in muscle CoQ10 in simvastatin-treated patients (Larsen et al. 2013). Nevertheless, some studies failed to show any decrease in muscle CoQ10 following statins treatment (Fukami et al. 1993; Laaksonen et al. 1996) or to correlate that decrease with mitochondrial dysfunction (Nakahara et al. 1998).

This study aimed to elucidate the mechanism underlying atorvastatin-induced myopathy in a rat model. Myopathic markers including biochemical, functional and histopathological alterations were assessed. Mitochondrial dysfunction parameters and ultrastructural changes were examined with special focus on type IIB fiber muscle. In addition, the elucidated changes in myopathic indices and mitochondrial parameters determinations were further assessed after co-administration of coenzyme Q10.

1. Materials and methods

1.1. Chemicals

Atorvastatin calcium was a gift form Borg pharmaceuticals, Alexandria, Egypt. Coenzyme Q10 was purchased from Selleckchem, Houston, USA. Both drugs were suspended in 0.5% carboxymethyl cellulose (CMC). All other chemicals or reagents are used with high grade and purity.

1.2. Animals and experimental design

Male albino Sprague Dawely rats weighing 200-230 gm. were obtained from the animal house of Pharos University, Alexandria, Egypt. Rats were housed under controlled temperature (25±2°C) and constant light cycle (12 h light/dark) and allowed free access to water and controlled diet (approximately 20 g standard rodent chow diet / 200 g rat). The procedures used for the care and euthanasia of animals complies with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and was approved by the Ethics Committee for Animal Experimentation at Faculty of Pharmacy, Cairo University.

Rats were divided into three groups. Group I (ATV) received atorvastatin (100 mg/kg). Group II received both atorvastatin (100 mg/kg) and coenzyme Q10 (100 mg/kg) (CoQ10+ATV). Groups III was the control group (C) receiving the vehicle (0.5% CMC). Group IV (CoQ10) received coenzyme Q10 (100 mg/kg) alone. All drugs were administrated by oral gavage for 21 days. The

dose of atorvastatin was used based on pilot study examining 10, 30 and 50 mg/kg. Only 50 mg/kg showed some myopathic signs in some rats, so the dose was escalated to 100 mg/kg.

At the end of the experiment (day 22), biceps femoris muscle was removed, weighed and frozen at -80°C. Frozen muscles were grinded and homogenized (homogenizer1600 MiniG, New Jersey, USA) in ice-cold phosphate buffer (pH=7.4). The supernatant was then separated and used for further biochemical determinations.

1.3. Myopathy indices

1.3.1. Body and muscle weight determination

Rats' body weights were determined on day zero, 7, 14 and 21 and percentage weight change was calculated compared to pretreatment weight. On the day of sacrifice, biceps femoris from the other hindlimb was isolated, washed in iced saline and weighed. The muscle was dried at 60°C till constant weight. Muscle weight was calculated as mg/g body weight. Percentage muscle weight change was calculated in both wet and dry conditions and compared among groups.

1.3.2. Motor activity assessment

An accelerating rotarod device designed for rats (Orchid scientific & Innovative, India) was used for motor function evaluation (Trapani et al. 2011). One week before the start of the experiment, rats were subjected to training for three days. At the start of the experiment, rats were allowed to accommodate then start running on a rotating rod at 24 rpm until falling down. Performance of rats was expressed as latency to fall, measured in seconds. Three different measurements were recorded and the average was calculated. Rats were allowed to rest and taken back to the cage for at least 10 min between each measurement. Percentage change in the performance was calculated for day 14 and day 21 with reference to day zero values.

1.3.3. Serum creatine kinase determination

Serum samples were separated on day zero, 14, and 21 and examined for creatine kinase activity using a Cobas autoanalyser (Roche diagnostics, USA) .The X-fold increase of creatine kinase activity was calculated by comparing day 14 and day 21 to day zero values.

1.3.4. Serum Myoglobin determination

Serum myoglobin content was measured on day 21 samples using ELISA kit (Cusabio, China) and expressed as ng/ml.

1.3.5. Muscle Lactate dehydrogenase enzyme content

Lactate dehydrogenase enzyme (LDH) was analyzed in muscle homogenate as a marker of cell damage using enzyme-linked immunosorbent assay ELISA kit (Cusabio, China).

1.3.6. Histopathological examination

Semimembranosus and extensor digitorum longus were isolated for histopathological examination. Muscles were fixed in buffered 10% formalin, processed to wax blocks. Paraffin embedded samples were sectioned transversely and longitudinally and stained with haematoxylin and eosin for examination by light microscopy. Necrosis was graded blindly into; grade 0= no necrosis; grade 1 = *mild* (up to 20% of fibers in section affected); grade 2 = *moderate* (20- 50% of fibers in section affected); grade 3 = *severe* (more than 50% of fibers in section affected) (Westwood et al. 2005).

1.4. Determination of mitochondrial dysfunction parameters in muscles

1.4.1. Determination of lactate and pyruvate contents

Determination of lactic acid (Sigma-Aldrich, Missouri, USA) and pyruvic acid (Megazyme, Ireland) was performed in muscle homogenate using colorimetric assay and expressed as ng/g tissue. The ratio of lactate to pyruvate was calculated and compared among groups as a measure of mitochondrial dysfunction (De Pinieux et al. 1996).

1.4.2. Determination of ATP content

The ATP content in biceps femoris muscle was measured using immunoenzymatic technique (Kamiya biomedical Co, Seattle, USA) according to the manufacturer instructions. The ATP content was expressed as pg/g tissue.

1.4.3. Determination of pAkt (Ser^{473})

The level of phosphorylated Akt (Ser^{473}) was quantified in muscle homogenate using ELISA kit (DRG International, Inc. New Jersey, USA). The phosphorylation levels were normalized to tissue weight. The values are expressed as unit /gm tissue. One unit of standard is defined as the amount of AKT [pS473] derived from 100 pg of Akt, which was phosphorylated by MAPKAP2 and PDK1.

1.4.4. Electron microscopic examination

Samples from biceps femoris were processed for ultrastructural examination. Samples were fixed in 2.5% glutaraldehyde. Glutaraldehyde-fixed samples were post-fixed in 1% osmium tetroxide and processed to Araldite resin blocks. Ultrathin, 70–90-nm, resin sections were cut, stained using uranyl acetate and lead citrate. Mitochondrial damage was examined using transmission electron microscope (Jeol JSM-5300 SEM, USA) using 80 kV accelerating voltage and photographed.

1.5.Statistical analysis

All data obtained were presented as mean ± S.E.M (n=8-10). Results were analyzed using one way analysis of variance test (one-way ANOVA) followed by Student-Newman-Keuls multiple comparison test. Body weight change, CK determination & rotarod test were subjected to two-way ANOVA followed by Bonferroni test, where the two factors considered were treatment and time. Statistical analysis was performed using GraphPad Prizm software (version 3.0). For all the statistical tests, the level of significance was fixed at $p < 0.05$.

2. Results

2.1.Myopathy indices

2.1.1. Body and muscle weights

Reduced body weight gain was observed in ATV-treated group on day 7, 14 and 21 compared to the control group ($p<0.05$). Body weight loss for all rats was less than 10 % of the pretreatment weights. In the group CoQ10+ATV group, the rats didn't show any sign of weight loss, however, the weight gain was slower than the control as shown in (Figure 1). Treatment with CoQ10 alone showed weight gain percent similar to that seen in the control group. Two-way ANOVA testing also showed significant interaction between time and treatment. Concerning muscle mass, the wet and dry weight of biceps femoris was significantly reduced (*-10.6%* and *-15.2%,* respectively) in ATV group compared to control. Biceps femoris wasting was prevented by coenzyme Q10 co-treatment in both wet and dry state.

2.1.2. Motor activity assessment

In atorvastatin-treated group, the rotarod performance was significantly reduced starting from day 14 (37.3 % ± 11.9) and progressively till day 21 (72.5 % ± 6.13), with respect to day zero values (Figure 2). Co-treatment with coenzyme Q10 significantly alleviated the reduction in rotarod performance on day 14 (0.93 % ± 0.63) and day 21 (45.4 % ± 11.1) observed in atorvastatin group, as shown in (Figure 2). The performance of rats treated with CoQ10 alone was comparable to control group. A significant effect of time was noted in ATV and ATV+CoQ10 groups in two-way ANOVA testing while interaction was found non-significant among groups.

2.1.3. Serum creatine kinase determination

The creatine kinase activity showed nearly two-fold increase in ATV-treated group compared to the control on both day 14 and day 21 ($p<0.05$). Creatine kinase level on day 21 was higher than day 14, however this increase was non-significant. This rise in CK level observed in ATV group was prevented by CoQ10 co-treatment (Figure 3A). By performing two-way ANOVA test, time and interaction was found insignificant.

2.1.4. Serum Myoglobin determination

The level of serum myoglobin increased sharply in ATV group (6-fold) with respect to control group. Co-administration of CoQ10 decreased the myoglobin level to about half its value in ATV-treated group ($p<0.05$) (Figure 3B).

2.1.5. Muscle Lactate dehydrogenase enzyme content

The level of LDH was significantly increased (5-fold) in atorvastatin treated group compared to control group as shown in (Figure 4). Co-treatment with CoQ10 induced a ~50% reduction in the rise of LDH induced by ATV treatment.

2.1.6. Histopathological examination

Compared to control, semimembranosus muscle showed necrosis in 60% of rats treated with atorvastatin, ranging from mild to severe necrosis as shown in (Figure 5).Co-treatment with coenzyme Q10 showed necrosis only in 40% of rats and reduced the mean score to 0.6 as presented in (Table 1). Atorvastatin also induced necrosis (33%) in extensor digitorum longus compared to the control, however to lesser degree than that observed with semimembranosus muscle (Table 1).

2.2.Mitochondrial dysfunction parameters in muscles

2.2.1. Biochemical parameters

Atorvastatin treatment showed marked changes in the parameters linked to mitochondrial functions. Lactate/pyruvate ratio showed significant increase (2.5-fold) with respect to the control ($p<0.05$) (Figure 6 A) .These changes were accompanied by a pronounced reduction of ATP levels (>80%) (Figure 6B). All these effects were alleviated by co-administration of CoQ10. Co-treatment with coenzyme Q10 showed ~30% reduction in the rise of lactate/pyruvate ratio observed in ATV-group. The muscle ATP content was significantly replenished (>45%) in CoQ10+ATV group (Figure 6B). The level of pAkt exhibited a significant (>50%) decline in ATV group compared to the control .This effect was partially prevented in CoQ10+ATV group ($p<0.05$) as shown in (Figure 6C). None of these parameters showed improvements in rats treated with CoQ10 only compared to the control.

2.2.2. Electron microscopic ultrastructure examination

Examination of biceps femoris muscle samples of atorvastatin-treated rats revealed multiple abnormal mitochondria compared to control. The sample showed unusually enlarged mitochondria with disrupted membrane and indefinite cristae as shown in (Figure 7 C, D). It should be mentioned that damaged mitochondria was in close proximity to unaffected mitochondria. Coenzyme Q10 treatment preserved mitochondria ultrastructure changes. Mitochondria appeared normal in size and shape and showing intact membrane and cristae (Figure 7 E, F).

3. Discussion

Results showed a significant and progressive reduction in body weights during atorvastatin treatment course. The wasting was reflected on biceps femoris muscle mass both in wet and dry conditions. Dry muscle showed higher weight reduction percent indicating a decrease in muscle fibers and not in water content. Biceps femoris is a large essential muscle in rat hindlimb consisting of 77% of type IIB fibers and devoid form type I fibers (Eng et al. 2008). This muscle was selected based on the reported fiber selective effect of statins myotoxicity. Statins only affect fast glycolytic type II fibers with almost total sparing of slow oxidative type I fibers. In addition, subtypes of type II fibers are differentially affected with type IIB the most sensitive followed by type IID and the least is type IIA (Seachrist et al. 2005; Westwood et al. 2005; Westwood et al. 2008). The wasting observed in type IIB fibers muscles could in part explain the decrease in body weight since 71% of total rat muscle mass consists of type IIB fibers (Delp and Duan 1996).

Muscle wasting was associated with diminished muscle strength and rapid fatigue manifested as reduced performance (latency to fall) in rotarod test on day 14 and progressive till day 21. In the same context, rats treated with simvastatin had previously shown reduced maximum speed and high latency to fall off the rotarod apparatus (Trapani et al. 2011). Atorvastatin treatment had also shown increased muscle fatigability and decreased performance of rats and mice using treadmill test (Bouitbir et al. 2011; Muraki et al. 2012). Reduced exercise tolerance could be referred to impaired muscle mitochondrial oxidative function or glycogen depletion (Bouitbir et al. 2011).

In the current work, administration of atorvastatin increased the level of serum CK on day 14, an effect that was progressive and persistent until day 21. The rise of serum creatine kinase was previously reported with atorvastatin (Bouitbir et al. 2011), cerivastatin (Seachrist et al. 2005) and simvastatin (Westwood et al. 2005) treatment. However, these studies measured CK level between day 14 and day 16 but none of them showed whether that increasing pattern was sustained for longer periods. The current results also showed a prominent rise in myoglobin (6-fold) level in atorvastatin-treated group. The increase of myoglobin in serum is a sign of muscle destruction and release of its content in the blood, indicating rhabdomyolysis (Armitage 2007).

The level of LDH enzyme showed a significant elevation in the muscles of atorvastatin-treated rats. This result indicates muscle-induced injury and cellular damage. Histopathological examinations further supported the previous results showing necrosis in the muscle fibers of rats treated with atorvastatin. It was previously reported that myopathy in rats is characterized by a rise in CK level along with muscle necrosis (Westwood et al. 2005). In the current work,

biochemical and functional alterations was further supported by graded degree of necrosis in fast-twitching muscles consisting mainly of type IIB fibers (Eng et al. 2008). Parallel results were previously reported with simvastatin and rosuvastatin treatment in rats (Westwood et al. 2005; Westwood et al. 2008)

In the current work, atorvastatin showed multiple biochemical alterations indicating mitochondrial dysfunction. Results showed a significant increase in lactate/pyruvate ratio outweighing a defect in mitochondrial respiratory reaction. Since blood lactate level could be related to a variety of disorders (Andersen et al. 2013), the present work has determined the lactate/pyruvate ratio in muscle homogenate. Previous studies reported an increase in blood lactate in animals (Bouitbir et al. 2011; Seachrist et al. 2005) and elevated lactate/pyruvate ratio in humans following statins treatment (De Pinieux et al. 1996; Neale et al. 2004). However none of them showed whether that increase was reflected on skeletal muscle or not. Elevated lactate/pyruvate ratio had been widely correlated with mitochondrial cytopathies (Bernier et al. 2002). Pyruvate, product of glucose metabolism, is usually reduced via the respiratory chain reaction. Any defect in cellular respiratory machinery shifts the pyruvate to anaerobic metabolism, giving rise to lactate/pyruvate ratio (Munnich et al. 1996).

The current data showed a prominent decrease in ATP content in muscles of rats treated with atorvastatin. Studies had previously reported a decrease in ATP content in rats' isolated myocytes (Kaufmann et al. 2006) and humans myocytes (Nishimoto et al. 2003) following statins treatment. The decrease in energy production is consistent with the observed results showing elevated lactate/pyruvate ratio. This could be explained by the fact that intracellular ATP content is in equilibrium with glycolysis and any defect in ATP production can lead to increase formation of pyruvate (Ivy et al. 1980). With the dysfunction of cellular respiration, pyruvate is shifted towards the production of lactate. Mitochondrial dysfunction parameters were further confirmed by ultrastructural deformities of muscle mitochondria following atorvastatin treatment. Numerous abnormal large mitochondria with disrupted membrane and cristae were observed. The change of mitochondrial size was previously reported with atorvastatin, simvastatin, fluvastatin, and cerivastatin treatment (Kaufmann et al. 2006; Seachrist et al. 2005) . Increase in mitochondrial size could indicate increased mitochondrial permeability resulting in mitochondrial swelling, release of cytochrome c and initiation of mitochondrial apoptotic process (Kaufmann et al. 2006).

The present work also showed a decrease in pAkt level of atorvastatin-treated rats' muscles. Phosphorylated Akt is an important signaling molecule involved in cell proliferation and survival, mitochondrial integrity and protein synthesis (Fanzani et al. 2012; Majewski et al. 2004; Parcellier et al. 2008). Former studies reported a decline in pAkt in myoculture treated with statins (Bonifacio et al. 2014; Mullen et al. 2011). Phosphorylated-Akt is thought to be essential for mitochondrial integrity. It acts through stabilizing mitochondrial membrane potential , depressing pro-apoptotic signs and preventing cytochrome c release (Parcellier et al. 2008). Furthermore, pAkt exerts an inhibitory effect on the transcription factor FoxO3 which mediates muscle protein breakdown and organelles autophagy (Fanzani et al. 2012). Hence, statins-induced decline in pAkt could have a dual effect: inducing mitochondrial disintegrity and accelerating muscle wasting via protein degradation and mitochondrial autophagy.

Coenzyme Q10 co-administration alleviated all the myopathic manifestations in rats treated with atorvastatin. The wasting observed in rats' body weights and biceps femoris muscle

mass was not detected with CoQ10 co-treatment. Muscle strength was significantly improved with co-treatment with coenzyme Q10. Similarly ,CoQ10 was reported to alleviate the reduction of exercise endurance in mice treated with atorvastatin (Muraki et al. 2012). The protective effects of CoQ10 were accompanied by a decline in serum CK and myoglobin. Previous studies showed that supplementation with high doses of CoQ10 normalized the CK levels in hereditary CoQ10 deficiency cases (Gempel et al. 2007; Mancuso et al. 2010). Consequently, these findings suggest that the rise of CK and myoglobin level accompanying statin myopathy could be refereed in part to CoQ10 depletion. Furthermore, cellular damage, manifested as increased LDH level, was reduced with CoQ10 co-treatment together with a reduction in the necrosis observed in rats 'muscles.

All the parameters showing mitochondrial dysfunction were significantly improved with the administration of CoQ10 with atorvastatin. The rise in lactate/pyruvate ratio was significantly reduced by CoQ10 co-treatment. In addition, CoQ10 replenished more than 45 % of ATP content compared to atorvastatin group. Similarly, ubiquinol was reported to rescue mitochondrial content and metabolic functions in human cell culture treated with simvastatin (Vaughan et al. 2013) . This pivotal protective effect of ubiquinone could be attributed to its role in repairing the defect in cellular respiration. Coenzyme Q10 lies on the inner membrane of the mitochondria and acts as an electron shuttle between complex I and III generating the electric gradient necessary for ATP formation (Crane 2001).

Administration of coenzyme Q10 ameliorated the decrease in pAkt following atorvastatin treatment. To our knowledge, this is the first study to examine the effect of CoQ10 on pAkt in rats 'skeletal muscle following statins. Previous studies showed that CoQ10 had restored the level of pAkt in rats' cardiac tissue (Huynh et al. 2012), endothelial cell line (Tsai et al. 2011) and in cultured neurons (Choi et al. 2013) following different depletive conditions. The effect of CoQ10 on the Akt level could be mediated via its strong antioxidant properties. The signaling pathway of Akt is reported to be negatively affected by oxidative stress which in turn reduces the phosphorylation of Akt. Administration of CoQ10 suppresses oxidative stress and subsequently alleviates the down regulation of pAkt formation (Huynh et al. 2012).

Taken together, these results clarified that multiple mitochondrial alterations are responsible for inducing myopathy in atorvastatin- treated rats. The current work also emphasized the mechanism of oral supplementation of CoQ10 in managing atorvastatin-myopathy. Owing to its role in restoring mitochondrial oxidative functions, CoQ10 significantly alleviated all the myopathic indices induced by atorvastatin. Up till now, the data reported about the benefits of CoQ10 are still not conclusive (Norata et al. 2014). Previous studies showed a decrease in myalgia score (Caso et al. 2007; Langsjoen et al. 2005) and muscle weakness in statins users (Kim et al. 2001) taking CoQ10 supplements. On the contrary , a meta-analysis and a randomized controlled trial disclaimed any advantages for ubiquinone administration to alleviate statin-induced myopathy symptoms (Banach et al. 2015; Taylor et al. 2015). However, although non-significant, some of the studies in the meta-analysis showed a decreasing tendency in the myalgia score with coenzyme Q10 supplementation (Banach et al. 2015).

Notably, administration of CoQ10 alone in healthy rats didn't show any improvement in weight gain, motor activity or in mitochondrial parameters compared to control rats. Similarly, a previous study showed that administration of CoQ10 increase its level in the tissues without any modulation in mitochondrial functions in healthy animals (Sohal et al. 2006). This could suggest

that CoQ10 reach a saturable level in the tissues and its physiological levels in the mitochondria does not exceed kinetic saturation of electron respiratory chain enzymes (Beal 1999; Sohal and Forster 2007). These findings further fortify the notion that the protective effects of CoQ10 are directly related to atorvastatin-induced deleterious effects on mitochondria. It was also noted that pAkt level was slightly but significantly decreased in CoQ10 group. This unexpected finding might be explained based on some studies reporting that pAkt signaling can be activated by ROS (Le Belle et al. 2011; Yalcin et al. 2010). As nontoxic levels of ROS are needed for normal cell functions, it can be assumed that low level of ROS might be necessary for maintaining pAkt level. Coenzyme Q10 has a powerful antioxidant effect that could lead to pAkt reduction. However, this was not in contradiction with its protective effect against atorvastatin-induced pAkt decline.

It should be mentioned that supra-pharmacological doses of statins are needed to induce myopathy in experimental animals. The doses used in rodents are usually near the maximum tolerated dose (Mallinson et al. 2009). Previous studies used large doses of statins to induce myopathy in rats; simvastatin 80 mg/kg (Sidaway et al. 2009) , simvastatin 100-150 mg/kg (Smith et al. 1991), rosuvastatin 120-160 mg/kg (Westwood et al. 2008), lovastatin 200-1000 mg/kg (Waclawik et al. 1993). In addition, atorvastatin dose used in this study was previously examined in other studies for longer periods without showing any toxicity signs (Garcia et al. 2011; Niederberger 2005).

4. Conclusions

In conclusion, this study emphasizes that mitochondrial dysfunction is the major contributor in the pathophysiology of atorvastatin-induced myopathy. The myopathy induced by atorvastatin was manifested, for the first time, with combined biochemical, functional, histopathological and ultrastructural examinations. In addition, the improvements observed on myopathic indices and mitochondrial dysfunction parameters with CoQ10 administration further emphasize the major role of mitochondria in statin-myopathy. The results also support the benefits of CoQ10 supplementation in the management of statin-induced myopathy.

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Figure 1: Effect of atorvastatin, coenzyme Q10 and atorvastatin plus coenzyme Q10 treatment on percentage body weight change.

Each value represents the mean of 8-10 experiments ± S.E.M.* p <0.05 compared to control, # p <0.05 compared to atorvastatin group.

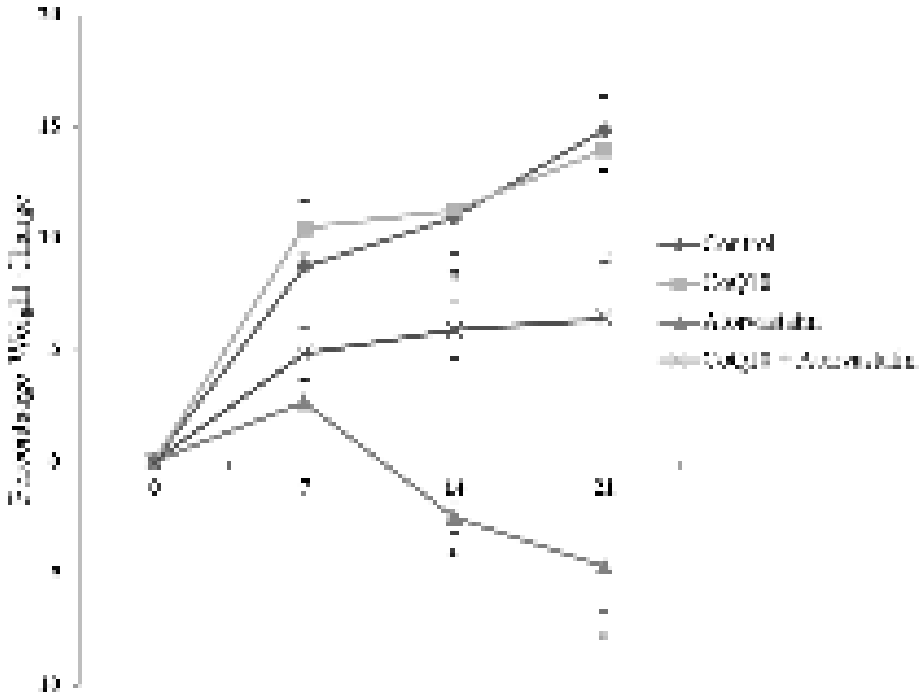

Figure 2: Percentage change in rotarod performance on day 14 and day 21 in control, coenzyme Q10, atorvastatin and atorvastatin plus coenzyme Q10 groups.
* $p < 0.05$ vs. control, # $p < 0.05$ vs. atorvastatin.

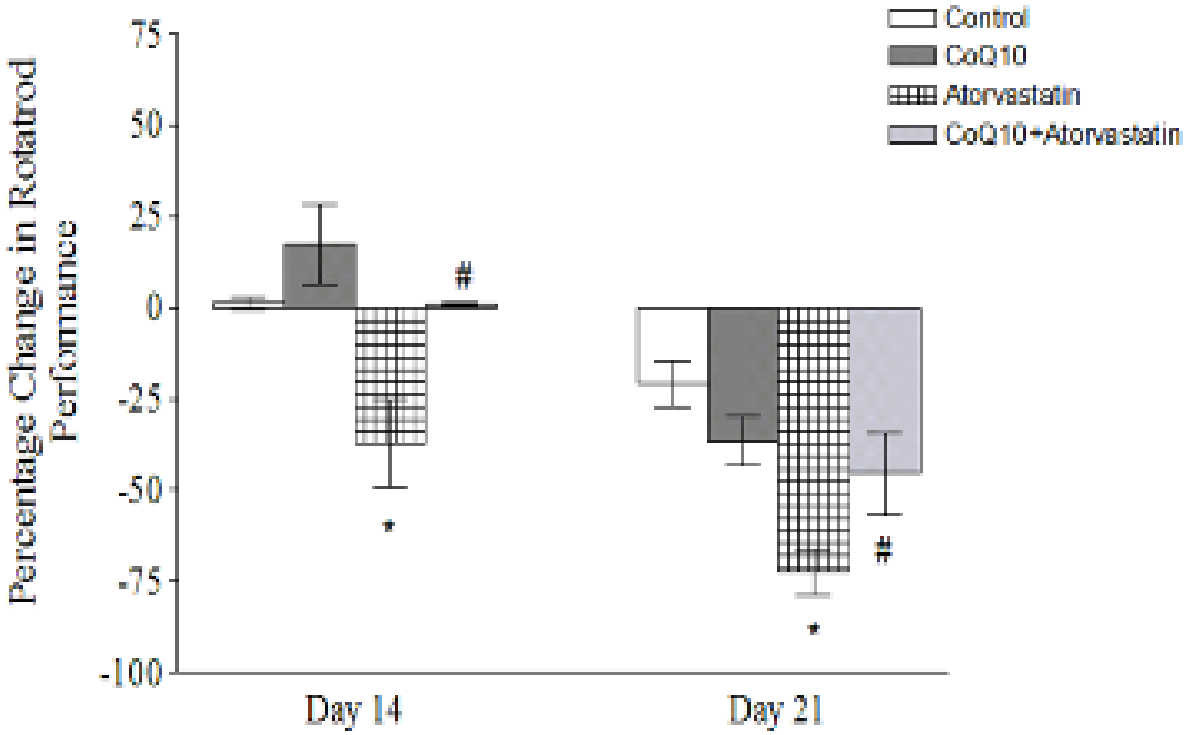

Figure 3: Effect of coenzyme Q10 on atorvastatin-induced changes in serum parameters: A. effect on the rise in serum creatine kinase on day 14 and day 21. B. Effect on serum myoglobin (day 21).

Each value represents the mean of 8-10 experiments ±S.E.M. *p < 0.05 vs. control, #p < 0.05 vs. atorvastatin.

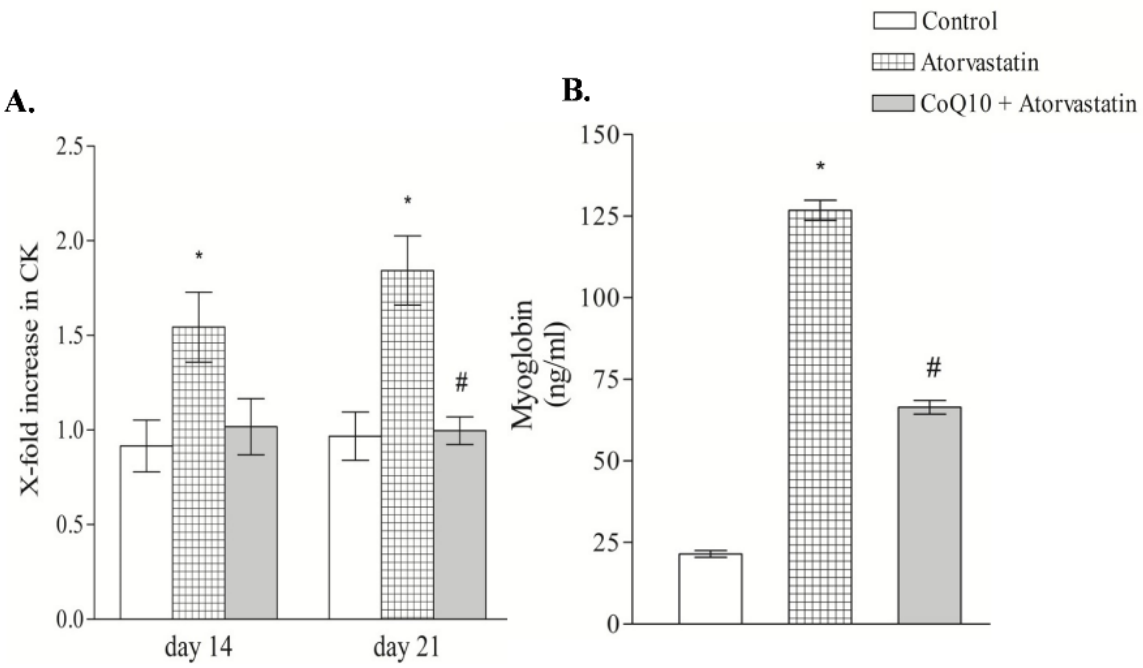

Figure 4 : Effect of atorvastatin and CoQ10 co-treatment on muscle LDH content.
Each value represents the mean of 8-10 experiments ±S.E.M. *p < 0.05 vs. control, #p < 0.05 vs. atorvastatin.

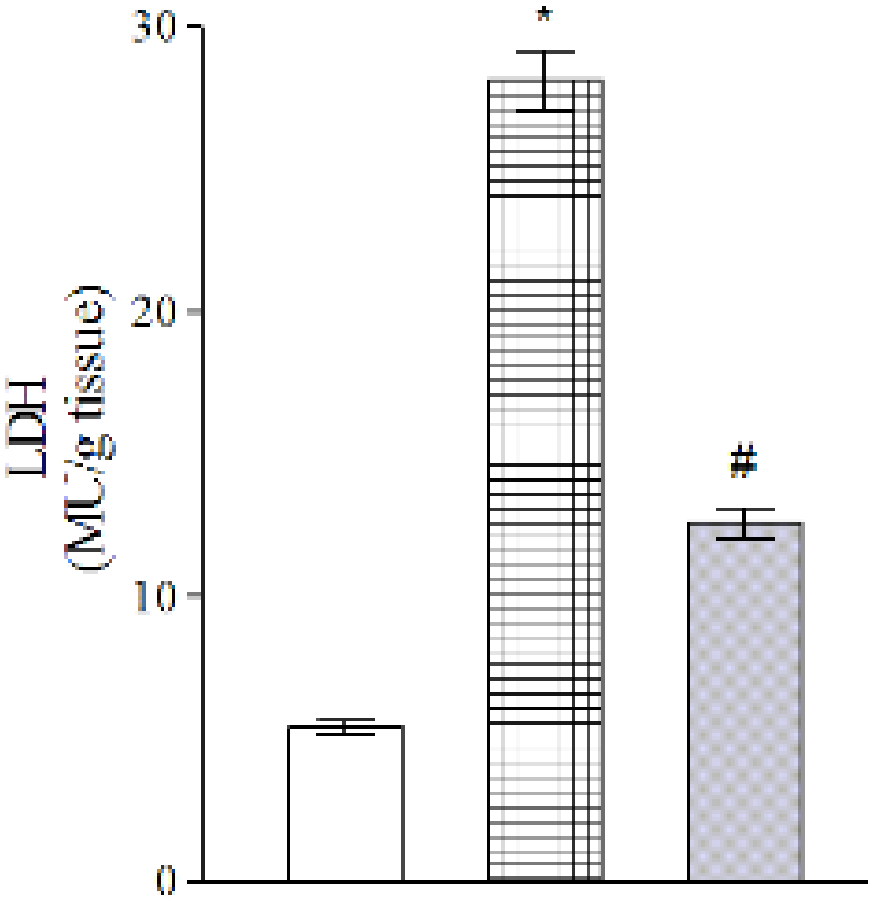

Figure 5: Representitve histopathological sections of semimembranosus muscle (H&E stained X 400) ; A. Control , B. Atrovastatin - treated rat showing mild necrosis (grade 1) , C. Atorvastatin - treated rat showing severe necrosis (grade 3) , D. Coenzyme Q10 + Atorvastatin - treated rat showing normal muscle (grade 0).

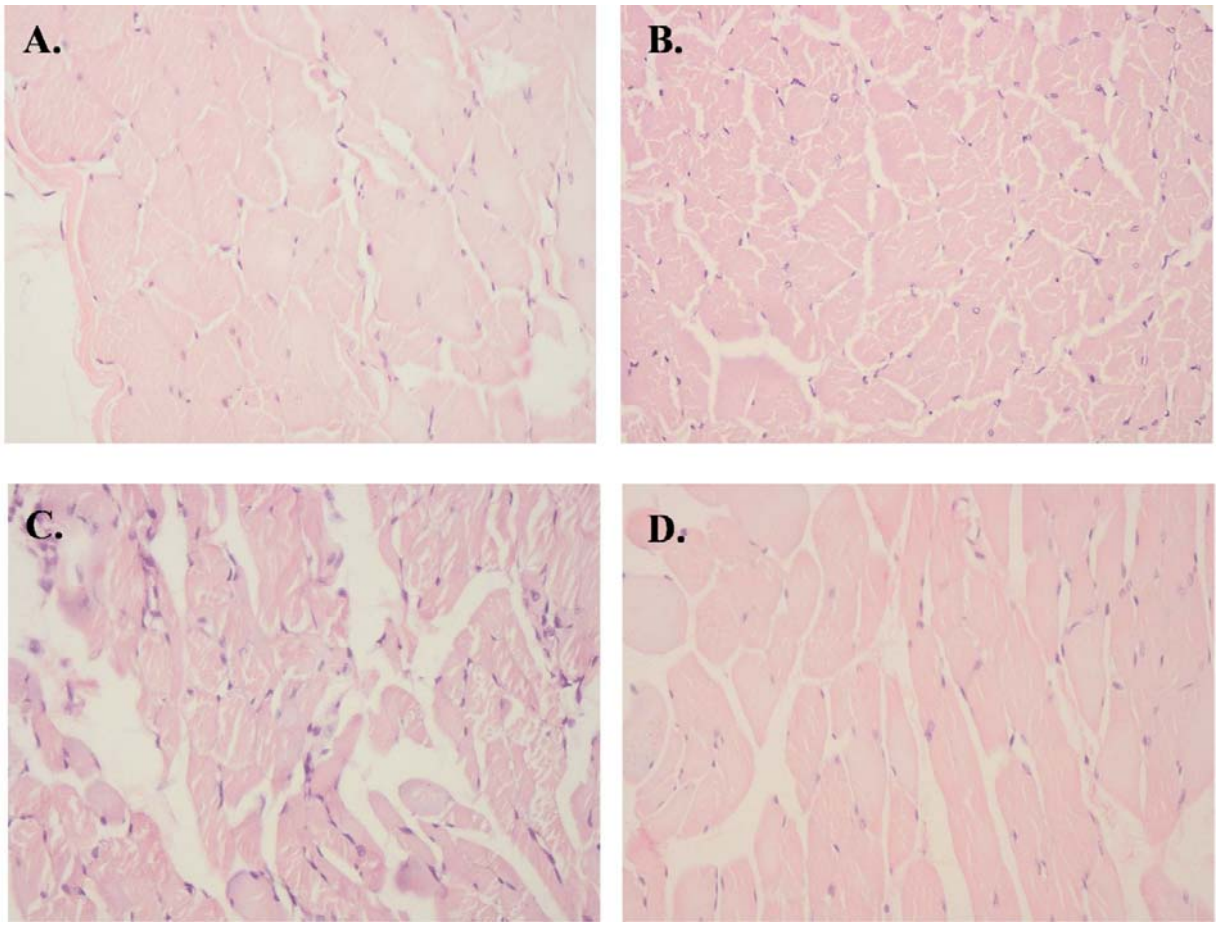

Figure 6: Effect of coenzyme Q10 on muscle mitochondrial function-related parameters in atorvastatin-treated rats: A. Effect on lactate/pyruvate ratio, B. Effect on ATP content, C.Effect on pAkt.

Each value represents the mean of 8-10 experiments ±S.E.M. *p < 0.05 vs. control, #p < 0.05 vs. atorvastatin.

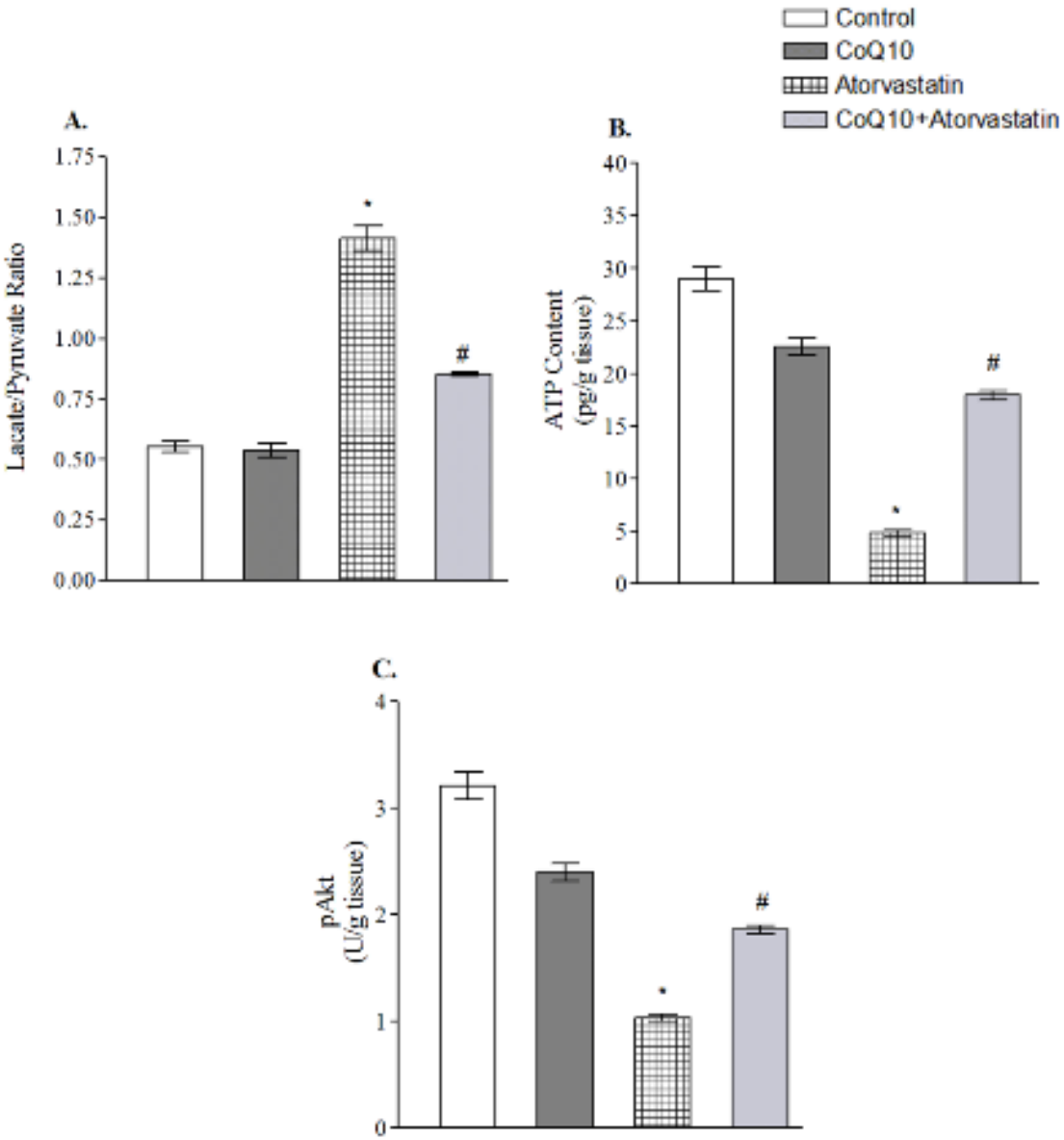

Figure 7 : Biceps femoris mitochondrial ultrastructural examination. Representative transmission electron micrographs of: control (A, B), atorvastatin - treated rats (C, D), atorvastatin-treated rats plus Coenzyme Q10 (E, F).

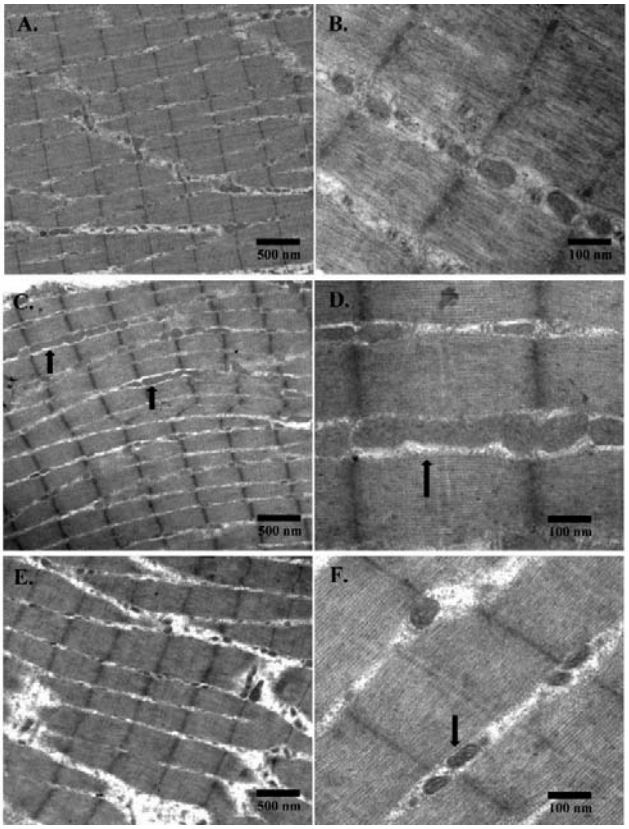

Table 1 : **Mean score of histopathological findings for control, atorvastatin and CoQ10 plus atorvastatin groups in different muscles.**

Muscle	Necrosis	Control		Atorvastatin		CoQ10 + Atorvastatin	
		%	Mean score	%	Mean score	%	Mean score
SEM	no	100	0.00 CI (0.0)	40	1.00 CI (0.37 - 1.63)	60	0.6 CI (0.0 - 1.20)
	yes	0		60		40	
EDL	no	90	0.2 CI (-0.25 - 0.65)	77	0.6 CI (0.02 - 1.18)	77	0.33 CI (-0.05 - 0.72)
	yes	10		33		33	

-Sem=semimembranosus, Edl=extensor digitorum longus.
- CI= confidence interval.