L-Carnitine and Vitamin D attenuation of diabetic nephropathy in streptozotocin diabetic rats

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ABSTRACT

Increased oxidative stress is one aspect of the multifaceted metabolic illness known as diabetes mellitus, which plays a role in the disease's etiology. This has led to numerous studies on the application of antioxidants as an additional treatment strategy. Due to its ability to balance anti-inflammatory cytokines and modulate the immune response, vitamin D may be an unexpected target for the development of novel treatment plans for individuals with autoimmune illnesses, particularly diabetes mellitus.

Through the inhibition of ceramide formation, carnitine can reduce oxidative stress and prevent apoptosis, or programmed cell death. This study was designed to investigate the possible beneficial effects of vitamin D and L carnitine and their combination to attenuate diabetic nephropathy in rats. Rats were classified into five groups; control, diabetic untreated, and three diabetic groups treated with carnitine, vitamin D and carnitine + vitamin D. Treatment with carnitine and vitamin D has been continued for 16 weeks. Treated group was found to significantly reduce the high levels of glucose, urea, creatinine, sodium, potassium, MDA, SOD, IL-6, and TNF-α in serum compared with the diabetic untreated group. Additionally, the treated animal groups showed improvements in the size of the Langerhans island. These studies indicate that by reducing oxidative stress and lessening diabetic nephropathy, L-carnitine and vitamin D therapy has a therapeutic protective impact in diabetes. As a result, vitamin D and L-carnitine may be clinically helpful in preventing oxidative stress in diabetic kidney patients.

1. Introduction

Hyperglycemia, a chronic condition that affects the metabolism of carbs, proteins, lipids, and electrolytes, is a hallmark of diabetes mellitus (DM). Due to the excessive build-up of glucose in these cells, it can cause damage to the capillary endothelium in a number of organs, including the retina, renal glomerulus, and central and peripheral nerves. This can create disruptions to the neurological and vascular systems[1-3]. Increased oxidative stress is a major factor in the pathophysiology of diabetic nephropathy (DN), a multifaceted metabolic
condition associated with diabetes mellitus (DM). The phrase "oxidative stress" refers to a change in the equilibrium between the production of oxidant species and antioxidant defenses, favoring an environment that is pro-oxidant [4].

If left untreated, diabetic mellitus can cause a wide range of illnesses and chronic problems that can be fatal [1]. Recent research demonstrates that oxidative stress and free radical production can be brought on by DM-induced persistent hyperglycemia in both human and animal models [5-8]. The primary cause of the secondary consequences of diabetes mellitus, including wounds and foot ulcers, is oxidative stress [8].

Vitamin D (Vit D) is being heralded as a cure-all for disease control and is currently at the forefront of therapeutic research [9]. It is now thought that vitamin D supplementation in the illness state improves patients' health in a variety of ways. Its antioxidant therapeutic potential, which has received less attention than its other activities, appears to be lacking in data [10–11]. Vitamin D is a fat-soluble vitamin that functions similarly to hormones and is essential for the metabolism of calcium and bone. The usually active form of vitamin D [1, 25(OH)2D3] has a biological role that includes influencing several systemic processes, including inflammation, immunological control, and cell differentiation [12–13]. As a non-enzymatic antioxidant molecule, vitamin D has gained increased attention recently [14]. Additional research revealed that vitamin D administration raised levels of total antioxidant capacity and dramatically reduced plasma levels of malondialdehyde (MDA) [15-16].

Long-chain fatty acids are transported across mitochondrial membranes by L-carnitine (LC), where they are oxidized to produce energy [17]. Apart from its crucial function, carnitine also buffers the CoA pool, eliminates potentially harmful acyl-CoA residues from cells, and controls the intramitochondrial ratio of CoA to acetyl-CoA [18]. Systemic carnitine deficiency disorders have been linked to defective mitochondrial-oxidation of fatty acids, decreased energy production, lipid storage myopathy, decreased exercise capacity, and cardiomyopathy [19–21], which is evidence of the relevance of carnitine. Athletes who are competitive, highly trained, and recreationally active take LC as a supplement, as it is one of the important proteins. Individuals who have low amounts of LC production in their bodies take supplements containing LC. For a variety of reasons, including skeletal myopathies, drug abuse, hypoglycemia, and hereditary disorders, some individuals have low levels of LC [22–24].

2. Material and methods

2.1 Experimental animals

Before the studies began, the animals were kept under observation for a week. All processes were carried out in compliance with Minia University of Egypt's Animal Ethics Committee. (No. MPEC230501). This study was carried out on forty physically healthy adult male albino rats weighing 190 - 220 gm that was obtained from the animal house at the National Research Centre (Giza, Egypt). Standard rat food was provided to the animals by (El-Nasr, Cairo, Egypt). The rats were kept in cages with light from 7:00 a.m. to 7:00 p.m., at a temperature of roughly 22–25°C.

2.2 Experimental design

Ten rats each were randomly assigned to one of five groups. As a control, group 1 animals received an injection of citrate buffer in an amount equivalent to the solvent used to dissolve streptozotocin (STZ) (Sigma Chemical Company). STZ (dissolved in citrate buffer, pH 4.5) was injected intraperitoneally (i.p.) into groups 2-4 (n = 40) at a dose of 65 mg/kg body weight [25-27]. Blood samples were taken and blood glucose levels were assessed following a 4-day STZ injection. Animals with blood glucose levels below 200 mg/dL (6 animals) were eliminated from the trial, whereas rats with blood glucose levels exceeding 225 mg/dL were classified as diabetic.

Animals of group 2 (n=10) were left as diabetic untreated animals. Animals of group 3 were given LC (n=8) (Carnitor, Sigma-Tau, Mary¬land, USA) was administered at a dose of 300 mg/kg b.w./day intraperitoneal for 16 week [28]. Animals of group 4 (n=8) was treated Concomitant administration of Vitamin D (Sigma–Aldrich Co., St. Louis, USA) (0.5μg/100g body weight) dissolved in distilled sesame oil [29-30]. Animals of group 5 (n=8) was treated LC and Vit D. Treatment of diabetic rats in groups 3–5 has been continued for 16 weeks.

2.3 Serum

Using urethane (1.5 g/kg i.p.) as an ether anesthetic, animals were slaughtered at the conclusion of the experiment, and each animal's heart was immediately punctured for the collection of blood. The following parameters were measured in serum using commercially available kits: glucose, urea, creatinine, sodium, and potassium (Biodiagnostic, Cairo, Egypt). The concentrations of TNF-α and IL-6 were measured using an ELISA kit from Labscience, Houston, Texas, USA.
accordance with the manufacturer's instructions. Using commercially available kits (Elabscience Co., Texas, USA), the activities of MDA and catalase as well as the quantities of SOD in the serum were measured in accordance with the manufacturer's instructions.

2.4 Histological study

For histological analysis, the pancreas was meticulously dissected, fixed in 10% formalin, sectioned into 5μm sections, and stained with eosin and hemoglobin.

2.5 Statistical analysis

The data was analyzed using the Statistical Package for Social Science (SPSS Chicago, IL, USA) version 15 software. Data were acquired using one-way analysis of variance (ANOVA) and presented as mean ± SD using a post-hoc test. At P < 0.05, the P-value was considered significant.

3. Results

3.1 The weights and kidney weight of the rats

At the start of the investigation, all groups' rats had similar starting weights. By the time the treatment ended, the animals with diabetes had lost a considerable amount of weight. Kidney weight did not differ between the groups (Table 1, Fig. 1). Rats with diabetes treated with LC, Vit D, or control did not differ in their starting weights (Table 1, Fig. 2). The diabetic mice that were not treated showed a substantial hyperglycemia (p <0.01) after receiving an injection of STZ (Table 1, Fig. 3).

Table 1 lists the following groups: group 1 (control), group 2 (diabetic untreated), group 3 (diabetic treated with LC), group 4 (diabetic treated with Vit. D), and group 5 (diabetic treated with LC + Vit. D). Body weight (g), kidney weight (g), serum glucose (mg/dl), serum creatinine (mg/dl), blood urea (mg/dl), serum potassium (mmol/L), and serum sodium (mmol/L) levels

<table>
<thead>
<tr>
<th>Parameters/groups</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial body weight</td>
<td>190.2 ± 0.73</td>
<td>196.2 ± 0.71</td>
<td>191.7 ± 0.58</td>
<td>195.5 ± 0.44</td>
<td>193.4 ± 0.68</td>
</tr>
<tr>
<td>Final body weight</td>
<td>194.5 ± 0.56</td>
<td>156.6 ± 0.88*</td>
<td>188.6 ± 0.49**</td>
<td>196.3 ± 0.50**</td>
<td>194.2 ± 0.41**</td>
</tr>
<tr>
<td>Kidney weight</td>
<td>3.1 ± 0.04</td>
<td>3.8 ± 0.12</td>
<td>3.6 ± 0.02</td>
<td>3.7 ± 0.08</td>
<td>3.6 ± 0.45</td>
</tr>
<tr>
<td>Initial serum glucose</td>
<td>98.2 ± 2.10</td>
<td>100.7 ± 2.52</td>
<td>99.2 ± 1.42</td>
<td>98.2 ± 1.05</td>
<td>99.2 ± 1.51</td>
</tr>
<tr>
<td>Final serum glucose</td>
<td>102.2 ± 1.45</td>
<td>254.3 ± 4.12***</td>
<td>178.3 ± 3.02**</td>
<td>188.8 ± 3.28**</td>
<td>167.2 ±2.34**</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>0.85 ± 0.05</td>
<td>2.3 ± 0.25*</td>
<td>1.7 ± 0.27**</td>
<td>1.4 ± 0.03**</td>
<td>1.2 ± 0.05**</td>
</tr>
<tr>
<td>Blood Urea</td>
<td>34.2 ± 2.75</td>
<td>93.7 ± 3.55*</td>
<td>51.3 ± 3.40**</td>
<td>56.2 ± 2.45**</td>
<td>45.3 ± 1.78**</td>
</tr>
<tr>
<td>Serum Potassium</td>
<td>4.6 ± 0.45</td>
<td>7.5 ± 0.88*</td>
<td>5.7 ± 0.55**</td>
<td>5.2 ± 0.26**</td>
<td>5.1 ±0.32**</td>
</tr>
<tr>
<td>Serum Sodium</td>
<td>146.4 ± 3.22</td>
<td>192.6 ± 3.12*</td>
<td>158.6 ± 3.32**</td>
<td>167.8 ± 3.26**</td>
<td>157.2 ±2.44**</td>
</tr>
</tbody>
</table>
animals in groups 3-5 significantly decreased all of these indicators (p<0.05; Table 1, Fig. 4, 5, 6, 7).

3.2 Kidney function after exposure of rats to LC and/or Vit D

In groups 3-5, treatment with LC, Vit D, and LC+Vit D significantly reduced the increased serum glucose (p <0.05; Table 1, Fig. 3). According to the study’s findings, group 2 had significantly higher blood urea, serum creatinine, serum sodium, and serum potassium levels (p<0.05). When compared to group 2, the treatment of the
3.3 Evaluation of lipid peroxidation parameters

Before beginning treatment, the values of MDA and SOD also show a considerable increase (P<0.05).

<table>
<thead>
<tr>
<th>Parameters/groups</th>
<th>Group 1</th>
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<th>Group 3</th>
<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>3.8 ± 0.07</td>
<td>7.8 ± 1.07*</td>
<td>4.8 ± 0.08**</td>
<td>5.4 ± 0.45**</td>
<td>4.1 ± 0.58**</td>
</tr>
<tr>
<td>SOD</td>
<td>903.2 ± 3.45</td>
<td>1442.3 ± 6.42*</td>
<td>1334.2 ± 5.02**</td>
<td>1402.1 ± 5.08**</td>
<td>1301.2 ± 4.45**</td>
</tr>
</tbody>
</table>

3.3 Effect of LC and/or Vit D on inflammatory markers

Additionally, the untreated diabetic group (group 2) had significantly higher levels of TNF-α and IL-6. When animals in groups 3–5 were treated with LC and vitamin D, the production of TNF-α and IL-6 was significantly reduced as compared to group 2 (p<0.01; Table 3, Fig. 10).

<table>
<thead>
<tr>
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<th>Group 4</th>
<th>Group 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>21.8 ± 0.65</td>
<td>52.4 ± 2.60*</td>
<td>39.5 ± 1.48**</td>
<td>41.8 ± 1.55**</td>
<td>35.8 ± 1.68**</td>
</tr>
<tr>
<td>TNF-α</td>
<td>41.5 ± 2.56</td>
<td>87.6 ± 2.88*</td>
<td>49.6 ± 1.49**</td>
<td>52.3 ± 1.50**</td>
<td>46.2 ± 2.41**</td>
</tr>
</tbody>
</table>
10, 11).

### 3.4 Histological results

The pancreas in the non-DM group had a normal islet of Langerhans and normal morphology, according to the histological section. The DM group displayed a shrinking and deteriorating Langerhans islet. On the other hand, LC+Vit D groups showed enhanced islet of Langerhans morphological features (Fig. 12A, 12B, 12C).

![Figure 12A: Pancreas representative photomicrograph: The non-DM group demonstrates a normal Langerhans islet with a normal size (red arrow).](image)

![Figure 12B: Pancreas representative photomicrograph: The DM group displays a smaller, shrunken Langerhans islet (green arrow).](image)

### 4. Discussion

The disruptions in beta cell function brought on by the toxin are reflected in the effects of streptozotocin on glucose and insulin homeostasis. Initially, there are effects on glucose metabolism (both glucose oxidation and oxygen consumption), insulin production, and
glucose-induced insulin secretion [31–34].

![Image](image.png)

**Figure 12C:** Pancreas representative photomicrograph: LC+Vit D groups demonstrating an improvement in the size of the Langerhans islet (blue arrow).

In this investigation, rats treated with STZ had far higher serum glucose levels than the rats in the control group. During the course of the 16-week investigation, the body weight of the diabetic rats who were not receiving treatment decreased, according to the data collected. These findings imply that the growth retardation in these animals was brought on by the blockage of glucose uptake brought on by the absence of insulin after the streptozotocin injection. When LC and vitamin D were administered to diabetic rats, their body weight increased with time. The collected statistics were consistent with earlier research [35–36].

Blood glucose levels in diabetic rats treated with LC and vitamin D were significantly lowered. These findings are based on another research that looked at the impact of vitamin D on insulin secretion, sensitivity, and fasting blood sugar levels [37]. Vitamin D works to lessen the inflammatory processes that lead to diabetes mellitus and insulin resistance [38–39].

Furthermore, research has shown that vitamin D controls calcium trafficking in pancreatic β-cells, which in turn controls insulin synthesis, secretion, and sensitivity. Additionally, vitamin D directly affects pancreatic β-cell function by binding the active form of 1, 25(OH)2D to its receptor, the vitamin D receptor, which is expressed in pancreatic β-cells [40].

After receiving L-carnitine, there was a decrease in serum glucose levels. This could be explained by the fact that L-carnitine increases the utilization of glucose by peripheral tissues [41] or that carnitine supplementation may improve glucose homeostasis by influencing the expression of genes related to the insulin signaling pathway, altering the expression of gluconeogenic and glycolytic enzymes, controlling the intra-mitochondrial acyl-CoA/CoA ratio, and modifying the activity of the pyruvate dehydrogenase complex [42].

According to certain research, supplementing with L-carnitine may influence and heighten the sensitivity of insulin receptors [43–44]. Through the overproduction of active carbonyl intermediates, the decrease in antioxidant enzyme activity, the creation of lipid peroxides and radicals, and the nonenzymatic glycosylation of proteins, oxidative stress is a significant factor in the pathophysiology of DN. Enzymes that are antioxidants guard against damage to cells and tissues. It is thought that DN is caused by an imbalance between the generation of reactive oxygen species (ROS) and antioxidants [45–46]. In this study, the induction of diabetes led to a considerable increase in MDA, SOD, urea, creatinine, sodium, and potassium levels. This may be related to the development of diabetic nephropathy and the pathophysiology of the rat kidney.

MDA and SOD levels were lowered in diabetic rats treated for 16 weeks with LC, Vit D, and LC+Vit D. Vit D supplementation markedly raised SOD and MDA levels. Depending on the type of cell, vitamin D can either inhibit oxidative stress or limit the production of free radicals via attaching to the nucleus's vitamin D receptor [47]. This vitamin alters antioxidant enzymes to provide its antioxidant effects and protects the cell membrane by preventing lipid peroxidation [48].

In the early stages, prior to the activation of mechanisms against oxidative stress, it functions as a scavenger of free radicals [49–50]. By lowering the level of oxidative stress, L-carnitine can enhance these cells' performance and efficiency [51]. When reactive oxygen species (ROS) are produced in excess, proinflammatory mediators are more likely to be expressed and the immune-inflammatory response is altered. This can lead to the release of inflammatory cytokines like TNF-α and interleukin (IL), which can trigger apoptosis and worsen inflammation [52–53].

Attenuation of advanced glycation end formation follows attenuation of NFκB activation when ROS production is reduced and antioxidant enzymes are activated. The expression of NFκB-regulated genes, such as IL-6, is regulated when the protein is attenuated. Diabetes, ROS, and DN all raised the level of IL-6 in the untreated diabetic group [14]. The administration of LC and vitamin D to diabetic rats resulted in a significant reduction in IL-6 and inflammatory levels, potentially linked to the enhancement of antioxidant enzymes. Our data are consistent with other organizations' earlier research [45–46, 54]. The primary organ impacted in this diabetic model is the pancreatic islet of Langerhan cells, which are destroyed by streptozotocin and lose their
functionality, impairing the synthesis and release of insulin [55].

In comparison to non-DM groups, the diabetic control rat's pancreas section histology revealed significant damage to the islet of Langerhans, as well as shrunken islet cells and a smaller islet overall. The LC+Vit D group's islets of Langerhans became larger than those of the control DM group, indicating some improvements in the pancreatic histology. Remarkably, the lower blood glucose level in the LC+Vit D group as compared to the DM group may imply that beta cells are functioning better in manufacturing and secreting insulin, which is supported by the improved histological finding. The collected data are consistent with our theory that vitamin D and LC have a protective impact on the kidneys against inflammatory and oxidative damage, which may be related to their combined antioxidant and anti-inflammatory properties.

Conclusion

The current study shows that LC and vitamin D have anti-oxidative and anti-inflammatory qualities that lower lipid peroxidation and IL-6 in diabetic rats. Antioxidant enzymes like SOD also showed an increase in activity. The detrimental biochemical and immunohistochemical effects of diabetes mellitus were lessened and ameliorated by vitamin D, most likely as a result of increased antioxidant efficiency, which also increased insulin sensitivity and secretion. In addition to other hypoglycemic medications, L-carnitine can be a helpful dietary supplement and medication. It lowers fasting plasma glucose levels and improves renal function by lowering urea and creatinine levels in the blood. We therefore hypothesize that LC and Vit D may act on diabetic nephropathy in diabetic rats by simultaneously mitigating inflammation and oxidative stress.

Conflict of interest

The author confirms that there are no conflicts of interests.

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References


