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Inflammation and autophagy in peripheral nerves. Metabolic syndrome versus type 2 diabetes in rodent models.

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Abstract: Obesity, metabolic syndrome (MetS) and type 2 diabetes (T2D) are closely associated with activation of inflammatory signaling pathways resulting in macrophage accumulation and abnormal cytokine production. The obesity-associated inflammation itself may significantly contribute to the increased risk of metabolic, cardiovascular, neurological (peripheral neuropathy, PN) diseases in the absence of overt diabetes. In the previous work, we investigated the increased inflammation with infiltrated macrophages in peripheral nerve and adipose tissue (AT) in animal models of MetS such as Wistar Ottawa Karlsburg W (RT1u) *WOKW* rat and *ob*/*ob* leptin-deficient mice with mild T2 D or *db*/*db* leptin receptor-deficient mice with severe T2D, respectively in comparison to non-obese, healthy control animals. Whereas the moderate- or high-grade inflammation observed in *ob*/*ob* or *db*/*db* mice was associated with neuropathic changes of sciatic nerve, the mild macrophage infiltration and up-regulated autophagy in WOKW rats did not lead to the development of neuropathic symptoms. The aim of the present communication is to identify the inflammatory phenotype in correlation to autophagy at the mRNA level in the peripheral nerves of rodent with MetS and mild and severe form of T2D (*ob*/*ob* and *db*/*db*). Since inflammation plays a causative role in neurodegeneration, the understanding its pathomechanisms and correlations is necessary to targeting PN.

Keywords: inflammation; autophagy; metabolic syndrome; T2D; PDN; *ob*/*ob* mice; *db*/*db* mice; WOKW rat

Citation: Lastname, F.; Lastname, F.; Lastname, F. Title. *Life* **2023**, *07*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Firstname Lastname

Received: date
Accepted: date
Published: date

1. Introduction

Obesity, dyslipidemia, hypertension, hyperinsulinemia, and impaired glucose tolerance, diagnosed in patients with the MetS and diabetes are predictors which activate inflammatory processes in adipose tissue. The inflammatory cytokines are secreted mainly by adipocytes and by the stromal vascular fraction of macrophages and play important role in lipid metabolism, fat mass, insulin resistance, glucose homeostasis [1, 2, 3]. It has been assumed that inflammation in diabetes simultaneously leads to secondary

complications such as peripheral neuropathy. However, clinical studies have shown that inflammation in obesity is already a risk factor for PN in the absence of overt diabetes [4, 5]. According to the Neuropathy Association, only 30% of neuropathies develop due to diabetes, the remainder being infectious, autoimmune or for unknown reasons [6]. Unexpectedly, patients with MetS develop peripheral neuropathic phenotype similar to that diagnosed in patients with overt diabetes including reduction of motor and sensory nerve responses, pain, microvascular dysfunction, small nerve fiber injury, macrophage infiltration in the endo/perineurium [7, 8, 9, 10, 11]. Baum et al. have investigated the extensive autonomic nerve dysfunctions, affecting both parasympathetic and sympathetic nervous systems with damage of thinly myelinated and C unmyelinated fibers already in the obese children without diabetes [12].

Notable, the activation of macrophages in the inflammation process could occur bidirectional [13]. Whereas the classically activated macrophages (M1) express a specific set of pro-inflammatory mediators, alternatively activated macrophages (M2) exhibit an anti-inflammatory phenotype [13]. The expression of pro-inflammatory mediators like interleukin -1ß and-6 (IL-1β, IL-6), tumor necrosis factor alpha (TNFα) and monocyte chemoattractant protein-1 (MCP-1) of obese individuals is among the biomarker for the diagnosis of PN [5, 11, 13, 14]. Recently, a successful anti-inflammatory treatment with M2 polarized macrophages in the peripheral nerves have been demonstrated [15]. Similarly, switching from M1 to M2 with niacin administration in patients with Parkinson's disease or ex vivo administration of M2 macrophages in patients with stroke have been shown to be beneficial for inflammation [16, 17].

Interestingly, both the stimulation and inactivation of inflammatory signaling pathway have been distinctly regulated by autophagy process [18]. Generally, autophagy performs a neuroprotective function in by clearance of aggregated and toxic proteins [19]. Genetic studies in mouse and human nerve cell models have shown that basal deficiency or shutdown of autophagy genes like Atg5, Atg7 causes neurodegeneration and cell death [20, 21]. The authors postulated that the mechanistic relationship between autophagy deficiency and nerve cell death could be the target for therapeutic interventions. Although post-inflammatory peripheral nerve regeneration corresponds to up-regulated autophagy [22], the alternation of the inflammatory pathways associated with autophagic activity is still poorly understood. Future studies of autophagy activity in correlation to the macrophage phenotype appear to be crucial in the treatment of PN.

2. Methods

2.1. Animals and experimental design

The following animals have been used for experimental studies: *ob*/*ob* leptin-deficient mice with mild type 2 diabetes (*B6.V-Lep ob*/*ob*) and *ob*/+ healthy control mice (*B6.V-Lep ob*/*J*), *db*/*db* leptin receptor-deficient mice with severe type 2 diabetes and *db*/+ healthy control mice, Wistar Ottawa Karlsburg W (*RT1u*) *WOKW* rats with metabolic syndrome and healthy *LEW.1W* control rats. *Ob*/*ob*, *ob*/+, *db*/*db* and *db*/+ mice were obtained from the Taconic Europe (Ry, Denmark) and *WOKW* and *LEW.1W* rats from the Department of Laboratory Animal Science of the University of Greifswald (Karlsburg, Germany) and transferred to Leipzig in 2010. All animals were adjusted to the local animal facilities and maintained on a 12 h light/dark cycle with free access to water and were fed with regular food (Global Rodent T.2018.R12; Harlan Teklad) containing 12% of calories from fat.

Animal studies were approved by the local authorities of the state of Saxony, Germany, as recommended by the responsible local animal ethics review board (Approval No: TVV10/11, TVV25/12, TVV63/12, T01/13, TVV65/15, T08/16, Landesdirektion Leipzig, Germany). The manuscript contains three figures quoted from own previous publications (Fig. 1-3). Phenotypes of the exanimated groups have been summarized in the Table

1. mRNA analysis presented on the Fig. 4 has been performed using the frozen tissue samples of sciatic nerves.

2.2. Blood glucose, HbA1c and serum insulin concentrations

Blood glucose concentrations were measured in whole blood taken from the ventral caudal vein using an Opticum Omega glucometer (GlucoMen, Menarini Diagnostics, Berlin, Germany) and HbA1c levels were analyzed using an automated chemical analyzer in the Institute of Laboratory Medicine and Clinical Chemistry. Serum insulin measurements have been performed using standard enzyme immunoassay kit (Rat Insulin ELISA, Mercodida AB, Uppsala, Sweden).

2.3. Immunostaining (Fig.1-3) and Western blot (Fig.3) methods are described in the previous publications [23, 24, 25, 26].

2.4. Molecular methods (Fig.4)

Sciatic nerve tissue samples were collected immediately after euthanasia by an overdose of isoflurane followed by cervical dislocation, as previously published [23-26]. Total RNA was isolated from sciatic nerves (n=3 per group) of *db*/*db*, *db*/+, *ob*/*ob* and *ob*/+ mice, *WOKW* and *LEW.1W* rats using TRIzol (Life Technologies, Grand Island, NY), and 1 μg RNA was reverse transcribed with standard reagents (Life Technologies, Grand Island, NY). Quantitative real-time PCR (qPCR) were performed using the standard curve method as previously described [27]. The mouse and rat primers probes, TNFα (Mm00443258_m1, Rn99999017_m1), MCP-1 (Mm00443258_m1, Rn00580555_m1), Il-6 (Mm00446190_m1, Rn01410330_m1), Il-10 (Mm01288386_m1, Rn99999012_m1), Atg5 (Mm01187303_m1, Rn01767063_m1), Atg7 (Mm00512209_m1, Rn01492725_m1), Beclin1 (Mm01265461_m1, Rn00586976_m1), mTOR (Mm00444968_m1, Rn00693900_m1) and 18sRNA (Hs99999901_s1, endogen reference) were purchased from Life technologies (Darmstadt, Germany).

2.5. Statistical analyses

The statistical data analyses were performed using GraphPad Prism 9 Software (Jandel Scientific, San Rafael, CA) and differences among the groups were performed using one-way-ANOVA, the Newman–Keuls test (Fig. 1-4). Results are presented as means ±SEM (Fig. 1-4) and means ±SD (Tables). The correlation analysis (Fig. 5) was calculated using Pearson correlation. p-Values were adjusted for multiple testing using the Benjamini and Hochberg method. Analysis was performed with R and the corrplot package. Corresponding scatter plots were generated with Python and the pandas, matplotlib and seaborn packages. The different degrees of significance were indicated as follows: *p≤0.05, **p≤0.01, ***p≤0.001.

3. Results

3.1. Parameters of MetS and T2D rodent models

Table 1. Characteristics of study subjects at an age of 3-months (n=10; mean±SD).

	***ob*/+**	***ob*/*ob***	***db*/+**	***db*/*db***	***LEW.1W***	***WOKW***
Body weight (g)	31.1 ± 1.7	50.5 ± 6.6	32.1 ± 2.1	45.4 ± 9.3	503.3 ± 12	673.4 ± 21
Blood glucose (mmol/l)	5.8 ± 1.4	12.5 ± 0.6	6.1 ± 0.7	18.5 ± 1.8	5.8 ± 0.5	6.5 ± 0.9
HbA1c (%)	3.9 ± 0.4	6.1 ± 1.5*	4.2 ± 0.2	8.2 ± 2.0*	3.7 ± 0.3	3.8 ± 0.2
Serum insulin (ng/ml)	0.9 ± 0.4	12.9 ± 4.1	0.9 ± 0.4	1.5 ± 0.6	1.3 ± 0.5	9.1 ± 0.6

*references: Type 2 Diabetes mellitus HbA ≥ 5.8.

3.2. Sciatic nerve inflammation and intraepidermal nerve fiber density in ob/ob, ob/+, db/db and db/+ mice and in WOKW and LEW.1W rats

Our previously studies have shown the significant infiltration of macrophages and T cells in the peripheral nerves of *WOKW* rats with MetS [24], *ob*/*ob* and *db*/*db* mice with mild and severe T2D [23, 25, 26], respectively (Fig. 1-3). Whereas, the inflammatory signs in *ob*/*ob* and *db*/*db* mice leaded to neuropathic changes like demeyelinisation, reduction of nerve conduction velocity (NLG), degeneration of endoneural microvessels and intraepidermal nerve fibers (IENF; Fig. 1, 2), the WOKW rats do not develop overt neuropathy (Fig. 3). Unexpectedly, the significant up-regulated autophagy with *atg5* and *atg7* protein expression, increased LC3-II/LC-I ratio (Fig. 3) and massive autophagosomes formation, has been determined in sciatic nerves of *WOKW* rats as compared to health *LEW.1W* control animals.

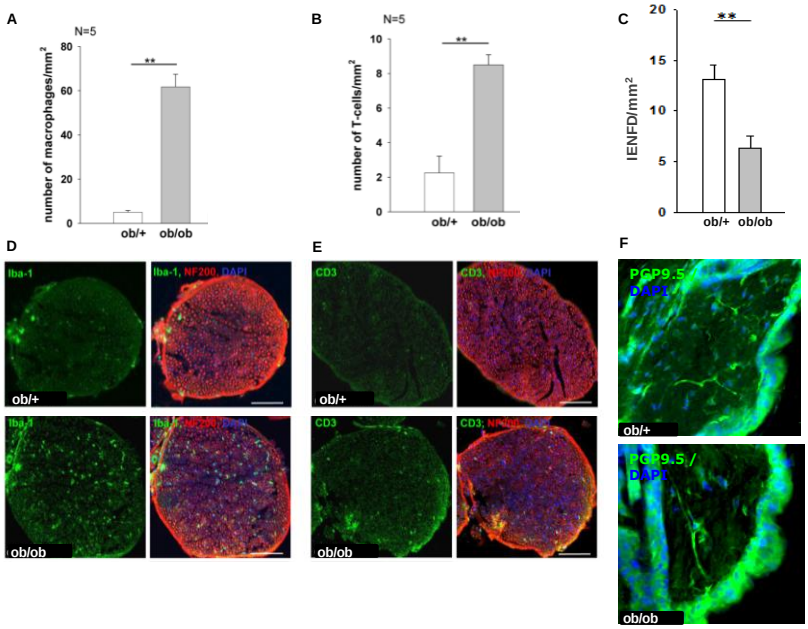

Figure 1. Macrophage and T cell distribution in sciatic nerves and intraepidermal nerve fiber density in the skin from *ob*/*ob* (mild T2D) and *ob*/+ healthy control mice. Quantification of Iba-1+ macrophages (**A**) and CD3+ T-cells (**B**) activation in sciatic nerves (n = 5). **D**: Double immunofluorescence staining against Iba1 (macrophages, green) or **E**: CD3 (T cells, green) and neurofilament 200 (nerve fibres, red). Cell nuclei were stained with DAPI (blue). A higher immunoreactivity of macrophages and T-cells was investigated in sciatic nerves of *ob*/*ob* mice compared to *ob*/+ control mice. Bar represents 100 mm (**D, E**). **C**: Analysis of intraepidermal nerve fiber density (IENFD) in the skin of the hind foot in *ob*/*ob* and *ob*/+ control animals. **F**: Immunofluorescence staining for PGP9.5 positive nerve fibers (green). Nuclei were counterstained with DAPI (blue, bar: 10 mm). Significant reduced IENFD has been found in skin of *ob*/*ob* mice as compared to healthy controls. Results are presented as mean ±SEM. * p≤0.05, ** p≤0.01, *** p≤0.001, according to the one-way analysis of variance together with the Newman-Keuls test. Adapted from *Kosacka et al. 2012, 2019* [23, 25].

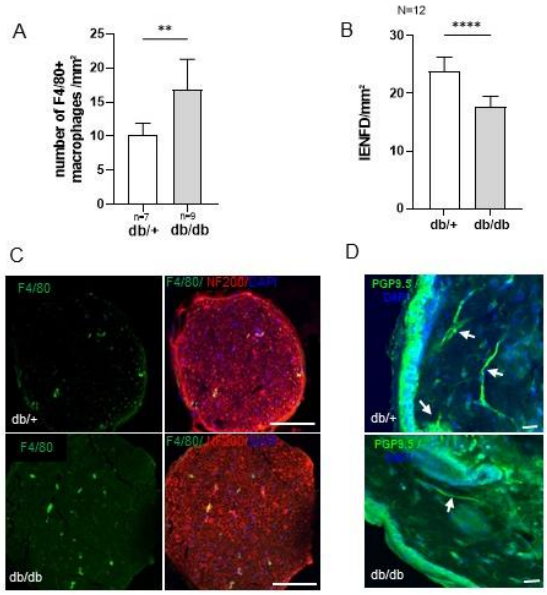

Figure 2. Macrophages distribution in sciatic nerves and IENFD in skin of *db*/*db* (severe T2D) and *db*/+ healthy control mice. **A**: Quantification of F4/80+ macrophages activation in sciatic nerves (n = 5). **C**: Double immunofluorescence staining against F4/80+ (macrophages, green) and neurofilament 200 (nerve fibres, red, bar represents 100 µm). Cell nuclei were stained with DAPI (blue). Statistical higher infiltration of macrophages was investigated in sciatic nerves of *db*/*db* mice compared to *db*/+ control animals (**A**). **B**: Analysis of intraepidermal nerve fiber density (IENFD) in the skin of the hind foot of *db*/*db* and *db*/+ control mice. A decreased IENFD has been found in *db*/*db* mice vs. control group. **D**: Immunofluorescence staining for PGP9.5 positive nerve fibers (green). Nuclei were counterstained with DAPI (blue, bar: 10 mm). Bar represents 10 µm. Results are presented as mean ±SEM. * p≤0.05, ** p≤0.01, *** p≤0.001, according to the one-way analysis of variance together with the Newman-Keuls test. Adapted from *Paeschke et al. 2019* [26].

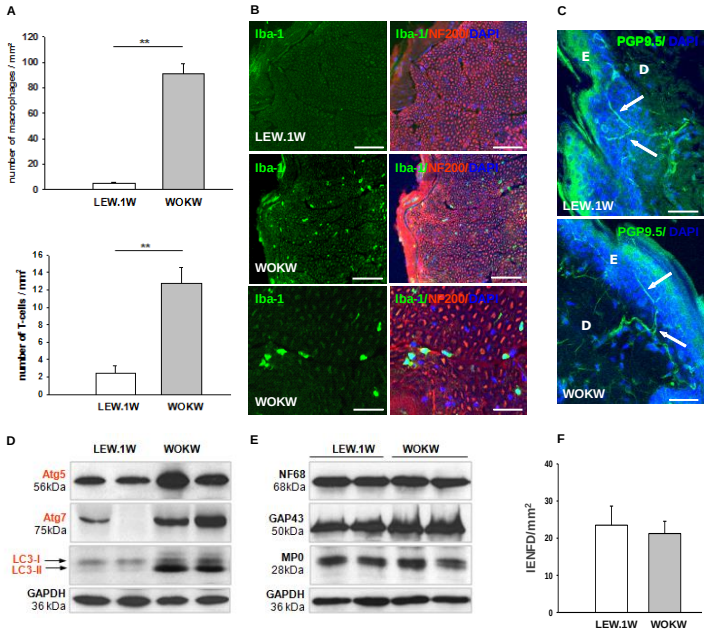

Figure 3. Inflammatory cells distribution, IENFD and protein expression of autophagy and neuronal marker in sciatic nerves of *WOKW* (MetS) and *LEW.1W* healthy control rats. **A**: Quantification of Iba-1+ macrophages and CD3+ T cells activation in sciatic nerves (n = 5). **B**: Double immuno-

fluorescence staining against Iba1 (macrophages, green) and neurofilament 200 (nerve fibres, red). Cell nuclei were stained with DAPI (blue). A higher immunoreactivity of macrophages was investigated in sciatic nerves of WOKW rats compared to LEW.1W control rats. Bar represents 100 µm (**B**). **C**: Immunofluorescence staining for PGP9.5 positive nerve fibers (green). Nuclei were counterstained with DAPI (blue, bar: 10 mm). **F**: Analysis of intraepidermal nerve fiber density (IENFD) in the skin of the hind foot between *WOKW* and *LEW.1W* control animals. No differences in IENFD have been found in both animal groups. E = epineurium; D = dermis. Bar represents 10 µm. **D**: Representative Western Blots (n=4) of the expression of the autophagy markers: Atg5, Atg7 and microtubule-associated protein light chain 3 (LC3) and neural structural proteins (**E**) such as: neurofilament (NF) 68, growth associated protein (GAP) 43, myelin protein zero (MP0) in sciatic nerves of *WOKW* and *LEW.1W* rats. Note the overexpression of Atg5, Atg7 and LC3-II in nerves of *WOKW* rats compared to the *LEW.1W* control animals (**D**). GAPDH was used as normalization control. Results are presented as mean ±SEM. * p≤0.05, ** p≤0.01, *** p≤0.001, according to the one-way analysis of variance together with the Newman-Keuls test. Adapted from *Kosacka et al. 2013* [24].

3.3. mRNA expression of pro- and anti-inflammatory cytokines and autophagy markers in the sciatic nerve of animal models with MetS and T2D

It has been proposed that activation of inflammatory signaling pathway and cytokine production could be stimulated by autophagy [28]. The mechanisms of balance between expression of pro-inflammatory and anti-inflammatory cytokines in peripheral nerves is still unclear. Here, we investigated whether pro- and anti-inflammatory cytokine expression corresponds with the expression of autophagy markers at the mRNA level.

We detected significantly higher expression of pro-inflammatory cytokines: *IL-6*, *MCP-1* and *TNFα* mRNA in sciatic nerves of *ob*/*ob*, *db*/*db* and *WOKW* animals as compared with healthy controls, respectively (Fig. 4A). However, the mRNA expression of anti-inflammatory cytokine, *IL-10*, has been significantly and about 4-fold upregulated in sciatic nerves of *WOKW* rats, exclusively vs. healthy *LEW.1W* control animals (Fig. 4A). Simultaneously, a significant increase in mRNA expression of autophagy marker *Atg7* (2.8-fold) and *Beclin-1* (1.8-fold) has been found in peripheral nerves of *WOKW* rats as compared with control animals (Fig. 4B). Similar, but less significant tendency was observed in the *Atg7* expression (1-fold) in peripheral nerves of *ob*/*ob* mice (mild T2D) versus healthy *ob*/+ controls (Fig. 4B).

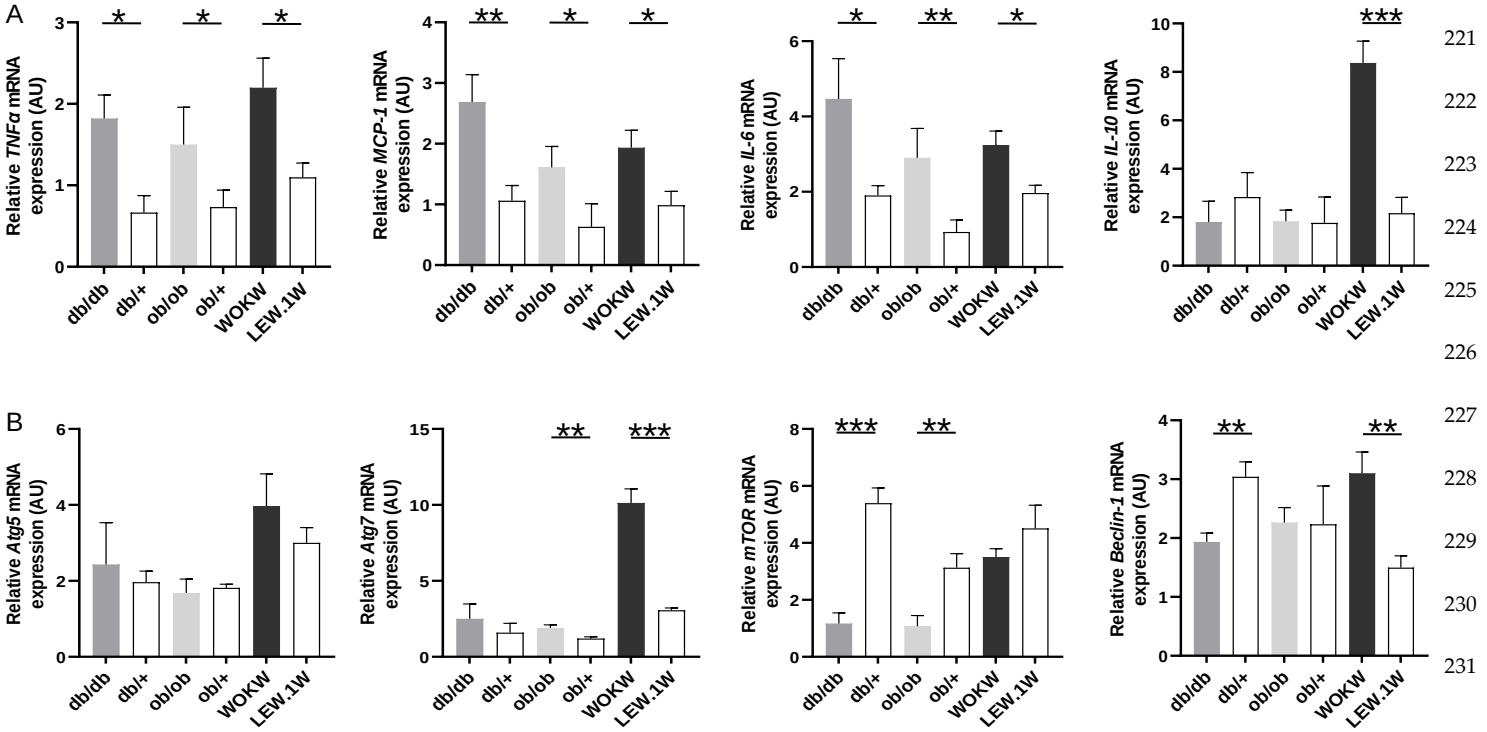

Figure 4. mRNA expression of pro-inflammatory, anti-inflammatory and autophagy related genes in sciatic nerves of animals with MetS and mild or severe T2D. **A**: *TNFα*, *MCP-1* and *IL-6* mRNA expression was increased in sciactic nerves in animals with MetS and T2D versus healthy controls. Anti-inflammatory *IL-10* and autophagy marker *Atg7* and *Beclin 1* mRNA expression (**B**) was significantly higher in the sciatic nerves of *WOKW* rats with MetS as compared to other groups. Data from N=3 animals per group are represented as mean ±SEM. * p≤0.05, ** p≤0.01, *** p≤0.001, according to the one-way analysis of variance together with the Newman-Keuls test.

3.4. The correlation analysis of pro- and anti-inflammatory and autophagy markers expression in the sciatic nerves of investigated animal models with MetS and T2D

Correlation analysis of the "co-expression" of inflammatory and autophagy markers in nerves of MetS and T2D donors has been used to determine the biological functionality of these genes in connection with the development of neuropathic inflammatory changes and their influence on the degree of neuropathy.

A positive correlation between up-regulated expression of autophagy markers (Atg7 and Beclin 1) and anti-inflammatory IL-10 cytokine has been found in sciatic nerves of *WOKW* rats with MetS without neuropathy. Simultaneously, moderate autophagic activity was shown corresponding to moderate expression of anti- inflammatory cytokines in correlation with mild and severe T2D with neuropathy compared to healthy controls (no changes). These results may confirm the neuroprotective function of autophagy by regulating the anti-inflammatory signaling pathway in peripheral nerves (Fig. 5)

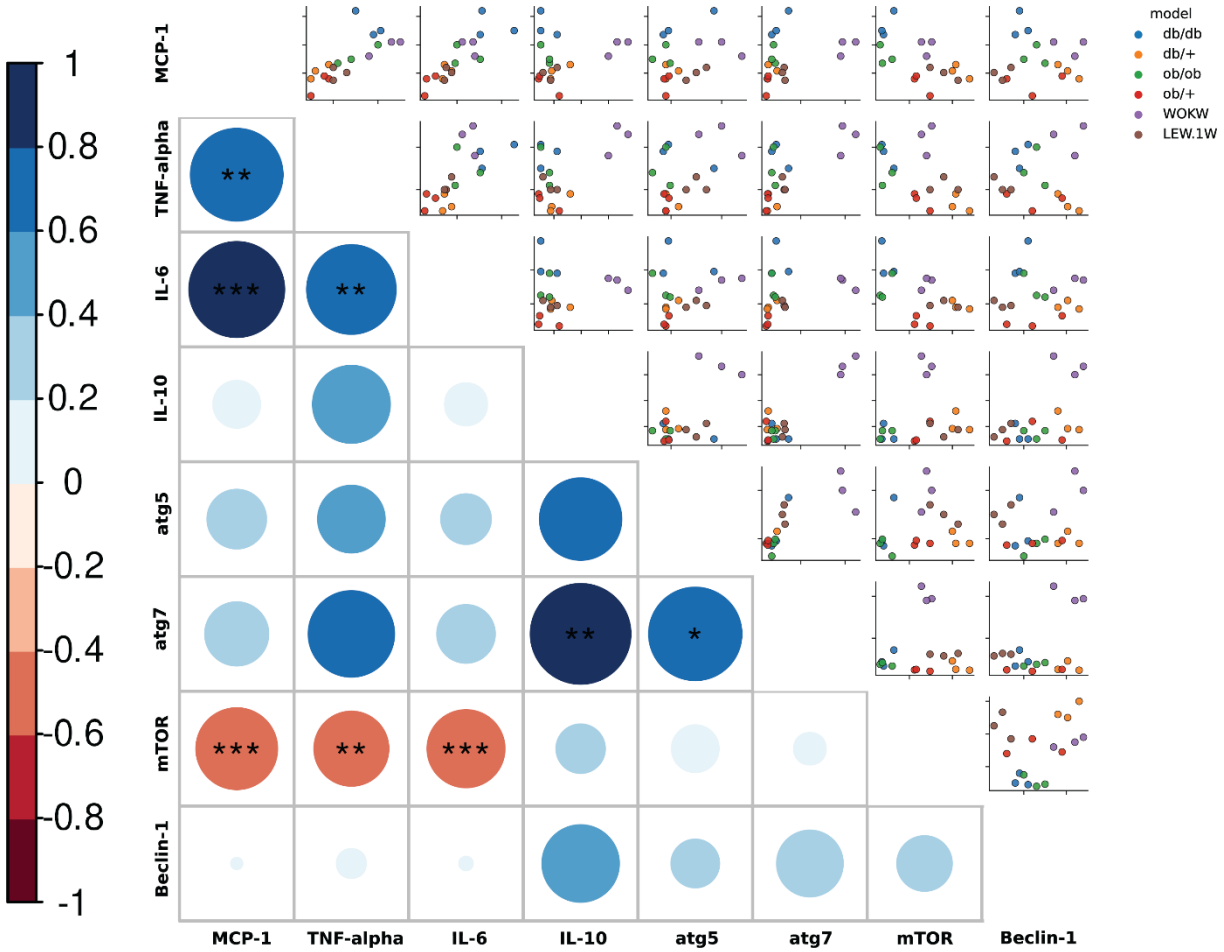

Figure 5. Correlation analysis between neuronal expression of pro/anti-inflammatory and autophagy markers in MetS (*WOKW* rats) and mild and severe T2D (*ob/ob*, *db/db* mice). A: Correlation matrix based on Pearson analysis with positive correlation in blue and negative correlation in red.

Areas of the circle are proportional to the correlation coefficient. Significance levels are *0.05, **0.01, and ***0.001, with p-values adjusted for multiple testing using Benjamini and Hochberg (BH). B: Corresponding individual scatter plots colored by animal model. .

4. Discussion

Although diabetic peripheral neuropathy (PDN) is classified as a multifactorial disease, active inflammation appears to be a determining factor in neurodegeneration. Increased oxidative stress and production of reactive oxygen species (ROS) in response to obesity and/or hyperglycemia lead to macrophage recruitment and induction of their pro-inflammatory M1 phenotype [29]. Massive expression of pro-inflammatory mediators, such as TNFα, IL-1, MCP-1, and an imbalance between pro- and anti-inflammatory answer, contribute to progressive inflammation and neuropathic pain [30]. The early symptoms of inflammation in PDN are degenerative changes in morphology of endoneural microvessels and small nerve fibers. It has been shown that microvascular damage precedes the degeneration of nerve fibers [31, 32]. Distal symmetric polyneuropathy, as the most common form of PN, not only develops symptoms such as weakness, unsteadiness, chronic pain, numbness of the hands and feet, but is also a decisive risk factor for diabetic foot ulcers and lower limb amputations [33, 34, 35].

Metabolic syndrome and type 2 diabetes are among the most important risk factors in the development of PN. Higher macrophage infiltration observed in the peripheral nerves of humans and animals with MetS and T2D is accompanied by increased synthesis of proinflammatory cytokines [24, 36, 37]. Similar plasma cytokine concentrations are also reported in these disease entities [36, 38]. Although infiltration of macrophages and T cells in peripheral nerves is a characteristic marker of inflammation, the macrophage phenotype informs about an active pro or anti-inflammatory pathway. Then the total number of macrophages will not be as significant as their phenotype, e.g. anti-inflammatory (M2), and the mechanisms responsible for this switch (M1/M2).

The present results suggest bidirectional changes in pro-inflammatory and anti-inflammatory mechanisms occurring in peripheral nerves in metabolic syndrome and type 2 diabetes depending on autophagy activity. However, the higher expression of pro-inflammatory cytokines TNFα, MCP1, and IL-1 has been found in the sciatic nerve of all examined animals with MetS (*WOKW rats*) and with mild and severe T2D (*ob*/*ob* and *db*/*db* mice), unexpectedly, the up-regulated expression of anti-inflammatory IL-10 and autophagy markers has been found only in nerves of *WOKW* rats as compared with healthy controls. In accordance, the unbalanced regulation of pro-/anti-inflammatory cytokines with lack of expression of anti-inflammatory marker, IL-10 in peripheral nerves of mice with T2D and neuropathy have been confirmed by Yanik and coauthors [38]. The exogenously administration of IL-10 signifantly reduced the inflammation of peripheral nerves [38, 39]. Previously, the authors have found a link beetwen low expression of anti-inflammatory cytokines in association with obesity, insulin resistance and glucose intolerance in animals with T2D [38].

Ziegler and co-authors found that prevalence of peripheral neuropathy is intermediate between diabetic and MetS subjects [8, 9]. It has been shown that subjects with MetS developed a small fiber neuropathy (Aδ thinly myelinated and C unmyelinated) involving sensory fibers and neuropathic pain. Our previous animal studies have shown that the neurodegenerative morphological changes in peripheral nerves like demyelinisation, degeneration of enoneural microvessels and intraepidermal nerve fibers or changes of the physiological parameters like reduction of motor and sensory nerve conduction velocity occured in the increasing gradient towards the mild and severe T2D phenotype. In addition, no significant neuropathy signs have been observed in peripheral nerves of animals with MetS (*WOKW* rats) [23, 24, 25, 26]. Overexpression of inflammatory cytokines in sciatic nerves in *WOKW* rats with up-regulated expression of autophagy markers did not result in neurodegeneration.

MetS and T2D, both are characterized by imbalance in the ATP levels, ROS expression and in the multiple cell functions of mitochondria including apoptosis. It has been postulated an important relationship between mitochondria and inflammation signal pathway. Alteration of mitochondria metabolic routes, such as oxidative phosphorylation and tricarboxylic acid cycle (TCA) can induced changes in gene expression [28]. Pro-inflammatory M1 macrophages are activated by the TCA cycle, while ß-oxidation induces M2 macrophages and anti-inflammatory response [28]. Recently has been provided that mitochondrial dysfunction and damage is the result of impaired autophagy [40, 41]. Since the autophagy is an evolutionarily conserved lysosomal degradation pathway, which controls cellular quality by eliminating protein aggregates, damaged organelles and intracellular pathogen [42] and regulating the antigen presentation and inflammatory signaling, so it plays a key role in protecting against inflammatory, metabolic and neurodegenerative disorders [43]. Notable, autophagy determines an important regulatory signaling pathway for MetS and T2D and their complications [40, 41] as well as its activation is therapeutically tested in preclinical studies [44].

In our previous works, we have investigated the protective role of autophagy in adipose tissue (AT) of obese patients without diabetes vs. T2D patients [45] and in AT and in peripheral nerves of *WOKW* rats with MetS [24, 46]. In this study, we shown the autophagy-dependent regulation of the anti-inflammatory cytokine IL-10 and its correlation with MetS in *WOKW* rats. This correlation links to our previously suggestion about the neuroprotective function of autophagy in MetS vs. T2D. The precise mechanism of "self-activation" of autophagy observed in animals with MetS in the direction of cell protection and anti-inflammatory processes needs further explanation. Rationale for the development of autophagy inducers for therapy have not only been given against neurodegenerative diseases [44, 47]. Drugs to improve autophagy are undergo tests, expectedly due to the form of application and against its side effects. In-depth knowledge of autophagy self-regulation may be suitable for use in clinical applications.

5. Conclusions

This comparative study focuses on the inflammation and autophagy process occurring in the peripheral nerves of both MetS and T2D animals and their contribution to the development of peripheral neuropathy. The correlation analysis presented here supports our previous findings of the neuroprotective function of autophagy in animals with MetS compared to animals with mild and severe T2D. This regulation included significantly higher mRNA expression of anti-inflammatory IL-10 and autophagy markers with moderately increased mRNA expression of inflammatory cytokines. Notably, the balanced via autophagy pro/anti-inflammatory phenotype in the peripheral nerves of *WOKW* rats did not lead to neuropathic changes, despite obesity, hyperinsulinemia, and other features of MetS. Conversely, a pro-inflammatory phenotype of peripheral nerve due to moderate or impaired autophagic activity in mild (*ob*/*ob*) and severe (*db*/*db*) T2D mice, respectively is an important factor in the development of PN.

Understanding the mechanisms of autophagy self-regulation in activating the anti-inflammatory signaling pathway could become a new strategy for therapies of peripheral neuropathy.

Supplementary Materials: Not applicable.

Author Contributions: Conceptualization J.K., P.B. and M.N.; methodology, S.P. and N.K.; software, M.B.; validation, M.Bu. and K.P.; formal analysis, M.K. and J.K.; investigation, T.E.; resources, N.K.; data curation, J.K. and M.N.; writing—original draft preparation, J.K.; writing—review and editing, P.B., M.N. and T.E.; visualization, P.B. and S.P.; supervision, J.K. and MN.; project administration, M.B.; funding acquisition, J.K. and N.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by German Research Foundation - project number 209933838 - SFB 1052/3; B4 to NK.

Institutional Review Board Statement: The animal study protocols were approved by the Institutional Review Board, the Landesdirektion Leipzig, local authorities of the state of Saxony, Germany (Approval No: TVV10/11, TVV 25/12, TVV 63/12, T01/13, TVV65/15, T08/16).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available in this manuscript.

Acknowledgments: The authors thank Viola Döbel, Lisa Gärtner, Jenny Schuster and Elisabeth Langer for the technical assistance.

Conflicts of Interest: The authors declare no conflict of interest.

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