ORIGINAL ARTICLE

# EFFECTS OF SIMVASTATIN AND ALPHA-TOCOPHEROL ON DISTURBED NERVE CONDUCTION IN OBESE SPRAGUE DAWLEY RATS

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**Background:** The incidence of obesity is increasing worldwide. The neuropathy associated with obesity, that is evident from disturbed nerve conduction, is one of the complications for which a number of treatment options are being considered. In this study, Simvastatin, a hydroxyl methyl glutaryl coenzyme A reductase inhibitor and alpha-tocopherol, a dietary antioxidant are compared for their effects on sciatic nerve conduction velocity. **Objectives:** To compare the effects of Simvastatin and alpha-tocopherol on sciatic nerve conduction velocity in obese rats. **Methods:** The study was a Randomised control trial conducted from December 2008 to November 2009. One hundred and twenty adult male Sprague Dawley rats were divided into four groups with 30 rats in each group. One group of rats was taken as control with normal diet while other three groups were given high fat diet (HFD) for the whole study period. Along with the high fat diet, group III and group IV were given Simvastatin and alpha-tocopherol supplemented diet respectively. At the end of study, conduction velocity of sciatic nerve was determined with the help of PowerLab® data acquisition system. **Results:** The three groups with HFD showed more than 25% increase in weight at the end of study compared to control group. The control group with high fat diet (Group II) showed decreased sciatic nerve conduction velocity when compared with control (Group I). Both the groups that were given Simvastatin and alpha-tocopherol each showed improvement in sciatic nerve conduction velocity ($p<0.001$) after four weeks when compared with the group that was given HFD without any supplementation. However with alpha-tocopherol, the nerve conduction velocity was improved more significantly. **Conclusions:** Simvastatin and alpha-tocopherol both are effective for improving sciatic nerve conduction velocity in HFD induced obesity.

**Keywords:** Obesity, Simvastatin, nerve conduction velocity, alpha-tocopherol, High Fat Diet

## INTRODUCTION

The increased prevalence of obesity worldwide is a serious public health as well as economic problem.[1] In Pakistan also, the prevalence is increased to 28% and the incidence in more than thirty years age group is 47%.[2] Genetic, metabolic, behavioural and environmental factors contribute in the aetiology of obesity.[3] The rapid increase in the incidence of obesity is related with increased energy intake and physical inactivity, both of which are influenced by socioeconomic status and environmental factors.[2] Dietary fat is considered to be one of the most important environmental factor in development of obesity, therefore high fat diet induced obesity may serve as effective model to investigate the pathophysiological mechanisms of obesity.[4]

A high BMI is a well recognised risk factor for median nerve sensory conduction slowing, and carpal tunnel syndrome.[5] Obese individuals are found to have decreased compound action potential amplitude of tibial and peroneal nerves and decreased sensory action potential amplitude of median, ulnar and sural nerves as compared to the non-obese individuals.[6]

There are a number of possible explanations for the neuropathy of obesity. Accumulation of lipids is one of the causes which results in decreased endoneurial blood flow leading to impaired sensory and motor function.[7] The peroxynitrite and superoxide levels are also found to be raised in subjects affected by impaired nerve function.[8] These abnormalities cause subclinical involvement of sensory nerve fibres leading to functional and not structural neuronal abnormalities.[6] In the pathogenesis of neuropathy, the potential contribution of increased oxidative stress was recognised several decades ago.[9] This is because of increase in free oxygen radicals and decrease in anti-oxidant defences. In addition superoxide dismutase which has an important role in neutralising superoxide radicals may be reduced in the peripheral nerve tissue thus enhancing the formation of free radical formation.[10] Slowing of the nerve conduction velocity is the earliest abnormality that occurs in patients with obesity and pre diabetes.[11]

In patients with obesity and at the stage of impaired glucose tolerance in the absence of clinical diabetes, there is endoneural microangiopathy

resulting in decreased endoneural oxygen tension and decreased endoneural blood flow.[12] Vitamin E can inhibit the free radical induced endoneural damage. It can also improve the impaired defences against the toxic oxygen radicals.[11] The role of alpha-tocopherol and other combinations of anti-oxidants is proved for partial prevention of decreased nerve conduction velocity in obese subjects.[13]

Among the treatment strategies for obesity and the associated neuropathy, there is a role of lipid lowering drugs like statins, bile acid sequestrants, fibrates and cholesterol absorption inhibitors.[14] There can be a possible role of antioxidants because literature supports the involvement of free oxygen radicals in development of neuropathy of obesity.[15] Alpha-tocopherol is the FDA approved dietary antioxidant. It can inhibit the free radical induced endoneural damage. It can also improve the impaired defences against the toxic oxygen radicals.[11] The role of alpha-tocopherol and other combinations of anti-oxidants is proved for partial prevention of decreased nerve conduction velocity in case of diabetic rats.[12] But its role in neuropathy of obesity is not known.

Simvastatin, an HMG Co-A reductase inhibitor, and alpha-tocopherol, a dietary antioxidant were compared in this study for their effects on disturbed conduction velocity of sciatic nerves in obese rats.

## MATERIAL AND METHODS

This was a randomised control trial, done at Islamic International Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad from December 2008 to November, 2009. One hundred and twenty male, adult Sprague Dawley rats weighing 200±25 grams were kept in animal house of NIH. Animals were placed in cages of 2×3 feet size, ten animals per cage. Room temperature of 24±2 ºC along with twelve hour light and twelve hour dark cycle was maintained. Rats were subjected to an adaptation period of one week in which the food intake and body weight was monitored before any intervention according to the experimental plan. Rats were then followed throughout the study for adequate intake of food and water and weight gain by weighing them weekly.

Rats were divided into four groups with thirty rats in each group (n=30). Group I served as a control and was given the normal standard rat diet. Group II was fed with a high fat diet for ten weeks with no intervention. High fat diet contained 230 gm/Kg diet of butter fat in addition to the standard contents of rat diet.[15] Group III was fed with a high fat diet for first six weeks and then given subcutaneous Simvastatin in the dose of 5 mg/Kg/day for next four weeks.[16] High fat diet was continued along with the drug. The dose calculated for each rat was 1 mg/day. Group IV was fed with a high fat diet for first six weeks and then alpha- tocopherol supplemented diet (300 mg/Kg diet) for next four weeks along with the high fat diet.[14] Diseased rat or any rat that developed disease during the course of study was excluded from the study. At the end of ten weeks of study, Sciatic nerve was dissected from each rat and the conduction velocity of nerve was calculated with the help of power lab data acquisition system. Statistical analysis was done with the help of SPSS-16. Mean values and standard deviations were calculated for all the data. Confidence interval was taken as 95%. For multiple comparisons, a one way analysis of variance (ANOVA) was used. When ANOVA showed significant difference, post-hoc analysis was performed with the Newman-Keuls multiple range test using SPSS and $p<0.05$ was taken as significant.

## RESULTS

All rats remained alive, healthy and active throughout the period of study. The initial weight of all the rats was noted. After ten weeks of study period, conduction velocity of sciatic nerve was determined using PowerLab® data acquisition system. The mean weight of control with normal diet was 214.57 gm with increase of 24%. The mean weight of control with HFD was 301.1 gm with increase of 75%. The weights of group III (HFD and Simvastatin) and group IV (HFD and alpha-tocopherol) on day 70 were 289.63 gm and 291.53 gm with increase of 68% and 69% in weight respectively (Table-1).

**Table-1: Weight of Sprague Dawley rats in groups at the start and end of study**

| Groups | Initial weight (g) | Final weight (g) | % increase |
|---|---|---|---|
| Group I (Normal diet) | 172.73 | 214.57 | 24% |
| Group II (HFD) | 172.2 | 301.1 | 75% |
| Group III (HFD+Simvastatin) | 172.9 | 289.63 | 68% |
| Group IV (HFD+alpha-tocopherol) | 172.83 | 291.53 | 69% |

The mean sciatic nerve conduction velocity of controls with normal diet was 88.67±5.35 m/Sec. The controls with HFD had the mean conduction velocity of 30.5±8.36 m/Sec. The comparison showed highly significant difference ($p$=0.000), among the values. The mean conduction velocity of group III (HFD and Simvastatin) and group IV (HFD and alpha-tocopherol) were 61.93±13.3 m/Sec and 73.37±16.22 m/Sec respectively. The values were compared with control with HFD. The comparison showed highly significant difference ($p$=0.000) among the values with highest sciatic nerve conduction velocity in group IV with alpha-tocopherol supplementation (Figure-1).

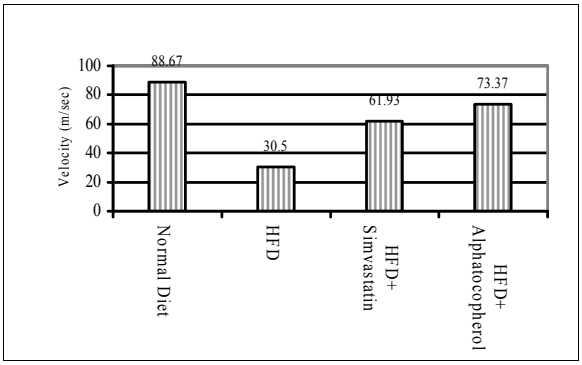


**Figure-1: Effects of HFD alone and in combination with Simvastatin and alpha-tocopherol on sciatic nerve conduction velocity of Sprague Dawley rats**

## DISCUSSION

Obesity and its complications are well recognised worldwide. This study was planned to compare the treatment options for one of the complications of obesity that is disturbance in nerve conduction which contribute to the morbidity and mortality associated with obesity. Effects of Simvastatin which belongs to HMG-CoA reductase inhibitor class of lipid lowering drugs and alpha-tocopherol which is a dietary antioxidant are compared for their effects in improving decreased nerve conduction in rats. In the present study, ninety-days-old male Sprague Dawley rats after ten weeks of high fat diet, gained more than 25% of weight compared to control with normal diet. This finding is similar to a study by Levin and Dunn-Meynell in which HFD was given to Sprague Dawley rats for 10 weeks.[17]

The parameter of this study was sciatic nerve conduction velocity that is decreased in obese subjects who often complaints of numbness, paresthesias and tingling in the extremities along with impaired sensations. Peripheral neuropathy can be the result of genetics, chronic diseases, environmental toxins, nutritional deficiencies or side effects of certain medications.[18] The cause of such neuropathy in obesity is the excess adipose tissue infiltration[7] and accumulation of reactive oxygen radicals with impaired antioxidant defence system. Oxidative stress is stated to be the biochemical trigger for sciatic nerve dysfunction and decreased endoneural blood flow in case of rats.[8] In the treatment options for peripheral neuropathy several vitamins including methylecobalamine, folate, pyridoxine and alpha-tocopherol have been used.[18]

The relationship of obesity and nerve conduction deficits was observed in a study conducted by Miscio *et al*, in which non-diabetic obese individuals even without symptoms suggestive of peripheral neuropathy were studied for nerve conduction deficits.[6] The obese group showed significantly decreased compound muscle action potential amplitude of tibial and peroneal nerves and decreased sensory action potential amplitude of all nerves. This showed a subclinical impairment of nerve conduction in obese individuals. It is reported that HFD fed female mice after 16 weeks developed obesity along with motor and sensory nerve conduction deficits.[13] This deficit in nerve conduction is similar to our study in which impaired nerve conduction developed after 10 weeks of HFD. The values of nerve conduction velocity are, however, different in the two studies because the rats were used in our study instead of mice. In another study of Coppey *et al*, male Sprague Dawley rats were studied for effects of anti-oxidants on impaired nerve conduction, the value of conduction velocity in sciatic nerve of control group was less compared to this study.[19] This difference can be due to the different methodology used, i.e., a non-invasive method in which the sciatic nerve of rat was stimulated by Grass S44® Stimulator. We used an invasive method after sciatic nerve dissection from the rat and the conduction velocity was automatically calculated on PowerLab®.

In the study by Oltman, vascular and neural dysfunction in sciatic nerves of Zucker diabetic fatty and Zucker rats was studied.[15] The rats in that study were obese and were glucose intolerant. The mean value of sciatic nerve conduction velocity was again less than our study because of different rat strain that was used and non-invasive method for determination of sciatic nerve conduction velocity.

Simvastatin, a lipid lowering drug used in our study has not been found for being studied directly as a treatment option for peripheral neuropathy but in a study conducted by Delbosc *et al* on male Sprague Dawley rats, it was proved that simvastatin markedly reduced the production of toxic oxygen radicals thus reducing the oxidative stress independent of its cholesterol lowering effects.[20] Therefore, we used this drug in a comparison with alpha- tocopherol for improving the nerve conduction velocity, one of the causes of which may be the involvement of free oxygen radicals. This could be a better treatment option for obese individuals who are having deranged lipid profile along with the impaired nerve conduction velocity. The results of this study after four week treatment with Simvastatin showed significant improvement of sciatic nerve conduction velocity of rats as compared to the control group who received HFD throughout the study period.

The alpha-tocopherol has been found beneficial in different studies for improving the peripheral neuropathy. In this study, the group of rats that was given alpha-tocopherol supplemented diet along with HFD for a period of four weeks showed a marked improvement in sciatic nerve conduction

velocity when compared to the control with HFD. However the improvement was not up to the normal values of control group with normal diet. In a double-blind, placebo –controlled trial, subjects with diabetic peripheral neuropathy were given vitamin E for six months. There was significant improvement in median and tibial nerve conduction velocity in the vitamin E group as compared to placebo.[18] In the study conducted by Tutuncu *et al* type II diabetic patients with clinically documented peripheral neuropathy were given vitamin E supplements for 6 months. The supplementation resulted in significant increase in median motor nerve conduction velocity.[21] In a study, the blood levels of various non-enzymatic anti-oxidants were seen in patients of hereditary optic neuropathy.[22] The conclusion of that study showed decreased alpha-tocopherol levels due to increased consumption as an antioxidant. That study suggested alpha-tocopherol to be the main scavenger molecule to react against the toxic oxygen radicals in patients of optic neuropathy. The study conducted by Khanna *et al* confirms the neuroprotective properties of anti oxidant vitamin E against the toxic oxygen radical mediated injury to neuronal cells.[23] In another study it was proved that the high dose alpha-tocopherol supplementation prevents the derangement in sciatic and tibial nerve conduction velocities in diabetic rats.[24]

One objective of this study was to compare the effects of Simvastatin and alpha-tocopherol on derangements in sciatic nerve conduction velocity in obese rats. While the two agents significantly improved the conduction velocity of sciatic nerve individually, the comparative analysis showed that alpha-tocopherol has caused even more improvement in the deranged values when compared with the control group of rats who were given HFD without any intervention. So it can be suggested that in obesity, the altered lipid levels that predispose an obese individual to a number of complications including the neuropathy can be improved by the use of Simvastatin. Alpha-tocopherol on one hand can markedly improve the decreased nerve conduction velocity in obese individuals while it has less effects on lipid profile when compared with Simvastatin. It is also evident that in neuropathy of obesity, there could be more involvement of the toxic oxygen radicals than the excessive lipid accumulation in sites particularly around the nerves.

## CONCLUSIONS

1. HFD induced obesity resulted in decrease in sciatic nerve conduction velocity.
2. Simvastatin and alpha-tocopherol both were effective for improving the sciatic nerve conduction velocity in obese rats but it was improved more significantly by alpha-tocopherol.

## REFERENCES


1. Eckersley ME, Losing the battle of the bulge: causes and consequences of increasing obesity. Med J Aust 2001;174:590–2.
2. Shafi S, Rao MH, Soomro IB. The effect of life style and socio-economic factors in the development of obesity in young adults. Pak J Med Res 2004;43(2):65–9.
3. Knobler H, Malnick SDH. The medical complications of obesity. Q J Med 2006;99:565–79.
4. Nishikawa S, Yasoshima A, Doi K, Nakayama H, Uetsuka K. Involvement of sex, strain and age factors in high fat diet induced obesity in C-57BL/6J and BALB/cA mice. Exp Anim 2007;56:263–72.
5. Ylitalo KR, Sowers M, Heeringa S. Peripheral neuropathy in individuals with cardiometabolic clustering and obesity. Diabetes Care 2011;34:1642–7.
6. Miscio G, Guastamacchia G, Brunani A, Priano L, Baudo S, Mauro A. Obesity and peripheral neuropathy risk: a dangerous liaison. J Peripher Nerv Syst 2005;10:354–8.
7. Hilton TN, Tuttle LJ, Bohnert KL. Mueller MJ, Sinacore DR. Excessive adipose tissue infiltration in skeletal muscle in individuals with obesity, diabetes mellitus and peripheral neuropathy: Association with performance and function. Phys Ther 2008;88:1336–44.
8. Ziegler D, Sohr CGH, Nourooz-Zadeh J. Oxidative stress and antioxidant defense in relation to the severity of diabetic polyneuropathy and cardiovascular autonomic neuropathy. Diabetic Care 2004;27:2178–83.
9. Low AP, Nickander KK, Tritschler HJ. The roles of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. Diabetes 1997;46:38–42.
10. Stahl W, Sies H. Antioxidant defence: vitamin E and C and carotenoids. Diabetes 1997;46:14–8.
11. Pace A, Giannarelli D, Galie E, Savarese A, Carano S, Della GM, *et al*. Vitamin E neuroprotection for cisplatin neuropathy: a randomized, placebo controlled trial. Neurology 2010;74:762–6.
12. Cameron NE. Metabolic and vascular factors in the pathogenesis of diabetec neuropathy. Diabetes 1997;46:31–7.
13. Obrosova IG, Inytska O, Lysogubov VV, Pavlov IA, Mashtalir N, Nadler JL, *et al*. High fat diet induced neuropathy of pre-diabetes and obesity. Effects of "healthy" diet and aldose reductase inhibition. Diabetes 2007;56:2598–608.
14. Sparrow CP, Burton CA, Hernandez M, Mundt S, Hassing H, Patel S, *et al*. Simvastatin has anti-inflammatory and antiatherosclerotic activities independent of plasma cholesterol lowering. Arterioscler Thromb Vasc Biol 2001;21:115–21.
15. Oltman CL, Coppey LJ, Gellett JS, Davidson EP, Lund DD, Yorek MA. Progression of vascular and neural dysfunction in sciatic nerves of Zucker diabetic fatty and Zucker rats. Am J Physiol Endocrinal Metab 2005;289:113–22.
16. Tawfik HE, El-Remessy AB, Matragoon S, Ma G, Caldwell RB, Caldwell RW. Simvastatin improves diabetes induced coronary endothelial dysfunction. J Pharmacol Exp Tther 2006;319:386–95.
17. Levin BE, Dunn-Meynell AA. Defense of body weight depends on dietary composition and palatability in rats with diet induced obesity. Am J Physiol Regulatory Integrative Comp Physiol 2002;282:46–54.
18. Head KA. Peripheral neuropathy: Pathogenic mechanisms and alternative therapies. Alternative Medicine Review 2006;11(4):294–329.
19. Coppey LJ, Gellett JS, Davidson EP, Dunlap JA, Lund DD, Yorek MA. Effect of antioxidant treatment of streptozotocin-induced diabetic rats on endoneurial blood flow, motor nerve conduction velocity and vascular reactivity of epineurial arterioles of the sciatic nerves. Diabetes 2001;50:1927–37.
20. Delbosc S, Cristol JP, Descomps B, Mimran A , Jover B. Simvastatin prevents angiotensin II induced cardiac alterations and oxidative stress. Hypertension 2002;40:142–7.

21. Tutunco NB, Bayraktar M, Varli K. Reversal of defective nerve conduction with vitamin E supplementation in type 2 diabetes. Diabetec Care 1998;21:1915–8.
22. Klivenyi P, Karg E, Rozsa C, Horvath R, Komoly S, Nemeth I, *et al*. Alpha-tocopherol/ lipid ratio in blood is decreased in patients with Leber's hereditary optic neuropathy and asymptomatic carriers of the 1178 mtDNA mutation. J Neurol Neurosurg Psychiatry 2001;70:359–62.
23. Khanna S, Roy S, Parinandi NL, Maurer M, Sen CK. Characterization of the potent neuroprotective properties of the natural vitamin E á-tocotrienol. J Neurochem 2006;98:1474–86.
24. Van Dam PS, Brvenboer B, Van Asbeck BS, Marx JJ, Gispen WH. High rat food vitamin E content improves nerve function in streptozotocin-diabetic rats. Eur J Pharmacol 1999;376:217–22.

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