

Morphometric analysis of the diameter and *g*-ratio of the myelinated nerve fibers of the human sciatic nerve during the aging process

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Abstract Myelinated nerve fibers suffer from different degrees of atrophy with age. The success of subsequent regeneration varies. The aim of this research was to analyze myelinated fibers of the human sciatic nerve during the aging process. Morphometric analysis was performed on 17 cases with an age range from 9 to 93 years. The outer and inner diameter of 100 randomly selected nerve fibers was measured in each of the cases evaluated, and the *g*-ratio (axonal diameter/outer diameter of the whole nerve fiber) of each was calculated. Scatter plots of the diameters and *g*-ratios of the analyzed fibers were then analyzed. Nerve fibers of each case were classified into three groups according to the *g*-ratio values: group I (*g*-ratio lower than 0.6), group II (*g*-ratio from 0.6 to 0.7) and group III (*g*-ratio higher than 0.7). Afterwards, nerve fibers of group II were further classified into small and large subgroups. The percentages of each group of nerve fibers were computed for each case and these values were used for correlational and bivariate linear regression analysis. The percentage of myelinated nerve fibers with large diameter and optimal *g*-ratio of the sciatic nerve declines significantly with age. This is accompanied by a simultaneous significant increase in the percentage of small myelinated fibers with *g*-ratio values close to 1 that occupy the upper left quadrant of the scatter plot. It can be concluded that aging of the sciatic nerve is associated with significant atrophy of large myelinated fibers. Additionally, a significant increase in regenerated nerve fibers with thinner myelin sheath is

observed with age, which, together with the large myelinated fiber atrophy, might be the cause of the age-related decline in conduction velocity. A better understanding of the changes in aging peripheral nerves might improve interpretation of their pathological changes, as well as comprehension of their regeneration in individuals of different age.

Keywords Ageing · Myelinated nerve fibers · Morphometry · Neuropathy · Sciatic nerve

Introduction

The myelinated nerve fiber of the peripheral nervous system (PNS) is comprised from the axon–Schwann cell complex. Several hundred Schwann cells arranged along the whole length of the nerve fiber compose its myelin sheath. Myelination of nerve fibers starts during embryonic development and continues postnatally. It also occurs during regeneration of injured nerve fibers during an individual's lifetime (remyelination). The relationship between Schwann cells and axon directly influences the conduction velocity of the nerve fiber as follows: an increase in axonal diameter stimulates thickening of its myelin sheath, which is associated with elongation of the internodal segment and a consequent increase in the saltatory conduction velocity of the nerve impulse (Thomas and Ochoa 1984). Nerve fiber diameters range from 2 to 18 μm and, according to their size, they can be classified into two groups (clusters): small and large fibers (Asbury and Johnson 1978; Thomas and Ochoa 1984). Rushton (1951) was the first to present the relationship between axonal diameter and the thickness of its myelin sheath as a *g*-ratio (*g*-ratio = axonal diameter/outer diameter of the

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whole nerve fiber). Rushton (1951) considered a g -ratio value of 0.6 to be theoretical optimal for the conduction velocity of nerve impulses. General opinion is that the g -ratio is optimized in such a way as to enable maximal efficacy and physiological optimization of the nerve fibers. Thomas and Ochoa (1984) upgraded Rushton's observations through their prediction of the internodal conduction velocity for the different deviations of the g -ratio from its theoretical optimum: a deviation of 0.1 (g -ratio 0.5–0.7) leads to an increase in internodal conduction velocity of 7 %; a deviation of 0.2 (g -ratio 0.4–0.8) increases conduction velocity by 28 %; while deviation of 0.3 (g -ratio 0.3–0.9) leads to an increase in internodal conduction velocity of 55 %. Thomas and Ochoa (1984) further cited that g -ratio values lower than 0.4 indicate the presence of degenerated nerve fibers with abnormal thickening of the myelin sheath, whereas values higher than 0.7 indicate either regenerated fibres with thinner myelin sheath, or demyelinated nerve fibers. Still today, g -ratio and diameter remain significant tools for the analysis of the myelinated nerve fibers in "healthy" peripheral nerves as well as in different types of neuropathies, and in evaluation of the success of the course of post-traumatic regeneration of injured nerve fibers and/or application of different techniques for their reparation.

Clinical and experimental studies have shown that the aging is associated with a decline in muscle strength, followed by a decrease in tactile, thermal and vibration sensitivity, as well as significant autonomic dysfunction (Melcangi et al. 2000; Verdu et al. 2000; Peters 2002; Kovačić et al. 2009). So-called senile neuropathy is most frequently accompanied by a decrease in the number and density of myelinated nerve fibers and by numerous irregularities in the myelin sheath. Senile atrophy of the myelinated nerve fibers is followed by more or less successful regeneration, the result of which depends on several factors. That is why its manifestation might be different in the central nervous system versus the PNS, in cranial versus spinal nerves, and between different spinal nerves. The aim of our research was to analyze the myelinated nerve fibers of the sciatic nerve as the largest nerve of the human body, during the aging process.

Materials and methods

Morphometric analysis of the myelinated nerve fibers of the sciatic nerve was performed on 17 cases of both genders, spanning an age range of 9 to 93 years. The cases analyzed had no history of diabetes mellitus or any neurological disorder that could alter peripheral nerve composition. Samples of the sciatic nerve 2 cm long were dissected from the middle part of the posterior femoral

region during routine autopsies performed at the Department of Forensic Medicine at Medical Faculty, University of Niš, Serbia. Afterwards, samples were fixed in 10 % buffered formalin, embedded in paraffin and then cut into sections 5 μm thick. The sections were further stained by Heidenhain's iron hematoxylin to allow visualization of the myelin sheath of the nerve fibers (Drury and Wallington 1980). Digital 24-bit RGB images (size 2560 \times 1920 pixels) of the stained sections were captured by a digital camera (Praktica DPix 3000) mounted on the eyepiece of a light microscope (Olympus CH2 K) under a 100x magnification lens using immersion oil. A total of 100 randomly selected nerve fibers was analyzed in each of the evaluated cases (5 myelinated fibers per each of 20 selected by an unbiased method of selected fields of vision in each case). Morphometric analysis was performed using ImageJ (<http://rsb.info.nih.gov/ij/>). Spatial calibration of the system was performed by an object micrometer (1:100). During the morphometric analysis, the outer (D) and inner (d) diameter of the each of the selected nerve fibers was measured at three different locations (along the vertical, horizontal and right oblique axis of the nerve fiber cross section) using straight line selection. The outer diameter represented the distance between two symmetrical points on the outer surface, while the inner diameter was the distance between two symmetrical points on the inner surface of the myelin sheath. The values obtained, i.e., the outer and inner diameters, were used to calculate mean values for each of the above cited parameters for each of the analyzed nerve fibers. The g -ratio for each of the analyzed nerve fibers was then calculated from the mean values of the outer and inner diameter obtained according to the formula $g = d/D$.

Scatter plots were used to analyze the distribution of the myelinated nerve fibers according to the values of their outer diameter and g -ratio. Statistical analysis was performed using SPSS version 16 (SPSS, Chicago, IL). Nerve fibers were classified into the three groups according to their g -ratio: group I included nerve fibers with thicker myelin sheath (g -ratio <0.6), group II were nerve fibers characterized by optimal myelin sheath thickness (g -ratio 0.6–0.7) and group III nerve fibers with thinner myelin sheath (g -ratio >0.7). Afterwards, nerve fibers group II were classified into small (average diameter $\leq 7.5 \mu\text{m}$) and large (average diameter $>7.5 \mu\text{m}$) subgroups. Further, the percentages of the above-cited groups of nerve fibers were computed for each case and the resulting values used for correlational and linear regression analysis. Three separate linear regression analyses were performed in which age was used as predictor and percentages of group I, II and III nerve fibers as outcome variables, respectively. Further, additional separate linear regression analyses were performed in which age was used as predictor and the

percentage of nerve fibers with optimal g -ratio with diameter $\leq 7.5 \mu\text{m}$, as well as the percentage of nerve fibers with optimal g -ratio with diameter $>7.5 \mu\text{m}$, were used as outcome variables, respectively.

Results

Figure 1a presents the endoneurium of a 9-year-old case, while Fig. 1b show the endoneurium of an 89-year-old. In the younger case, larger myelinated nerve fibers with higher diameter predominate, whereas in the older case a predomination of nerve fibers with lower diameter and thinner myelin sheath can be observed. Figure 2 presents combined scatter plots of the g -ratio and diameter of the myelinated nerve fibers of all analyzed cases. A scatter plot of the nerve fibers of the 9-year-old case reveals a homogenous distribution of nerve fibers with diameters ranging from $3 \mu\text{m}$ to $14 \mu\text{m}$, and g -ratio values ranging from 0.5 to 0.7. Scatter plots of the 18- and 21-year-old cases reveal the presence of two distinct clusters of nerve fibers (small and large fibers), with clusters of large myelinated fibers being obviously larger. The g -ratios of nerve fibers in both clusters ranged from 0.5 to 0.8. Scatter plots of the nerve fibers of cases aged from 27 to 53 years presented a similar distribution of nerve fibers. Two distinct clusters with approximately equal numbers of nerve fibers can be observed on each of the scatter plots. In these cases, groups of small nerve fibers in which the g -ratio ranged from 0.5 to 0.7 can also be observed. Nevertheless, the g -ratio of the large nerve fibers of these cases increased, and ranged from 0.6 to 0.8. Two groups of nerve fibers still can be observed on the scatter plots of cases with an age range from 69 to 80 years. Large nerve fibers, in which the g -ratio was close to 1 and ranged from 0.7 to 0.9, are less numerous and are positioned in the upper right quadrant of the scatter plot. The number of smaller nerve fibers with g -ratio values from 0.4 to 0.8 is significantly higher, and these are positioned mostly in the upper left quadrant of the scatter plot. In scatter plots of cases older than 80 years,

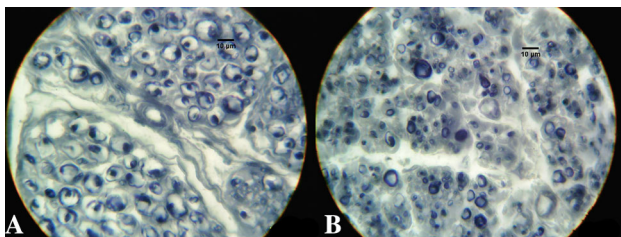


Fig. 1 a, b Endoneurium of the sciatic nerve in a 9-year-old case (a), and in an 89-year-old (b). Myelinated nerve fibers are observed as black stained annular structures with different size. Heidenhain's iron haematoxylin, 100x lens magnification. Bar $10 \mu\text{m}$

nerve fibers of smaller diameter and g -ratio values from 0.7 to 1 predominate and form a homogenous cluster in the upper left quadrant of the scatter plot.

Table 1 presents the percentages of group I, II and III nerve fibers, then the same information for nerve fibers with optimal g -ratios for which the diameter is $\leq 7.5 \mu\text{m}$ as well as the percentage of nerve fibers with optimal g -ratio and a diameter of $>7.5 \mu\text{m}$. The percentage of sciatic nerve type I and II myelinated nerve fibers, in which the g -ratio was lower than 0.6 and ranged from 0.6 to 0.7, respectively, decreased significantly during the aging process (type I nerve fibers: $R = -0.79$, $n = 17$, $P < 0.0001$; type II nerve fibers: $R = -0.87$, $n = 17$, $P < 0.0001$) (Fig. 3a, b). Simple linear regression analysis showed that age significantly predicts both the percentage of the type I [$F(1,15) = 24.56$, $P < 0.0001$] and type II [$F(1,15) = 46.03$, $P < 0.0001$] nerve fibers of the sciatic nerve. These relationships were identified by the following two models: type I nerve fibers $\% = 41.51 - 0.35 \times \text{age}$ and, type II nerve fibers $\% = 48.93 - 0.44 \times \text{age}$. The adjusted R square value was 0.6 for type I fibers, leading to the conclusion that age explains 60 % of the type I nerve fiber's percentage variance, while R^2 was even higher (0.74) for type II nerve fibers, consequently showing that 74 % of the type II nerves' fibers percentage variance can be explained by the age of the cases evaluated. In contrast, however, the percentage of type III myelinated nerve fibers increased significantly during the aging process ($R = 0.88$, $n = 17$, $P < 0.0001$) (Fig. 3c). This is also significantly predicted by age [$F(1,15) = 49.89$, $P < 0.0001$] and the identified equation that explains this relationship is: type III nerve fibers $\% = 9.56 + 0.75 \times \text{age}$. In this case the adjusted R^2 was 0.75, which means that 75 % of the type III nerve fibers percentage variance can be explained by age.

In group II myelinated nerve fibers, we observed that the percentage of small nerve fibers with a diameter $\leq 7.5 \mu\text{m}$ increased significantly [$F(1,15) = 57.41$, $P < 0.0001$] (Fig. 4a), while the percentage of large nerve fibers with diameter $>7.5 \mu\text{m}$ [$F(1,15) = 28.20$, $P < 0.0001$] decreased significantly (Fig. 4b) during the aging process. It is interesting that these two relationships were identified with the following two equations which are identical, but have the opposite direction compared to the relationships cited above: $\%$ normal nerve fibers with $D \leq 7.5 \mu\text{m} = 15.26 + 0.74 \times \text{age}$, and, $\%$ of normal nerve fibers with $D > 7.5 \mu\text{m} = 84.74 - 0.74 \times \text{age}$. These two models explain 78 % of the small and large nerve fibers percentage variance during the aging process (adjusted $R^2 = 0.78$ in both cases). This might lead to the conclusion that these two processes, i.e., the loss of large nerve fibers and the increase in small nerve fibers during aging, are mutually connected.

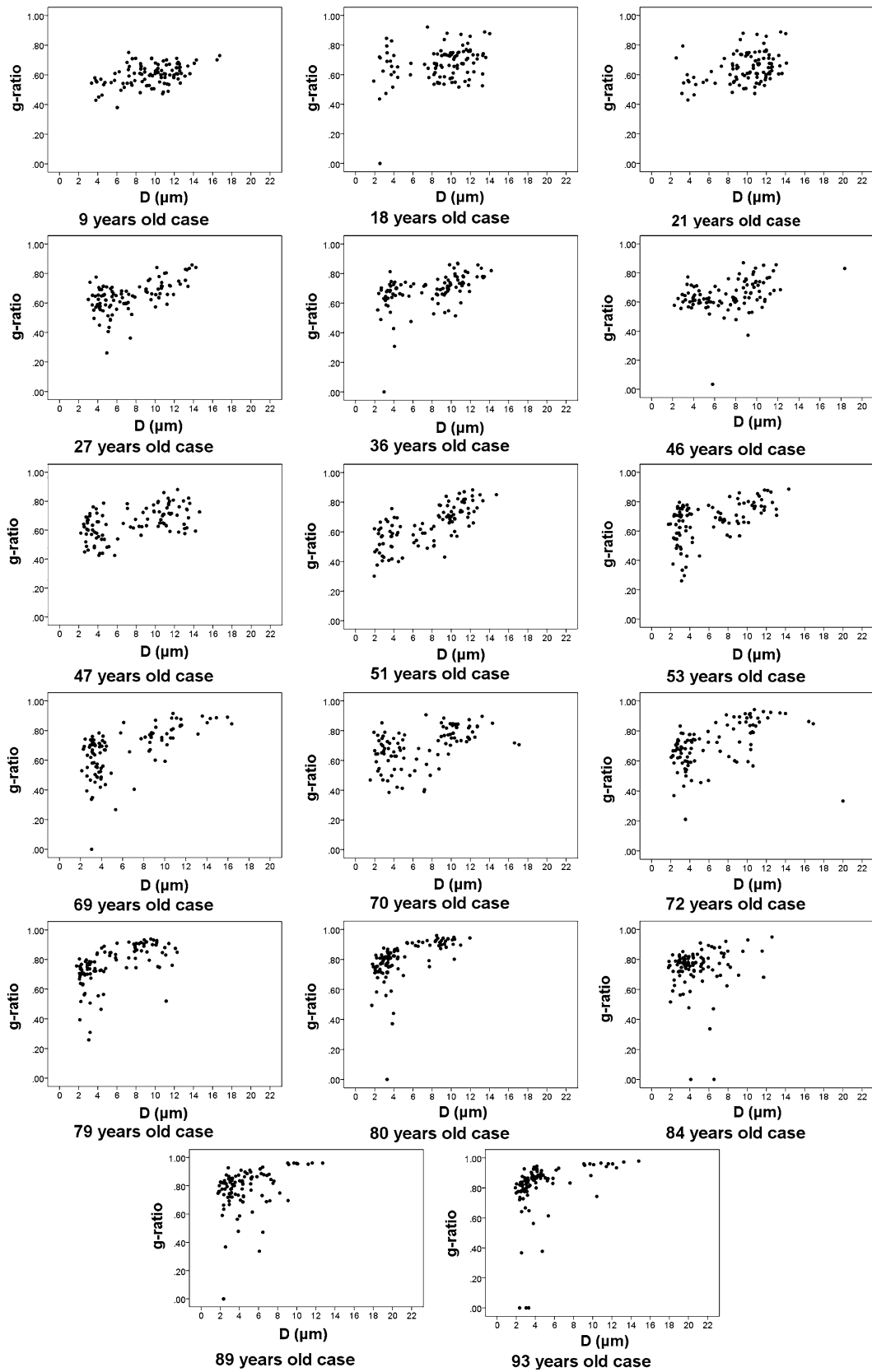


Fig. 2 Scatter plots of the g-ratios and diameter of the nerve fibers of all evaluated cases

Table 1 Percentage of all types of evaluated myelinated nerve fibers in evaluated cases

Case	Age	Type I nerve fibers (%)	Type II nerve fibers (%)	Type III nerve fibers (%)	% of type II ($D \leq 7.5 \mu\text{m}$)	% of type II ($D > 7.5 \mu\text{m}$)
1	93	6	4	90	82.40	17.60
2	89	8	10	82	82.40	17.60
3	84	10	12	78	88.20	11.80
4	80	7	4	89	67.60	32.40
5	79	11	7	82	82.40	17.60
6	72	21	24	55	67.60	32.40
7	70	21	23	56	52.90	47.10
8	69	29	20	51	70.60	29.40
9	53	23	25	52	52.90	47.10
10	51	37	28	35	50.00	50.00
11	47	28	34	38	50.00	50.00
12	46	26	44	30	47.10	52.90
13	36	14	36	50	41.20	58.80
14	27	31	40	29	64.70	35.30
15	21	34	36	30	14.70	85.30
16	18	25	28	47	23.50	76.50
17	9	47	43	10	23.50	76.50

Discussion

Analysis of scatter plots of nerve fibers of the sciatic nerve in our study showed a progressive loss of large myelinated nerve fibers and predominance of small fibers with thinner myelin sheath in cases older than 70 years. According to Friede and Beuche (1985), such small fibers with thinner myelin sheath represent regenerated nerve fibers with disproportionately thin myelin sheath. These latter authors considered the characteristics of the regenerated nerve fibers to be as follows: (1) a distinct increase in the g -ratio (meaning thinner myelin sheath in relation to normal fibers); (2) a slight shift in the distribution of the fibers to the left on the scatter plots (which is the sign of the subtotal restitution of the diameter of the fibers); and (3) the parabolic shape of the distribution of nerve fibers in the upper left quadrant of the scatter plot (empirically, it is confirmed that this indicates the presence of a group of immature, e.g., regenerated, nerve fibers). Scatter plots can also provide information about the selective damage to one and/or both groups of fibers. Thus, scatter plots of patients suffering from alcoholic neuropathy show loss of fibers with small and large diameter, while their g -ratios retain optimal values, indicating damage to both groups of fibers without their subsequent regeneration (Friede and Beuche 1985).

The results of statistical analysis of the myelinated nerve fibers of the sciatic nerve revealed a significant decrease in the percentage of nerve fibers with optimal g -ratios. Additionally, within the latter cited group of nerve fibers

(group II), a decrease in the percentage of nerve fibers with large diameters, accompanied by a simultaneous increase in nerve fibers of the smaller diameter, was also observed. It is obvious that, during the aging process, myelinated nerve fibers with large diameter are damaged more severely than those of small diameter in the sciatic nerve. Jacobs and Love (1985) established that, in the human sural nerve at the age of 10 years, the g -ratio of small diameter nerve fibers ranges from 0.65 to 0.80, while the values of the g -ratio of large diameter nerve fibers ranges from 0.55 to 0.65. Such g -ratio values remain unchanged until the age of 60, after which the g -ratio continues to increase to reach final values close to 1. Jeronimo et al. (2008) and Soltanpour et al. (2012) observed a decrease in the axonal diameter, the thickness of the myelin sheath and the g -ratio, as well as a shift in the scatter plot of the g -ratio to the left in the hypoglossal and sural nerve of rats. In contrast to the latter authors, Chentanez et al. (2010) did not establish any changes in the morphometric parameters of myelinated nerve fibers in the superficial branch of the human radial nerve. Fahrenkamp and Friede (1987) detected that, in rats, the g -ratio of the splanchnic is 0.78; that of the vagal nerve 0.73; glossopharyngeal nerve 0.78; oculomotor nerve 0.8; hypoglossal and facial nerve 0.69. Taking into the consideration the fact that scatter plots of the g -ratio of different nerves in rats differ, it can be assumed that there is "specific" pattern for the dynamics of the axonal diameter and thickness of the myelin sheath of each peripheral nerve during the aging process (Fahrenkamp and Friede 1987; Azcoitia et al. 2003; Jeronimo et al. 2008; Soltanpour et al.

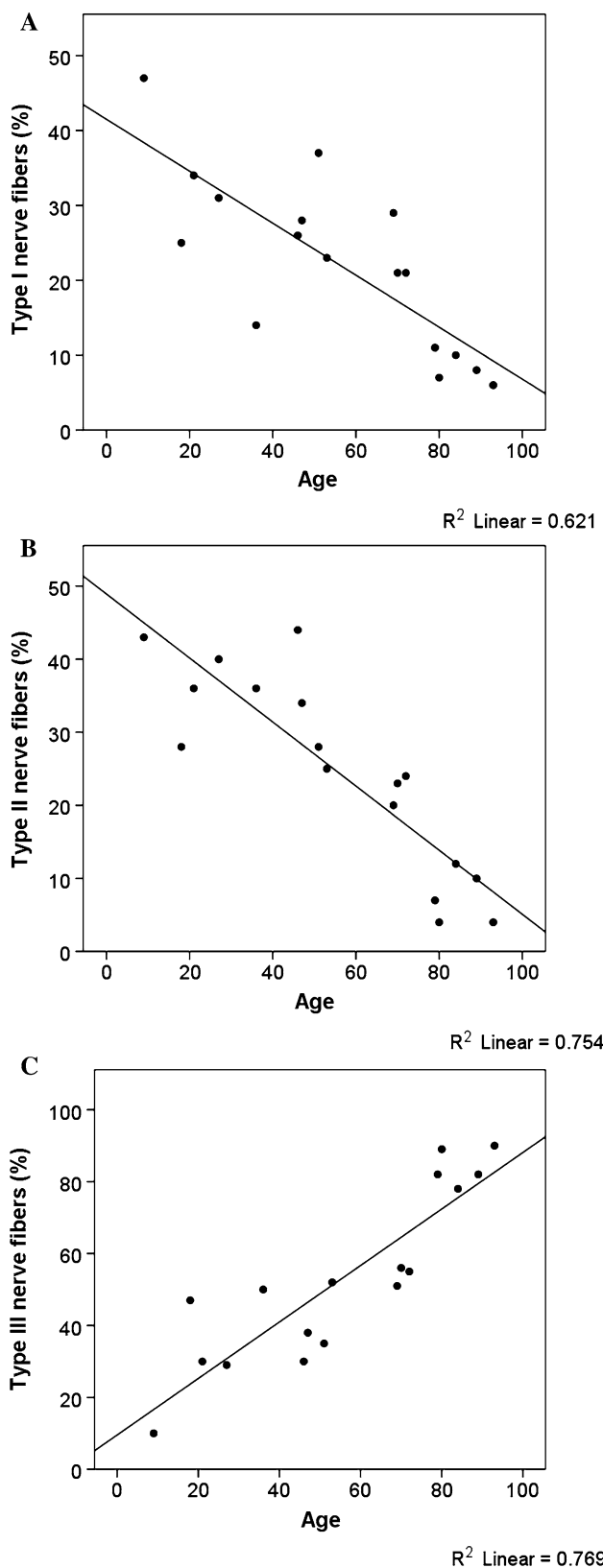


Fig. 3 Percentage of **a** type I, **b** type II, and **c** type III myelinated nerve fibers of the evaluated cases of the human sciatic nerve during the aging process

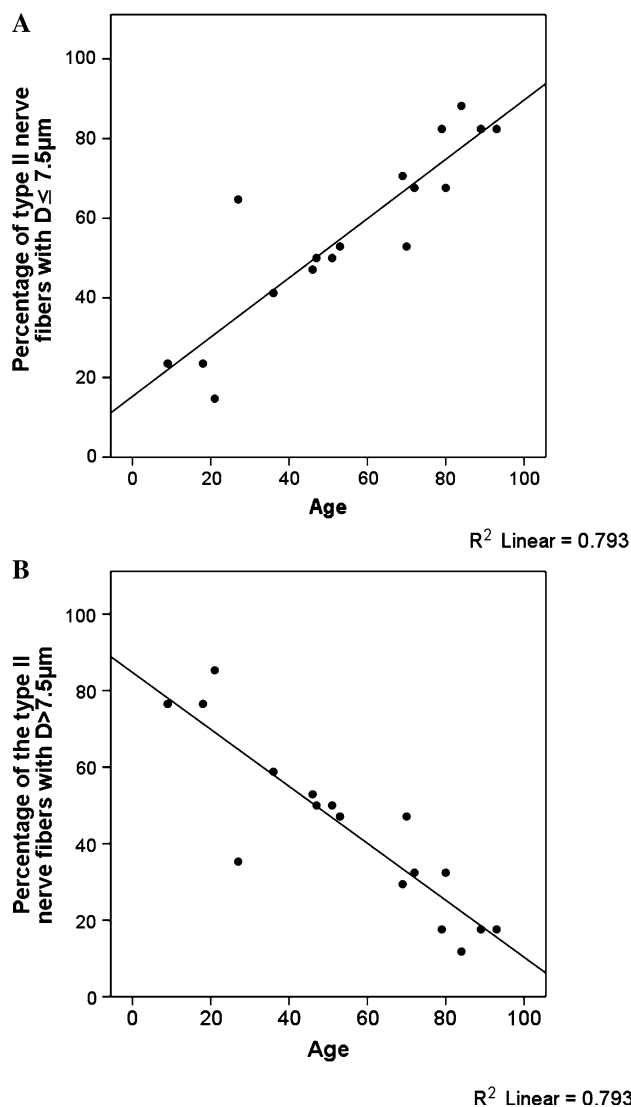


Fig. 4 **a** Percentage of optimal myelinated nerve fibers with diameter $\leq 7.5 \mu m$. **b** Percentage of nerve fibers with diameter $> 7.5 \mu m$ during the aging process

2012). Bosboom et al. (2001) analyzed bioptic material of the sural nerve and detected significantly lower *g*-ratio values in patients with chronic inflammatory demyelinating polyneuropathy than in patients with chronic idiopathic axonal polyneuropathy and control cases. In contrast to the latter finding, value of the *g*-ratio of the sural nerve in diabetic patients did not differ significantly from those of autoptic control samples (Malik et al. 2001). Sanada et al. (2012) reported that, in rats, neuropathy associated with hypertension is accompanied by a decrease in the *g*-ratio, which was followed by a shift in the histogram to the right due to the significant atrophy of myelinated nerve fibers with small diameter. It seems that aging also influences, through the different models for the motor, sensitive and autonomic nerve fibers, regeneration of the peripheral

nerves and reinnervation of target organs (Ceballos et al. 1999).

Melcangi et al. (2000) and Verdu et al. (2000) considered that one possible cause for the destruction of the large myelinated fibers with age might be a reduction in axonal transport. Taking into the consideration that a reduction in axonal transport of different substances might lead to the destruction of neurofilaments, which are the structures responsible for the preservation of axonal caliber, a consequent decrease in the axonal caliber and, finally, its degeneration. Age-related changes to internodal geometry are not distributed homogeneously. For example, they are less obvious in the facial nerve than in the ulnar and lateral popliteal nerve of the rat. Thus, Melcangi et al. (2000) considered that the cause of this variability between different peripheral nerves might be the consequence of recurrent mechanical disorders of certain nerves. More profound aging changes in the plantar nerves, which are more exposed to such mechanical traumas, in relation to the more protected tibial nerve in elderly rats, confirmed the latter assumption. The differences observed between the intensity of aging changes in peripheral nerves of the lower extremities in relation to the same regions of the upper extremities might be explained by the higher susceptibility of the peripheral nerves of the lower extremity to trauma and vascular disorders (O' Sullivan and Swallow 1968; Tohgi et al. 1977; Atkinson and Santer 1999). Atkinson and Santer (1999) analyzed the effects of aging on cardiac nerve fibers and concluded that they are protected from age-related changes because the majority of such fibers retain *g*-ratio values of between 0.4 and 0.6 with age. Besides mechanical damage of the nerve itself, Johansson et al. (1996) concluded that aging also affects the target structures that such nerves innervate. Namely, the latter authors found that aging changes are more profound in the inferior alveolar than in the mental nerve of rats, which they explained through differences in their target structures: intense aging changes in the mucocutaneous structures innervated by the mental nerve are less compared to the same changes in teeth. The theoretical speculations of Chomiak et al. (2009), who correlated optimal *g*-ratio values of the myelinated nerve fibers of the rat with their volume as a significant limiting factor for the optimization of the *g*-ratio, are very interesting. It is possible that, in the PNS, the space constraint is less of a limiting factor than in the brain, as axonal myelination in the PNS tends to be optimized for maximizing conduction velocity so that long projection axons can ensure rapid sensory and motor responses. Muratori et al. (2012) tested the ability of nerve fibers to restore their normal prelesional structure spontaneously. The results of their study showed that, in the median nerve of rats, the number of nerve fibers

was restored to prelesional values after a sufficiently long period of regeneration, but diameter and *g*-ratio were not restored.

Analysis of the myelinated nerve fibers of the sciatic nerve pointed to the significant atrophy and loss of large myelinated nerve fibers with age. An increase in the number of small nerve fibers with a thinner myelin sheath might point to the regeneration of nerve fibers during the aging process. Such fibers probably do not reach prelesional diameter and therefore prelesional function. There is a lack of literature data on sciatic nerve age-related changes. We consider that the results of our study might be significant for the interpretation of the results of peripheral nerves biopsies during certain neuropathies and for a better understanding of the insufficient regeneration of peripheral nerves after injury in elderly individuals.

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