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BASIC RESEARCH

The leptin receptor mutation of the obese Zucker rat causes sciatic nerve demyelination with a centripetal pattern defect

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ABSTRACT

Young male Zucker rats with a leptin receptor mutation are obese, have a non-insulin-dependent diabetes mellitus (NIDDM), and other endocrinopathies. Tibial branches of the sciatic nerve reveal a progressive demyelination that progresses out of the Schwann cells (SCs) where electron-contrast deposits are accumulated while the minor lines or intermembranous SC contacts display exaggerated spacings. Cajal bands contain diversely contrasted vesicles adjacent to the abaxonal myelin layer with blemishes; they appear dispatched centripetally out of many narrow electron densities, regularly spaced around the myelin annulus. These anomalies widen and yield into sectors across the stacked myelin layers. Throughout the worse degradations, the adaxonal membrane remains along the axonal neuroplasm. This peripheral neuropathy with irresponsive leptin cannot modulate hypothalamic-pituitary-adrenal axis and SC neurosteroids, thus exacerbates NIDDM condition. Additionally, the ultrastructure of the progressive myelin alterations may have unraveled a peculiar, centripetal mode of trafficking maintenance of the peripheral nervous system myelin, while some adhesive glycoproteins remain between myelin layers, somewhat hindering the axon mutilation.

Heading title: Peripheral neuropathy and myelin

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Leptin receptor; myelin; NIDDM – obesity; sciatic nerve – Schwann cell; Zucker rat

Introduction

In basic neuropathology texts, demyelination could be acute or chronic. However, the etiology of the degenerative process related to the nourishing layer of nerve fiber's myelin, either involving the central or the peripheral nervous system (PNS), is complex and still poorly understood. [1–6] In dealing with peripheral neuropathies, textbooks bring the topic along with neuromuscular anomalies. [7] The defects are classified either as (a) axonal neuropathies in which insults of the axons often consist in degeneration occurring distally and secondarily to damage the myelin or (b) as demyelinating neuropathies characterized by Schwann cell (SC) changes wherein myelin would display abnormal conduction velocities. This latter type of neural defect is apparently short-sized and can appear randomly to reduce the internode myelin sheaths while maintaining the axonal content. There, changes occurring in the PNS endoneurium have been seldom investigated. [8–10] Recent advances about cooperativity between SC basal lamina components and axon have revealed paracrine and juxtacrine interactions with at least one of the neuregulins. [11,12]

This report encompasses the fine structure of sciatic nerve demyelination injuries in the young male Zucker rats. A preliminary study of this topic [13] followed investigations that have dealt with other endocrinopathies, such as thyroid gland dysfunctions (hypothyroidism [14–27] and hypercalcemia [14–16]), motricity [27] along with a non-insulin-dependent diabetes mellitus (NIDDM) or diabetes type 2 [14–17] In this rat strain, these defects have been linked to a leptin receptor mutation [28–36], comforted by pancreatic changes. [37–39] Additionally, this leptin receptor defect provokes other hypothalamo-pituitary axis

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Dedicated to our colleague Dr Joseph Allan Tucker jr Louise Lenoir Locke, Professor of Pathology, University of Southern Alabama Medical School, Mobile, AL, USA
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failures. [40–42] Because PNS demyelination defects are often viewed by light microscopy (LM) and not well illustrated with fine structure, we have aimed to document further ultrastructural information on diabetes-related neuropathy.

Interestingly, the Zucker obese rats bore myelin anomalies resembling the ones found in toxicant-induced diabetes in animals [43–52] and probably also those – not studied by fine structure – found in unusual human cases of diabetes where leptin receptor was similarly incompetent. [53–69] Therefore, the discussion of our demyelination data includes diabetes type 2 considerations along with leptin-linked endocrine interactions.

Our micrographic illustrations have been arranged in a progressive peripheral nerve defects sequence that could supplement those found in human diabetes biopsies or those of testing animal for diabetes and treatments. Additionally, both a preliminary report presented in Lisbon meeting [70] and the analysis of the myelin defects collected could have unveiled another possible molecular dynamic mechanism, dealing with the maintenance of the PNS myelin membranes and components that could involve a centripetal diffusion out of either the SCs, marked by an excessive content in electron-contrasted species.

Materials and methods

The Institutional Animal Care and Use Committee of the Northeastern Ohio Universities College of Medicine (now named 'Northeast Ohio University'), Rootstown, Ohio, USA have approved the procedures of animal care, anesthesia, euthanasia, and tissue's collection of this study and concomitant ones. [40,41]

Terminology: Lean Zucker rats have a possible genotype of the dominant trait Fa homozygous (Fa/Fa) or heterozygous (Fa/*fa*), hence called Fa/?, where the interrogation mark indicates whether fa or Fa trait is associated with another *fa* trait (weight) without being unable to verify the corresponding leptin receptor genotype. [12–18] Phenotypically, the obese Zucker rats possesses both recessive traits (*fa/fa*) and consistently showed significant overweight at matching age.

Five young obese male Zucker rats (*fa/fa*; 3 month of age, 398 ± 21.2 g) and five lean littermates (Fa/?) (201 ± 13.5 g) obtained out of a colony originally purchased from Charles River Laboratories (Raleigh, NC) and derived from an original stock [10–13] were housed individually and maintained on a 12 h-light/12 h-dark cycle (light from 06.00 to 18.00 h). Rats fed rodent chow (Purina, St Louis, MO) and water ad libitum. Anesthetized with ether [71] rats were perfused with warm saline (38°C) through aorta for 5 min; then saline was then replaced by an ad hoc fixative to allow other studies. The fixative was a mixture of 3% glutaraldehyde–paraformaldehyde (1:1) buffered by phosphate buffer (pH 7.3–7.4) [72] for 30 min in cold temperature because tissues were primarily used for immunohistochemistry investigations and one not necessarily ultrastructure. Excision of sciatic nerve branches, other organs, and tissues occurred after brain removal was performed by others [40,41] as exploratory investigations with the aim of potential other studies on these rodents. At the time, no quantitation was planned or performed.

Out of all the lean (Fa/?) and obese (*fa/fa*) perfused rat carcasses, several (5–12 mm) segments excised from the sciatic tibial nerve branches were not blind-collected; they were fixed another hour in the same fixative [72], washed in cacodylate buffer for 30 min (0.1M Na cacodylate buffer, pH 7.35 and sucrose) and post-fixed 2 h by 2% OsO_4 aqueous solution. Samples were dehydrated, cleared and processed in PolyBed epoxy resin (Polysciences, Warrington, PA). One-micrometer thick sections stained with toluidine blue were examined in an Olympus BX51 photomicroscope (Olympus America, Melville, NY). Selected areas of LM were ultrathin sectioned, collected on 50- and 75-mesh hexagonal copper grids (SPI, West Chester, PA), stained in uranyl acetate and lead citrate before they were examined in a Zeiss EM-10 transmission electron microscope (TEM; Carl Zeiss, Thornwood, NY).

Results

Light microscopy

Comparisons between the 1-µm thick sections of lean (Figure 1(a,b)) and obese (Figures 4(b) and 5(a,b)) rat sciatic tibial nerve specimens reveal that, in both lean and obese nerves, the population of large and small myelinated and unmyelinated fibers can be viewed in all the samples of

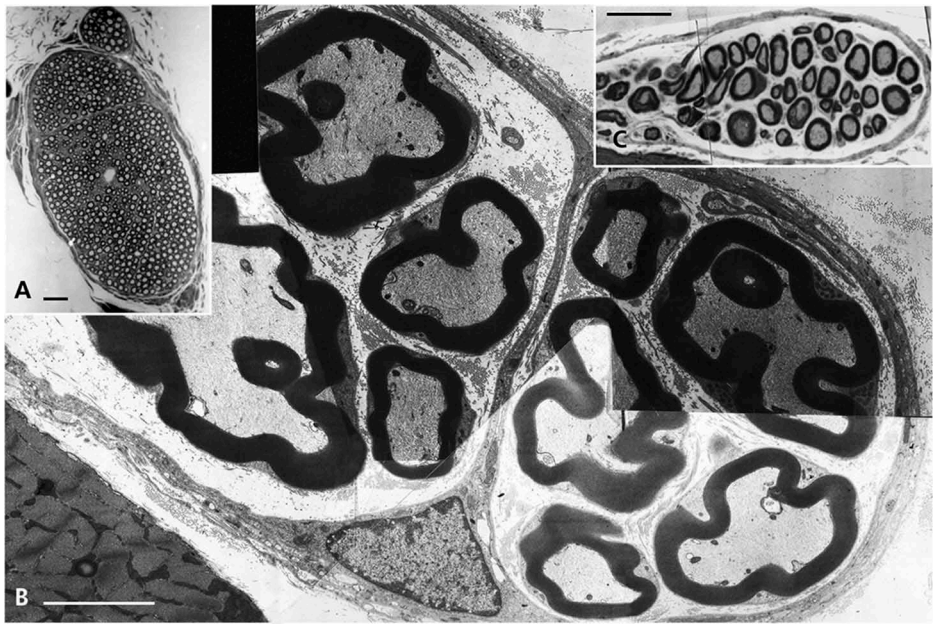

Figure 1. (a–c) Lean Fa/? Zucker rat sciatic nerve. One-micrometer thick sections of tibial branches of the sciatic with its epineurium (a,c). Toluidine blue stain. (a): Example of a large branch with tight fascicles contained in the perineurial sheath and two adjacent small branches. The central region contains an obvious vasa nervorum. In (c): A small intramuscular nerve subdivision. Scale in (a) and (b) is 10 µm. (b): TEM pane mounted out of nine micrographs of a further intramuscular branch of (c) demonstrating two small fascicles of five fibers each, surrounded by the epi- and perineurial fibers and an endoneurial loose connective tissue where nerve fibers show their densely contrasted myelin; the most folded ones likely denote their near- or paranodal region. Scale is 5 µm.

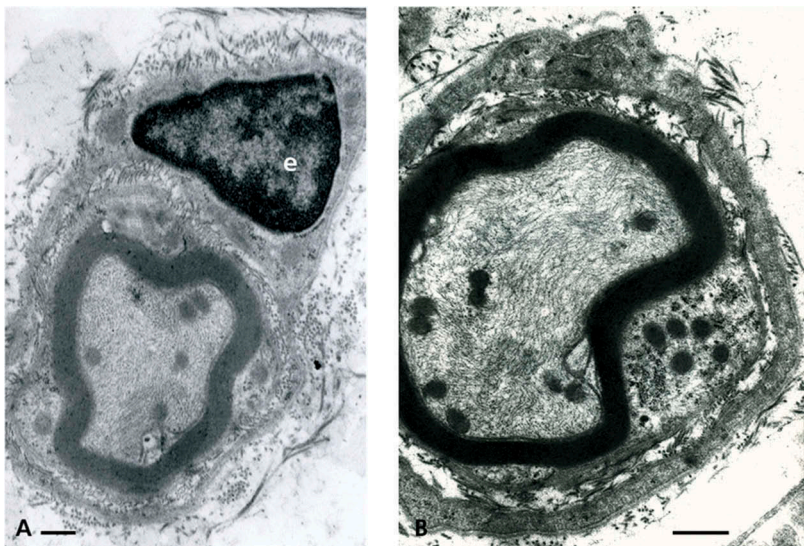

Figure 2. (a–b) Lean Fa/? Zucker rat. TEM of cross-sections of isolated intramuscular nerve fibers both surrounded by their endoneurial connective fibers (e). The small part of Schwann cell viewed in intermodal cross-section is the Cajal band, its intranodal myelin and narrow cytoplasm, surrounded by its basal lamina Scale is 1 µm.

nerve branches, including a few single intramuscular ones. However, LM aspects poorly resolve differences at the highest magnifications or by enlarging the micrographs through computerized captures. The oblique to longitudinal sections of the *fa/fa* sciatic nerve branches and intramuscular fiber profiles, stained with toluidine blue, displays a myelin layer with peculiar whorls or sieve-like aspects. Additionally, in the obese rat nerves, swollen axons and a less dense internode myelin staining can be found compared with the lean ones. It is only by

ultrastructure examination that differences between lean or Fa/? nerves can be verified (Figures 1(c), 2(a,b), and 3(a,b)), such as the fine and vacuole-like blemishes revealed along the myelin of the nerve fibers of obese *fa/fa* nerves (Figures 4(a) and 5(a)–16).

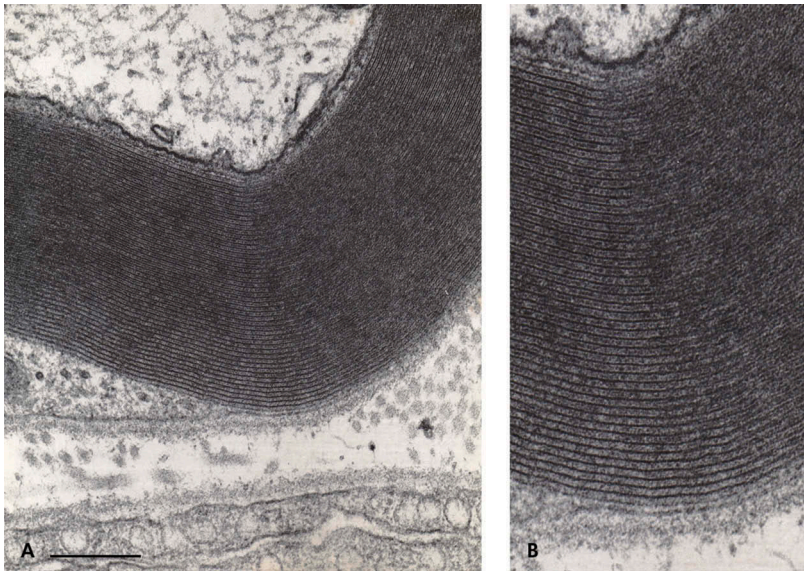

Figure 3. (a–b) Lean Fa/? Zucker rat. Both TEM views of an internodal segment of myelin from either Figure 1(c) or 2(a or b) showing its typical basal lamina and the characteristic layering of myelin insulation with 12.5 nm periodicity of the major dense lines spaced by a middle 6.0–6.3 nm minor dense line or intraperiod, corresponding to the external leaflets of the Schwann cell neurilemma. A vasa nervorum endothelium, rich in endo-exocytotic vesicles and its basal lamina is also shown in (a) where the axoplasm contains its neurofibrils and a few neuroreticulum saccules adjacent to adaxonal membrane. (b) is (a) magnified. Scale in (a) is 500 nm and in (b) 12.5 nm is the major dense line periodicity.

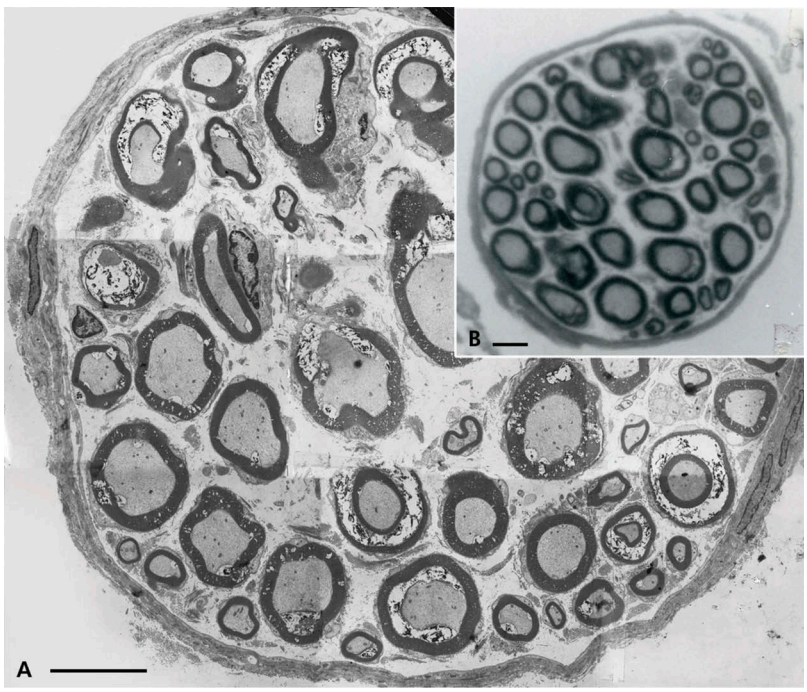

Figure 4. (a–b) Obese Zucker rat. (a): LM view of 1 µm-thick cross of a sciatic tibial nerve branch. Toluidine blue stain. Scale is 10 µm. (b): TEM micrograph montage reconstituting a view of (a) section. This pane depicts diverse myelinated fiber damages, in oblique and cross-sections. Some of them were enlarged to further illustrate this study. Note the myelinated and a few Remak nerve fibers accompanied by a loose endoneurial and perineurial supportive tissue. Scale is 1 µm.

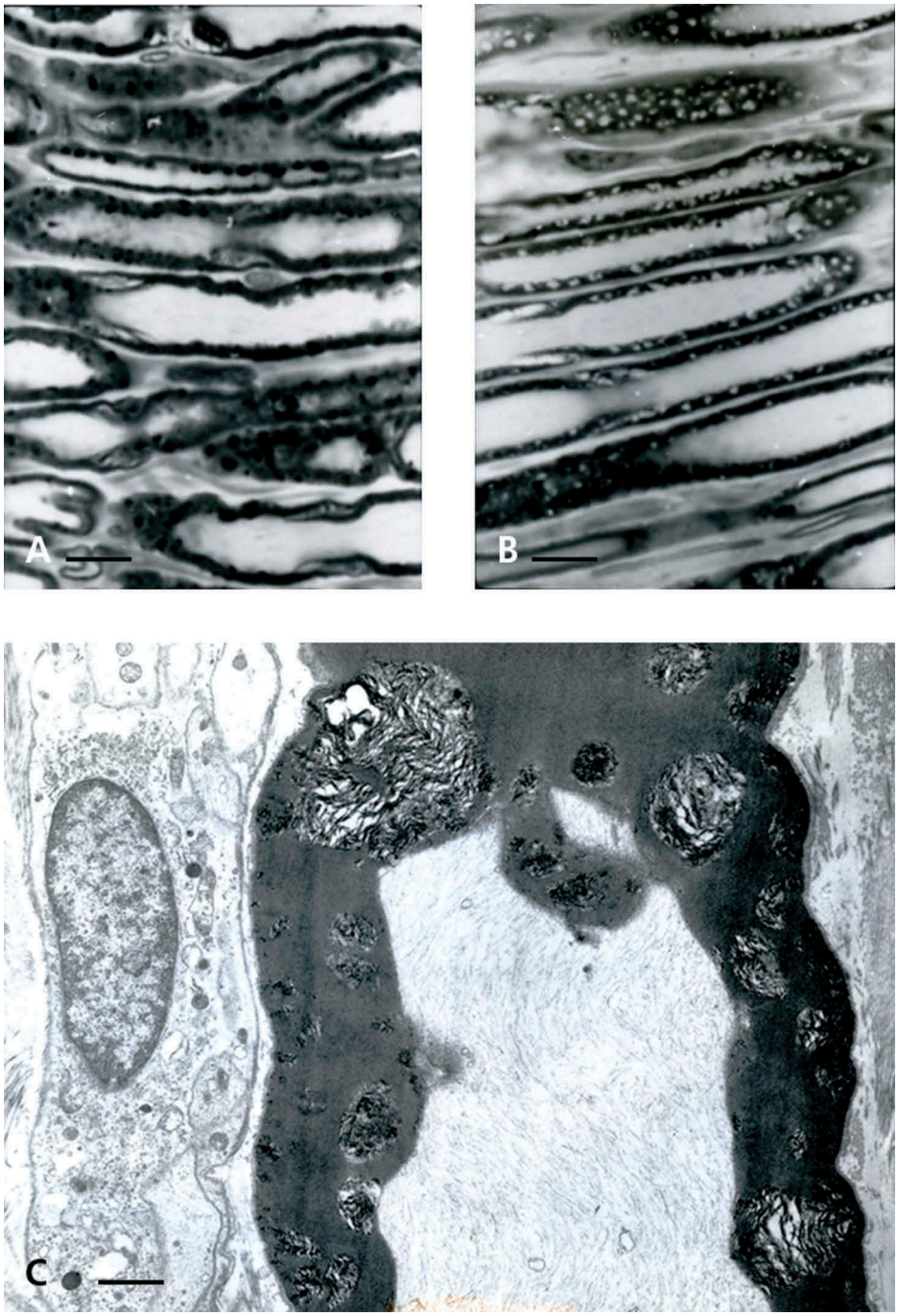

Figure 5. (a–c) (a–b) One-micrometer thick longitudinal to oblique section of a sciatic tibial nerve branch of an obese Zucker rat. Toluidine blue stain. Scales are 10 µm. (a): Example of field of view that shows how difficult is LM resolution to verify whether alterations have occurred but the tiny, poorly stained bulges (*) while nerve fibers' internodes appear swollen. (b): Tangential views of most myelinated fibers of a fascicle denote changes in myelin with spaced vacuolizations of the insulating myelin that can appear as dark Swiss cheese (upper area). In both (a) and (b) Schmidt-Lantermann areas can be noticed as if oblique gashes in the myelin (both left middle areas). (c): TEM detail of an oblique to longitudinal aspect of one nerve fiber of (b) that resolves the spaced vacuoles and the bulging segments in the myelin to be sectors of focal, demyelination. An endoneurial fibrocyte is adjacent to this nerve fiber section and demonstrates multiple contrasted deposits. Scales equal 10 µm in (a) and (b), in (c) is 1 µm.

Transmission electron microscopy

TEM observations further confirm that no alterations affect the lean rat nerves (Figures 1(c), 2(a,b), and 3(a,b)), while all the obese rat myelinated nerve samples display some demyelinating damages and worse are found to be proportional

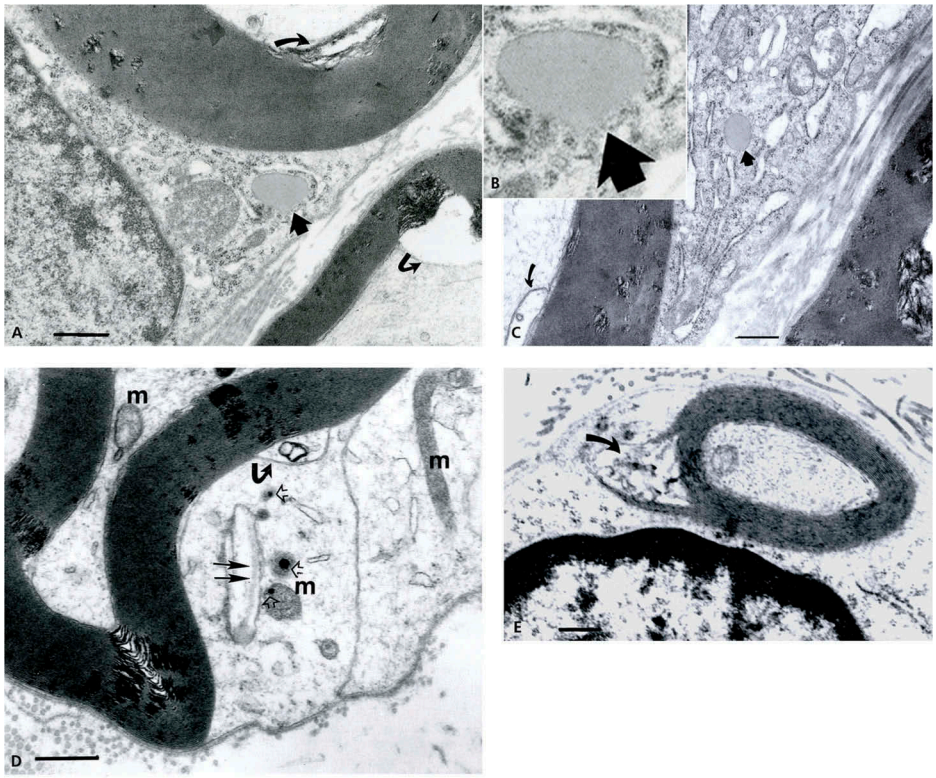

Figure 6. (a–d) TEM of obese rat sciatic nerve. In (a–c): Semi-serial TEM views of perikaryal SC (or parts of Cajal bands containing a mitochondrium, smooth and rough endoplasmic reticulum, polyribosomes and a lipid deposit (dense arrow)) enlarged in insert near (b) showing that no membrane lines the lipid-like inclusion, i.e. not a 'vesicle'. (b): similar area of Cajal band; TEM view as in (a) showing the same lipid deposit. (c): Cajal band perikaryal area of an obese nerve fiber showing a mesaxon area. Long mitochondria cut (m) are shown adjacent to electron dense vesicles or deposits adjacent to a Golgi cistern containing a fibrillar striated content (prepro-collagen?). (d): Small nerve fiber with its SC nucleus and perikaryon with a disorganized mesaxon complex. The curved arrows mark defects in the mesaxon, abaxonal myelin, leaving intact the adaxonal membrane. Scales in (a–d) are all equal to 1 µm.

to their diameter size (Figures 4(b) and 5(b)–16). In the following paragraphs, descriptions of nerve fiber injuries of the obese rat nerves are organized to depict the progression of the damages, from the smallest to the worst, comforting the neuropathic changes associated with the NIDDM-associated leptin receptor defect of this rat strain.

Lean (Fa/?) Zucker rat sciatic nerves

Myelinated fibers. In these fibers, the neurolemnocyte or SC cytoplasm of some small or large nerve fibers reveals typical indent of the perikaryal areas (not shown here); it contains typical cell organelles. The SC nucleus, with its perikaryon, is in the median internode regions of each peripheral nerve fiber, thus the internodal cytoplasm is most often viewed as a narrow band with the random TEM sections, named Cajal band. This 'band' of the SC contains the abaxonal region with its outermost myelin layer and sheaths where, by place, outer mesaxons can be viewed (Figures 1(c) and 2(a,b)). The axoplasms show mitochondria, neurotubules, neurofibrils, and a few adaxonal neuroreticulum cisternae. In the rat tibial branches, most nerve fibers are typically myelinated and organized by SCs in concentric tight layers of neurilemma forming an electron dense Fermat-like spire enclosing the neurites, whether found in cross or oblique, or low-magnification sections (Figure 3(a)).

Viewed at a higher resolution, the myelin displays the internodal myelin tightly organized, with its regularly distanced membranous architecture and adhesive contacts. There, the typical major dense lines are viewed as highly contrasted thick 'lines' which are observed ranging between 12.5 and 15.5 nm distance periodicity. There, the thinned SC cytoplasm is spaced by weakly contrasted intraperiod lines (or minor lines) that appear with poor contrast, containing

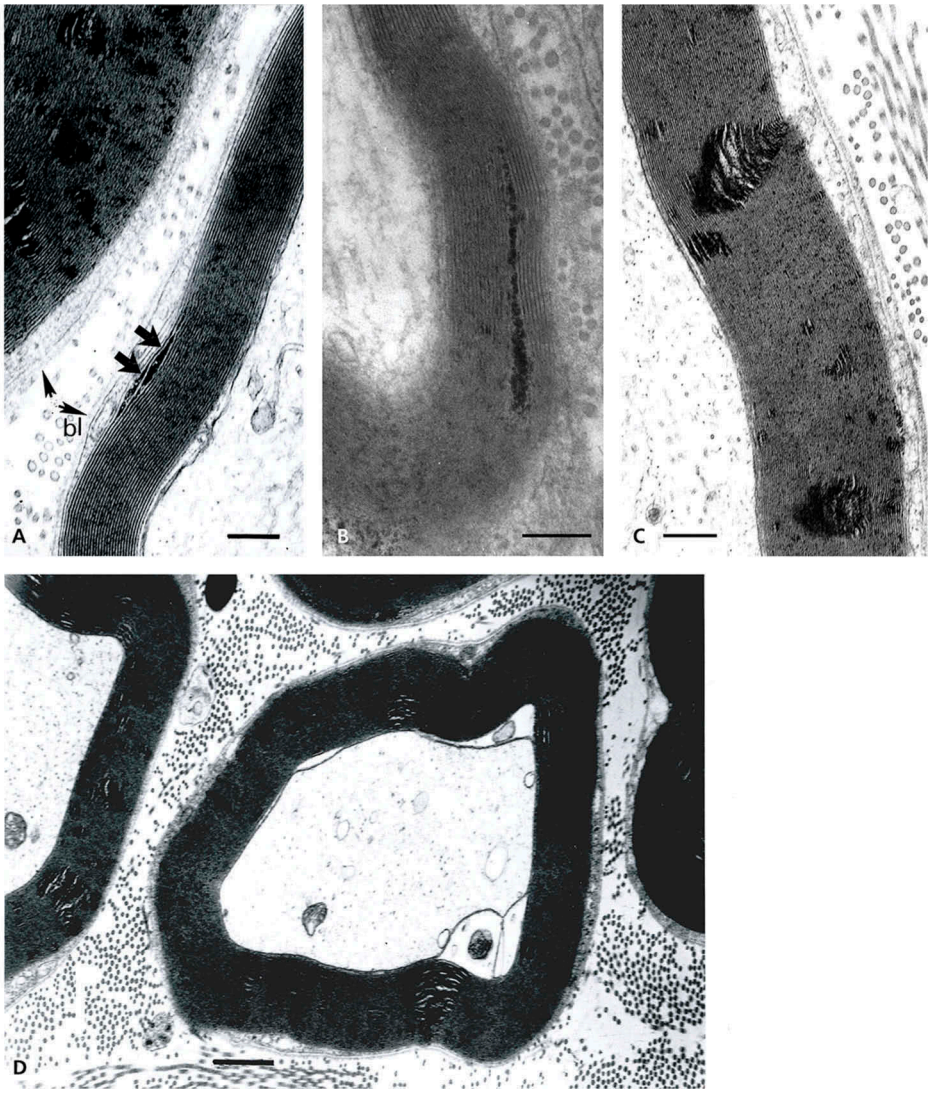

Figure 7. (a–d) TEM of sciatic nerve fibers of obese Zucker rat with initial myelin damages. (a): Twin dense arrows respectively mark the first and second intraperiod lines spaced with electron dense eposits adjacent to the Cajal band; small arrows and bl: indicate the basal laminae of adjacent nerve fibers. (b): Electron dense deposit enlarges and widen along 9–10th intraperiod line level. (c): Example of small abaxonal cisterns in Cajal band along the outermost myelin sheath. These damages continue as cone-like profiles to reach the adaxonal membrane as discrete to wide intermembranous spaces. (d): example of internodal cross-section with narrow Cajal band where discrete myelin blemishes initiate and a deformed adaxonal membrane, as shown on adjacent fibers. All the scales are equal to 1 µm.

the adjacent contacting membranes of one internodal wrapping SC. The periodical distance between major lines can reach between 20 and 300 nm in width, the widest often located at the level of Schmidt–Lantermann (S-L) and the nodal zones (Figure 3(b)). In cross-section, each entire myelin insulating profile shapes like an annulus, somewhat circular but folded up near and at the Ranvier's nodes. The adjacent to axolemmal with its adaxonal SC cytoplasm, or so-called Mauthner's layer, is quasi inexistent due to the compaction of myelin.

Unmyelinated nerve fibers. A few unmyelinated (Remak-like) fibers can be found among the endoneurial stroma adjacent to the myelinated ones but are not illustrated in Figure 1, especially when one has enlarged the small intramuscular sciatic nerve branches.

The supportive stroma including endoneurium. This stroma, associated with the basal laminae produced by the SCs, surrounds every fiber whether myelinated or unmyelinated. The endoneurium reveals its loose endoneurium containing

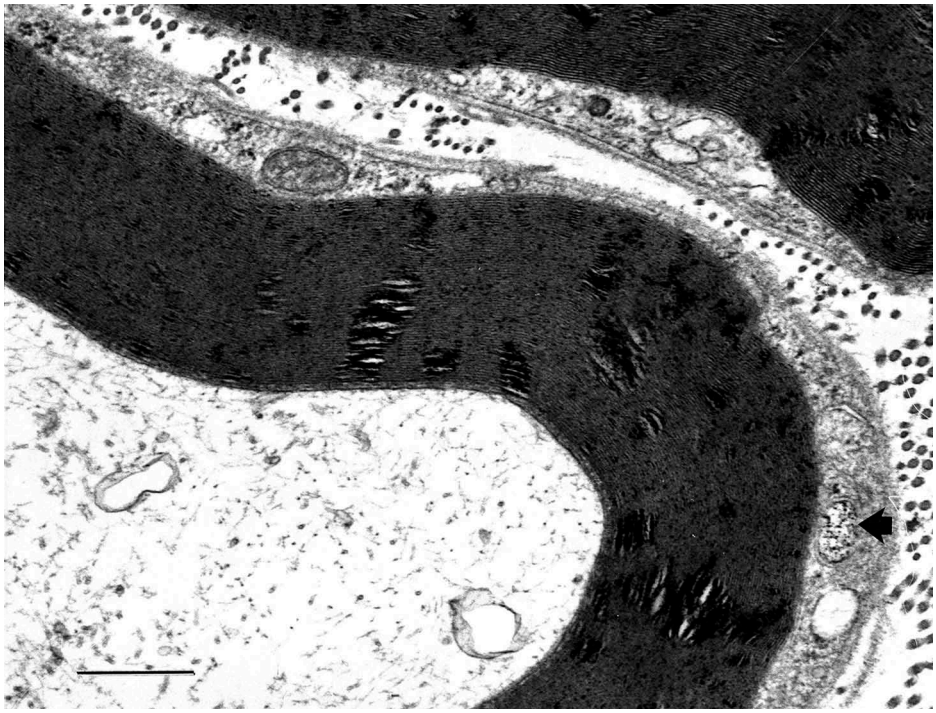

Figure 8. Enlarged sector of a fa/fa sciatic nerve fiber showing the Cajal band contains either marbled (arrow) or emptied-like vacuoles facing the abaxonal membrane (arrow) seemingly in contact with the abaxonal myelin, displaying ovoid-shaped alterations rupturing locally the periodicity of the packed myelin annulus. Note along its perimeter and through that annulus the aligned dense component that contrasts as electron dense striped lines into the initial myelin layers that reach deep in it. The basal lamina surrounds the entire SC with its noted extracellular matrix. Scale equals 500 nm.

Figure 9. TEM of obese Zucker rat. A narrow sector of a sciatic nerve with at high magnification showing intraperiod lines or spaces containing densely contrasted elongated hyphen-like buttons in the mid-regions, corresponding to adhesive 'rivets' that holds myelin major lines or attachments between the fissuration damages. Scale is 15 nm between two major dense lines.

scattered fibroblasts, dispersed bundles of collagen fibers, in the interstitial, extracellular matrix loose connective tissue, and few small blood vessels (Figure 1(a)). The perineurium is constituted by adjacent fibrocyte-like cells providing nerve fascicle or even single-nerve fiber external support, as epineurium subdivisions branch and resolve into perimysium. This one is a thin fibroblastic sheath, creating a surrounding channel around each nerve fiber, as endoneurium with the

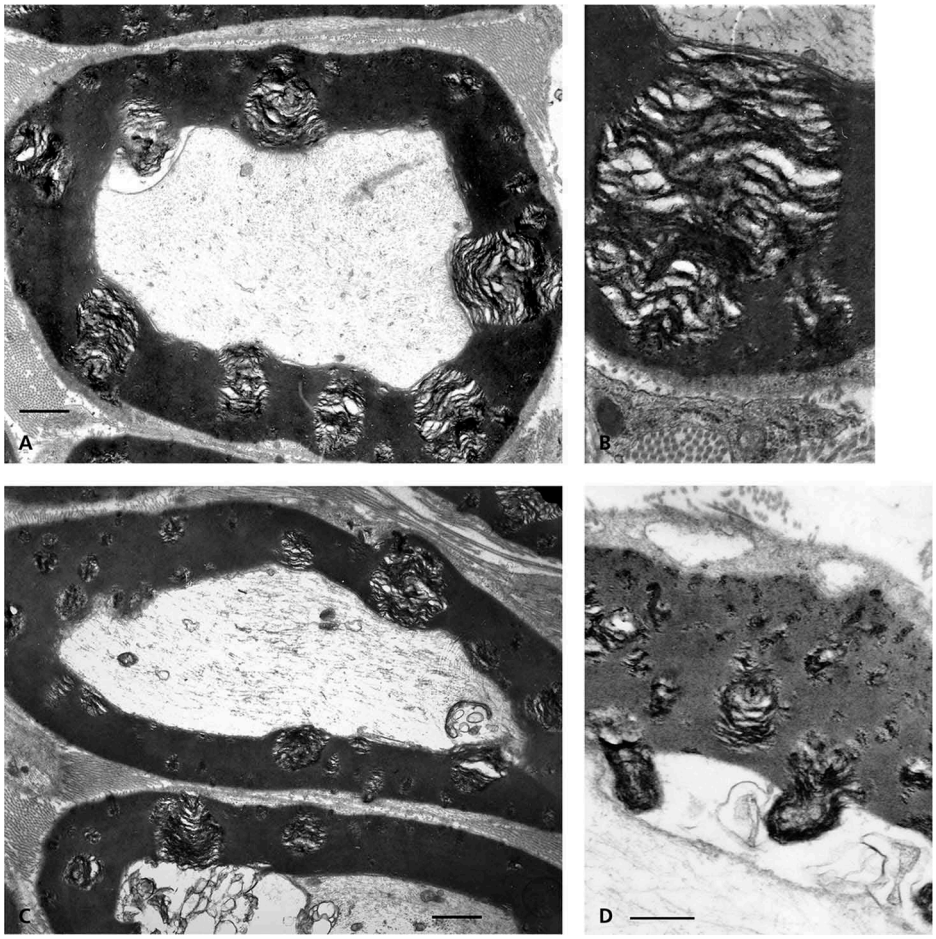

Figure 10. (a–d): TEM views of obese Zucker rat sciatic nerve fibers cross (a, b, and d) and oblique (c) internode sections demonstrating the spaced vacuolated defects revealed across the width of the myelin annulus sheaths displaying a sort of conical shape, narrow in abaxonal side and enlarged in the adaxonal region. The interval spaces can branch into smaller defects. Either damages favor bulges of the adaxonal membrane and can reveal an axonal content vacuolated. In (d), Cajal band shows several vacuoles as noted in Figure 8. Scales are 1 µm.

intercellular basement membrane-like and the basal lamina of the SCs (Figures 1(a–c) and 2(a, b)).

Obese rat (fa/fa) sciatic nerves

Myelinated fibers. Low magnification demonstrates that all the samples from obese nerves have damaged nerve fibers shown with LM in a small branch of the sciatic nerve (Figures 4(a,b) and 5(a,b)). Among the smallest fibers alterations, some nerve fibers display defective myelin tight organization in the outer mesaxon of the SC cytoplasm where altered wrapping membrane can be seen while the axoplasm content seems untouched (Figures 4(b) and 5(c)–13). Further away from the perikaryon, internodal SC zones show other disruptions or anomalies in the outer and inner mesaxons with adjacent debris to the tight myelin (Figure 15) even though SCs appear to reveal typical nucleus with perikaryal organelles, clusters of dilated cisterns of rough and smooth endoplasmic reticulum, Golgi parts, intermingling polysomes, and mitochondria are recognized (Figure 6(c,d)). In Figure 6(c), an example of a lucky field of view displays an elongated deposit droplet (no limiting membrane) seemingly or faintly striated but of unclear nature can accompany other few endoplasmic cisterns where sometimes a fibril of collagen precursor (pro-collagen?) is viewed; one could interpret it to be later secreted as part of the basal lamina (Figure 6(d)). At all stages of damage, the nerve fibers show inner mesaxon changes or anomalies in alignment as well as for the adaxonal lining. The neuroplasm reveals swelling of the neuroreticulum but no apparent fibrillar or microtubular changes (Figures 4(b) and 9–13). Near one Golgi cistern,

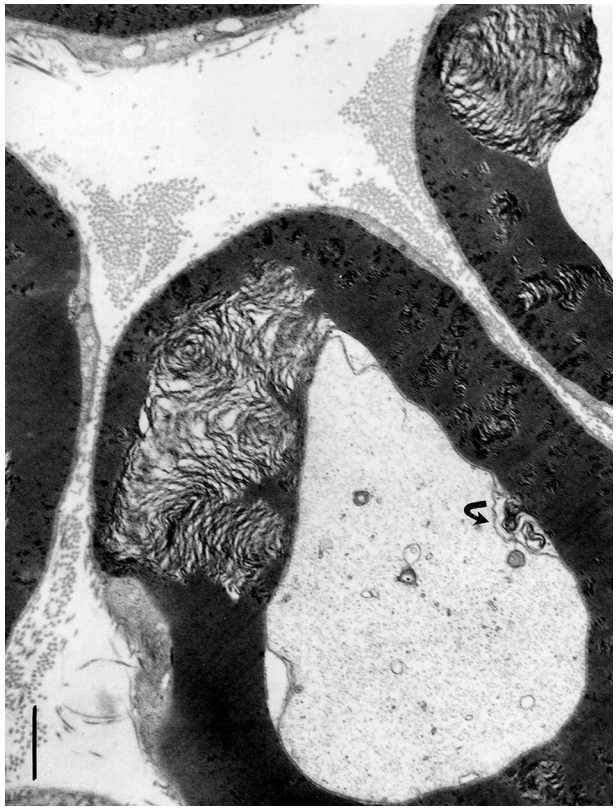

Figure 11. TEM of adjacent obese Zucker rat sciatic nerve fibers with internodal, variable size, enlarged demyelinating sectors and their narrow Cajal bands with pale vacuoles. Defects appear initiated at the abaxonal Cajal band myelin layer with regularly spaced narrow, electron contrasted 'sinks' enlarged toward the adaxonal layer with obvious onion-like rifts. A curved arrow marks altered adaxonal myelin to be compared with the adjacent fiber where the onion-shape blemish bulges into the axonal space. Scale equals 1 µm.

adjacent electron dense vesicles (lysosomes?) are noticed (Figure 6(d)). The basal lamina always tightly surrounds all SCs and does not appear with any discontinuities in all TEM views throughout the nerve. In the endoneurium, collagen eventually shows erratically organized fibers and bundles (Figure 4(b)).

Initial damages. At first, thought to be artifacts, minute myelin changes appear as if narrow broadenings of a few abaxonal and outermost major dense lines with exaggerate electron dense content or deposits; these appear made of fine granular-like aspect caused by tissue processing, revealing their anionic content (Figure 7(a–c)). Noticed in the first and second major abaxonal lines, these peculiar deposits appear to also widen the cytoplasmic compartment of the SCs and the intermembranous spaces separating the intraperiod or minor dense lines, i.e. extracellular faces of adhering SC's membranes. The defects correspond with a disjointing of adhering membranes of the internodal myelin. Similar SC deposits are noticed at the mid-level of the myelin (Figure 7(b)) made of unwrapping adjacent myelin sheaths, then enlarged slits rip them apart in an apparent centripetal way. Initiated in the Cajal band nurturing the myelin, the membrane defects appear as tiny teared sectors that 'diffuse' by accretion into each innermost adjacent layer of myelin minor lines. There, membrane separation expansions broaden and disorganize the tight concentric myelin layering by accumulated contrasted (and, maybe, poorly contrasted) materials between adjacent, intervening cytoplasmic tongues thus create rifts within the myelin annuli. Overall damages create injuries in the shape of conical pockets pointing outwardly. Therefore, the morphology of the damages reinforces the idea of a centripetal progression toward the adaxonal membrane, ending brutally as a wide elongated slit at this membrane or, earlier, within the myelin sheaths. These alterations then appear to branch as sectors with inward

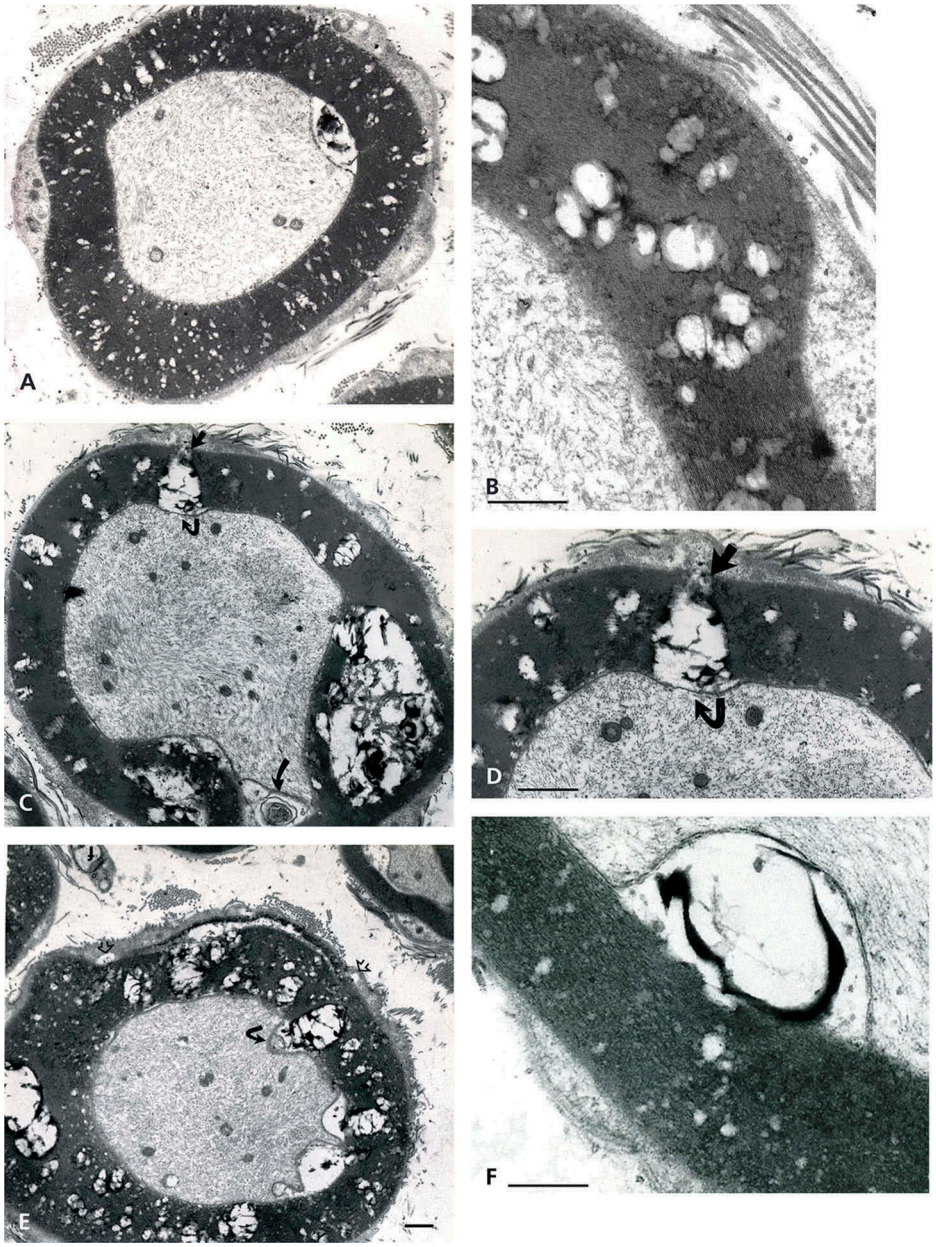

Figure 12. (a–f): TEM aspects of obese Zucker rat sciatic nerve obtained out of thick ultrathin (>500 nm). (a–d): Near entire cross-sections show damages of myelin to appear as 'bubbles' across several layers of myelin sheaths with higher resolution in (b), (d), (e), and (f). Fusing with each other, myelin degrades throughout with stacked faults or crevices, rupturing sectors with cracks out of internal pockets or wide gashes, leaving intact the adaxonal membrane and the content of the axoplasm. Debris includes waxy, electron dense deposits in the large spaces. Small fatty-like vacuoles in the Cajal bands can be seen in (b)–(f) (arrows). All scales are equal to 1 µm.

progress, thus widen the defect zones, as noted according to the randomness of the examined nerve fibers sections (Figures 5(c), 7(c,d), 8, and 9–11).

Demyelination progress. The myelin degradations appear to be the worst at the level of the largest diameter fibers. Again, the defects begin in the narrow regions of the Cajal bands. There, oblong vesicles, ranging from 60 to 150 nm in diameter, with marbled content or similar sized vacuoles can be viewed adjacent to or in contact with the outermost sheath of the abaxonal myelin. Interestingly, most vesicles face the sites where the initial series of myelin blemishes or sector fissures occur (Figures 8, 9, 10(a–d), and 12(a–f)). Other views of the fissures can also resolve into

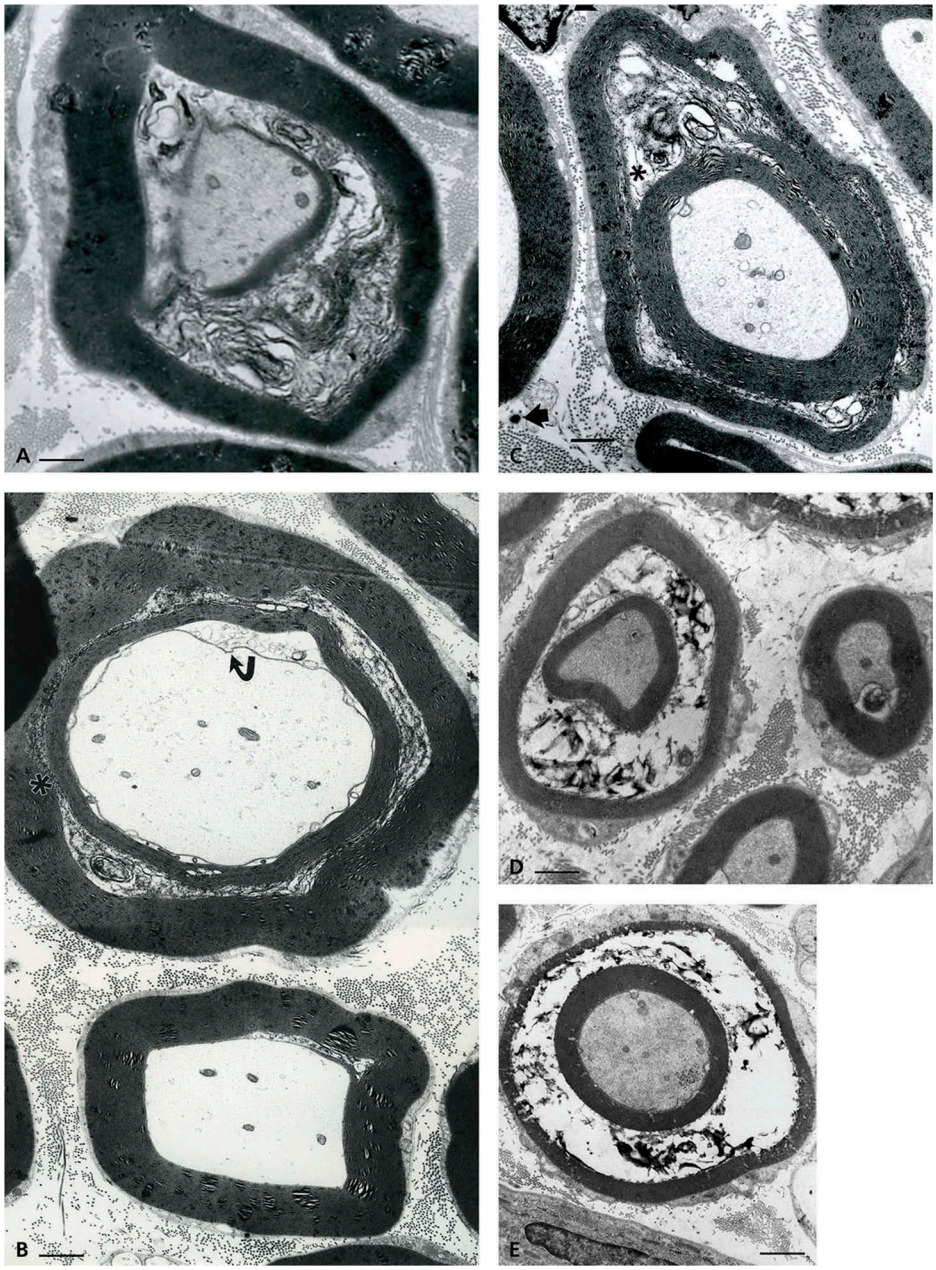

Figure 13. (a–e): Obese Zucker rat sciatic nerve fibers with aspects of demyelination found as circumferential fissuring of the internodal and paranodal zones as thick onion peeled. Complex whorls with waxy debris are noted in the spaces formed. (a–c): Almost complete splits with adaxonal membrane partly detached from the damaged myelin (curved arrow in (b)). (d–e): complete splits of myelin annulus form quasi two encircling rings out of the intact, single myelin annuli built as spiral (i.e. suggested in (c)). Scales are all equal to 1 µm.

further emptied spaces separating myelin layers' pile-ups. At first, the aligned defect distribution reminds of widenings of the radial lines in central nervous system (CNS) as they are regularly spaced along the myelin annulus profiles (Figures 5(c) and 9–12(b)). These accumulated, piled-up mutilations are sometimes aligned in the oblique and near longitudinal sections suggestive of sorts of intraperiod damages propagated along the outermost layer of the myelin in a periodic fashion of growing faults toward the adaxonal membrane. The degradation pockets appear as sieve-like with LM (Figure 5(b)) and confirmed with TEM (Figure 11(a–f)). They create inner curved bulges in transverse sections, also with onion-like aspect limited by the inner adaxonal membrane, initially viewed as minor bulges (Figures 5(c), 7(d), 8, 10(a–d), 11, and 13

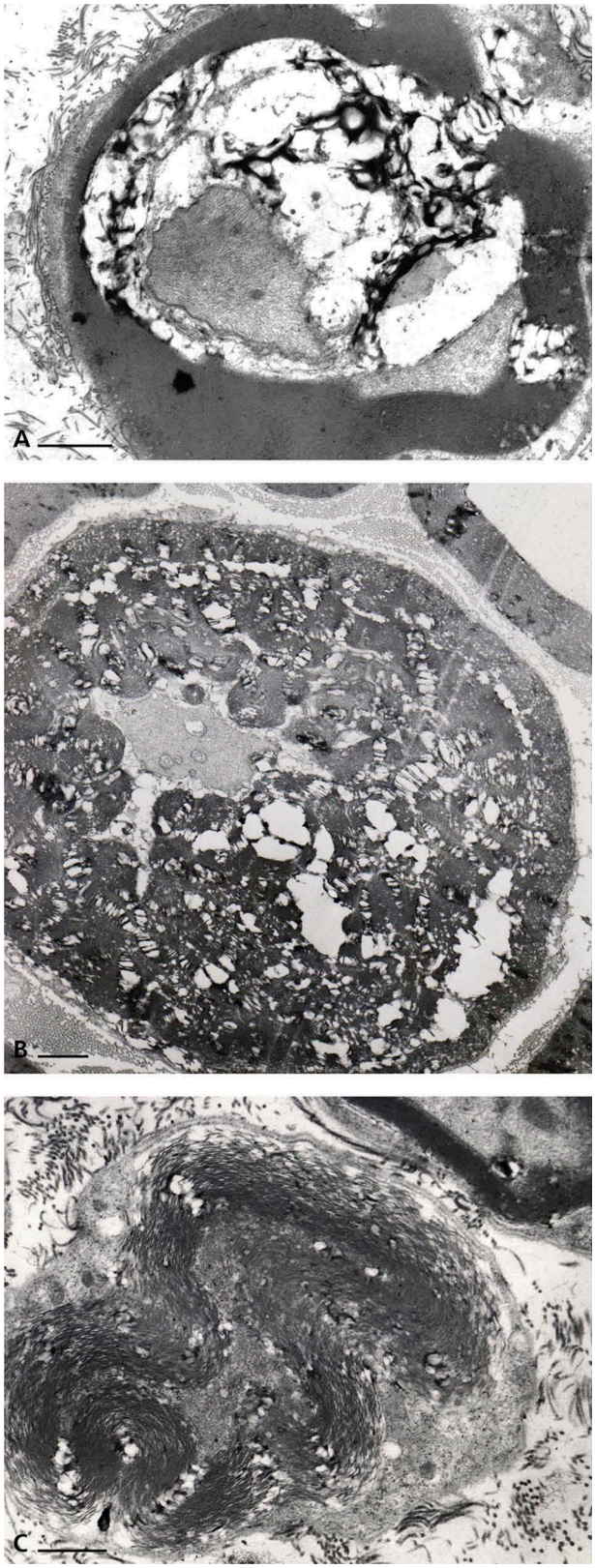

Figure 14. (a–c): Pane with paranodal (a–b) to nodal (c) cross-sections of sciatic nerve fibers of an obese Zucker rat with the worse demyelination. (a): Cracks of the myelin annulus with diverse debris and shredded axonal content. (b): Peculiar aspect of paranodal zone with myelin layers retaining points of adhesion (torn in small linkers) creating a peculiar labyrinthine pattern caused by the processing and infoldings of the myelin. (c): Folded node of Ranvier's region in cross-section with highly contrasted myelin layers with loosen circumferential fissures making the appearance of onion-like aspect and showing a detached adaxonal membrane. In all views, the chaffed SC basal lamina and the axoplasm is reduced into a minute central target-like zone. Scales are 1 µm.

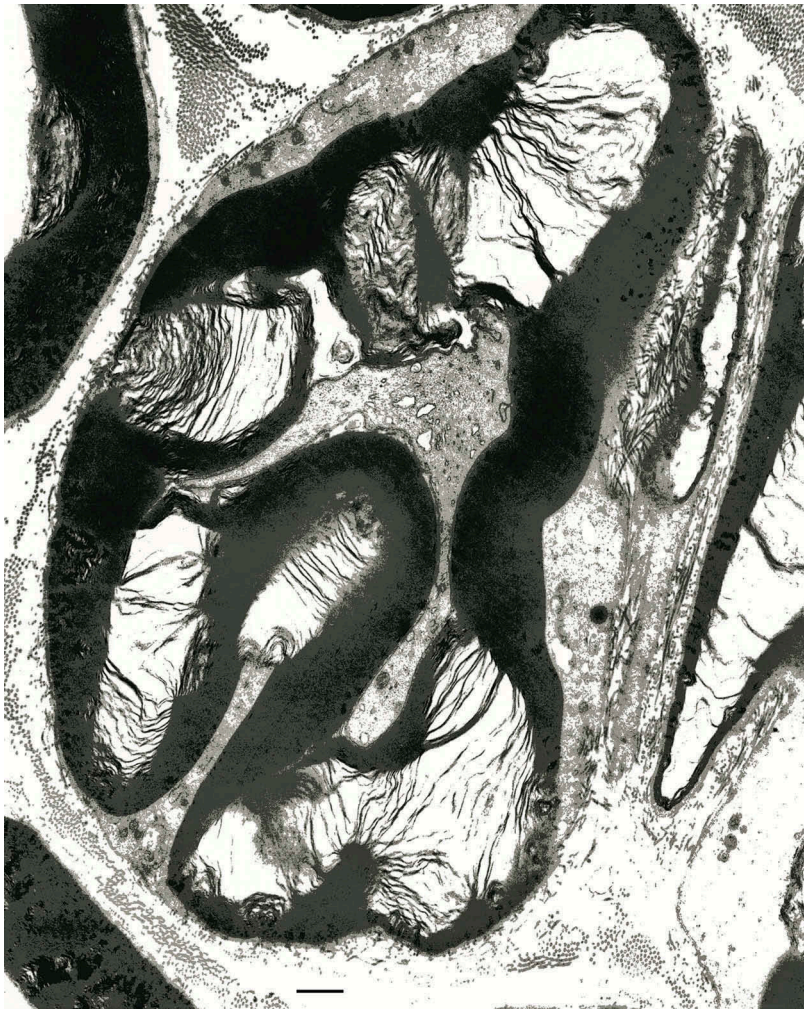

Figure 15. Near nodal region of an obese Zucker fa/fa rat sciatic nerve fiber. This typical folded region of a myelinated fiber demonstrates apparent myelin adhesion defects not so different that typical node fine structure but appear as large, loosened, open onion-like sectors along with interstices of obliquely-cut tight myelin appears packed, as electron dense stripes. The axoplasm content is vacuolated and the myelin depicts a large star-shaped overall aspect due to partial unwrapping of the myelin layers near the Ranvier node. Scale is 1 μm.

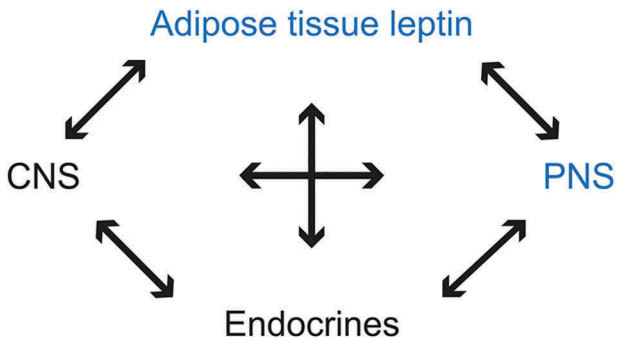

Figure 16. Schematic representation of adipose tissue's leptin influences with feedbacks on CNS, PNS, and endocrines.

(b)). The partial or quasi-complete altered myelin now encompasses dissecting damages through expanded fissures into vacuolated spaces that progress as segments with intervening exaggerated spaces along the circumference of the myelin annulus sections (Figures 4(b) and 13(a–e)). Finally, more fused or coalescent fissures peel off layers of still adherent sheaths of the insulating inner layer (Figures 13(a–e) and 14(a–c)).

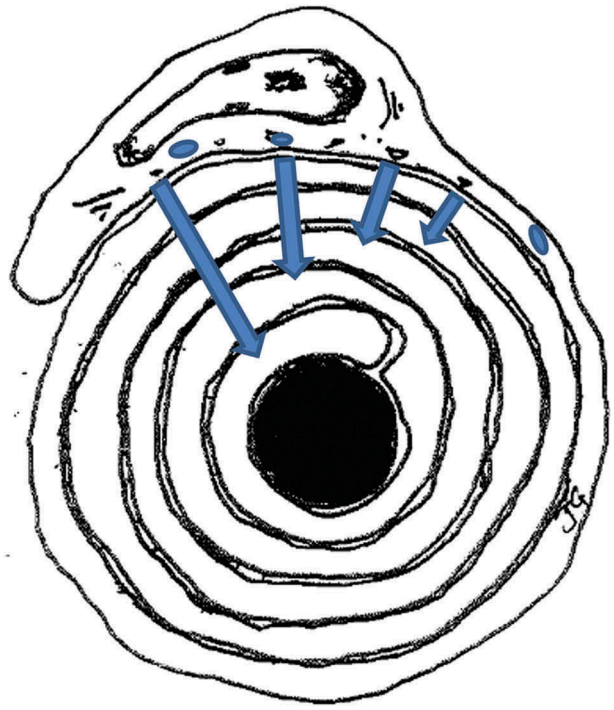

Figure 17. Diagrammatic representation of a suggested, unexpected peculiar centripetal diffusion (blue arrows) of myelin compound(s) dispatched by Schwann cells. Axon in black.

Either erratic or wavy layers reveal the sector's rough devastation that eventually completely dissects the myelin, hacking the entire myelin annulus (Figure 14(c)). The gashes perforate or ruin the entire myelin layer (Figure 14(a–c)).

Altogether, these defects do not usually include the adaxonal membrane (Figures 5(c), 10(d), 11, and 12(a–f)). These micrographs with important tearing of the myelin show inward vacuole-like spaces lined by the adaxonal membrane, leaving separated the intact neuroplasm and the axonal content. These myelin tearings feature all sorts of membranous debris, including some waxy, electron dense remnants (Figures 4(b), 11, 12(a–f), 13(a–e), and 14(a)). Further, the complex degradation of the same myelin leaves large adaxonal spaces and an axonal content compressed to totally unwrapped myelin in the same area where typical, undulating tight myelin occurs and identifies the juxta- and paranodal zones (Figures 4(b), 13(a–e), 14(a–c), and 15). In the same paranodal zones, myelin keeps some of its interconnected membranes leaving remaining ones attached across the annulus with clear intermembranous, somewhat punctate junctions. These encompass SC's outer membrane leaflet contacts albeit most of it is fissured by small intraperiod elongated vacuoles, (Figures 9 and 14(b,c)). There, even though the myelin ravages tear apart the entire width of its annulus morphology, it remains form a distorted, multicurved outline where displaced layers of membranes are still retained together. Cross-sections of those teased membranes, amassed with defects, appear as if they were bales of wires (Figure 14(b)). Following the most ultimate disengagement of the myelin ring in the near internode and paranode regions, the adaxonal membrane that has maintained the neurolemma out of the insulating defects can show breaches without that of the neuroplasm (Figures 13(b) and 14(a–c)).

Again, demyelination would likely interfere or obliterate some of the Zucker nerve conductivity, as it was suggested by exercise tests. [27] It is interesting to view an enlarged small sector out of a typical myelin damage micrograph to verify that intraperiod line densities remain as small interperiod, elongated contrasted dots, or line-like densities spaced between the major dense lines unless they become excessively displaced by some intercellular gaps (Figure 9). The gaps can correspond with intercellular charged components admixed onto the glycocalyx and rafts. These alterations change the typical myelin stratification and stiffness thus causing demyelinating defects with excess in extracellular accumulated repelling charges contributing to separate them by narrow to large gaps (Figures 7(a) and 9).

Unmyelinated fibers. Even though most of the nerve fibers of the tibial branches of the sciatic nerves are motor neurons [67], only a few Remak fibers' membranes examined appear to contain higher electron-contrasted zones when compared with lean ones (Figure 4(b)). However, more data are needed to comment on these scarce observations.

Endoneurium and supportive stroma. Figure 4(b) reveals a very loose endoneurium, maybe brought by some surrounding changes. Figure 5(c) also demonstrates the endoneurium layer, charged with numerous vacuoles – including many densely contrasted ones – suggests some lipid content getting in close contact with the outer Cajal band,

probably spaced by the fixative processing, wherein no basal lamina is displayed. This observation signals further investigations could be done along with old data suspecting this layer to be involved in myelination by SCs and their association in peripheral neuropathy associated with diabetes or in NIDDM condition.

Discussion

Based on the ultrastructural data of this study, a main specific cause for the myelin defects points to and comforts the leptin receptor mutation. However, how and which myelin component(s) is (are) involved cannot be pinpointed. The enormous literature dealing with myelin, added to the leptin receptor mutation, suggests this myelinopathy can be caused by NIDDM but also by coexisting endocrinopathies. We tried in the following paragraphs to explain the myelin changes collected in this rodent that probably disclose comparable nerve defects to be found in human neuropathology. Surprisingly, our ultrastructural findings on demyelination may justify us to claim to have unravelled a peculiar centripetal mode of myelin maintenance in PNS nerves.

Diabetes and peripheral neuropathy

Even though known since Antiquity [6,73], a clinical descriptive of diabetes has been made by Dobson [74] and throughout the years followed by numerous authors (e.g. reviews in [4], [6], [42], [55], [75], [76]). In Ref. [6], one reads that "… before Marchal de Calvi [77], diabetes mellitus was believed to be secondary to a lesion of the nervous system rather than the reverse"…" Since that time, most investigators have accepted that diabetic polyneuropathy is secondary to the metabolic disorder …". This excerpt could summarize the etiology of diabetes type 1 or insulin-dependent diabetes mellitus (IDDM) and of diabetes type 2 or NIDDM. However, such 50-year-old etiology has been reconsidered and surveyed repeatedly [6,10,46,69,75–86] but not necessarily with illustrations of the PNS-associated damages to demonstrate the myelinopathy progression. [6,79,87,88] Nowadays, specialists of diabetes can reappraise the old clinical neuropathy explanation [75] in NIDDM because the Zucker rats [40–42,89,90], other animal studies [6,44,46–53,87–94], and several clinical cases [6,54–64,78,83–90,95–99] showed the disease to arise out of perturbations in the hypothalamic-pituitary axis interacting with other endocrine tissues due to faulty receptors. These findings make room for new avenues to understand and fight diabetes because not only SC's changes in metabolism with a genetic origin alters myelin and, maybe, both that of SCs and connective tissues could be influenced by some other CNS dysfunctions, thus also contribute or cause PNS myelinopathy, as shown here with ultrastructure.

The Zucker rat sciatic nerve defects are not artifacts

At first, the nerve structures found with TEM have been thought to be artifacts caused by the sample's processing because some texts have shown mechanical manipulations of excisions could injure myelin. However, after LM examination of diverse parts of the sciatic nerves, alterations affect all obese nerve fibers of tibial small branches including those intramuscular fibers out of dissected muscles obtained after the fixing perfusion. None of the lean rat nerves of the same tissue regions show damage while simultaneously processed. Furthermore, the architectural damages of the myelin form discrete and irregular density of the myelin sheaths. Spotty and distributed throughout along the length of the insulating myelin (e.g. Figure 5(b,c)), the defects cannot be caused by mechanic manipulations, friability or edema of the samples, or processing as they carry on in diverse directions with delaminating aspects.

The compression damages reviewed [e.g. 100,101,102,103] are segmental, unique and appear with a more injurious pattern than the ones found in this study or even than the ones described recently in rat sciatic with crushed injury. [104] In the largest branches of the obese rat nerves, most nerve fibers are myelinated [105] and bore injuries (not totaled in this report) across all the similarly processed obese nerve samples. In addition, some of the diamond knife traces obtained out of sectioning samples show tiny disruption of the myelin (Figures 5(c) and 14) but all other micrographs obtained the same knife, including lean nerves, are artifact-free and compared well with the micrographs found in other publications. [105–108] Several studies where

diabetes has been induced, including with sciatic nerve cross-sections, have defects in both animal data [43,44,46–53,55,71–87,91,109,110] and in human biopsies and of other peripheral nerves [46,73–75,77–89,106–108,111,112] where myelin is showing some aspects of ours.

Having established that the myelin injuries are not artifactually made, one can assume that young male obese NIDDM rats live with peripheral neuropathy and one would have to relate this PNS defect with their complex endocrinopathy. The advances of new information published on this rodent defects, including some molecular markers, have stimulated us to submit data collected and introduced a while ago. [12]

PNS myelin and sciatic nerve

Myelin aspects

Throughout the micrographs displayed, the myelin periodicity of the major dense lines typically ranges between 12.5 and 17.5 nm for both lean and intact regions of the obese rat sciatic nerves after perfusion fixation and the supplementary fixation. There, the major dense lines and intraperiod lines are preserved, indicating that the constitutive element has been preserved to retain the myelin lamellar periodicity, except in the blemished internodal and paranodal regions. In addition, some of the intact regions of the obese nerve fibers show intermembranous, hyphen-shaped electron dense ticks (Figures 10 and 13–15) identical to those of the typical sciatic nerves of rat. [105–108,111,112]

Our findings about periodicity can be favorably compared with the data resolved by cryofixation, vitrification, and X-ray diffraction studies [113–115] as well as those pioneering studies with TEM where the insulation sheath compared to a kind of 'jelly roll' formed by the SCs surrounding nerve fibers whether rats, other animals, or human samples [3,4] and monographs dealing with structure, molecular organization, and physiopathology. [1–4,7,9,87,105–109,111–113,116]

SCs and sciatic nerve changes

Even though the dynamic mechanisms by which SC cytoplasm wraps constitute the enveloping major dense lines and the extracellular surface contacts form the minor lines of myelin can now be better understood than in the earliest investigations, the distribution and maintenance of many of the myelin components wrapped in associated sheaths are still unclear in the PNS [1–6,76,87,117–125]; this is especially true in the diabetes types. [76,78–82,87,88,123,126,127] Recalling numerous investigations, some of them have clarified the single molecular marker's turnover during growth *in vivo* and *in vitro*. [3,6,108,121–123,126,127] However, many PNS nerve fibers owe large number of membrane wrappings; this more complex architecture leaves unresolved questions, compared with those now obtained with some CNS myelinated ones [3,5,102,106,109,111,116,128–134] and others found with demyelination diseases especially linked with autoimmune or genetic defects. [1–7,124,125,131–144]

The choice of using sciatic nerve samples in the Zucker rats has been influenced by the frequent studies done on this nerve throughout the literature [1–5,87,109,111,116,124,125,136–141] and the easy access of rat carcasses obtained from local investigators to initiate our study. The PNS nerve myelin, like the one made by the oligodendrocytes, contains a huge proportion of cholesterol. It is thus without surprise that cholesterol was one of the first lipid investigated with radiolabeled-chase radioautography and found to be provided by the SCs through longitudinal diffusion mode along the cytoplasm and its extension, the major dense lines, out of the S-L spaces or incisures, in addition to the neuroplasm, in a centrifugal fashion. [113,125,129,138–141] If cholesterol with specific lipoproteins rafts imparts the large part of the essential myelin architecture or morphology (layering, curvature with stiffness combined; 125,140,141,142,143,144,132), many other myelin components have not seen such clear resolution in provenance and placement because they can be randomly distributed throughout the sheaths or even via the axoplasm as well as the SCs. [1–4] In the lipid matrix of myelin, some of the constitutive glycoproteins (PGs) or proteolipids proteins are capable of translational diffusion within this bilayer. [1–5,125,133,134,140]

It is quite amazing to consider that the SCs huge management and homework to express and dispatch properly intrinsic, extrinsic proteins, plasmalogens, adhesive PGs, and glycolipids and dynamically construct, maintain, and repair a proper myelin membrane's integrity. [1–5,109,111,116,133,134,141,145–150] Even though, there are probably other still undiscovered regulations to be deciphered in the nerve system,

especially those that would cause defects in PNS disease-related myelin components to clarify the etiology and possible treatment of their associated neuropathies, such as diabetes type 1 (IDDM) differing from type 2 (NIDDM). [116,127,131] Many studies have emphasized the CNS defects [109,111,131,134] and reduced PNS disease's diagnostics into biopsy studies. [1–6,87,88] In PNS myelin, among all its non-phospholipid metabolome, large amounts of PGs or plasmalogens contribute to its architecture, integrity of adherence of the neuroplasm, and to nerve protection and insulation. [150–154] Further, the neuroplasm interacts with myelin both ways [155–159], with cadherins [160–166] and others still to be found [165–168] as well as to place periaxin [169] with connexin 32 (Cx32). [170]

The alignment and points of adherence of the defects can suggest one of the most abundant myelin PGs involved, such as myelin protein zero (P0) which core integral protein replaces the proteolipid protein or PLP found in the CNS myelin. [148,149,171–180] P0 makes homophilic contacts across major dense and minor lines and contacts others, such as the peripheral myelin protein 22 (PMP22) [178–180] altogether potent stabilizers of the myelin [160,179–181] along with the adaxonal myelin-associated glycoprotein (MAG). [160–164,181] Among them, the discovery of neuregulins in CNS [182] triggered a series of observations in favor of its neurotropic activities on myelin differentiation, adherence, layering, and interactions with the SC basal lamina in the sciatic nerve in vivo knockout mice and in vitro [12,183–192], thus engaging possible new interactions between the immediate endoneurial layer made by the SCs and possibly an external influence of components of this endoneurium with the abaxonal activities to constitute a proper myelin. Notwithstanding, if a form of neuregulin favors myelination and it alteration can cause for hypomyelination, hypothyroidism has been already a factor of the etiologic nerve damage considered in NIDDM Zucker rats. [13,14,27,70,193]

Out of old data about diabetes autopsies [194], analyses of nerves from limb amputations [195], other human and animal data [47,196–198], cholesterol–phospholipid balance have been noted to be lower than of normal nerves as well as in components of the myelin. [47,194–200] Thus, the observations support that in obese Zucker rat nerves, similarly to other animal models and human, changes in junctional carbohydrate's SC's coating of phospholipids, PGs, glycolipids could be caused by altered expression due to a combined hypothyroidism and diabetes followed by an excessive Golgi sorting to membrane negatively charged phospholipids [198], sphingomyelins [47,199–201] and sulfatides [199] initiated with endoplasmic reticulum stresses. [203–205] In fact, alone, insulin treatment shows that altered utilization of glucose and some lipogenic activities can be restored. [204–206] Other possible changes may be associated with the progressive alterations in nerve vascular supply and, in some experiments, metabolic changes even suggest ROS injuries. [202–207] This etiologic functional maze of interactions reminds us about previous surveys [76–85,110] and clarifications of human, Zucker rat, or other animal pathology involving PNS demyelination in diabetes type 2 could still come out from further modern verifications, wherever necessary, utilizing knockout murine models.

The myelin and possible causes of the damages

The minor (extracellular) dense lines excessive hydrophobic, inositol-proton rich charges could 'unzip' attachment sites of the major dense lines (containing phospholipids and plasmalogens such as the MAGs, heavily phosphorylated glycosaminoglycans -i.e. anion- charged- like the myelin basic protein in CNS [160–164,181,208], galactosylceramides and sulfatides) constitute a large proportion of the total membrane glycolipid mass [75–85,110,126,127,162,209–215] that can be altered in NIDDM, in addition to the rafts [208,215–216] and along with the S-L incisures. [209]

One of the potent supplier for ceramides is the sphingomyelin synthase 2 (SMS2) [218–223] that is normally stimulated by leptin. [214,218,221] In fa/fa Zucker rat tissues, including SCs, they bear a leptin receptor defect which could dull a typical expression of SMS2 and typical sorting of ceramide into sphingomyelin from inner to outer myelin membrane, protracted to support the renewal of the minor dense line. Insulin insensitivity or resistance makes a deficient conversion of ceramides into sphingomyelins to be incorporated in the myelin glycosphingolipids [217]. This could mean that an excess of slow-used, perhaps even of peroxidated ceramides with no charge may overload and can disturb the major dense lines,

extensions of the S-L. Reaching the NIDDM glycocalyx, these ceramides add peculiar damages [219] and, following processing of tissues, leave the myelin with stores of lingering, waxy, electron dense deposits among gaps in myelin extracted altered lipids.

Compounded with abaxonal origin, any myelin membrane spacing disruption could also modify not only its rigid curvature but also create an onion-like membrane structures through unbalanced lipid-phospholipid-cholesterol content. This failed turnover with accumulation of the initial defect along with other minor PGs [224] can compromise the integrity of the myelin. Excess of ceramides can result in a sort of Wallerian degeneration. [109,111,116,225] However, the Wallerian degenerative morphology does not fit with the type of injuries found here since axons are not segmented [226] and the described injuries do not compare with other recent diabetes findings. [227] Additionally, traumatic crush defects found in a recent ultrastructural study, dealing with sciatic nerve in rodent, are different than our data. [228]

Disturbances in the minor dense line with facing glycocalyx can be caused by some acquisition of excessive anionic repelling charges [198,223] out of a sequential, decreasing anionic amounts of residues (phosphorylated > sulfated > carboxylated). [168,195–199,223] Repelling spaces can be revealed by clear, unstructured rifts in the fine morphology of the minor lines, where sciatic myelin external surfaces with excessive similar charges (sialic acid or associated) thus could cause other adherence faults, increasing disturbances in cooperative PG interactions. Narrow sectors ultimately generate large sectors of undulating onion layers or extracted since the rafts could now have modified membrane components and, thus, alter the tight wrapping of the normal myelin, as noted in diabetes nerves [226] but with further, more intense damages.

The demyelination changes of the obese nerve myelin, observed at first as narrow spaces or nicks to channel-like into cones with electron dense contrast, could originate from the overloads of ceramides as sphingomyelin precursors in the Cajal bands. Ceramide channels have been illustrated in model membranes and other cells [229,230] and correspond with ceramidase inactivity in obese rats. [231] It can be hypothesized that the maintenance of myelin layers by the high need of ceramides to form sphingomyelins produces sorts of molecular trans- and, then, intermembranous throughs or channels that could widen toward the adaxonal myelin zones. Each of generated directrix comes to an end at the axoplasm surface where the axolemma appeared preserved. This type of channeling could be injurious because of the peculiar myelin maintenance, delimited to the myelin insulating layer alone. [155–159,166–168] The transmembranous passageways across the sort of liquid crystal-like phase between myelin strata could be created by a sort of Rayleigh–Taylor instability. [232,233] In this case, at first, accumulated molecular species, passing through undetected pores, appear by processing as channel-like, with centripetal orientation, widen into sectors caused by the progressively changed myelin composition and processing extraction of the samples.

Other interactions exist between lipid rafts and ceramide or sphingomyelin precursors due to external glycanized moieties [225–227] and other membrane glycolipids. [224] Even though these plasmalogens belong to the family of immunoglobulins and can provide with injuries potent antigens toward autoimmune myelin defects akin to those noted in CNS (e.g. 87, 134) The absence of inflammatory reactions in sciatic nerve defects points to a main metabolic insult toward either SCs and/or axoplasm (i.e. neural) origin in cooperativity with changed connective stroma of Zucker NIDDM and some other endocrinopathies discussed in the following paragraph.

The detection of small ‘marbled’ or ‘pale, fatty-like’ vesicles in the Cajal bands adjacent to or abutting the myelin abaxonal layer in the obese nerves, not found in the lean ones, could be another clue linking the damages to a ‘distribution’ of excessive lipid-containing to the myelin. At first, one thought to identify the vesicles with lysosomes, where excessive loads of low pH and acid phosphatases with mannose could produce electron densities with the Golgi sorting processes but they are not membrane-bound. Therefore, the nature of granular-like deposits in the initial myelin defects cannot make them Reich or Π (lysosome) bodies parts of the PNS appearing through the dense line-extensions of the S-L spaces. [234–237]

For the sake of completion about myelin defects, neuregulin-1 [12,182–192,234,238], [243] that has been shown to control myelin thickness in relationship with the axoplasm through a suggested ‘centrifugal diffusion’. [193] However, out of the electron micrographic

illustrations of the same [193] publication, the knock-out 'control' murine strain' demonstrates myelin regularly spaced defects that appeared similar with those observed in this study. It is near or at the level of the Ranvier's nodes, or the juxtanode areas that the teared or 'unzipping' of the usual tight adhesion occurs between the myelin membranes and the axolemma. [1–6,107,108,119,134,146,239–242]

Out of all the main points discussed, myelination and its maintenance require the activation and high-level expression of myelin-specific genes of the SCs producing numerous specialized membrane components [190] with minor cooperative influences of the axon. [126,140,147,148] As such, demyelination that has been found in this investigation with diabetes type 2/ NIDDM Zucker rats is mainly caused by a single amino acid change in the leptin receptor, limited to internodal and juxtanodal zones without involvement of the axon components. However, as noted in a previous paragraph, this genetic etiology results not only out of NIDDM but also can be caused by convoluted associated endocrinopathies where the incapacitated central control further influences several organ's functionality. Among those, one cannot exclude the interstitial endoneurium metabolism and its vascular supply as these components still need further clarifications [8–10,86] Indeed, diabetes type 2 damages differ from those of diabetes type 1 because in IDMM more widespread peripheral neuropathic changes would be found and include nodal defects, whether in animal [48,49,84,93,127] or human observations [53,55,79] or in both. [47,96,97,134,197]

The obese Zucker rat leptin receptor mutation defect is exacerbated by other endocrinopathies

The adipokine leptin is without receptors in obese Zucker rat

The circulating adipokine leptin has been identified in wild and obese mice, and cloned. [35,88,244–247] Then, leptin became important in clinics to understand biology of obesity and diabetes with a considerable bibliography. [248–251] Leptin receptors have been cloned and localized in rat CNS areas, including the lean and obese Zucker strains. The arcuate nucleus, the choroid plexus, and the hypothalamus-pituitary-adrenal axis have the receptors even though unresponsive in obese ones. [244,251–255] In obese Zucker rats with NIDDM, the chromosome 5 bears a missense recessive homozygous mutation of the gene Fa into fa controlling the expression of a defective leptin receptor (named OB-R) caused by a substitution in position 269 of glutamine to proline of the extracellular domain of that receptor, also expressed in many tissues while the expression of the receptor long (active) form is found at normal concentrations in the lean Fa/? Zucker and most other laboratory or wild rats. [29–35]

The obese mice are unable to express leptin but they have adequate receptors for leptin, therefore, after injected with it, obesity can be eliminated. [244–250] However, obese Zucker *fa/fa* rats have no such suitable receptors, including in the stomach, where ghrelin is expressed and is known to suppress the hunger stimulus of the CNS arcuate nucleus. [244,256–260] The leptin receptor flaw makes *fa/fa* rats subjects to an agonistic autocrine activity inciting gluttony that further amplifies adipose tissue's leptin expression. They live with a chronic sevenfold normal serum leptin level. [28,31,32,36,246,256] This leptin plethora without receptor severs the normal hypothalamo-pituitary (and pineal?) axis functions (endocrines, circadian rhythms, etc.). It is quite possible that, similarly in similar human cases, an equivalent defective receptor mutation, also localized in chromosome 7q, translates into faulty leptin receptor causing congenital obesity [54–66,88,244,246,261–264] that can influence insulin activity. [38,54–66,263,264] In these human cases, demyelination defects have yet to be found and studied.

Central signals to periphery changes include PNS and adrenal maintenance along with inadequate reproductive activities and metabolism. [244,262–264] These changes have impact on myelin maintenance due to thyroid [14,15,19–25,43,265], growth hormone/prolactin [40–42,266,267] deficiencies in addition to gonadotropic [41,42,58,266,267], corticotropic [264–269], and POMC signaling defects [60,264,266],

A diminished external and SC's neurosteroid progesterone activity, along with that of adrenal secretion, can contribute to restrain the expressions of P0 and PMP22 plasmalogens. [160–162,165,167,171–181,184,270] However, there are some remaining linkers in the worse damaged myelin.

The SCs in the obese nerves and neuroprogesterone

A lack of leptin receptors in the adenohypophysis can also disturb activities of most basophils,

especially the gonadotrophs and POMC-making cells, thus alters SCs endogenous SC steroids production that further impact on peripheral neuropathy found with NIDDM of Zucker rats, and in similar human defects [262,263] when compared with normal subjects. [271] If neurosteroids can prevent myelin alterations caused by diabetes [272], verifications of neurosteroid changes are yet to come in Zucker rat to comfort this influence on the sciatic nerve defects. Repairs can be promoted through an external neuroactive steroid-like progesterone (P) thus this sex steroid could then become a preventative or repair way to reestablish the lipid myelin alterations in diabetics. [272–275]

The expression and metabolism of neurosteroids in the vertebrate nervous system have been studied in the PNS by Melcangi's laboratory and others. [276–286] In PNS, SCs are a major local source of growth factors and neurosteroids with internal P receptors. [278,279] P acts as an autocrine regulatory mechanism involved in myelination. [270,277,281] Neuroprogesterone synthesis by SCs in the PNS were confirmed with cytochrome P450scc (ssc: side-chain cleavage enzyme) and 3β-hydroxysteroid dehydrogenase/Δ5-4 isomerase mRNA (or 3β-HSD mRNA) found to be markedly regulated in myelinating cocultures of dorsal root ganglion neurons and SCs. These mRNAs were exclusively localized in the SCs. [282–284] Importantly, after sciatic nerve lesion, the upregulation of 3β-HSD mRNA correlated with the expression of plasmalogens P0 and PMP22 mRNAs which are also stimulated by P and by 5α-dihydroprogesterone treatment in the sciatic nerve in vivo and in cultured SCs. Isolated from neonatal rat sciatic nerves SCs in vitro the previous enzyme activities convert 25-hydroxycholesterol, a cholesterol metabolite which easily crosses cell membranes, into pregnenolone. [283] Pregnenolone was found to be higher in male rat sciatic nerves than in plasma; those levels were not reduced by castration and adrenalectomy, strongly suggesting a local synthesis of the direct precursor of progesterone, independent of external endocrine sources. In consequence, one could add internal SC's neurosteroid anomalies in the list of defects associated or caused by leptin receptor's inactivation.

Therefore, it seems coherent to propose, in addition to NIDDM [47,133,134,194–197,226], that leptin receptor defect of the young male obese Zucker rats can associate with a demyelination due to lack or depletion of sustaining neurosteroids. [285–287] However, it is not currently proven whether leptin could directly influence the SC's functions. [288,289]

Endoneurial support and myelin

Leptin receptors can modulate signals of the endoneurial fibroblast's lipid metabolism. This possibility is only supported by a few studies. [9,289] It is not too surprising since the endoneurium and SC basal lamina are linked with the ground substance to sustain myelin growth. [8,10,11,84,148,290,291] This endoneurium also influences myelin reconstruction and can be astutely instilled by recent contributions complementing old ones because SCs also reveal ownership of a plasticity for myelin reconstruction due to epithelio-mesenchymal transition. [288] Through this means, steroid hormones (androgens and thyroid ones), other factors [126,149,168,182–184,292–294], and still unknown signals can control SC's transcript expressions in maintaining a 'correct' PNS myelin composition and architecture.

Conclusions

The opportunity to have collected sciatic nerve long segments of the obese Zucker rat carcasses that bore a single amino acid mutation in the adiponectin leptin receptor not only makes this cytokine able to influence CNS tissues but also affects PNS nerve myelin directly or indirectly on the SCs metabolome [75,84,85,89,90,194,295–296] (Figure 15). This unique genetic defect has multiple entwined systemic/metabolic alterations that allow us to have observed and described progressive and large PNS ultrastructural damages limited to myelin (Figure 16). The myelinopathy seems mainly caused by the altered membrane content.

With NIDDM disease [78,130], the data suggest that the membrane's maintenance appears to have failed because membranes seem to have 'liquefied' due to lack of adequate cholesterol and modified PLPs (rafts [297]) in the internodal sectors. Instead, some unsaturated lipids accompanied by intermembranous locks – i.e. PGs or glycolipids but mostly sphingomyelin types – have been maintained. [95,97]

The SC defects could be amplified by changes in neurosteroids linked with this leptin receptor mutation. As shown in the previous paragraphs, several questions remain to verify the regulation of the myelin metabolome in normal vs. diabetes type 2 condition.

Furthermore, the imagery unveiled with this NIDDM model hopefully contributes and supplements by their details the few ultrastructural data reported in the PNS to diagnose this endocrine defect in animals [1–6,47–53] and, maybe, in human diabetes type 2 and/or some complex forms of the same disease. [54–58,75,97–99,194,195,295,296,298] Additionally, to assist in understanding how some pattern of damages can alter the peripheral neural conduction. Thus, another direct and indirect role of leptin, an adipokine, on PNS maintenance and with CNS interactions, [295,296] can be proposed as one has schematically illustrated in Figure 17.

Finally, the nerve defects described may have divulged an additional mode of maintenance of the PNS myelin by SCs through a kind of penetration-diffusion, diagrammatically depicted in Figure 16: some peculiar, myelin components, detected and illustrated in several micrographs appear in excess, faulty moieties or charges (maybe ceramides metabolites?). Issued from the SC's abaxonal regions they would be centripetally inserted in the myelin membrane layers and, through instable diffusion, transferred across myelin membranes, using flipping channels with spillage across the myelin layers. In so doing, the mechanism alters the intrinsic membrane rafts in addition to the diffusion already demonstrated in the CNS myelin with longitudinal-spiral diffusion out of the near nodal (juxta- and paranodal) Ranvier's zones toward the internodal region. [128–130]

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Declaration of interest

The authors declare that there is no conflict of interest.

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