Effect of Simvastatin on the Skeletal Muscles of Senile Male Albino Rats and Possible Protective Role of L-Carnitine. A Histological Study

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ABSTRACT

Background: Hyperlipidemia is a disorder in metabolism which means an abnormal increase in levels of lipids (as cholesterol) and lipid-protein in the blood. It is one of the risk factors that mainly cause atherosclerosis, myocardial infarction, coronary heart disease, cerebral stroke, and renal failure. Simvastatin (S) is one of the most prescribed drugs frequently all over the world due to its excellent performance as hypolipidemic and its relatively low price. L-carnitine (LC) is a nutritional supplement supporting with clinical challenges such as dyslipidemia, anorexia & physical performance. This study aimed to evaluate the protective role of LC on the skeletal muscle of hyperlipidemic rats treated with (S).

Materials and Methods: Thirty senile male albino rats were used, divided into five equal groups as follows: control group (GI) and the other four groups were fed the high-fat diet for three weeks till the occurrence of hyperlipidemia. GII: was fed the high-fat diet for three weeks (hyperlipidemic) then sacrificed. GIII: hyperlipidemic rats were then treated orally with (S; 1.5 mg/rat/day) for 4 weeks. GIV: hyperlipidemic rats were then treated orally with (LC; 20 mg/rat/day) for 4 weeks. GV: hyperlipidemic rats were then treated with (S) and (LC) at the same time. Samples were taken and processed for light and electron microscopic studies.

Results: Application of (S) induced multiple changes of muscles as loss of transverse striations, splitting of myofibrils, and presence of central nuclei. Treatment with (LC) causes improvement of these changes.

Conclusion: Application of (LC) improved the degenerative changes of muscle obtained by administration of (S).

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Key Words: Electron microscope; hyperlipidemia; L-Carnitine; simvastatin; skeletal muscles.

INTRODUCTION

Hyperlipidemia is a metabolic disorder that involves abnormally increased levels of lipids [such as cholesterol] and lipid-protein in the blood. It is one of the focal hazards of atherosclerosis, cerebral stroke, coronary heart disease, myocardial infarction and renal failure[1]. An effective way to decrease the associated morbidity and mortality is to treat the high blood cholesterol level with changes in the lifestyle and if that is not sufficient enough, with medications[2].

Simvastatin is one of the worldwide frequently prescribed drugs because of its excellent hypolipidemic performance and relatively low price as a generic drug. They are used in clinical practice for hypercholesterolemia treatment, for primary and secondary decreasing incidence of cardiovascular disorders and stroke, due to their capability to lowers up to 55% of low-density lipoprotein in plasma (LDL)[3,4]. Statins are one of the world's most prescribed drugs[5]. In addition, statins have multiple effects that assist in improving endothelial function, stabilize plaques and decrease oxidative stress and inflammation[6]. Simvastatin acts by many mechanisms; it inhibits β-Hydroxy β-methylglutaryl-CoA (HMG-COA) reductase, reduces production of cholesterol, and increases LDL receptor sensitivity in the liver and High-density lipoprotein (HDL) level, meanwhile decreases triglycerides (TG) level in blood circulation[7]. However, statins are associated with some side effects although it is known to have a good safety record; the most common of which are related to muscle[8–10].

Simvastatin is known for its higher association with myopathy than other statins[11] and this adverse effect was pharmacokinetics related[12]. The pharmacokinetics of simvastatin is mainly determined by its disposition [i.e., distribution and metabolism] rather than its absorption[13]. Simvastatin, as a pro-drug, undergoes extensive metabolism (including first-pass metabolism) to the extent that its absolute bioavailability is less than
5%{[14]}. Distribution (e.g., influx and efflux across the liver cell membrane) of simvastatin is mainly through passive diffusion due to its lipophilic property{[15]}

The clinical evidence of statin-associated muscle disorders ranges from benign myalgia to severe myopathy, up to the life-threatening rhabdomyolysis. Advanced age patients, suffering from senile muscle atrophy and loss of performance is an increased risk of statin-induced muscle disorder{[16]}. Although the pathological mechanism of the myopathy associated with a statin was studied in detail and many theories existed, there is still no real consent{[17]}. The currently dominant ideas include genetic predisposition, mitochondrial dysfunction, lack of prenylated proteins and coenzyme Q10, abnormal cell membrane function and altered calcium homeostasis{[18]}. Statin-tempted myotoxicity happens mostly as a result of mitochondrial injury and oxidative stress{[19,20]}

Statins toxicity is thought mostly to be caused by mitochondrial oxidative strain and lowering of mitochondrial respiration. L-Carnitine (LC), has antioxidant properties and can reduce formation of superoxide radicals{[21]}. LC is mostly concentrated in skeletal muscles [about 95% of all body stores], and its convenience is important for the physiological processes of it{[22]}. The risk of myopathy persuaded by statin therapy seems to be dose-dependent and independent of decrease in LDL cholesterol{[23]}

The mechanism of myopathy caused by statins is not completely clear. A dose-related and pro-apoptotic effect, mitochondrial direct-effects, drug interactions and genetic features, or mixtures may be elaborated. Recently, a rare immune-mediated myopathy created by statin use has been designated{[24]}. Mitochondria play a central role in cellular energy provision and metabolism through the tri-carboxylic acid cycle, beta-oxidation of fatty acids, and production of reactive oxygen species, oxidative phosphorylation, cell division, and apoptosis. Mitochondrial dysfunction is believed to be the main cause of statin-induced myopathy{[25]}

L-carnitine (LC), an endogenous mitochondrial membrane compound, has a role in enabling the conveyance of long-chain fatty acids into mitochondrion for ingoing β-oxidation trail. It may be formed by amino acids, methionine and lysine or taken by food supply{[26]}. Many studies suggested that LC lowers oxidative stress and guards mitochondria from fatty acid stress and apoptosis initiation through the stoppage of mitochondrial swelling and Cytochrome-c release{[27]}. Carnitine plays a critical role in energizing mitochondrial membrane potential, cardiolipin component and oxygen consumption in mitochondrial dysfunction related to age{[28]}. Additionally, LC effectively augmented the endogenous antioxidant protection capacity, modified bioenergetics supported by mitochondrial and attenuates age-related changes{[29]}. Recent signs propose that carnitine intake may act as radical scavenger, thus helping in protection from statin-made oxidative muscle damage{[30,31]}. The present study was designed to study to evaluate the effect of simvastatin drug on the histological structure of skeletal muscle fibers of senile male albino rats and the possible protective effect of LC.

MATERIALS AND METHODS

Material

Drugs

a. Simvastatin commercially known as (Simvacor 40 mg tablets), purchased from (Sigma company for pharmaceutical and chemical industries). The daily single oral dose was (7.2 mg /kg body weight /day) ~ (1.5 mg /rat /day), dissolved in distilled water and given orally once by a gastric tube for 4 weeks. The dose was calculated in accordance with{[32]}. This dose was equivalent to the human high therapeutic dose of 80 mg once daily. It was selected according to{[33,34]} who recommend this dose for greater lipid goal achievement and more effective cardiovascular prevention.

b. L-Carnitine commercially known as (Carnitol 500 mg capsules); it was purchased from Global Napi Company for pharmaceutical and chemical industries. The daily single oral dose was (100 mg/ kg bodyweight/day) ~ (20 mg/rat/day) dissolved in distilled water and given orally by gastric tube for 4 weeks. It is the recommended dose for rats{[35]}

Animals

The present study was performed on thirty senile male albino rats, their average weight was 200-250 gm. and their ages were about 18-24 months old. They were handled according to the guidelines and ethics of the animal protocol of faculty of Medicine Al-Azhar University, Cairo, Egypt. They were kept in well-ventilated stainless-steel cages, at normal room temperature, and 12 hours light/dark cycle with strict care and hygienic measures. All rats were freely provided with water and rat chow. They were left for one week before the starting of the experiment for acclimatization.

Methods

Induction of experimental hyperlipidemia

After one week of acclimatization Animals were divided into five groups, six rats each. The first served as control group which fed on standard rodent chow. All other groups had free access to high fat diet (Vanaspati ghee and coconut oil) in a ratio of 3: 2 respectively in addition to normal diet for 3 weeks{[36,37]}

Animal groups

a. Control group (G I): Included 6 rats, fed on standard rodent chow all through the experiment.

b. Hyperlipidemic group (G II): Included 6 rats, were daily fed on high fat diet for 3 weeks only to induce hyperlipidemia, and then sacrificed.
c. Hyperlipidemic, Simvastatin treated group (G III): Included 6 rats, were daily fed on high fat diet for 3 weeks, and then treated with simvastatin for 4 weeks.

d. Hyperlipidemic, LC treated group (G IV): Included 6 rats, were daily fed on high fat diet for 3 weeks, and then treated daily for 4 weeks with LC.

e. Hyperlipidemic, Simvastatin and LC treated group (GV): Included 6 rats, were daily fed on high fat diet for 3 weeks, and then treated for 4 weeks with both Simvastatin and LC at the same time.

**Biochemical parameters**

Blood samples of all groups were obtained after 3 weeks and at the termination of the experiment. All the animals were kept for next overnight fasting, 2 ml of blood was collected at the morning from the orbital sinus with the help of a capillary tube by pressing the thumb behind the angle of the jaw resulting in the engorgement of retro-orbital plexus\(^{(3)}\). The blood was centrifuged; serum was collected and the parameters of lipid profile were estimated as the following: 1) TC\(^{(30)}\), 2) TG\(^{(30)}\), 3) HDL\(^{(41)}\), and 4) LDL\(^{(42)}\).

**Histological techniques**

(A) Light microscopic techniques: The animals were anaesthetized by ether inhalation, the gastrocnemius muscle of the right leg of each rat was dissected and cut into smaller pieces. The specimens fixed in 10% formalin, dehydrated in ascending grades of ethyl alcohol, cleared in xylene, impregnated and embedded in pure molten paraffin wax, sectioned on a rotary microtome at 5 µm thicknesses and mounted on an albumenized glass slide. Sections were then stained with the following methods:

- Hematoxylin and Eosin (H&E) for studying the general structure, Mallory’s trichrome stain for staining the collagen fibers, Periodic acid Schiff’s (PAS) technique for detection of glycogen content of skeletal muscle.

(B) Electron microscopic technique: The specimens taken from the rats were alive under anesthesia. Longitudinal muscle strips from the gastrocnemius muscle of the left leg from all groups were collected, trimmed into small pieces approximately 0.5 mm\(^3\), immediately fixed by immersion in 5 % glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.3 at 0 - 4°C for 8 hours\(^{(46)}\). Post-fixation was carried out in 1% osmium tetro-oxide, dehydrated in ascending grades of ethyl alcohol cleared in propylene oxide, embedded in epoxy resin then left for 24 hours in an oven at 40°C for resin polymerization\(^{(46)}\). The sections were cut on an LKB ultra-microtome using glass knife into 0.5 µm semi-thin sections stained with toluidine blue then ultrathin sections were cut (80 nm) and picked up on a 200 mesh copper grids\(^{(49)}\). Sections were examined and photographed by JEOL 1010 transmission electron microscope (Jeol; Tokyo, Japan).

**Morphometric study**

Data were obtained using "Leica Qwin 500 C" image analyzer computer system Ltd. (Cambridge, England) to measure the following parameters:

1. Area percent of collagen fibers in Mallory’s trichrome stained sections was done in 10 non overlapping microscopic fields at magnification x400.

2. Optical density of PAS +ve reaction in PAS stained sections was done in 10 non overlapping fields in each section at magnification x400.

**Statistical analysis**

Data analyzed using statistical package for social science (SPSS) version 21. Results were expressed as mean ± standard error. Different results were analyzed statistically using: ANOVA (Analysis of Variance): for comparison of quantitative data of more than two groups. The probability (P-value) of < 0.05 was considered statistically significant.

**RESULTS**

**Biochemical results**

In the current study the successful induction of hyperlipidemia was detected by the significant increase of serum cholesterol, LDL and triglyceride in rats.

The mean values of serum cholesterol, TG, LDL and HDL (mg/dl) in all rats of experimental groups after induction of hyperlipidemia for 3 weeks and by the end of the experiment were illustrated in (Table 1, Histogram1&2).

We observed that the levels of serum cholesterol, triglycerides and LDL were significantly decreased in all groups in comparison to GI (p ≤ 0.05); however, the serum level of HDL was significantly decreased in comparison to GI (p ≥ 0.05).

Comparing the lipid profile by the termination of the experiment discovered that the levels of serum cholesterol, triglycerides and LDL were significantly decreased in GIII, GIV and GV in comparison to GII; however, the serum level of HDL was increased significantly if compared to GII.

**Histological results**

**Light microscopic results**

Examination of H&E stained longitudinal section (LS) in the gastrocnemius muscle of the control group (G I) showed the structure of the skeletal muscle. The muscle fibers appeared parallel, non-branching and cylindrical in shape. They have acidophilic sarcoplasm and clear transverse striations of alternating dark and light bands. The nuclei were multiple elongated, peripheral just beneath the sarcolemma (Figure 1a). Examination of (GII) revealed segmental degeneration of the muscle fibers,
a part of the fibers showed loss of striations in the affected regions. Nuclei were obviously seen shifted to the central part of fibers in some areas (Figure 1b). Some muscle fibers appeared with wavy sarcolemma, wavy myofibrils having in-between areas of hemorrhage with splitting of some muscle fibers (Figure 1c).

Examination of (GIII) showed more muscular degenerative changes in the form of complete loss of transverse striations, more splitting of myofibrils, and presence of pyknotic peripheral nuclei (Figure 1d). Degeneration extended to massive destruction with dramatic loss of muscle architecture, loss of transverse striations, central small dark nuclei and some reached to the extent of complete destruction of the muscle fibers with mononuclear cell infiltration (Figure 1e).

Examination of (GIV) showed improvement in the structure of the skeletal muscle; Restored transverse striations in many areas with aggregation of central vesicular nuclei, however splitting of some fibers was still observed (Figure 1f).

Examination of (GV) showed multiple histological changes; some fibers appeared striated with peripheral nuclei, others showed patchy loss of striation and splitting; however, other muscle fibers appeared intact (Figure 1g).

Examination of sections stained with Mallory's trichrome of the control group (GI) revealed the presence of bundles of muscle fibers (fascicles) separated by thin connective tissue (perimysium). The fibers were surrounded by delicate CT which enclose fibers and connect them together (endomysium). Few blue stained collagen fibers were seen in-between the muscle fibers and around the blood vessels (Figure 2a).

In hyperlipidemic rats, (GII) the gastrocnemious muscle showed mild to moderate increase in the collagen fibers in-between the muscle fibers (Figure 2b). Marked increase in the collagen fibers was obviously seen in (GIII) in-between the muscle fibers as well as filling the wide degenerated areas (Figure 2c). Nearly normal appearance could be seen in rats treated with L-carnitine, (GIV).

The blue collagen fibers were few in-between the muscle fibers as well as around the blood vessels, as compared with Group II (Figure 2d). GV showed mild to moderate decrease in the collagen fibers in-between the muscle fibers as well as around the blood vessels as compared with Group III (Figure 2e).

Examination of sections stained with PAS of the control group (GI) declared the normal distribution of PAS +ve reaction in the skeletal muscle fibers; there were noticed two types of muscle fibers. Strong PAS +ve reaction appeared in some fibers while others appeared faint both appeared with obvious normal transverse striations (Figure 3a).

In (GII) moderate decrease in PAS + ve reaction was seen, however patchy areas appear with weak reaction in the skeletal muscle (Figure 3b). Examination of (GIII) showed marked decrease in PAS +ve reaction in the skeletal muscle however, marked increase of PAS +ve reaction obviously seen at the margins of the muscle (L.S and T.S) (Figure 3c).

Marked restoration of PAS +ve reaction could be detected in the skeletal muscle of rats of GIV, near to normal (Figure 3d). In Group V some muscle fibers appeared with marked increase in PAS +ve reaction while others showed faint reaction (Figure 3e).

**Electron microscopic results**

Electron microscopic examination of sections from GI showed that the myofibrils were seen filled the sarcolemmal settled parallel to the long alliance of the myofibers. Regular architecture of alternating light (I) and dark (A) bands was noticed. An electron-lucent narrow region, the H band, was seen at the center of the A band with a dark electron-dense line (M line) within it. At the middle of the light band Z-line was seen. Sarcomere extends between two consecutive Z-lines. Flat heterochromatic nuclei were present beneath the sarcolemma (Figure 4).

In GII there is Irregularities in myofibrils, congested blood capillarized with RBCs inside its lumen, presence of fibroblast nucleus and increased collagen fibers is also noticed (Figure 5).

In GIII there was focal degeneration of myofibrils, deviation of Z-lines, together with corrugated nuclear membrane. There is also increased collagen fibers, appearance of autophagic vacuoles and accumulation of glycogen granules (Figure 6).

In Group IV an improvement in the arrangement of the myofibrils with light and dark bands was noted (Figure 7).

In Group V some myofibrils appeared normal having in-between well detected disorganized areas, degenerated parts with loss of Z line also observed (Figure 8).

**Morphometric results**

**Collagen area percentage in all the experimental groups**

A significant difference was detected in the percentage of collagen fibers of the gastrocnemius muscle in the different groups, the highest mean value of collagen area% was observed in GIII and GII, while the least mean value of collagen area% was observed in GI, GIV and GV respectively. The results were statistically significant (p ≤ 0.05), (Table 2, Histogram 3).

**Changes in the optical density of PAS reaction in all the experimental groups**

A significant difference was present in the optical density of PAS + ve reaction in the skeletal muscle fibers in the different groups when compared to the control group especially in GIII as (p ≤ 0.05). The least mean value was observed in GIII, while the highest mean value was observed in GI and GIV. The mean value in GII and GV was reduced respectively being marked in GII, (Table 3, Histogram 4).
Table 1: Comparison of the lipid profile after 3 weeks and at the end of the experiment among the different experimental groups

<table>
<thead>
<tr>
<th>Lipid profile (mg/dl) after 3 weeks</th>
<th>Serum cholesterol (mg/dl) Mean ±SD</th>
<th>Serum triglyceride (mg/dl) Mean ±SD</th>
<th>Serum LDL (mg/dl) Mean ±SD</th>
<th>Serum HDL (mg/dl) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (N= 6)</td>
<td>95.6±3.8</td>
<td>79.7±7.4</td>
<td>64±6.97</td>
<td>29.6±4.83</td>
</tr>
<tr>
<td>Group II (N= 6)</td>
<td>143.6±2.5*</td>
<td>139.8±9.5*</td>
<td>123.8±10.1*</td>
<td>28.8±4.43</td>
</tr>
<tr>
<td>Group III (N= 6)</td>
<td>139.8±6.72*</td>
<td>143.5±4.5*</td>
<td>128±8.42*</td>
<td>26.6±3.72</td>
</tr>
<tr>
<td>Group IV (N= 6)</td>
<td>141.3±4*</td>
<td>140.4±4*</td>
<td>123.9±5.99*</td>
<td>24.4±2.5</td>
</tr>
<tr>
<td>Group V (N= 6)</td>
<td>143.5±4.2*</td>
<td>139.3±7.2*</td>
<td>127.3±4.92*</td>
<td>26.7±3.46</td>
</tr>
<tr>
<td><strong>P value</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.000&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.269 (NS)</td>
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</table>

<table>
<thead>
<tr>
<th>Final lipid profile (mg/dl)</th>
<th>Serum cholesterol (mg/dl) Mean ±SD</th>
<th>Serum triglyceride (mg/dl) Mean ±SD</th>
<th>Serum LDL (mg/dl) Mean ±SD</th>
<th>Serum HDL (mg/dl) Mean ±SD</th>
</tr>
</thead>
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<td></td>
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<tr>
<td></td>
<td>0.000&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.000&lt;sup&gt;*&lt;/sup&gt;</td>
<td>0.269 (NS)</td>
</tr>
</tbody>
</table>

* p-value ≤ 0.05 was considered as statistically significant.  
N: number of rats in each group  
SD: standard deviation  
NS: non-significant

Table 2: Changes in the percentage of collagen fibers among the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10</td>
<td>1.412</td>
<td>0.102</td>
<td>0.032</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>6.571*</td>
<td>2.001</td>
<td>0.633</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group III</td>
<td>10</td>
<td>16.520*</td>
<td>2.432</td>
<td>0.769</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group IV</td>
<td>10</td>
<td>2.610*</td>
<td>0.383</td>
<td>0.121</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group V</td>
<td>10</td>
<td>4.498*</td>
<td>1.072</td>
<td>0.383</td>
<td>≤0.05</td>
</tr>
</tbody>
</table>

*statistically significant P value ≤ 0.05
N: number of rats in every group. SD: standard deviation. SE: standard error.

Table 3: Changes in the optical density of PAS reaction among the different experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>S.E</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>10</td>
<td>1.087</td>
<td>0.214</td>
<td>0.068</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group II</td>
<td>10</td>
<td>0.400*</td>
<td>0.213</td>
<td>0.067</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group III</td>
<td>10</td>
<td>0.229*</td>
<td>0.096</td>
<td>0.030</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group IV</td>
<td>10</td>
<td>0.910*</td>
<td>0.080</td>
<td>0.025</td>
<td>≤0.05</td>
</tr>
<tr>
<td>Group V</td>
<td>10</td>
<td>0.662*</td>
<td>0.231</td>
<td>0.073</td>
<td>≤0.05</td>
</tr>
</tbody>
</table>

*statistically significant P value ≤ 0.05
N: number of rats in every group. SD: standard deviation. SE: standard error.
Fig. 1: (G1) (a) Non-branching, cylindrical and parallel muscle fibers. Multiple peripheral nuclei beneath the sarcolemma (arrows). Deep acidophilic sarcoplasm with clear transverse striations. (G2) (b): regular striations (green arrow), central nuclei (blue arrows), segmental degeneration (asterisk), splitting, loss of striations and loss of nuclei (black arrow), (c) wavy sarcolemma and myofibril (w.m), Splitting (black arrow) regular striations (asterisks), pyknotic nuclei (blue arrow) and hemorrhage (green arrow). (G3) (d): Loss of architecture of some fibers (green arrow), splitting (arrow heads) pyknotic nuclei (blue arrow), faint transverse striations (asterisk) & mononuclear cell infiltration is seen (black arrow). (e) Loss of architecture, homogenous deep acidophilic sarcoplasm (green arrows), Pale degenerated areas (black arrow), loss of striations although, faint striations (asterisk), small dark peripheral & central nuclei (blue arrow) & area of necrosis with mononuclear cell infiltration (yellow arrow). (G4) (f) restored transverse striations (asterisk) with peripheral nuclei (blue arrows) aggregation of nuclei in the center (black arrow) and Splitting of muscle fibers (green arrows). (G5) (g) restored striations(asterisk), peripherally located nuclei (green arrows), splitting (blue arrows) and segmental degeneration (black arrow). (H&E.  x 400)
Fig. 2: (GI) (a) few collagen fibers in between the muscle fibers (black arrow) and around the blood vessels (yellow arrow). (GII) (b) moderate increase in the collagen fibers (black arrows). (GIII) (C) marked increase in the collagen fibers (black arrows). (GIV) (D): few collagen fibers in-between the muscle fibers (yellow arrow) and around the blood vessels (black arrows). (GV) (E) mild to moderate decrease in collagen fibers in comparison to GIII (black arrow) (Mallory's trichrome stain x 400)
Fig. 3: (GI) (a) Some muscle fibers showed intense PAS +ve reaction (black arrow), while others showed faint PAS +ve reaction (blue arrow). (GII) (b) moderate decrease in PAS reaction and patchy areas of weak reaction (black arrow). (GIII) (c) marked decrease in PAS reaction, however intense PAS reaction is seen at the periphery of the muscle fibers (black arrows). (G IV) (d) marked decrease in PAS reaction, however intense PAS reaction is seen at the periphery of the muscle fibers (L.S&T.S) (black arrows). (GV) (E) moderate increase in PAS reaction however intense PAS reaction is seen at the periphery of the muscle fibers (blackarrow). (PAS X 400)

Fig. 4: An electron micrograph of (GI) showing: parallel arrangement of the myofibrils with alternating light (I) and dark (A) bands. Z line (yellow arrow) is seen bisecting the light band. Elongated heterochromatic nuclei (N) are seen under the sarcolemma. Sarcoplasmic reticulum is also seen (SR). Inset showing higher magnification. (Uranyl acetate and Lead citrate X 6000, 20000)

Fig. 5: An electron micrograph of (GII) showing: distorted sarcomere with irregular Z-line, Congested blood capillary with RBCs inside it, accumulation of collagen fibers (red arrow) and appearance of fibroblast nucleus(N). (Uranyl acetate and Lead citrate X 5000)

Fig. 6: An electron micrograph of (GIII) showing irregularities of Z lines & distorted sarcomere (yellow arrow), degenerated parts of myofibrils & irregular nuclear membrane. Inset showing accumulation of collagen fibers (red arrow), discontinuity of myofibril (MF) with accumulation of autophagic vacuoles (V). (Uranyl acetate and Lead citrate X 5000, 40000)

Fig. 7: An electron micrograph of (GIV) showing normal parallel arrangement of myofibril (MF), and nuclei of fibroblast N. (Uranyl acetate and Lead citrate X 15000)
This study was performed on senile male rats because myopathy is higher in aged than in adult subjects. Thus, muscular manifestations accompanied with statin treatment may lower their use and may be linked with termination of a needed therapy. The motive of the high risk of myopathy in the old age is not recognized but the deterioration of muscle function associated with age and diminishing of regenerating capability with metabolic insinuations may play an important role.

At first; induction of hyperlipidemia in rats by high fat diet for 3 weeks, followed by administering simvastatin. It was reported that 80 mg/kg/day of simvastatin is the maximum tolerated dose that causes wide spread muscle degeneration in rats and thus provides a relevant model for myopathy.

Selection of gastrocnemius muscle in this study as most of its fibers are formed of type II white fibers. It has been reported that statins affects fast type II muscle fibers and causes necrotic changes in them. Degenerated muscle can grossly seem either pale or dark. Histologically, degenerated myofibers will exhibit a variation of microscopic changes, such as swollen cell, hyper eosinophilia, vacuolization, loss of striation, fragmentation, and rupture of fibers.

In the current study, light microscopic examination of H&E stained sections in (G II) revealed evident degeneration of muscle fibers which is noted to be segmental with evident loss of striations in the affected regions. Nuclei were shifted to central part of fibers in some areas, some muscle fibers showed wavy appearance of sarcolemma and wavy myofibrils, areas of hemorrhage and splitting of some muscle fibers. These findings were in agreement with Abdou et al., who reported that large areas of degenerating muscle fibers were pale stained with evident loss of transverse striations, and wide interfascicular spaces were shown in hyperlipidemic effect of diazinon – treated rats.

That was also consistent with El Rabey et al., who reported that the cardiac muscle showed some sorts of hemorrhages and wavy appearance of myofibres, as well as the sarcoplasm of some fibers was characterized by granular appearance, large areas of degenerated muscle fibers with evident loss of transverse striations and wide interfascicular spaces in hyperlipidemic group compared to the control group.

These findings were in agreement with Haschek et al., who reported that degeneration often only affects a segment or several segments of the fiber, as opposed to the entire myofiber due to the long length of muscle fibers. Several studies attributed these changes to excessive production of reactive oxygen species (ROS), results in alteration in the balance between ROS and endogenous antioxidants and creates oxidative stress which is implicated in many pathological conditions.

In the current study, Mallory's trichrome stained section of (G II) showed increased collagen fibers in between skeletal muscle fibers. These findings were in accordance with Novitsky et al., who mentioned that hypercholesterolemia was considered a risk factor for hepatic fibrosis. Studies found that oxidation products such as lipid peroxidation products can stimulate α-collagen expression and collagen synthesis.

PAS stained sections in (G II) showed moderate decrease in PAS reaction. This is in agreement with Alkhalfy et al., who reported that 9 weeks of a high fat Western-style diet induced defects in skeletal muscle substrate concentrations, including glycogen and lipid contents.

In the present work the ultra-structural analysis in the gastrocnemius muscle of hyperlipidemic group (GII) showed distorted sarcomeres, irregular Z-lines, congested blood vessels increased collagen with appearance of fibroblast. These findings were in agreement with Tomazoni et al., who reported that high fat diet in mice showed great muscle structural abnormalities in HFD group. Furthermore, it appeared weak and was on the border of degenerations. The bands were diminished with loss of connections among myofibrils and these changes due to excessive production of reactive oxygen species and insulin resistance.

In the current study, H&E stained sections (GIII) showed loss of the normal skeletal muscle architecture, myofibers degeneration, sarcoplasmic fragmentation, inflammatory cellular infiltration, small dark nucleus, myofiber splitting and shift of nucleus to a central position. These results also were similar to the finding of Meregalli et al., who reported that animals treated with simvastatin exhibited significantly greater morphological and structural skeletal muscle damage.

Westwood et al., reported that after application of elevated doses of statin in rats the necrosis was more widespread categorized by cytoplasmic eosinophilia with loss of cytoplasmic construction and there was...
mononuclear cell and polymorph nuclear cells infiltration, edema and vacuolation with disintegration and loss of cytoplasm.

Meregalli et al.,[85] explained why the damaged muscle fibers following injury undergo hyalinization as a result of over contraction precipitated by excessive intracellular Ca2+. The fibers became necrotic or apoptotic and initiate inflammatory reaction via releasing of mitogenic chemo-attractants. It could be suggested that the degenerative changes that affected the muscle fibers might be the stimulus that initiated the inflammatory reaction which explained by Stevnes et al.,[66], who suggested that the degenerated fibers release different inflammatory mediators that lead to mononuclear cellular infiltration.

The splitting of the muscle fibers might be due to the insufficient oxygen supply and metabolites exchange to the enlarged and hypertrophied fibers. The central located nuclei have been used also as a marker for muscle fiber damage[67]. Centra located nuclei were also reported in cases of muscle fiber regeneration[68].

Abundant collagen fibers appeared (GIII) in-between skeletal muscle fibers. These findings were supported by Fukushima et al.,[60] and Li et al.,[70], who mentioned that fibrotic process that occurs after muscle injury is promoted by the local release of growth factors such as transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor, which stimulate the cells of the extracellular matrix to produce local collagens and trigger differentiation of myogenic cells to myofibroblasts, which start collagen deposition.

GIII also showed marked decrease of PAS reaction with accumulation of PAS reaction at the margin of muscle fibers. These finding was explained by Al-Doaiss et al.,[71] who reported that administration of statins caused depletion of glycogen stores in the hepatocytes. This alteration might be due to the effect of statins on glucose absorption or on the enzymes involved in the process of glycogenesis or/and glycolysis.

E/M examination of (GIII) showed disorganized sarcomere, irregularities of Z line, nuclear membrane indentation increased collagen and presence of autophagic vacuoles. These results were in agreement with Obayashi et al.,[21] who reported that swollen mitochondria, dilated sarcoplasmic reticulum and accumulation of autophagic vacuoles followed by degenerated myofibrils occur in rats after cervistatin treatment.

Presence of autophagic vacuoles related to alterations to autophagy and/or lysosomal function which are observed in many skeletal muscle diseases[73-78] and have also recently been implicated in myofibrillar myopathy[70].

The underlying mechanisms of statin myopathy were explained by Boutibir et al.,[73], who declared that statins have been associated with high oxidative stress in skeletal muscle and this is responsible for transcriptional deactivation of mitochondrial biogenesis together with mitochondrial dysfunction. Bitzur et al.,[70] and Stroes et al.,[10] suggest that the toxic effect of statins is associated with mitochondrial dysfunction by decreasing energy production and altering muscle protein degradation.

In our study, an improvement was obviously detected in the structure of skeletal muscles of GIV in the form of restored transverse striation of muscle fibers in many areas with aggregated central vesicular nuclei. Splitting of some fibers was also observed. Similar results were reported by Farouk et al.,[79] and Artioli et al.,[80] who declared that the hypercellularity and central nuclei were prominent features for muscle regeneration. These findings reflected a protective role of L-carnitine.

Murad et al.,[81] mentioned that L-Carnitine occupy a vital role in the mitochondrial transport and oxidation of fatty acids. Shehata et al.,[82] stated that L-carnitine defend against mitochondrial malfunctions accompanying oxidative strain caused by many factors such as aging, ischemia reperfusion, inflammation, degenerative diseases, carcinogenesis and drug toxicity, in vivo or in vitro. Its mechanism of action was explained by Oyanagi et al.,[23] who mentioned that it avoids apoptosis pathway by stimulating mitochondrial respiration and encouraging of cell energy manufacture via β-oxidation in the mitochondria.

In (GIV) minimal collagen fibers were seen among muscle fibers. These finding are similar to Mazroa et al.,[67]. There is also restored PAS reaction with focally increased reaction at the periphery of the muscle fibers. These results are reinforced by several studies which reported that L-carnitine halts age-related surges in reactive oxygen species (ROS), lipid peroxidation and DNA strand disruptions in heart, skeletal muscle and brain, concomitant with enhancement in mitochondrial enzyme and respiratory chain activities[83,84].

E/M examination of (GIV) showed normal parallel appearance of myofibrils with dark and light bands which reflected a protective role of L-carnitine. L-Carnitine beside its metabolic role has an antioxidant effect and can directly decrease superoxide radical generation[83].

In the current work, (GV) showed areas of intact muscle fibers and others with remaining histological changes as patchy loss of striation, central nuclei and splitting of some fibers. These results are explained by La Guardia et al.,[21] who stated that statins myopathy may occurred 2ry to carnitine levels abnormalities as L-carnitine acts as radical scavenger which prevent oxidative mitochondrial damage and protect muscles. Also Costa et al.,[81] mentioned that L-Carnitine guard against simvastatin-induced cell necrosis in prostate cancer-3 cell line by declining the simvastatin induced mitochondrial hyperproduction of the superoxide anion and preventing oxidative mitochondrial dysfunction.

In (GV) moderate amount of collagen fibers was seen in our study. Tateno et al.,[83] said that fibrosis hinders the regenerative ability of skeletal muscle after injury and slow
their healing process. This leads to incomplete recovery after injury and even formation of disorganized scar tissue that often replaces damaged myofibers and may be a cause of muscular re-injury.

In (GV) E/M examination showed some muscle fibers appear normal while others disorganized. These changes are due to L-Carnitine protective effect against simvastatin-induced cell necrosis through preventing oxidative mitochondrial dysfunction[31].

CONCLUSION

Application of (LC) improved the degenerative changes of muscle obtained by administration of (S).

CONFLICTS OF INTEREST

There are no Conflicts of interest.

REFERENCES


SIMVASTATIN INDUCED MYOPATHY AND L- CARNITINE

الملخص العربي

تأثير عقار السيمفاستاتين على العضلات الهيكلية للفئران البيضاء المسنة والدور الوقائي المحتمل لعقار (ال) كارنيتين. دراسة نسيجية

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زيادة الدهون في الدم هو عبارة عن اضطراب في التمثيل الغذائي وهو ما يعني زيادة غير طبيعية في مستويات الدهون (مثل الكوليسترول) والبروتينات الدهنية في الدم. إنه أحد عوامل الخطر التي تسبب بشكل رئيسي تصلب الشرايين وجلطات القلب وأمراض السرطانات التاجية والسكته الدماغية والفشل الكلوي. يعد عقار (سيمفاستاتين) أحد أكثر الأدوية الموصوفة بشكل متكرر في جميع أنحاء العالم نظرًا لدراسته الذاتية للكارنيتين في نقص نسبة الدهون في الدم وسرعته المنخفض نسبيًا. (ال- كارنيتين) هو مكمل غذائي يصحح الكثير من التحديات الإكلينيكية مثل عدم انظام مستوي الدهون في الدم، وفقدان الشهية والأداء العضلي.

هدفت هذه الدراسة إلى تقييم الدور الوقائي لـ (ال – كارنيتين) على العضلات الهيكلية للفئران المسنة التي تعاني من زيادة مستوي الدهون في الدم وتعرض بالسيمفاستاتين.

المواد والطرق: تم استخدام ثلاثين من ذكور الفئران البيضاء المسنة، مقسمة إلى خمس مجموعات متساوية (الخاصة: 1) المجموعة الضابطة و باقي المجموعات تغذت على نظام غذائي غني بالدهون لمدة ثلاثة أسابيع حتى حدوث زيادة في مستوي الدهون في الدم، المجموعة الثانية (عالية الدهون): تغذت على نظام غذائي غني بالدهون لمدة ثلاثة أسابيع ثم تم التضحية بها، المجموعة الثالثة: تغذت على نظام غذائي غني بالدهون لمدة ثلاثة أسابيع ثم عولجت عن طريق الفم (سيمفاستاتين) (1.5 مجم / يوم) لمدة أربعة أسابيع. المجموعة الرابعة: تغذت على نظام غذائي غني بالدهون لمدة ثلاثة أسابيع ثم عولجت عن طريق الفم (سيمفاستاتين) (20 مجم / يوم) لمدة أربعة أسابيع.

المجموعة الخامسة: كانت على نظام غذائي غني بالدهون لمدة ثلاثة أسابيع ثم عولجت بـ (ال – كارنيتين) في نفس الوقت لمدة أربعة أسابيع. ثم أخذ العينات ومعالجتها للدراسات المجهرية الضوئية والإلكترونية.

النتائج: إعطاء (سيمفاستاتين) أدى إلى تغييرات متعددة في العضلات كفقدان الخطوط العرضية، وتفتيت ألياف العضلات، ووجود نواة مركزية. بينما العلاج باستخدام (ال – كارنيتين) أدى إلى تحسين هذه التغييرات.

الخلاصة: إعطاء (ال – كارنيتين) أدى إلى تحسين التغييرات العضلية التي حدثت نتيجة لإعطاء (سيمفاستاتين)