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The role of oxidative stress and inflammatory response in high-fat diet induced peripheral neuropathy

Rafet Ozay [a], Ertugrul Uzar [b], Abit Aktas [c], Mehtap Erkmen Uyar [d], Bora Gürer [a,*], Osman Evliyaoglu [e], Nuri Eralp Cetinalp [f], Cansel Turkay [g]

[a] Ministry of Health, Diskapi Yildirim Beyazit Education and Research Hospital, Neurosurgery Clinic, Turkey
[b] Dicle University, Department of Neurology, School of Medicine, Diyarbakır, Turkey
[c] Istanbul University, Faculty of Veterinary Medicine, Department of Histology and Embriology, Istanbul, Turkey
[d] Başkent University, Medical School, Department of Nephrology, Ankara, Turkey
[e] Dicle University, Department of Biochemistry, School of Medicine, Diyarbakır, Turkey
[f] Etlik Ihtisas Research and Educational Hospital, Department of Neurosurgery, Ankara, Turkey
[g] Fatih University, Department of Gastroenterology, School of Medicine, Ankara, Turkey

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ABSTRACT

Objective: Earlier studies suggest that high-calorie diet is an important risk factor for neuronal damage resulting from oxidative stress of lipid metabolism. In our experimental study of rats under high-fat diet, oxidative stress markers and axonal degeneration parameters were used to observe the sciatic nerve neuropathy. The aim of this study is to evaluate the pathophysiology of neuropathy induced by high-fat diet.

Methods: A total of 14 male rats (Wistar albino) were randomly divided into two experimental groups as follows; control group ($n = 7$) and the model group ($n = 7$); while control group was fed with standard diet; where the model group was fed with a high-fat diet for 12 weeks. At the end of 12 weeks, the lipid profile and blood glucose levels, interleukin-1β (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and transforming growth factor-β (TGF-β) levels were studied. Tissue malondialdehyde (MDA), nitric oxide (NO) levels and super-oxide dismutase (SOD), paraoxonase-1 (PON-1) and glutathione peroxidase (GPx) activities were studied. The distal blocks of the left sciatic nerves were evaluated for histomorphological analysis (including mean axon area, axon numbers, nerve fiber diameters, axon diameters, and thickness of myelin sheets).

Results: Body weights, serum glucose and high-density lipoprotein (HDL) levels of rats were found not statistically significantly different compared between the model and the control groups ($p > 0.05$). Serum cholesterol, triglyceride, TGF-β and TNF-α levels were significantly higher in the model group when compared with the control group ($p < 0.05$). IL-1 and IL-6 levels were not statistically significantly different compared between the model group and the control group ($p > 0.05$). The MDA and NO levels and the SOD and GPx activities of the sciatic nerves in model group were statistically significantly higher than the control group ($p < 0.05$). In addition, the activities of PON-1 were statistically significantly lower in the model group when compared with the control group ($p < 0.05$). The difference in the total number of myelinated axons between the control group and the model group was not statistically significant ($p > 0.05$). The nerve fiber diameter and the thickness of the myelin sheet were statistically significantly lower in the model group when compared with the control group ($p < 0.05$). The axon diameter and area were significantly decreased in the model group when compared with the control group ($p < 0.05$).

Conclusion: Our results support that dyslipidemia is an independent risk factor for the development of neuropathy. In addition, we postulated that oxidative stress and inflammatory response may play an important role in the pathogenesis of high-fat diet induced neuropathy.

* Corresponding author at: İrfan Bastug cad. S.B. Diskapi Yildirim Beyazit Egitim ve Arastirma Hastanesi Beyin Cerrahi Servisi, Turkey. Tel.: +90 506 316 42 01; fax: +90 312 318 66 90.
E-mail address: boragurer@gmail.com (B. Gürer).

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1. Introduction

Simple carbohydrates and saturated fats as of the most important elements of our modern life style nutrition are the prime cause of obesity and insulin resistance (Gross et al., 2004). Earlier studies suggested that high-calorie diet is an important risk factor for Alzheimer disease due to neuronal damage resulting from oxidative stress of lipid metabolism (Luchsinger et al., 2002; Cutler et al., 2004). The diet-induced hyperlipidemia had been shown as the cause of neuropathy via the cholesterol accumulation, galactosylceramide, sulfatide, sphingomyelin in the neurons (Arboleda et al., 2009; Molteni et al., 2002; Stranahan et al., 2011). These deposits further cause oxidative stress resulting with apoptosis of the neurons (Arboleda et al., 2009; Molteni et al., 2002; Stranahan et al., 2011).

High fat diets with high-calories have been accepted as the cause of obesity, dyslipidemia and insulin resistance in rodents for many decades. The complications of high-fat diet are similar to the human metabolic syndrome such as: myocardiopathy, hepatic steatosis, endothelial dysfunction and renal pathology (Buettner et al., 2006; Deji et al., 2009; Kobayasi et al., 2010; Woods et al., 2003). High fat diet had been shown to cause oxidative stress by increasing reactive oxygen species in humans (Dandona et al., 2001; Erhardt et al., 1997). These reactive oxygen species were found to be associated with over expression of proinflammatory mediators (Kobayasi et al., 2010; Smith and Adams, 2011).

As the prevalence of obesity increases, the related metabolic problems such as diabetic neuropathy (DN) are gaining importance (Edwards et al., 2008; Zochodne, 2008). The pathophysiological mechanisms of DN include distal axonal degeneration; therefore, nerve dysfunction leads to sensorimotor deficits, decreased epidermal innervations and reduced nerve conduction velocities (Hammes, 2003; Sinnreich et al., 2005; Zochodne, 2007). The role of high-fat diet in the development of both diabetes and diabetic complications were studied in several studies (Steemburgo et al., 2007; Young et al., 1993). Indeed, recent studies suggested that; dyslipidemia is the main and progressing risk factor for DN associated with diabetes mellitus (Fox and Kester, 2010; Vincent et al., 2009a).

In our experimental study of rats under high-fat diet, oxidative stress markers and axonal degeneration parameters were used to observe the sciatic nerve neuropathy. The aim of this study is to evaluate the pathophysiology of neuropathy induced by high fat diet.

2. Materials and methods

The animals included in this study were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. The Medical Faculty Experimentation Ethics Committee approved the experimental procedures of the study. A total of 14 male, Wistar albino rats were used. Their ages were between 8 and 12 weeks; and the average of weight was 230 ± 20 g. The rats were placed in a controlled room, where the temperature was 22 ± 2 °C and humidity was 60 ± 5%. Before starting the experiment, 12 h light/12 h dark cycle was maintained for 2 weeks. The rats were randomly divided into two experimental groups as follows: Group I, the control group (n = 7), and Group II, the model group (n = 7). The control group was fed with standard diet (BilYem, Turkey) and tap water was provided ad libitum for 12 weeks. Similarly, the model group was fed with a high-fat diet [standard diet (contains 24% protein, 6.4% cellulose, 5.6% lipid, 5.75% ash, 1.25% lysine, 0.94% calcium, 0.74% phosphorus, 0.6% methionine, 0.05% sodium) + 20% pure lard and 2% cholesterol (≥95% lanolin, cholesterol)] (Sigma-Aldrich, St. Louis, Missouri) and tap water was provided ad libitum for 12 weeks (Chen et al., 2006). At the end of 12 weeks, all rats were anesthetized with an intramuscular injection of 50 mg/kg ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey) and 10 mg/kg xylazine (Rompun, Bayer, Turkey) then weighted. Before the rats were sacrificed 5–10 mL blood samples were taken from vena cava inferior for the measurement of lipid profile and blood glucose levels, interleukin-1β (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and transforming growth factor-β (TGF-β) levels. All the rats were fastened 12 h prior to blood collection. Than bilateral 3 cm incisions in gluteal muscles were performed with dissection. Bilateral sciatic nerves were exposed and quickly removed without damaging the tissues. Sciatic nerve tissues taken from the right side were stored at −30 °C until the analysis of malondialdehyde (MDA), nitric oxide (NO) levels and super-oxide dismutase (SOD), paraoxonase-1 (PON-1) and glutathione peroxidase (GPx) activities were performed.

2.1. Stereological analysis

The distal blocks of the left sciatic nerves were removed. The nerves were stretched to in situ length by pinning onto a card and then fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 6 h in 4 °C. Once they were fixed, they were rinsed in a phosphate buffer (pH 7.4). After these steps, specimens were post fixed in 1% osmium tetroxide for 2 h, and dehydrated in an ascending alcohol series than put into propylene oxide for 16 min. After these procedures, the tissues were embedded in Epon Embedding Kit (Fluka Chemie Gmbt, Switzerland). An ultramicrotome (Leica RM 2155; Leica, Nubloch, Germany) was used for examining the obtained semithin sections (1-μm thick). Sections were stained with 1% toluidine blue for stereological analysis. Stereological analyses of the sciatic nerves were done according to the principles described in earlier studies (Canan et al., 2008; Kaplan et al., 2010; Turgut et al., 2005). A manual stereological workstation composed of a digital camera (Nikon COOLPİX5400 Tokyo-Japan), a manual dial indicator controlled specimen stage (Kaplan et al., 2001, 2005), and a light microscope (Nikon Microphot-FX, Tokyo-Japan) were used for axon number counting. To obtain the estimation of total axon number in an unbiased manner from nerve cross-section we used two-dimensional dissector technique. In this application the area of the unbiased counting frame was 1600 μm^2. A counting frame was placed on to a monitor and the sample area was chosen by a systematic uniform random manner via dial indicator controlled specimen stage.

Meander sampling of each sectioned nerve profiles were performed by 70 μm × 70 μm step size in a systematic-random manner. This ensures that all locations within a nerve cross section were equally represented and that all axon profiles were sampled with an equal probability regardless of shape, size, orientation and location (Geuna et al., 2000, 2001; Gundersen, 1986).

Another stereological workstation composed of a CCD digital camera, image capture card (Flash Point 3D, Integral Technologies, Indianapolis, Ind.), personal computer, and computer-controlled motorized specimen stage (Prior Scientific, Cambridge, United Kingdom), a microcator (Heidenhein Traunreut, Germany), and a light microscope (Leica, Wetzlar, Germany) were used for stereological analyses of myelin thickness, axon cross sectional area, nerve fiber diameter and axon diameter. The software program (CAST-GRID®-Computer Assisted Stereological Toolbox-Olympus, Copenhagen, Denmark) was used to control, measure and record stereological data and to capture digital images of the sections. This system reproduced microscopic images (obtained through a 60×, NA 1.40) on the computer monitor at a final magnification of 3209 that allowed accurate recognition and quantifying the myelinated nerve fibers.

A two-dimensional isotropic uniform random nucleator was used for estimation of axon area, nerve fiber diameter, axon diameter and the thickness of myelin sheet using an oil objective

(60×, NA 1.40) at a final magnification of 3209×. Meander sampling of each sectioned nerve profiles were performed over successive, systemic-random 70 μm × 70 μm step sizes.

In order to show myelin degeneration in selected cross-sections, electron microscopic images were obtained by using determined electron microscopy study techniques. In both groups myelin thicknesses and structures were examined for compatibility of the data obtained from light microscope. Neither numerical measurements nor statistical analysis were done from electron microscopic images.

2.2. Biochemical analyses

2.2.1. Total cholesterol, HDL, triglycerides, fasting blood glucose analyses

Blood samples were centrifuged at 5000 rpm for 3 min, than total cholesterol, HDL, triglycerides, fasting blood glucose levels were determined with photometric method by an autoanalyzer (Cobas Integra 800, Roche, Switzerland).

2.2.2. Inflammatory cytokine analyses

Inflammatory cytokines IL-1β, IL-6, TNF-α and TGF-β levels (Biosource rat IL-1β ELISA kit, Biosource rat IL-6 ELISA kit, Biosource rat TNF-α ELISA kit and Biosource rat TGF-β ELISA kit) were studied utilizing ELISA method.

2.2.3. Tissue biochemical analyses

The excised nerve tissue samples were weighed, and immediately stored at −30 °C. The left sciatic nerve tissues perfused with 1.15% ice-cold KCl, minced, then homogenized in five volumes (w/v) from the same solution. Assays were performed on the supernatant of the homogenate that is prepared at 14,000 rpm for 30 min at +4 °C (Senoglu et al., 2009). The protein concentration of the tissue was measured by the method of Lowry et al. (1951).

2.2.3.1. Tissue malondialdehyde (MDA) analyses. Tissue MDA levels were determined by a method based on the reaction with thiobarbituric acid (TBA). Briefly, the samples were mixed with two volumes of cold saline solution containing 0.001% butylated hydroxytoluene (BHT) (200 μL of 0.01% BHT solution in methanol) and 0.07% sodium dodecyl sulfate (SDS) (20 μL of 7% SDS). Then 1 mL of samples was added to 500 μL of 0.01 NH_2SO_4 and 500 μL of the thiobarbituric acid reagent (0.67% thiobarbituric acid in 50% acetic acid) to precipitate protein. Then the samples were heated in boiling water for 60 min. After cooling, an equal volume (2 mL) of *n*-butanol was added to each test tube and mixed. The mixture was centrifuged at 4000 rpm for 10 min at room temperature. The absorbance of the organic layer in 1 mL cell was read at 535 nm (Molecular Devices Corporation, Sunnyvale, CA, USA). MDA concentrations were expressed as *n* moles per gram tissue wet weight.

2.2.3.2. Tissue nitric oxide (NO) analyses. The level of NO was estimated by the method based on the diazotization of sulfanilic acid by NO at acidic pH and subsequent coupling to N-1-napthyl-ethylene diamine (Griess reaction) as described before (Cortas and Wakid, 1990). Since nitrate anion does not give a diazotization reaction with sulfanilic acid, the samples were treated by cadmium (a reducing agent) to reduce nitrate anions into nitrite anions before the NO estimation. The results were expressed as mcmol/mg-proteine.

2.2.3.3. Tissue superoxide dismutase (SOD) analyses. Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method described previously (Sun et al., 1988). The principle of the method is based on the inhibition of nitrobluetetrazolium reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 mL ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Superoxide dismutase activity was expressed as U/mg-protein.

2.2.3.4. Tissue paraoxonase-1 (PON-1) analyses. Paraoxonase-1 levels were measured spectrophotometrically by modified Eckerson method (Eckerson et al., 1983). Initial rates of hydrolysis of paraoxon (0.0-diethyl-0-p-nitrophenylphosphate; Sigma Chemical Co., London, UK) were determined by measuring liberated-p-nitrophenol at 405 nm at 37 °C (Eckerson et al., 1983).

2.2.3.5. Tissue glutathione peroxidase (GPx) analyses. Glutathione peroxidase activity was measured by following changes in NADPH absorbance at 340 nm (Paglia and Valentine, 1967). In the activity calculations (IU, international unit), extinction coefficients NADPH were used for GPx. The results were expressed as IU/mg-protein.

2.3. Statistical analysis

The data was analyzed by using Statistical Package for the Social Sciences version 11.5 (SPSS 11.5 for Windows, Chicago, IL, USA). Normality of the variables was assessed by Shaphiro–Wilk test. After confirming that the variables show a normal distribution, differences between the groups were tested using independent groups-test (Student *t*-test and Mann–Whitney *U* test). A value of $p < 0.05$ indicates a significant difference.

3. Results

3.1. Biochemical results

Body weight, glucose and plasma lipid parameters of rats are shown in Table 1. Body weights, serum glucose and HDL levels of rats were not statistically different between the model group and the control group ($p > 0.05$). Serum cholesterol and triglycerides levels were significantly higher in the model group when compared with the control group ($p = 0.003$).

Plasma proinflammatory cytokine parameters of rats are shown in Table 2. Despite, IL-1 and IL-6 levels were higher in the model group when compared with the control group, this difference was not statistically significant ($p > 0.05$). However, TGF-β and TNF-α

Table 1
Body weight, glucose and lipid parameters of the study groups.

	Control group ($n = 7$) (mean ± SD)	Model group ($n = 7$) (mean ± SD)	*p*-Value
Body weight (g)	344.4 ± 30.8	342.16 ± 34.6	0.83
Serum cholesterol (mg/dl)	57 ± 10.06	149 ± 23.56	0.003
HDL (mg/dl)	49 ± 13.4	55 ± 8.47	0.352
Serum triglycerides (mg/dl)	62 ± 20.11	113 ± 45.9	0.03
Serum glucose (mg/dl)	114 ± 40.48	136 ± 36.04	0.775

SD: standard derivation; HDL: high-density lipoprotein.

Table 2
Plasma pro-inflammatory cytokine levels of the study groups.

	Control group ($n = 7$) (mean ± SD)	Model group ($n = 7$) (mean ± SD)	*p*-Value
IL-1 (pg/ml)	16.2 ± 6.9	18.1 ± 7	0.943
IL-6 (pg/ml)	13.2 ± 5.4	19.8 ± 8.8	0.116
TGF-β (pg/ml)	6.5 ± 3.1	42.2 ± 13.4	0.003
TNF-α (pg/ml)	8 ± 4.7	20.3 ± 7	0.003

SD: standard derivation; IL-1: interleucin-1; IL-6: interleucin-6; TGF-β: transforming growth factor-β; TNF-α: tumor necrosis factor-α.

Table 3
Oxidant/antioxidant levels of the study groups.

	Control group ($n=7$) (mean ± SD)	Model group ($n=7$) (mean ± SD)	p-Value
MDA	15.6 ± 2.8	26.9 ± 4.6	0.001
NO	0.10 ± 0.02	0.20 ± 0.04	0.001
SOD	62.5 ± 8.3	80.2 ± 10.6	0.002
GPx	7.49 ± 0.89	9.47 ± 1.33	0.003
PON-1	0.145 ± 0.014	0.129 ± 0.013	0.03

SD: standard derivation; MDA: malondialdehyde; NO: nitric oxide; SOD: superoxide dismutase; GPx: glutathione peroxidase; CAT: catalase; PON-1: paraoxonase-1.

levels were statistically significantly higher in the model group when compared with the control group ($p = 0.003$).

Oxidant/antioxidant parameters in sciatic nerve of rats are shown in Table 3. Tissue MDA and NO levels of the sciatic nerve in the model group were statistically significantly higher than the control group ($p = 0.001$). In addition, the SOD and GPx activities were statistically significantly higher in the model group than the control group ($p = 0.002$ and 0.003, respectively). The PON-1 activity in the sciatic nerve was significantly lower in the model group when compared with the control group ($p = 0.03$).

3.2. Stereological analyses and histological findings

The quantitative results of the sciatic nerves including mean axon area, axon numbers, nerve fiber diameters, axon diameters, and thickness of myelin sheets in the study groups are shown in Table 4. The difference in the total number of myelinated axons between the control group and the model group was not statistically significant ($p > 0.05$). The nerve fiber diameter and the thickness of the myelin sheet were statistically significantly lower in the model group when compared with the control group ($p = 0.004$ and 0.02, respectively). The axon diameter and area were significantly decreased in the model group when compared with the control group ($p = 0.04$ and 0.01, respectively). The histopathological sections and electron microscopic images are shown in Fig. 1.

Table 4
Comparison of the results of stereological analyses of nerve fiber diameter, axon diameter, myelin thickness, axon number, and axon cross-section area measurements between the study groups.

	Control group ($n=7$) (mean ± SD)	Model group ($n=7$) (mean ± SD)	p-Value
Fiber diameter (μm)	12.23 ± 0.83	10.51 ± 0.82	0.004
Axon diameter (μm)	9.09 ± 0.80	7.51 ± 0.53	0.04
Myelin thickness (μm)	1.56 ± 0.10	1.41 ± 0.12	0.02
Axon area (μm^2)	62.27 ± 13.1	46.12 ± 8.30	0.01
Axon number	5516 ± 207	5329 ± 375	>0.05

SD: standard derivation.

4. Discussion

The etiology of DN involves numerous complex mechanisms including hyperglycemia, accumulation of glycation end products,

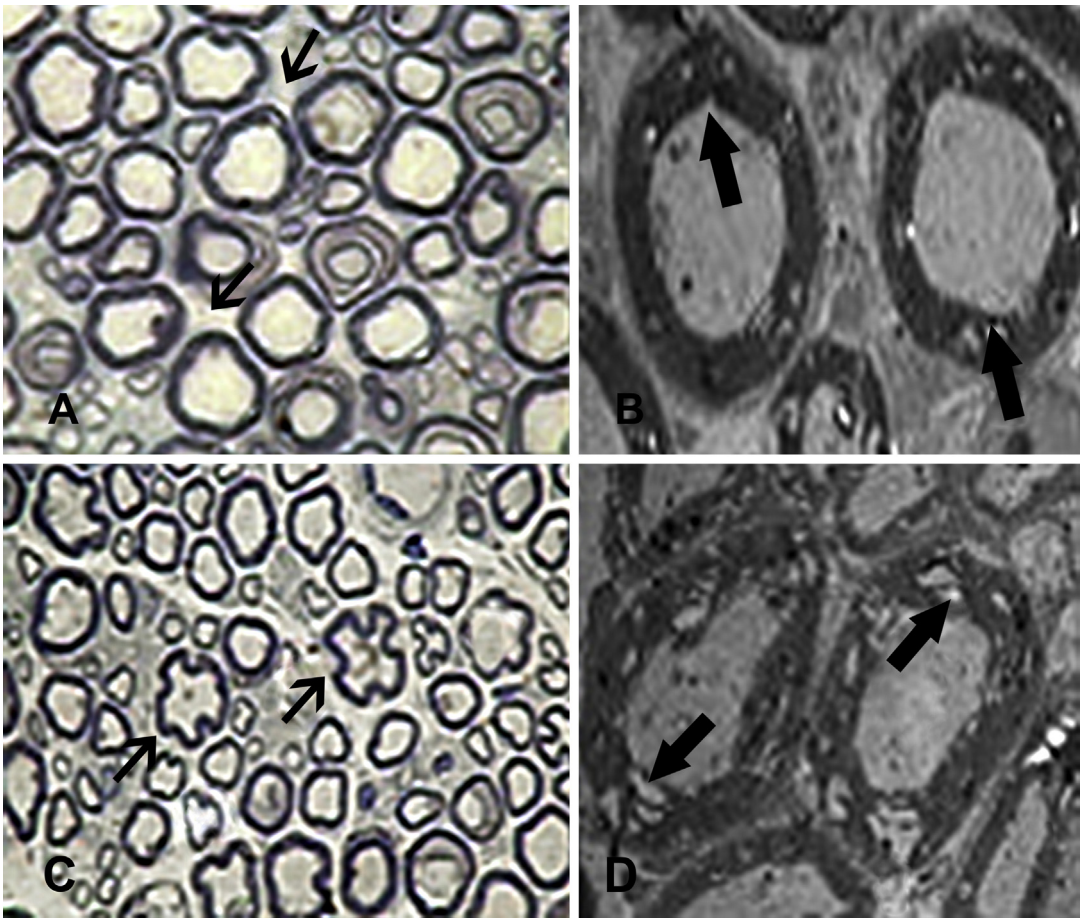

Fig. 1. Photomicrograph showing the light microscopic appearance of the control group revealed that regular form and thickness of the myelin sheath (arrow) (A). Electron microscopy of the control group revealed minimum irregular fiber shape and separation of lamellae (arrow) (B). Photomicrograph showing the light microscopic appearance of the model group revealed irregularly shaped fibers (arrows) (C). In the electron microscopic examination of the method group, myelin degeneration, myelin sheath swelling and shrinkage in axons and general separation of lamellae are revealed (arrows) (D).

systemic oxidative stress, and altered growth factor equilibrium (Ikemoto et al., 1996; Petro et al., 2004; Surwit et al., 1995; Yao et al., 1980a). Earlier studies concluded that hyperglycemia due to insulin resistance triggers systemic and neuronal oxidative stress mechanisms which further leads to peripheral nerve injury (Tomlinson and Gardiner, 2008; Vincent et al., 2007). Despite the proposed mechanisms of the peripheral DN had been widely studied, reliable biochemical and histopathological markers of DN are needed.

A new elaborating theory is dyslipidemia plays an important role in the development of DN (Leiter, 2005). In this context, some studies suggest that a high-fat diet produces neuropathy independent of hyperglycemia (Obrosova et al., 2007; Wiggin et al., 2009). Development of neuropathy before hyperglycemia formation was shown with electrophysiological parameters in mice on a high-fat diet (Vincent et al., 2009b). The first article about concurrency of painful peripheral neuropathy and essential hyperlipidemia was published as a case report by Fulton (1952) and followed by Fessel (1971) who asserts that peripheral neuropathy may cause peripheral nerve infarct via fat embolism or lipid induced thrombocyte aggregation by reporting 6 disease series with lipid disorders and peripheral neuropathies in 1971. In a prospective study with 6 patients, McManis et al. (1994) reported that increasing blood triglycerides are related with peripheral neuropathy and by returning to normal blood levels, signs and symptoms regress. Drory et al. (1999) had demonstrated electrophysiological data that supports this observation by showing the mild axonal (motor-sensory) involvement in cases with triglyceride levels above 400 mg/dL. As relevant with the above hypothesis, our experimental study revealed that high-fat diet caused significant axonal damage.

Lipid profiles are usually abnormal early in the course of type-2 diabetes that correlates with the presence of diabetic neuropathy, and it was recently reported that elevated triglyceride levels are the predictors of a more rapid progression of the disease (Drory et al., 1999; Yao, 1985). In addition, several well-designed trials reported that type-2 diabetic patients represent early dyslipidemia as a major independent risk factor for the development of diabetic neuropathy (Vincent et al., 2009a; Yao et al., 1980b).

Some studies suggest that inflammation may play a role in high-fat diet-induced neuropathy (Cameron and Cotter, 2008; Watcho et al., 2010). Indeed, obesity is associated with chronic inflammation (Smith and Adams, 2011; Xu et al., 2003). In addition, adipose tissue is an important source of proinflammatory cytokines that may up-regulate cytokine production due to high-fat diet (Kern et al., 2001; Samad et al., 1997; Sampey et al., 2011; Todoric et al., 2006). Also, proinflammatory cytokines are mediators to the course of inflammatory demyelinating neuropathies. They can induce inflammatory response; increase vascular permeability of the blood-nerve barrier and transmigration of leukocytes into the nerve (Creange et al., 1997; Lisak et al., 1997). In addition TNF-α, TGF-β and IL-6 substantially contribute to the insulin resistance on the peripheral tissue (Kern et al., 2001; Samad et al., 1997; Uysal et al., 1997).

Despite, hyperglycemia plays a key role in oxidative stress in the diabetic nerve, the contribution of other factors such as endoneurial hypoxia, transition metal imbalances, and hyperlipidemia have also been accused (Pop-Busui et al., 2006). A high-fat diet alone has been shown to increase oxidative stress (Kempler et al., 2002; Young et al., 1993) and by this pathway they developed sensory and motor nerve conduction velocity deficits prior to impaired glucose tolerance in rats (Vincent et al., 2009b). Increased oxidative stress has clearly been shown to contribute to the pathology of neural dysfunction in diabetes and it has been proposed as a mechanism that contributes to pathogenesis of DN (Pop-Busui et al., 2006; Vincent et al., 2004). For that reason, we investigated the oxidant/antioxidant parameters of sciatic tissues in rats. Our study revealed that, pro-inflammatory cytokine levels such as TNF-α and TGF-β were higher in the high-fat diet group than in the control group. These data suggest that inflammation may play a key role in the pathophysiology of the neuropathy induced by high-fat diet.

Malondialdehyde is an oxidative metabolite and an oxidant parameter of the lipid peroxidation in peripheral nerve (Wang et al., 2008). Both MDA and NO may activate neurotoxic cascade accumulation of calcium in the cells, apoptosis and eventually cell death in peripheral nerve when their levels get higher than physiological level (Ghafourifar and Cadenas, 2005; Wang et al., 2008). Superoxide dismutase acts as one of the primary defense mechanisms against reactive oxygen metabolites (McIntyre et al., 1999). Also, GPx has been shown to be an important compensatory response to oxidative stress and the increase of peroxides is mediated by SOD action (Varija et al., 2009; Wang et al., 2008). Superoxide dismutase and GPx were shown to be increased significantly in polyneuropathy (Naik et al., 2006; Wang et al., 2008). Although, PON-1 is a serum enzyme that prevents oxidation of low-density protein by hydrolyzing lipid peroxides; also it has an effect on hydrogen peroxide. Paraoxonase-1 reduces hydroperoxides and hydroxyl radicals have been thought to have peroxidase-like activities, correspondingly; in conditions with high oxidative stress, the activity of PON-1 is reduced (Serhatlioglu et al., 2003). In the present study; the MDA, NO levels, SOD and GPx activities were significantly higher in the high-fat diet group than the control group. The increased enzyme activity of SOD and GPx in the high-fat diet group may be an adaptive response to the elevated oxidative stress. Similar results for the positive correlation between activity of SOD and level of MDA were demonstrated in the earlier study (Ben Abdallah et al., 2009). In the sciatic nerve of rats, high-fat diet caused a significant decrease in the activity of PON-1 in our study, in which there was a negative correlation between MDA, NO levels and PON-1 activity. Therefore, we suggest that the decreased activities of PON-1 in the sciatic nerves might be related to high-fat diet induced oxidative stress.

Stereological methods are commonly used in researches regarding experimental nerve injuries, repair and regeneration (Geuna et al., 2000, 2001; Gundersen, 1986). Quantitative features of the formal characteristics at the nerve fibers, such as; axon number, axonal area and myelin thickness are usually parameters for determining experimental nerve injury types. Earlier studies showed that the stereological analysis technique could supply correct and trustworthy estimates of histomorphological data (Canan et al., 2008; Geuna et al., 2000, 2001). Therefore, in our study; we performed a stereological morphometric analysis of the sciatic nerves obtained from both groups. Mean nerve fiber diameter, axon diameter, axon area and myelin sheet thickness in the sciatic nerve were lower in rats with high-fat diet than in the control group. However, no significant differences in the axon numbers were found between the groups. Electron microscopic images showed that; while regular form and thickness of myelin sheaths were protected in the control group, myelin degenerations and shrinkage in axons were seen in high-fat diet group.

On the other hand, this study has some limitations. First of all functional outcome measures are needed. The number of the rats in each group may be augmented. Quantitative results of the electron microscopic evaluation are needed.

5. Conclusions

In conclusion, all these data support that high fat diet creates an inflammatory environment with high oxidative stress, which causes neuronal degeneration. We postulated that; oxidative stress and inflammatory response might play an important role in

the pathogenesis of high-fat diet induced neurotoxicity. In addition, our results support that dyslipidemia is an independent risk factor for the development of diabetic neuropathy. So, the clinicians should keep in mind the strict control of lipid levels for the protection and the treatment of patients with DN.

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