

Nerve Conduction Velocity of Sciatic Nerve in High Fat Diet Induced Obesity in Rats: Effect of Corn Oil and Omega 3 Fatty Acids Supplement

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Abstract: Background: Obesity is a major susceptibility factor leading to the development of various conditions of the metabolic syndrome. In obese rats, slowing of motor nerve conduction velocity was observed. Fatty acids metabolism disturbance is very important in the occurrence of peripheral neuropathy. The aim of this work is to consider the role that balanced diets high in omega 6&9 PUFA (corn oil) or supplying rats with omega 3, play in modulating the impaired nerve function in obese rats. **Methods:** Thirty two adult male albino rats were randomly assigned to receive normal chow (NC) (n=8) or high fat diet HFD (n=24), for 12 weeks. After 12 weeks, body weight and body mass index(BMI) were measured and the NC group(n=8) continue their normal chow diet, **Group 1 (NC)** and served as a control group and the obese rats were randomly divided into 3 groups, 8 rats each: **Group 2:** Ob + HFD group, they continue their high animal fat diet, **Group 3:** Ob+HFD + corn oil group, they are obese rats received high fat diet containing corn oil and **Group 4:** Ob + HFD + Omega 3 group, they are obese rats, fed high animal fat diet supplemented with omega 3 (0.4 g/kg) daily. After five weeks, the final body weight was measured and BMI was calculated and blood samples were collected for measuring fasting plasma glucose level and insulin level and homeostasis model assessment of insulin resistance (HOMA-IR) test were evaluated. Plasma cholesterol, triglycerides and free fatty acids (FFAs) were measured. The rats were then killed and sciatic nerves were carefully dissected for measuring the nerve conduction velocity (NCV). Superoxide dismutase activity (SOD), malondialdehyde (MDA) and tumor necrosis factor alpha (TNF α) were estimated in the nerve tissue of the 4 groups. **Results:** The results of this study showed a significant increase of body weight (gm) and BMI (kg/m²) in high fat diet group ($p < 0.05$) after 12 weeks of the start of the diet when compared to the control group (NC). There were significant elevations in the final weight (gm) and BMI (kg/m²), a significant elevation in insulin level (μ IU/l) and HOMA-IR test, a significant increase in nerve malondialdehyde (MDA), and tumor necrosis factor alpha (TNF α) and a significant decrease in superoxide dismutase activity (SOD) and nerve conduction velocity (NCV) (m/s) after 5 weeks of high fat diet in (Ob+HFD) group, when compared to NC group. Changing diet composition for 5 weeks in Ob+ HFD+corn oil and Ob+HFD+omega 3 groups, did not induce any significant variation in body weight, BMI, or fasting blood glucose level as compared to Ob+HFD group. Insulin level (μ IU/l) and HOMA-IR test were significantly decreased in Ob+ HFD+corn oil and Ob+HFD+omega 3 groups compared to Ob+HFD group. Plasma cholesterol levels (mg/dl), triglycerides (mg/dl), and free fatty acids (FFA) (mmol/l) were significantly decreased after 5 weeks diet in Ob+ HFD+corn oil or Ob+HFD+ Omega 3 groups when compared to mean values of Ob+HFD group. Tissue malondialdehyde (MDA) and tumor necrosis factor alpha (TNF α) were significantly decreased but superoxide dismutase (SOD) activity was significantly increased in Ob+HFD+corn oil and Ob+HFD+omega3 groups compared to Ob+HFD. NCV(m/s) in Ob+HFD+ corn oil group was significantly increased compared to Ob+HFD and their values in Ob+HFD+ corn oil group showed no significant variation as compared to NC group. While there was a significant increase in NCV in Ob+ HFD+Omega 3 group as compared to Ob+ HFD group, there was still a significant decrease compared to NC group. **Conclusion:** The results of this study may have important clinical and speculative implications. Corn oil or omega 3 supplementation may be effective in obesity induced neuropathy. The mechanism of their effects is multifactorial including improving insulin sensitivity, correction of dyslipidemia, reducing oxidative stress and an anti-inflammatory effect. This possibility should be carefully considered and examined in future trials of essential fatty acid supplementation.

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Key words: nerve conduction velocity, obesity, oxidative stress, inflammation, corn oil, omega3, insulin resistance.

1. Introduction

Obesity is a pathological condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to

reduced life expectancy and/or increased health problems [1]. Obesity is a major susceptibility factor leading to the development of various conditions of the metabolic syndrome. A recent study from the

World Health Organization approximates that, globally, 1.6 billion adults are overweight with at least 400 million adults classified as obese [2].

In obese rats, slowing of motor nerve conduction velocity was observed. Obese Zucker rats develop neural deficits independently of hyperglycemia [3]. Evidence for the development of neuropathic changes at the prediabetic stage, prior to development of overt hyperglycemia and diabetes mellitus, is emerging from both clinical [4] and experimental [5] studies. The pathophysiologic basis of this relationship is not well understood. A number of pharmacological agents that showed promise in animal studies have been withdrawn from clinical trials because of a lack of efficacy or adverse side-effects [6].

The induction of obesity may be performed in animals by neuroendocrine, dietary or genetic changes. [7]. High-fat diet (HFD)-fed rats with alimentary obesity and hyperinsulinemia, develop nerve conduction velocity deficit, therefore, represent an ideal model for evaluating effects of changing dietary composition on manifestations of neuropathy[8]. The contributions of insulin resistance, hypertriglyceridemia and/or increased nonesterified fatty acids (NEFA), and hypercholesterolemia to this condition remain unknown [9].

Fatty acids metabolism disturbance is very important in the occurrence of peripheral neuropathy. Low plasma omega-6 and omega-3 fatty acids levels were associated with accelerated decline of peripheral nerve function with aging [10]. Evidence has emerged suggesting that both omega-6 and omega-3 fatty acids are also important for peripheral nerve health and function [11].

The administration of unsaturated fatty acids especially omega-3 has gained considerable attention recently. The effect of omega-3 fatty acid on the treatment of coronary arteries atherosclerosis has been shown [12]. Consumption of Omega-3 fatty acids in animal models could be effective in restoring nerve conduction velocity [13]. Also, omega 6&9 poly unsaturated fatty acids (PUFA) supplied from corn oil has also been reported to be beneficial in systemic diseases, such as hypertension [14], cardiovascular disease [15] and cancer [16], however, little is known about the possible benefits that dietary omega-3 or omega 6&9 may have for the nerve conduction disorders in obesity. In this study, we propose that the lipid changes associated with obesity might partially explain the reported neural dysfunction. We consider the role that balanced diets high in omega 6&9 (corn oil) or supplying rats with omega 3, can have in modulating the impaired nerve function in obese rats.

2. Material and methods

Experimental animals and groups

Thirty two adult male albino rats of body weight 80-100 gm, 3 to 4 weeks old, were included in this study. The rats were supplied by the Animal House Unit of Kasr Al-Ainy, Faculty of medicine, Cairo University, housed in cages at room temperature with normal light & dark cycle. The rats were randomly assigned to receive normal chow; control group (NC) [16] (n=8), and HFD [17] (n=24), for 12 weeks. The composition of the different experimental diets used is shown in table 1.

Table 1: The composition of experimental diets

Ingredients Contents (g/kg diet)	HFD (animal fat)	HFD (animal fat) +corn oil	Control diet (NC)
Carbohydrates			
-Corn starch	100	100	480
-Sucrose	100	100	100
Fats			
- Soybean oil	50	50	50
- Animal fat	500	300	120
- Corn oil		200	
Protein			
- Casein	190	190	190

This study was carried out in the Physiology and Biochemistry Departments, Faculty of Medicine, Cairo University. After 12 weeks, the rats were classified into:

Group 1: they are the normal rats, received normal chow and served as normal control. They continued free access to laboratory rat chow and tap water and received 4 mL of normal saline through gavage daily for another 5 weeks (NC). The HFD-fed rats were divided into 3 groups, 8 rats each:

Group 2: Ob + HFF group, they are obese rats those continue their high animal fat diet and received 4 mL of normal saline through gavage for 5 weeks.

Group 3: Ob +HFD+ corn oil group, they are obese rats, received high fat diet. In this group of rats, corn oil was administered as 20% of the diet, replacing same percent of animal fat and received 4 mL of normal saline through gavage daily. Corn oil contains high omega 6&9 polyunsaturated fat [18]. FA analysis of corn oil showed that corn oil contained mono unsaturated fatty acids (27.576%), poly unsaturated fatty acids (PUFA) (57.36%),

omega 6 (58%) and omega 9 (28%), fatty acids in the percent shown in table 2 [19].

Table 2: The fatty acid analysis of corn oil

	Saturated fatty acids	MUFA	PUFA	Omega 3	Omega 6	Omega 9
Corn oil	12.948	27.576	57.36	1	58	28

Values are expressed as weight percent (%) of total fat.

Group 4: Ob + HFD + Omega 3 group, they are obese rats, fed high fat diet rich in saturated animal fat for 5 weeks and received omega 3 (0.4 g/kg BW) daily through gavage [20]. Each Omega-3 capsule contains Fish Oil (Omega-3 Docosahexaenoic acid DHA 12%, eicosapentaenoic acid EPA 18%) 999 mg, Vitamin E 1 mg, Gelatin (food grade) 371 mg. Alpha tocopherol is included in the capsule to avoid auto-oxidation.

Weight measurements:

All rats were weighed in grams and naso-anal lengths were measured in cm at the end of the 12-weeks study and at the end of the 5 weeks of different diet trial. The body mass index (BMI) was calculated (by dividing the body weight in kilograms by the length in meters squared) [21] at the end of the study period (after 12 weeks of NC or HFD and after 5 weeks of corn oil or omega 3 supplementation).

Rats were fasted overnight for at least 6 hours and blood samples were obtained by introducing a fine heparinized capillary tube at the inner canthus of the eye into the venous plexus. The blood samples were delivered into centrifuge tubes to which anticoagulant was added then centrifuged at 10,000 rpm for 20 minutes and plasma was separated and stored at -70°C for measuring fasting blood glucose level and insulin level and HOMA-IR test were evaluated. Plasma cholesterol, triglycerides and FFAs were measured. The rats were then killed and sciatic nerves were carefully dissected for measuring of nerve conduction velocity, tissue superoxide dismutase activity (SOD), malondialdehyde (MDA) and tumor necrosis factor alpha (TNF α).

Biochemical measurements

Measurement of fasting plasma glucose level

Plasma glucose in blood samples was measured using oxidase- peroxidase method [22].

Measurement of plasma insulin

Plasma insulin levels were analyzed using enzyme-linked immunosorbent assay ELISA (Dako, Carpinteria, CA) according to the manufacturer's instructions [23].

HOMA-IR test

To estimate insulin resistance, the homeostasis model assessment for insulin resistance (HOMA-IR: insulin resistance index) [24] was used, calculated as the product of fasting insulin (in μ IU) and fasting glucose (in mmol/l) divided by 22.5. A lower index indicates greater insulin sensitivity.

FFA detection

FFA was measured in plasma samples using Free Fatty Acid Quantification Kit supplied by Abcam USA according to manufacturer guide [25].

Measurement of lipid

Plasma total cholesterol was assayed as described by Siedel *et al.* [26], while the protocols of Jacobs and Van Denmark [27] was adopted for the determination of triglycerides (TAG).

Measurement of MDA

To measure the MDA concentration, 100 mg of sciatic nerve tissue in 1 mL PBS, pH 7.0 was homogenized with micropestle in microtube. 20 % TCA was added to nerve homogenate to precipitate the protein, and centrifuged. Supernatants were collected and thiobarbituric acid (TBA) solution was added to the supernatants. After boiling for 10 minutes in water bath, the absorbance was measured. Concentration of MDA in supernatants of nerve homogenate was calculated using the standard curve [28].

Measurement of SOD activity

Superoxide dismutase (SOD) activity in nerve homogenate was measured through the inhibition of nitroblue tetrazolium (NBT) reduction by O₂⁻ generated by the xanthine/xanthine oxidase system. One SOD activity unit was defined as the enzyme amount causing 50% inhibition in 1 mL reaction solution per milligram tissue protein and the result was expressed as U/mg protein [29].

Measurement of TNF- α

TNF- α was measured by in nerve tissues using ELISA (quantikine R&D system USA) according to the manufacturer's instructions [30].

Nerve conduction velocity measurements:

Electrophysiological Recording:

The Sciatic nerve was mounted in a nerve chamber designed for recording of action potential from isolated nerve. It contains 15 stainless wire electrodes. The nerve was dissected free without any muscles remnants. About 2 cm of the nerve was positioned over the electrodes and embedded in paraffin oil to maximize signal amplitude and prevent drying. The proximal part of the nerve was stimulated by 2 platinum stimulating hook electrodes and the recording electrode was placed 1 cm apart from the stimulating one.

Electrophysiological measurements were performed using an AD instruments Power Lab 4/25

stimulator and Bio AMP amplifier followed by a computer assisted data analysis. Sciatic nerves were stimulated with square wave pulses of 200 μ sec duration at 1-10 volts for conduction velocities. Conduction velocity is measured by dividing the distance between the stimulating and recording electrodes by latent period, which is the time elapsed between the application of stimulus until the peak of the maximum compound action potential(CAP) [31].

Statistical analysis:

Data were analyzed using the statistical package SPSS version 15. Values were expressed as mean + standard deviation (SD). Comparisons between groups were done using analysis of variance (ANOVA) with multiple comparisons post hoc test in normally distributed quantitative variables while non para metrical Mann- Whitney test was used for non normally distributed quantitative variables. *P*-values less than 0.05 were considered as statistically significant [32].

3. Results

Effect of 12 weeks of HFD on body weight and BMI in rats:

The results of this study showed a significant increase of body weight (gm) and BMI (kg/m^2) in high fat diet group ($p < 0.05$) when compared to control group (NC) after 12weeks of the start of the diet, indicating presence of obesity in HFD group (Table 3, Figures 1,2).

Effect of 5 weeks of HFD (without supplementation) on body weight and BMI:

The results reported a significant elevation in the mean values of final weight measurements (gm) and BMI(kg/m^2) in rats after 5weeks of high animal fat diet (Ob+HFD), when compared to corresponding values of normal chow group(NC).(Table 4, Figures 3,4)

Effect of 5 weeks of HFD on plasma glucose, insulin, HOMA-IR in obese rats:

Table 5 and figures 5-7 show that HFD yielded an insignificant elevation in the mean value of fasting blood glucose level (mmol/l), while there was a significant elevation in insulin level ($\mu\text{IU}/\text{l}$) and HOMA-IR test after 5weeks of HFD compared to NC group. This reflects the effect of HFD on induction of insulin resistance.

Effect of 5 weeks of HFD on cholesterol levels, triglycerides and free fatty acids.

When observing levels of plasma cholesterol levels (mg/dl), triglycerides (mg/dl), and free fatty acids (mmol/l) after 5weeks of high animal fat diet (Ob+HFD) in table 6 and figures 8-10, the present results recorded a significant elevation in their plasma levels compared to NC group, denoting the effect of

obesity and HFD on dyslipidemia and elevation of plasma lipid levels.

Effect of 5 weeks of HFD on oxidative stress and inflammation:

Our results recorded a significant increase in nerve tissue malondialdehyde (MDA), and tumor necrosis factor alpha (TNF α) and a significant decrease in superoxide dismutase activity (SOD) after 5weeks of high animal fat diet in obese rats compared to NC group (Table 7, Figures 11-13). This reflects impairment of antioxidant activity and the effect of HFD in obese rats on elevation oxidative stress and increased the inflammatory marker.

Effect of 5 weeks of HFD on nerve conduction velocity in obese rats:

Interestingly, the current results recorded a significant decrease in nerve conduction velocity (NCV) (m/s) after 5weeks of high animal fat diet (Ob+HFD), compared to values recorded from normal chow group of rats (NC) ($p < 0.05$) (Table 8, Figure 14).

Effect of changing diet composition in obese rats on different parameters: Ob+ HFD+corn oil versus Ob+HFD+ Omega 3:

When observing the values of final body weight (gm) and BMI measurements (kg/m^2) in rats after changing diet composition for 5W, we can observe that there was no significant variation in these values in Ob+HFD+ corn oil group or in Ob+HFD+omega 3 groups compared to values recorded in Ob+HFD group (Table 4 and Figures 3&4).

Furthermore, as shown in table (5) and figures (5-7) there was no significant variation in fasting blood glucose level in Ob+HFD+ corn oil and Ob+HFD+ Omega 3 group compared to Ob+HFD group. As regarding the mean values of fasting insulin level ($\mu\text{IU}/\text{l}$) and HOMA-IR test after 5 weeks, there was no significant variation between Ob+HFD+corn oil group when compared to Ob+HFD+Omega 3, however, the plasma levels of these parameters in the Ob+HFD+ corn oil and Ob+HFD+ Omega 3 groups were significantly decreased when compared to Ob+HFD. Thus, these results reflect that changing the formula of diet from HFD only to addition of corn oil or of omega 3 to HFD improved insulin sensitivity and decreased the exposure to insulin resistance condition.

When observing levels of plasma cholesterol levels (mg/dl), triglycerides (mg/dl), and free fatty acids (mmol/l) after 5weeks diet (Ob+HFD+ corn oil or Ob+HFD+ Omega 3), table 6 and figures 8, 9 & 10 show no significant change in the mean values recorded between these 2 groups, but the mean values of these parameters in the 2groups were significantly decreased when compared to mean values recorded in

Ob+HFD group. This indicates that the corn oil or omega3 protected against dyslipidemia.

When nerve tissue malondialdehyde level (MDA), superoxide dismutase activity (SOD) and tumor necrosis factor alpha level(TNF α) were estimated after 5weeks of high corn oil diet (Ob+HFD+ corn oil), or high fat diet supplemented with omega 3 (Ob+HFD+Omega 3) in male rats, it can be observed that there was no significant variation in mean values of MDA, SOD activity or TNF α in Ob+HFD+ corn oil group compared to Ob+HFD+ Omega 3 group, but the 2 groups showed significantly decreased tissue malondialdehyde and (MDA) and tumor necrosis factor alpha (TNF α) levels and significantly increased superoxide dismutase activity (SOD) when compared to Ob+HFD (Table 7, Figures 11-13).

Regarding NCV(m/s) values, both in Ob+HFD+ corn oil and Ob+ HFD+Omega 3 groups, there was a significant increase compared to Ob+HFD and their values in Ob+HFD+ corn oil

group showed no significant variation as compared to control group. In contrast in Ob+ HFD+Omega 3 group, there was a significant decrease in values of this group as compared to the control group and Ob+HFD+ corn oil (Table 8, Figure 14).

Table 3: Body weight measurements (gm) and body mass index (Kg/m²) in rats after 12weeks of normal chow (NC) (n=8), high animal fat diet (HFD)(n=24), in male rats.

	NC Group	HFD group
Body weight (gm) Mean \pm SD	163.88 \pm 11.52 ^a	324.25 \pm 28.07 ^b
BMI (Kg/m²) Mean \pm SD	5.93 \pm 0.40 ^a	11.69 \pm 1.09 ^b

Results with different letters in the same raw are significant ($p < 0.05$)

Results with the same letter in the same raw are insignificant ($p > 0.05$).

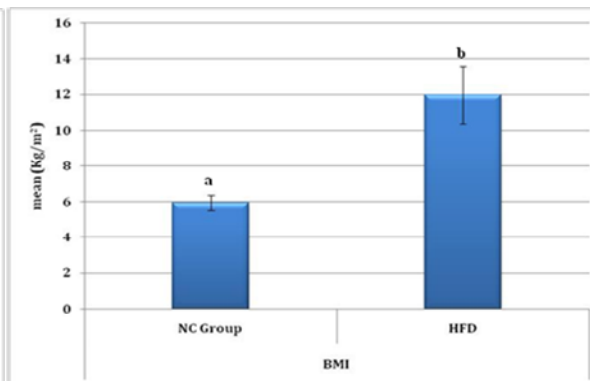
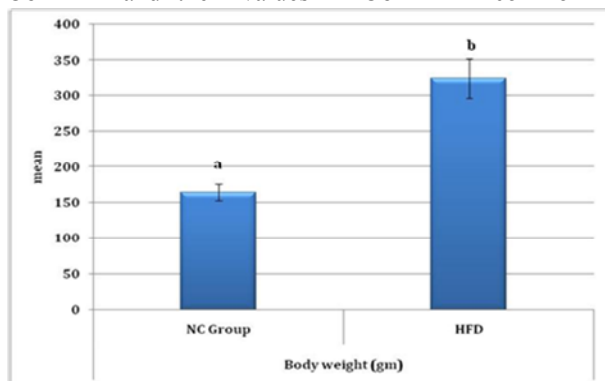


Figure 1: Body weight measurements (gm) and figure 2: body mass index in rats after 12weeks of normal chow(NC) (n=8), high animal fat diet (HFD)(n=24), in male rats.

Results with different letters are significant ($p < 0.05$)

Results with the same letter are insignificant ($p > 0.05$).

Results with the same letter in the same raw are insignificant ($p > 0.05$).

Table 4:Final body weight measurements (gm) and body mass index(Kg/m²) in rats after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (Ob+HFD+Omega 3) in male rats.

	NC group	Ob +HFD	Ob+ corn oil	Ob+HFD +Omega 3
Final weight (gm)	189.50 \pm 8.50 ^a	359.38 \pm 38.53 ^b	347.25 \pm 25.76 ^b	358.13 \pm 26.16 ^b
BMI (kg/m²)	5.93 \pm 0.40 ^a	11.96 \pm 1.62 ^b	11.70 \pm 0.55 ^b	11.90 \pm 0.43 ^b

(n=8)

Results are mean \pm SD.

Results with different letters in the same raw are significant ($p < 0.05$)

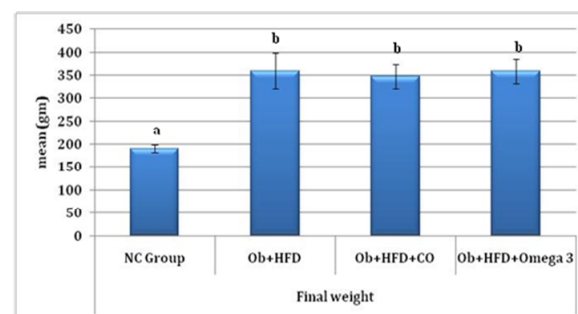


Figure 3: Final body weight measurements (gm) in rats after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

(n=8) -Results with different letters are significant ($p < 0.05$).-Results with the same letter are insignificant ($p > 0.05$).

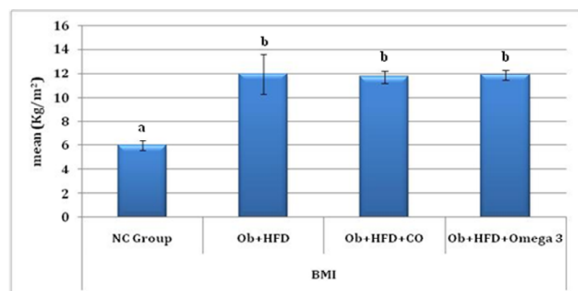


Figure 4: Body mass index (kg/m²) in rats after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats. n=8

Results with different letters are significant ($p < 0.05$)
Results with the same letter are insignificant ($p > 0.05$).

Table 5: Fasting plasma glucose level (mmol/l), insulin level(μ IU/l) and HOMA-IR test after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet rich in polyunsaturated fatty acids(Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

	NC group	Ob+HFD	Ob+HFD +corn oil	Ob+HFD + Omega 3
Glucose mmol/l	4.69 \pm 0.37	5.41 \pm 0.30	5.05 \pm 0.18	5.45 \pm 0.39
Insulin μ IU/l	11.64 \pm 0.78 ^a	20.59 \pm 1.20 ^b	11.68 \pm 0.74 ^a	11.55 \pm 0.76 ^a
HOMA-IR	2.43 \pm 0.26 ^a	4.95 \pm 0.40 ^b	2.63 \pm 0.23 ^a	2.80 \pm 0.31 ^a

(n=8)
Results are mean \pm SD.
Results with different letters in the same raw are significant ($p < 0.05$)
Results with the same letter in the same raw are insignificant ($p > 0.05$).

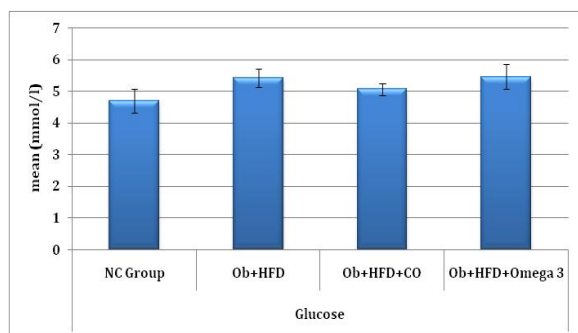


Figure 5: Fasting plasma glucose level (mmol/l) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet rich in polyunsaturated fatty acids (Ob+HFD+corn oil), or high fat diet supplemented

with omega 3 (HFD+Omega 3) in male rats. Results in the different groups are insignificant to each other ($p > 0.05$).

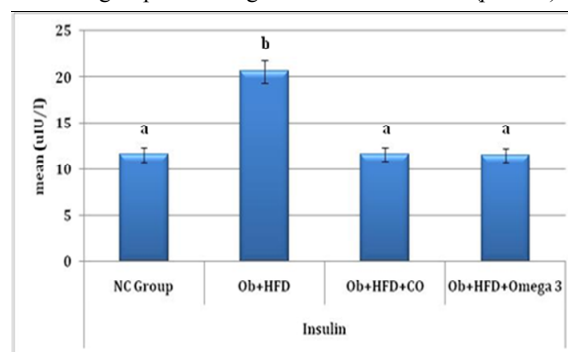


Figure 6: Insulin level(μ IU/l) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet rich in polyunsaturated fatty acids(Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$)
Results with the same letter are insignificant ($p > 0.05$).

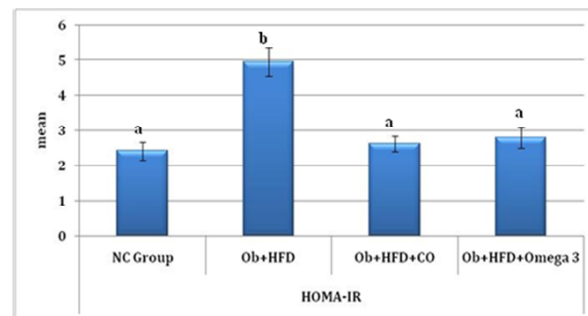


Figure 7: HOMA-IR test after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet rich in polyunsaturated fatty acids(Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$)
Results with the same letter are insignificant ($p > 0.05$).

Table 6: Plasma cholesterol levels (mg/dl), triglycerides (mg/dl), and free fatty acids (mmol/l) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (Ob+HFD+Omega 3) in male rats.

	NC group	Ob+HFD	Ob+HFD +corn oil	Ob+HFD + Omega 3
Cholesterol mg/dl	128.79 \pm 2.86 ^a	188.44 \pm 6.3439 ^b	140.86 \pm 5.36 ^c	153.11 \pm 2.00 ^c
Triglycerides mg/dl	72.43 \pm 6.92 ^a	105.75 \pm 2.80 ^b	84.53 \pm 1.84 ^a	81.25 \pm 1.64 ^a
FFA mmol/l	0.17 \pm 0.04 ^a	0.52 \pm 0.04 ^b	0.24 \pm 0.02 ^a	0.27 \pm 0.02 ^a

(n=8)
Results are mean \pm SD.
Results with different letters in the same are significant ($p < 0.05$)
Results with the same letter in the same raw are insignificant ($p > 0.05$).

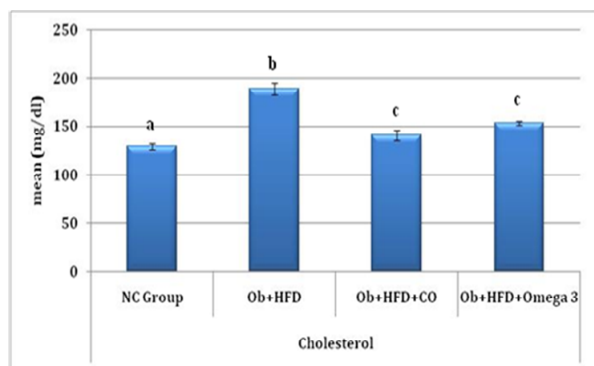


Figure 8: Plasma cholesterol levels (mg/dl) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (Ob+HFD+Omega 3) in male rats. Results with different letters are significant ($p < 0.05$) Results with the same letter are insignificant ($p > 0.05$).

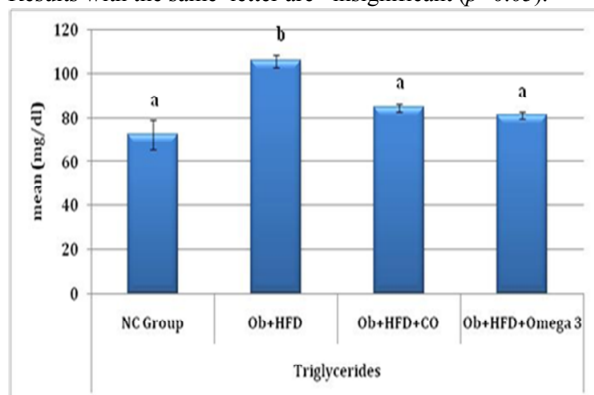


Figure 9: Plasma triglycerides (mg/dl) after 5weeks of normal chow (NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (Ob+HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$). Results with the same letter are insignificant ($p > 0.05$).

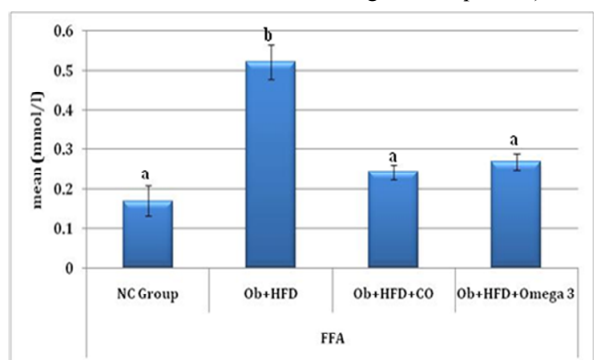


Figure 10: Plasma free fatty acids (mmol/l) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (Ob+HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$). Results with the same letter are insignificant ($p > 0.05$).

Table 7: Tissue malondialdehyde level (MDA), superoxide dismutase activity (SOD) and tumor necrosis factor alpha level (TNF α) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

	NC group	Ob+HFD	Ob+HFD +corn oil	Ob+HFD + Omega 3
MDA nmol/mg ptn	111.36 \pm 5.51 ^a	173.10 \pm 7.07 ^b	148.51 \pm 3.93 ^c	156.30 \pm 4.84 ^c
SOD activity U/mg ptn	2.03 \pm 0.17 ^a	0.45 \pm 0.02 ^b	1.26 \pm 0.15 ^c	1.60 \pm 0.26 ^c
TNF α pg/ml	112.15 \pm 2.07 ^a	234.89 \pm 4.78 ^b	187.79 \pm 4.62 ^c	207.23 \pm 5.03 ^c

(n=8)

Results are mean \pm SD.

Results with different letters in the same raw are significant ($p < 0.05$)

Results with the same letter in the same raw are insignificant ($p > 0.05$).

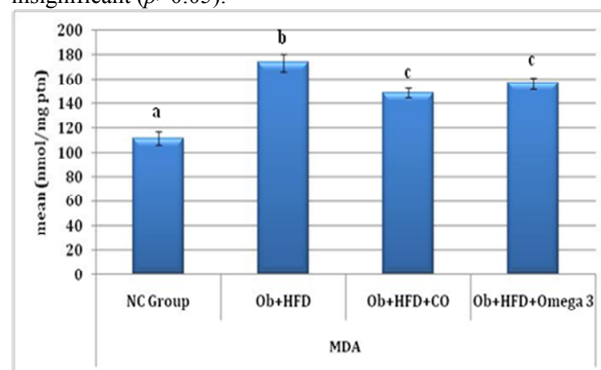


Figure 11: Tissue malondialdehyde (MDA) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats. Results with different letters are significant ($p < 0.05$). Results with the same letter are insignificant ($p > 0.05$).

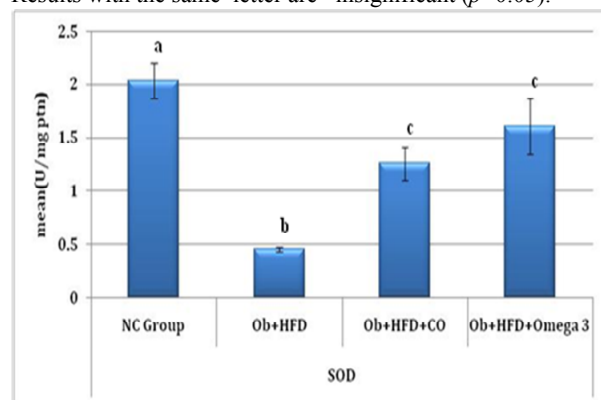


Figure 12: Tissue superoxide dismutase activity (SOD) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$). Results with the same letter are insignificant ($p > 0.05$).

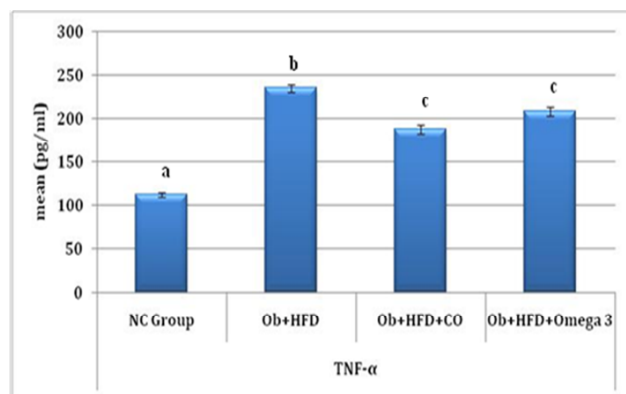


Figure 13: Tissue tumor necrosis factor alpha (TNF α) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+HFD+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$). Results with the same letters are insignificant ($p > 0.05$).

Table 8: Nerve conduction velocity (NCV) (m/s) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

	NC group	Ob +HFD	Ob+HFD +corn oil	Ob+HFD + Omega 3
NCV m/s	8.28 \pm 0.44 ^a	6.49 \pm 0.43 ^b	7.85 \pm 0.33 ^a	7.22 \pm 0.43 ^c

(n=8)

Results are mean \pm SD.

Results with different letters in the same raw are significant ($p < 0.05$)

Results with the same letter in the same raw are insignificant ($p > 0.05$).

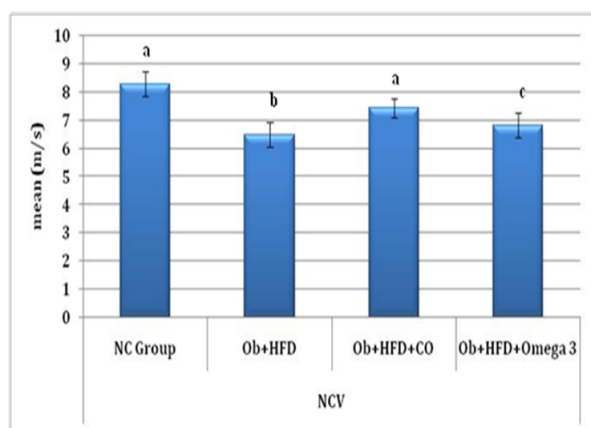


Figure 14: Nerve conduction velocity (NCV) (m/s) after 5weeks of normal chow(NC), high animal fat diet (Ob+HFD), high corn oil diet (Ob+corn oil), or high fat diet supplemented with omega 3 (HFD+Omega 3) in male rats.

Results with different letters are significant ($p < 0.05$)

Results with the same letter are insignificant ($p > 0.05$).

4. Discussion:

Obesity is a strong risk factor for developing dyslipidemia [33,34], diabetes mellitus [35], fatty liver [36], cardiovascular (CV) diseases such as heart failure (HF) and coronary heart disease (CHD) [37].

Feeding of (HFD) to rats was proved to be a useful model of putative effects of dietary fat in humans [38]. In the present study, obesity was induced in rats by using a high fat diet. Obesity was induced in 12 weeks. The weight of rats fed HFD was significantly more than that of rats fed the normal diet. Rat models are therefore useful tools for inducing obesity as they will readily gain weight when fed high-fat diets [39]. Many workers were able to induce obesity in rats using different formulas of high fat diets [40-42].

An evidence for insulin resistance was recorded in the present study. Although fasting blood glucose levels were not significantly different between NC rats and Ob+HFD fed rats, insulin levels were significantly increased in Ob+HFD fed rats compared to NC rats and HOMA-IR test showed a significant increase in Ob+ HFD fed rats compared to NC rats. In agreement with our results, Oltman *et al.*[3] reported that obese Zucker rats are insulin resistant. Also Davidson *et al.* [43] reported that the obese Zucker HFD fed rats were not hyperglycemic; however, they were insulin resistant.

In contrast, Watcho *et al.* [8] and Obrosova *et al.* [44] reported that a 16-week HFD feeding resulted in a modest (14.5%) increase in non-fasting blood glucose concentrations compared with the mice fed NC which they described as being consistent with increased serum insulin concentrations as well as insulin resistance and impaired glucose utilization previously described in this model[45]. Moreover, Ishii *et al.* [46] reported that after 16 weeks of age, the group on a standard diet showed an increase in serum glucose levels and a decrease in serum insulin levels compared with high fat diet fed rats. Unexpectedly, in the group on the high-fat diet, they observed a suppressed of the progression of hyperglycemia and hypoinsulinemia. This might be explained in part by different animal species, variable duration of diet or by measuring non fasting blood glucose level.

We can see from these results that HFD fed obese rats developed insulin resistance, but did not developed diabetes or hyperglycemia.

It was also recorded from the results of this study that HFD in obese rats resulted in dyslipidemic changes as illustrated by increasing serum levels of triglycerides, total cholesterol, free fatty acids as compared to control; a finding in accordance with that of Woo *et al.* [47], and Kamal and Mohamed [48].

Dyslipidemic changes occurs in obesity may be due to the increased triglycerides content of the liver due to increased influx of excess non esterified fatty acids (NEFAs) into the liver[49]. Lipid alterations affect the structure and function of the nerve membrane and have been considered as contributory factors to oxidative stress in obesity [50].

In the present study, it was found that nerve MDA level was significantly elevated with a significant decrease in the enzyme superoxide dismutase activity in HFD fed obese rats compared to NC rats and this was an indication for increased oxidative stress in these obese rats. Increased production of reactive oxygen species as well as reduced antioxidant defense mechanisms have been suggested to play a role in both humans and animal obesity induced pathology [51,52].

Interestingly, oxidative stress, the key metabolic abnormalities previously thought to be caused primarily by high glucose and shown to contribute to diabetic neuropathy, clearly manifest in this Ob+HFD model of pre-diabetic neuropathy characterized by insulin resistance in the absence of overt diabetes or hyperglycemia.

In this study, our obese rats also developed nerve disorder, demonstrated as NCV slowing. The finding that rats fed a high fat diet develop indices of neuropathy is consistent with studies of obese Zucker rats [3, 53], and also with clinical studies in which pre-diabetes and impaired glucose tolerance have been associated with an early-onset neuropathy [54, 55]. It was previously suggested that impaired glucose tolerance can directly cause nerve injury [56], however, it appears that NCV slowing is simply a covariant with other factors related to obesity.

This study shows that velocity of sciatic nerve conduction in obese rats could depend on dietary fat modification. The addition of omega 3 to high animal fat diet or consumption of corn oil rich in omega 6&9 PUFA in addition to animal fat was associated with increased sciatic nerve conduction velocity in obese rats, whereas high animal fat diet in obese rats caused a significant slowing of sciatic nerve conduction velocity. In addition, we found that omega 6&9 PUFA supplemented food (corn oil) induced a significant improvement of nerve conduction velocity compared to the enriching food with omega 3. These data show that omega 3 enrichment or corn oil could be associated with improved nerve conduction velocity in obese rats.

The present study provides evidence of the therapeutic efficacy of omega 6&9 PUFA and omega 3 on NCV deficits, in the model of neuropathy associated with obesity. It should be noticed that the improvement in NCV was not associated with weight reduction. As seen from the

results of the present study, there is no significant change in final body weight or BMI between the obese groups fed HFD with animal fat, corn oil or supplemented with omega 3 fatty acids.

Some previous studies suggested an association between insulin resistance, compensatory hyperinsulinemia, and peripheral neuropathy in human. Also, higher insulin resistance was independently associated with the presence of cardiac autonomic neuropathy (CAN) in Korean type 2 diabetes mellitus (T2DM) patients [57].

In the present study it was shown that either omega 3 or corn oil supplementation was associated with improved insulin sensitivity and decreased blood insulin level and this may play a role in improving nerve conduction, however the exact mechanism for this relation is not clear and needs further investigations.

One study provides evidence that insulin receptor substrate (IRS) proteins are expressed in the dorsal root ganglia (DRG) and could play an important role in the ability of insulin to support peripheral neurons. Elevated serine phosphorylation of IRS proteins reported in their study to be a major contributing mechanism underlying the effect of insulin resistance on neurons [58].

Insulin resistance is an important risk factor for endothelial dysfunction, and impairment of vascular function of epineurial arterioles precedes nerve dysfunction in obese normoglycemia Zucker rats [59]. It has been shown that improving insulin sensitivity improves vascular resistance in obese Zucker rats [60].

We can see from the results of the present study that dyslipidemia may be a contributing factor to reductions in peripheral nerve conduction velocity. This dyslipidemia was shown to be mostly corrected by dietary supplements and this correction appears to play a role in improvement of nerve conduction velocity, may be in part by a normalization of fatty acid composition of nerve membrane and eicosanoid synthesis, which is depressed in neuropathy and/or by a direct effect on incorporation of these fatty acids into the plasma membranes [61]. By changing membrane properties, omega 6&9 or omega 3 PUFA can modify the activity of transmembrane enzymes, such as Na,K- Atpase, which is implicated in the propagation of nerve impulses[62].

Our findings are consistent with studies showing that high dietary intake of fatty acids prevents the development and clinical progression of nerve conduction deficits in diabetic animals as well as in the general human population [63,11]. In diabetic rats, the administration of linoleic acid, an n-6 fatty acid, improved sciatic NCV [11]. In patients with generalized peroxisomal disorders, congenital

diseases with impaired myelinogenesis, the administration of the n-3 fatty acids, DHA, significantly improved myelin formation alleviating the symptoms in these patients [64].

PUFA are the major structural components of the neuronal membrane phospholipids [65] and therefore, their structural and chemical characteristics influence membrane functions, such as the activity of membrane bound proteins, signal transduction and also neurotransmission [66-68]. It was also reported that supplementation with sunflower oil, which contains high quantity of linoleic acid, restored NCV in diabetic rats, and this effect was accompanied by a modification of phospholipid fatty acid composition in nerve membranes [10].

In particular, the electrophysiologic effect of the omega-3 fatty acids seems to be the result of specific modulation of ion currents, particularly of the voltage-dependent sodium current and of the L-type calcium currents across sarcolemmal phospholipids membranes [68].

Mammals synthesize the long chain PUFA from linoleic acid [18:2(n-6)] and α -linolenic acid [18:3(n-3)], which are the 2 precursors of (n-6) and (n-3) fatty acids families provided by the diet. Specific enzymes, desaturases and elongases, are involved in this pathway, but the conversion of precursors to long chain PUFA is generally low in humans. Consequently, the decrease in bioavailability of PUFA, affects the fatty acid composition of membrane phospholipids (PL) with repercussions on membrane protein functionality [69], eicosanoid production [70, 71], and peroxisome proliferator-activated receptor (PPAR) regulation [72, 73].

It was suggested that the rate-limiting nature of ω -6-desaturation contributes to the development of neuropathy. Bypassing the rate-limiting step by using gamma-linolenic acid (GLA) may have desirable effects and anti-inflammatory effects [74]. Because essential fatty acids (EFAs) and their metabolites are exceptionally important in both the structure and function of nerves [75], it seemed possible that neuropathy might be particularly responsive to PUFA supplementation.

An important observation in the results of this study is that omega-6 fatty acids, supplied by corn oil, appear to have a beneficial effect on peripheral nerve function than omega-3 fatty acids, requires consideration. In fact, omega-6 PUFAs are generally more highly represented in the nerve membrane than omega-3 fatty acids and have major effects of excess than the n-3 fatty acids [76].

The beneficial effects of omega 6&9 or omega 3PUFA may at least partially be related to inhibition of oxidative stress in peripheral nerve as evidenced in the present results by improving the antioxidant

enzyme superoxide dismutase activity and decreasing oxidative stress marker MDA.

Oxidative stress is closely linked to upregulation of 12/15-lipoxygenase (12/15LO), an enzyme converting arachidonic acid to 12-Hydroxyeicosatetraenoic acid (12(S)-HETE), 15(S)-HETE, and a number of derivatives of these acids. These lipid-like compounds undergo spontaneous lipid peroxidation, which leads to induction of oxidative nitrosative stress, activation of mitogen-activated protein kinases (MAPKs), and proinflammatory response [77, 78]. MAPK activation has been demonstrated to play an important role in peripheral diabetic neuropathy [79, 80].

It was demonstrated that reducing oxidative stress in epineural vessels improved vascular relaxation to acetylcholine as well as NCV [52, 81-83]. The increase in superoxide in the aorta of high fat fed rats is likely due to increased NAD(P)H oxidase activity and/or expression, which has been linked to increased activity of angiotensin in obesity [84].

Finally, the results of the present study show that diet supplemented with omega 3 or PUFA rich in omega 6&9 fatty acids induce an anti-inflammatory effect as indicated by decreased TNF alpha content in the sciatic nerve of the obese rats.

Evidence for the importance of low grade inflammation in diabetic neuropathy is also emerging from both experimental and clinical studies [85,86].

Our results are in agreement with Ferrucci *et al.* [87] and Kapoor and Huang [88] who reported from their studies that n-3 PUFAs and the gamma linolenic acid (GLA), an n-6 fatty acid, have been shown to have significant anti-inflammatory properties. PUFAs inhibit the production of proinflammatory cytokines, i.e., IL-1 β , IL6 and tumor necrosis factor-alpha by activating transcription factors, such as the peroxisome proliferator-activated receptors and nuclear factor kB [89]. As inflammation is one of the main pathophysiologic processes involved in peripheral polyneuropathy, it could be extremely relevant in progression of axonal damage [90].

Studies in normal volunteers indicate that omega-3 fatty acid supplementation reduced the ability of monocytes to produce IL-1 β upon stimulation with endotoxin. The effect was most pronounced 10 weeks after stopping the supplementation and suggests prolonged incorporation of omega-3 fatty acids into a pool of circulating monocytes [91]. The capacity of the monocytes from these donors to synthesize IL-1 β returned to the pre-supplement level 20 weeks after ending supplementation. Similar results were observed for IL-1 α and TNF [92].

Previous studies suggested that in patients affected by peripheral neuropathy, a supplementation with PUFA may positively influence the axonal degeneration of the nerve [10].

Conclusion

The results of this study have important clinical and speculative implications. Based on our findings, we suggest that corn oil or omega 3 supplementation may be effective in treatment of obesity induced neuropathy. The mechanism of their effects is multifactorial including improving insulin sensitivity, correction of dyslipidemia which could reflect on fatty acid composition of the nerve membrane structure and function, reducing oxidative stress and an anti-inflammatory effect. This possibility should be carefully considered and examined in future trials of essential fatty acid supplementation.

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