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Role of calorie restriction in alleviation of age-related morphological and biochemical changes in sciatic nerve

Mona G. Amer[a,b,*], Nehad F. Mazen[a], Nader M. Mohamed[c]

[a] Histology & Cell Biology Department, Faculty of Medicine, Zagazig University, Egypt
[b] Histology Department, Faculty of Medicine, Taif University, Saudi Arabia
[c] Pediatrics Department, Faculty of Medicine, Taif University, Saudi Arabia

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ABSTRACT

Background: Aging is associated with structural, functional and biochemical alterations in the nervous system. Calorie restriction (CR) was found to retard most physiological indices of aging.
Objectives: This work aimed to investigate the effect of CR on age-related changes in sciatic nerves.
Materials and methods: Thirty male albino rats aged 1 month were equally divided into three groups; Group I [control adult-ad libitum AL]: fed a regular diet and sacrificed at the age of 6 months, group II (aged-AL group): fed a regular diet AL and sacrificed at the age of 18 months, and group III (aged CR) fed a 40% calorie restricted diet and sacrificed at the age of 18 months. Rats were anesthetized and sciatic nerves were processed for light, electron microscope and morphometric studies. Oxidative stress in sciatic nerves was investigated by estimation of lipid perioxidation by product malondialdehyde (MDA) tissue level and antioxidant enzyme; superoxide dismutase activity (SOD).
Results: The aged (AL) sciatic nerves appeared disorganized, with thick perineurium and increased collagen fibers associated with decreased *g*-ratio. Abnormal myelin forms were seen as outfolded myelin loops, thin denuded myelin, splitting of myelin into myelin figures and interlamellar vacuoles. Schwann cells revealed vacuolated cytoplasm. There was also significant increase in MDA level and a significant decrease in SOD activity in comparison to control adult (AL). Apparent structural and histomorphological improvement were noticed after CR in aged rats.
Conclusion: Aging caused structural and biochemical alterations in sciatic nerves with alleviating effect of calorie restriction on such effects.

1. Introduction

Aging is associated with structural, functional and biochemical alterations in the nervous system. Neuromuscular system function declines with age and manifests as dramatic decreases in muscle strength and size, often referred to as sarcopenia (Vandervoort, 2002). Skeletal muscle atrophy and weakness lead to the loss of functional mobility and independence for many older adults (Roubenoff, 2001). Age-related changes in the central nervous system (CNS) are well documented and include neuronal loss, demyelination, and deficits in cognitive function; however, little has been reported concerning age-related changes in the peripheral nervous system beyond a decline in nerve conduction velocities (Peters, 2002). Neurons with long processes are particularly vulnerable to degeneration which makes peripheral nerves (PN) susceptible to age-related modifications (Mattson and Magnus, 2006). Signal propagation along axons is facilitated by myelin, a lipid-rich membranous structure formed by Schwann cells (SC). Distinct domains within the myelin and the axonal plasma membrane are maintained by complex signaling events between neurons and glia (Garbay et al., 2000). Therefore, degenerative changes in either cell type have global influences on overall nerve structure and function.

A clear understanding of the mechanisms underlying age-related changes in the peripheral nervous system is necessary to fully understand and prevent the decline in neuromuscular function that often accompanies aging (Sims-Robinson et al., 2013).

The health benefits of calorie restriction (CR) have been known to the scientists for decades. In the recent literature, CR or dietary restriction has been generally defined as consumption of nutritious diet that is 40% less in calories compared to ad libitum diet

* Corresponding author at: Histology & Cell Biology Department, Faculty of Medicine, Zagazig University, Egypt. Tel.: +20 1223333863.
E-mail addresses: mona_amer@rocketmail.com (M.G. Amer), nehadmazen@yahoo.com (N.F. Mazen), nadermmm333@yahoo.com (N.M. Mohamed).

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(Wolf, 2006). Reduced calorie intake without malnutrition extends lifespan in rodents and delays the onset of multiple age-associated diseases (Colman et al., 2014). Although there have been extensive studies on the ability of CR to reduce age-related changes in the central nervous system, peripheral organs and lifespan (Mattson et al., 2001; Jolly, 2004), little is known about the effects of such approach on peripheral nerves. Previous studies (Rangaraju et al., 2009; Opalach et al., 2010) showed that dietary restriction is an efficient means of defying age-related oxidative damage in peripheral nerves associated with reduction in the expression of the major myelin proteins and widening of the nodes of Ranvier. However, no available data on the effect of such mechanisms on the age associated histopathological changes in peripheral nerves.

We hypothesized that the mechanism of aging is mainly attributed to oxidative damage. Therefore, the present study examined the effect of a 40% caloric restriction (CR) versus ad libitum (AL) feeding on the structural, ultrastructural changes and oxidative parameters associated with aging in sciatic nerves.

2. Materials and methods

All experimental procedures were carried out in accordance with the research protocols following the recommendations of the Institute Review Board Instruction of Care and Use of Laboratory Animals, Faculty of medicine, Zagazig University.

2.1. Animals

This study was carried out on 30 male albino rats, aged 1 month, weighing 50–75 g. They were obtained from the Animal House, Faculty of Medicine, Zagazig University, Egypt. Rats were housed in stainless steel cages with a 12-h light/dark cycle and allowed tap water ad libitum. They were allowed to acclimatize for 2 weeks before starting the experiment and observed for general health and suitability for induction in the experiment and included in the study.

2.2. Experimental design

Rats were equally divided into three groups as follows:

(1) Group 1 [adult-ad libitum (AL) group]: Animals were fed a regular diet AL (Table 1A) and sacrificed when they reached the age of 6 months (Bhattacharyya and Thomas, 2004). They served as the control group.
(2) Group 2 (aged-AL group): Animals were fed a regular diet AL and sacrificed at the age of 18 months.
(3) Group 3 (aged-calorie restricted (CR) group) (Obin et al., 2000): Animals were fed a 60% calorie diet (Table 1) started at 6 weeks age until the age of 18 months, when they were sacrificed.

The 40% calorie restriction diet was formulated so that the animals received 40% fewer calories than the ad-libitium-fed animals.

At the time of sacrifice, rats were anesthetized by intraperitoneal injection of pentobarbital sodium (55 mg/kg bw). A longitudinal skin incision was performed at the upper hind limb level with blunt separation of gluteal muscles to expose the sciatic nerve and 10 mm long nerve fragments were collected and processed for histological and biochemical analysis.

Table 1
Regular diet (A) and 60% caloric diet (B) according to the feeding protocol of the Faculty of Veterinary Medicine, Zagazig University.

Regular diet (A)	Caloric diet (60%) (B)
Yellow corn (620 g)	Yellow corn (372 g)
Soyabean (250 g)	Soyabean (250 g)
Molasses (20 g)	Molasses (12 g)
NaCl (10 g)	NaCl (10 g)
Vitamin mix (100 g)	Vitamin mix (100 g)

2.3. Histological study

For light microscopy, specimens were fixed in 10% formalin solution and processed to prepare 5 μm thick paraffin sections that were stained with hematoxylin and eosin (Bancroft and Gamble, 2008).

Parts of sciatic nerves of all groups were processed using simple pre-embedding protocol for staining myelin sheaths, described by Di Scipio et al. (2008). The method involved immersing the specimens in 2% osmium tetroxide for 2 h after paraformaldehyde fixation, followed by routine dehydration and paraffin embedding. Sections were then counterstained using Masson's trichrome counterstain, which permitted the imaging of connective structures in nerves.

Specimens for electron microscope examination were cut into 1 mm^3 pieces and stored in the same fixative overnight at 4° C (2.5% glutaraldehyde buffered with 0.1 mol/L phosphate buffer at pH 7.4 for 2 h). They were postfixed in 1% osmium tetroxide for 1 h, dehydrated through graded alcohol series, and embedded in epoxy resin. Ultrathin sections (50 nm thick) were collected on copper grids and stained with uranyl acetate and lead citrate (Hayat, 2000).

2.4. Quantitative morphometric analysis

The measurements were obtained using computer-based image analysis software (Leica Qwin 500; Imaging Systems, Cambridge, UK). Sections from all groups were analyzed for certain parameters (Inserra et al., 2000): The nerve cross-sectional area (in micrometer square) was determined by manually outlining the nerve image at 10× magnification using H&E-stained sections. Total number of nerve axons, number of regularly organized myelinated axons, number of irregular and degenerated myelinated axons and area percentage of blue colored collagen fibers was determined using osmic acid and MT-stained sections at 40×.

Fiber and axon diameters were measured using osmic acid and MT-stained sections at 1000× magnification in five different fields for each specimen (rat), following which they were stored and summarized for statistical analysis. The *g*-ratio (The ratio of axon diameter to fiber diameter) was calculated (Arbuthnott et al., 1980). All results were statistically analyzed.

2.5. Biochemical analysis

Sciatic nerves were rinsed in ice-cold saline solution and frozen in liquid nitrogen after removal of the adherent tissue. On the day of the homogenate preparation, sciatic nerve segments were measured, weighed and rinsed in ice-cold saline solution. Sciatic nerves were cut into small pieces and then homogenized at 4 °C in 2 mL of ice-cold saline (11 mmol L^{-1} Tris buffer, pH 7.4) with glass homogenizer. The resulting homogenate was passed through a cellulose filter to remove impurities and divided into aliquots for biochemical analysis. All sciatic nerve samples were analyzed for determination of the following parameters:

(a) Determination of lipid perioxidation: Lipid peroxide was estimated by measurement of malondialdehyde (MDA) levels spectrophotometrically in sciatic tissue homogenate according to method done by Buege and Aust (1978). MDA in the supernatant can react with freshly prepared thiobarbituric acid (TBA) to form a colored complex which has maximum absorbance at 535 nm. The nmol MDA/g wet tissue was