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RESEARCH ARTICLE

Nervonic acid, a long chain monounsaturated fatty acid, improves mitochondrial function in adrenomyeloneuropathy fibroblasts

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Abstract

Background and Purpose: Nervonic acid plays a vital role in maintaining normal brain and neuronal function. Nervonic acid has gained increasing attention because of its potential neuroprotective and anti-inflammatory properties. Nonetheless, the beneficial effects of nervonic acid are yet to be fully investigated. Adrenomyeloneuropathy (AMN), a type of X-linked adrenoleukodystrophy (ALD), is a progressive inherited metabolic disease characterised by accumulation of saturated very long-chain fatty acids (VLCFAs) in plasma and tissues, leading to increasing oxidative stress, mitochondrial dysfunction, neuroinflammation, cognitive dysfunction and disability. We previously found that nervonic acid can biochemically reverse the accumulation of saturated VLCFAs and increase cellular ATP production in ALD. Here, we investigated nervonic acid as a potential therapy for ALD by assessing its impact on mitochondrial function.

Experimental Approach: We assessed the effect of nervonic acid on cellular bioenergetics and oxidative stress in AMN patient-derived fibroblasts. We employed Seahorse real-time cell metabolic analysis and imaging of cells treated with increasing concentrations of nervonic acid. Normal dermal fibroblasts served as the healthy control.

Key Results: AMN cells demonstrate significantly impaired basal respiration, ATP production, maximal respiration and spare respiratory capacity compared to healthy fibroblasts. These mitochondrial respiration parameters significantly improved on treatment with nervonic acid in a concentration-dependent manner. Nervonic acid treatment also significantly reduced mitochondria-derived and total cellular reactive oxygen species, indicating mitigation of total oxidative stress.

Conclusion and Implications: Our findings indicate a new mechanism of action for nervonic acid in ALD and other mitochondrial dysfunction-associated diseases. This can also indirectly prevent downstream inflammation, thus altering disease progression.

Abbreviations: ALD, Adrenoleukodystrophy; AMN, Adrenomyeloneuropathy; cALD, Cerebral adrenoleukodystrophy; MEM, Minimum essential medium; VLCFA, Very long-chain fatty acid.

KEYWORDS
adrenomyeloneuropathy, metabolic disorder, mitochondrial function, monounsaturated fatty acid, nervonic acid, oxidative stress, X-linked adrenoleukodystrophy

1 | INTRODUCTION

Nervonic acid (NA, C24:1n-9) is a 24-carbon very long-chain fatty acid (VLCFA) that plays an essential role in human health (Li et al., 2019). As a naturally occurring fatty acid in human milk, nervonic acid helps with myelination in the early stages of life (Yu et al., 2019). Further, as the major monounsaturated fatty acid in sphingomyelin that contributes to the formation of the myelin sheath, nervonic acid is crucial for the development and maintenance of the brain and nervous system (Lewkowicz et al., 2019; Martínez & Mougan, 1998). Preclinical and clinical investigations have indicated that nervonic acid holds the potential to alleviate motor, cognitive and neurological impairments (Aihaiti et al., 2023; Song et al., 2022; Zhang et al., 2024). Preliminary evidence suggests that nervonic acid can also modulate cellular antioxidant defence systems and inflammation in neurodegenerative conditions, rendering it a promising therapeutic candidate for neurological diseases (Aihaiti et al., 2023).

X-linked adrenoleukodystrophy (ALD) is one such inherited neurodegenerative disorder with an incidence of approximately 1 in 14,700 to 17,000 births in the United States (Moser et al., 2016; Turk et al., 2020). It is a rapidly progressive and devastating condition that typically results in fatality within a few years from disease onset, particularly affecting boys in the first decade of life (Santosh Rai et al., 2013). Substantial phenotypic variability has been shown in ALD, ranging from the milder form of adrenomyeloneuropathy (AMN, 40–46%) to the more severe form of cerebral ALD (cALD, 40–45%), with over 50% of AMN and cALD patients developing adrenocortical insufficiency (Addison's disease) (Engelen et al., 2012; Ronghe et al., 2002). In addition, women who have ALD will also eventually develop symptoms in adulthood, with over 80% developing spinal cord disease (Huffnagel et al., 2019). Current approved therapies are targeted toward young boys with cerebral ALD in the early stages of the disease to arrest disease progression (Gupta et al., 2022). However, to date, there are no FDA-approved treatments available for all patients with ALD, especially those who will ultimately develop one of the ALD phenotypes in the later decades of life.

ALD is caused by mutations in the ***ABCD1*** gene and is characterised by the accumulation of saturated VLCFA, predominantly hexacosanoic acid (C26:0), in plasma, brain white matter, adrenal cortex and testis (Ferrer et al., 2010; Gordon et al., 2018; Kemp & Wanders, 2010). Elevated levels of VLCFA are the predominant driving factor of disease progression in ALD. VLCFA toxicity can increase reactive oxygen species (ROS) production, leading to oxidative stress, impaired mitochondrial function, thereby inducing neuroinflammation in ALD models (Fourcade et al., 2008, 2015; Singh & Pujol, 2010). Dysfunction in mitochondria by itself can further result in issues such as accelerated lipid peroxidation of neuronal membranes, decreased mitochondrial inner membrane potential and ATP production, axonal degeneration and ultimately neuronal death (Fourcade et al., 2015; Zhao et al., 2022). These processes, triggered by toxic VLCFAs can occur in all individuals harbouring *ABCD1* mutations, thus serving as a major therapeutic target for developing disease-modifying interventions.

Considering the neuroprotective properties of nervonic acid, we postulate that this fatty acid can directly and indirectly benefit the treatment of ALD. Our previous publication demonstrated a direct effect of nervonic acid, by biochemically reversing the accumulation of free and complex C26:0 in fibroblasts derived from patients with ALD. In addition, we observed nervonic acid to concurrently increase ATP production and indirectly offer cytoprotection in these cells (Terluk et al., 2022). However, the underlying mechanisms by which nervonic acid mitigates oxidative damage and enhances ATP production have not been comprehensively investigated. In this paper, using patient-derived fibroblasts and live cell analyses, we elucidate the therapeutic benefits of nervonic acid, specifically targeting mitochondrial dysfunction and cellular oxidative stress in AMN. The enhanced comprehension of the pharmacological benefits of nervonic acid in

What is already known?

- In X-linked ALD, the accumulation of VLCFA triggers oxidative stress, mitochondrial dysfunction and neuroinflammation.
- Nervonic acid can significantly decrease VLCFA levels in ALD patient-derived fibroblasts.

What does this study add?

- Adrenomyeloneuropathy (AMN) cells demonstrate impaired mitochondrial function, which can be normalised by nervonic acid treatment.
- Additionally, nervonic acid treatment can mitigate cellular and mitochondrial oxidative stress.

What is the clinical significance?

- Nervonic acid has therapeutic benefits for ALD through multiple mechanisms of action.
- The effect on mitochondrial function supports its clinical use in other related conditions.

AMN will facilitate its future development as a potential therapy for all forms of ALD and may extend to treating a broader range of neurological diseases associated with mitochondrial dysfunction and oxidative damage.

2 | METHODS

2.1 | Fibroblast culture and treatments

Normal human dermal fibroblasts (NHDF) were purchased from Lonza Bioscience (cat. no. CC-2509, Walkersville, MD, USA). This cell line, derived from the dermis of the normal human neonatal foreskin, was used as the healthy control for evaluating mitochondrial function. Normal human dermal fibroblasts were generously provided by Dr. James Dutton (Stem Cell Institute, University of Minnesota, Minneapolis, MN, USA). AMN fibroblasts (GM17819, RRID:CVCL_W059) derived from the forearm skin of an adult AMN patient were purchased from Coriell Institute (Camden, NJ, USA). Fibroblasts were seeded on either 8-well chamber slides or 96-well plates using MEM supplemented with 5% FBS, 1% MEM non-essential amino acids solution and 1% penicillin–streptomycin in a humidified incubator at 37°C with 5% CO_2. The cells were treated with various concentrations of freshly prepared nervonic acid solutions (5, 20 and 50 μM), EtOH (vehicle control) or medium only (control), using 2% FBS medium with supplements. Following 5 days of treatment, cells were subjected to mitochondrial function analysis or cell imaging.

2.2 | Live cell staining and cell imaging

Oxidative stress in AMN fibroblasts was quantified utilising live cell staining and cell imaging techniques. Cells were seeded on chamber slides at a density of 1.25×10^4 cells per well. After 5 days of treatment, the cellular ROS, mitochondrial superoxide and content of mitochondria were measured by staining the cells with specific fluorescent dyes. CellROX™ Green reagent (5 μM) was employed for the detection and quantification of total ROS in AMN cells. MitoSOX™ Red reagent (5 μM), which is selectively targeted to the mitochondria, was utilised for the detection and quantification of mitochondrial superoxides. MitoTracker™ Green reagent (200 nM) was used for labelling and localisation of mitochondria. **DAPI** (5 μg ml^{-1}) was used to stain cell nuclei by binding to DNA, facilitating the visualisation and localisation of cells. Following the staining procedure, cells were fixed with 4% paraformaldehyde (PFA) and promptly used for cell imaging. Cell imaging and fluorescence visualisation were performed using the All-in-One Fluorescence Microscope BZ-X800 (Keyence, Osaka, Japan) with appropriate excitation filters as recommended by the manufacturer. Quantification analysis of fluorescence intensity was performed using the BZ-X800 Analyser (Keyence, Osaka, Japan) with single extraction mode. The total fluorescence intensity was quantified for each image and the corresponding total cell numbers were counted. The average fluorescence intensity was calculated by dividing the total fluorescence intensity by the total cellular content.

2.3 | Mitochondrial function analysis

Mitochondrial function was assessed on normal human dermal fibroblasts and AMN cells using the Mito Stress Test (MST) in an XFe96 Extracellular Flux Analyser (Agilent Technologies, Santa Clara, CA, USA). Cells (1.25 and 2.25×10^4 cells per well) were seeded overnight on Cell-Tak™ coated XFe96 microplate and then treated with nervonic acid (5, 20 and 50 μM) for either 24 h or 5 days. For the Mito stress test measurements, the culture medium was replaced with XF DMEM medium supplemented with glutamine 4 mM, sodium pyruvate 1 mM and glucose 24.9 mM, and set to pH 7.4 and 37°C. Oxygen consumption rate (OCR) was detected at baseline and after the following injections: (1) oligomycin (2.5 μM), (2) carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone/FCCP (2 μM) and (3) rotenone (1 μM) combined with antimycin A (1 μM). This protocol allowed the analysis of the following parameters: basal respiration, ATP production, proton leak, maximal respiration, spare respiratory capacity, non-mitochondrial respiration, coupling efficiency percentage and spare respiratory capacity percentage. The Mito stress test assay protocol was conducted following the manufacturer's instructions (Agilent). Oxygen consumption rate data were normalised by the number of cells determined by CyQUANT™ NF Cell Proliferation Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and using a fluorescence BioTek Synergy 2 Multimode Reader, along with BioTek Gen5 software (Agilent). Oxygen consumption rate results were generated by Seahorse Wave Pro (Agilent), analysed with the Seahorse Analytics software (Agilent) and expressed as the oxygen consumption rate in pmol min^{-1} $cells^{-1}$.

2.4 | Data and statistical analysis

All results are presented as the mean ± standard error of the mean (SEM). All the analyses were based on data from at least 5 independent experiments performed on different days. Data were analysed using one-way analysis of variance (ANOVA) with Tukey's test or Fisher's Least Significant Difference (LSD) test for multiple comparisons and the unpaired Student's *t*-test. A significance level of $P < 0.05$ was adopted as the threshold for determining statistical significance. Post hoc tests were run only when F achieved $P < 0.05$ and there was no variance inhomogeneity. All statistical analyses were performed using GraphPad Prism 8 software (GraphPad Software, Boston, MA, USA). Randomization or blinding were not carried out as they were considered not applicable for this study. Graphical abstract was generated using BioRender (Created in https://BioRender.com). This paper complies with *British Journal of Pharmacology* recommendations and requirements on experimental design and analysis (Curtis et al., 2022).

2.5 | Materials

2.5.1 | Cell culture medium and reagents

Minimum essential medium (MEM), heat-inactivated foetal bovine serum (FBS), MEM non-essential amino acids solution, penicillin–streptomycin (5000 units ml^{-1} of penicillin and 5000 μg ml^{-1} of streptomycin) and trypsin–EDTA (0.05%) were obtained from Thermo Fisher Scientific (Carlsbad, CA, USA); phosphate-buffered saline (PBS) was acquired from Genesee Scientific (San Diego, CA, USA). Cell-Tak™ Cell and Tissue Adhesive was purchased from Corning Inc. (Corning, NY, USA).

2.5.2 | Cell treatment

Nervonic acid (purity ≥ 99%) was purchased from Sigma-Aldrich (Burlington, MA, USA). Ethanol (EtOH, 200 proof) that was used for the preparation of nervonic acid stock solution was purchased from Decon Laboratories Inc. (King of Prussia, PA, USA). The concentration of EtOH in the medium was maintained below 0.1% for all treatment groups.

2.5.3 | Cell staining reagents

CellROX™ Green reagent, MitoSOX™ Red mitochondrial superoxide indicator and MitoTracker™ Green FM were obtained from Thermo Fisher Scientific (Carlsbad, CA, USA). DAPI (4′,6-Diamidino-2-Phenylindole, Dilactate) was purchased from Sigma-Aldrich (Burlington, MA, USA).

2.5.4 | Reagents for Seahorse assay

Seahorse XFe96 FluxPak, Seahorse XF DMEM medium and Seahorse XF 1.0 M glucose solution were purchased from Agilent.

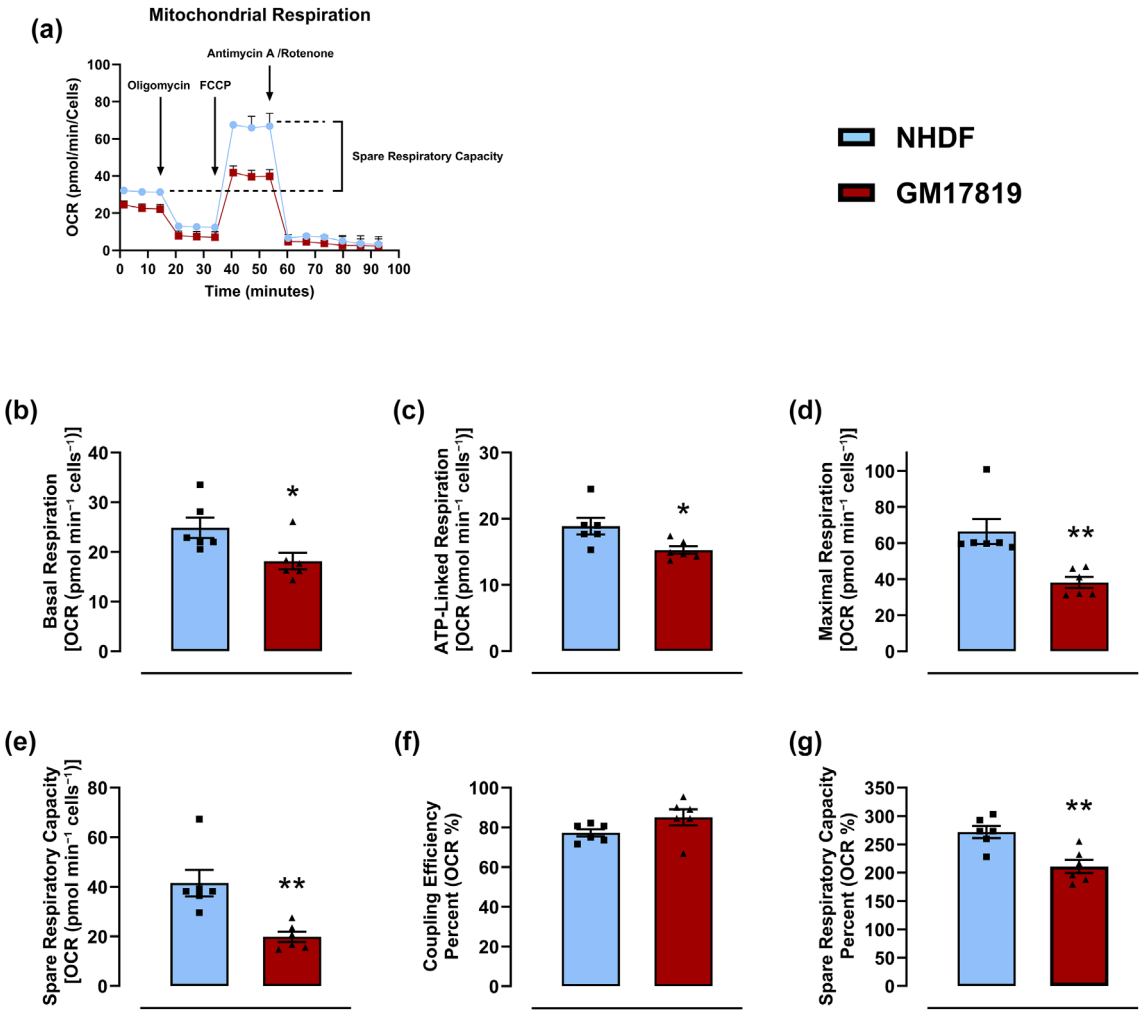

FIGURE 1 Mitochondrial function in patient-derived fibroblasts from adrenomyeloneuropathy (AMN; GM17819) compared to normal cells (NHDF) was assessed using the mitochondrial stress test (MST) conducted by Seahorse Analyser. (a) The mitochondrial respiration and (b–g) mitochondrial parameters were calculated from oxygen consumption rates (OCR). Data are presented as individual values and means ± SEM for NHDF ($n = 6$) and AMN cells ($n = 6$). Statistical analysis was performed using Student's t-test (*, $P < 0.05$).

L-Glutamine was obtained from Thermo Fisher Scientific (Waltham, MA, USA). Sodium pyruvate was sourced from Life Technologies (Carlsbad, CA, USA). Oligomycin complex, 2-[2-[4-(trifluoromethoxy) phenyl]hydrazinylidene]-propanedinitrile (FCCP), antimycin A and rotenone were acquired from Cayman Chemical (Ann Arbor, MI, USA). Additionally, the 0.1% gelatine in water was procured from Stem Cell Technologies (Vancouver, British Columbia, CA).

Details of other materials and suppliers are provided in specific sections.

2.6 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in the IUPHAR/BPS Guide to PHARMACOLOGY http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24 (Alexander et al., 2023).

3 | RESULTS

3.1 | Mitochondrial function is impaired in adrenomyeloneuropathy (AMN) fibroblasts

We first assessed the extent of mitochondrial dysfunction in AMN in relation to normal fibroblasts (NHDF) using a real-time cell metabolic analysis. Mitochondrial respiration profiles of normal human dermal fibroblasts and AMN cells were determined (Figure 1a), and key respiration parameters were calculated from oxygen consumption

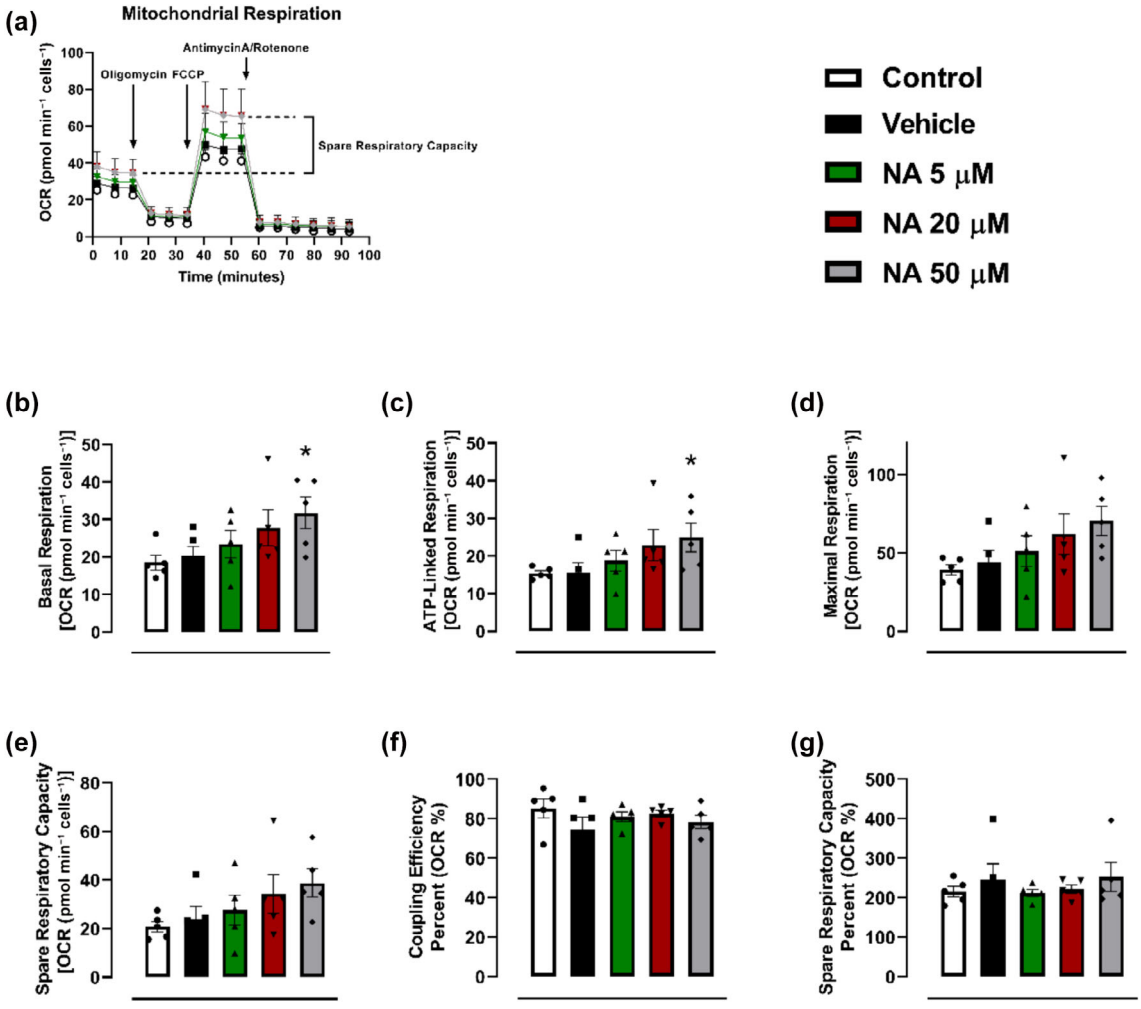

FIGURE 2 Effect of nervonic acid (NA) on mitochondrial bioenergetics in adrenomyeloneuropathy (AMN) fibroblasts. Cells were treated with increasing concentrations of NA (5, 20 and 50 μM), growing media only or ethanol (vehicle) plus growing media (control) for 5 days, and subsequently analysed using the mitochondrial stress test (MST) by Seahorse analyser. The (a) mitochondrial respiration profile and (b–g) mitochondrial parameters were calculated from oxygen consumption rates (OCR). Data are presented as individual values and means ± SEM ($n = 5$). Statistical analysis was conducted by one-way ANOVA with Fisher's LSD test (*, $P < 0.05$).

rate measurements before and after the sequential injections of oligomycin, FCCP and rotenone with antimycin A, as utilised in the Mito stress test. This approach allows for the calculation of mitochondrial respiration parameters illustrated in Figure 1b–g, including basal respiration, ATP production, maximal respiration and spare respiratory capacity. Additionally, coupling efficiency and spare respiratory capacity percentages were calculated. Our results indicate that basal respiration, ATP-linked respiration, maximal respiration, spare respiratory capacity and spare respiratory capacity percent in AMN cells are significantly lower than those in normal cells. However, coupling efficiency percentage was found to be elevated in AMN compared to normal fibroblasts. Additionally, oxygen consumption rate values for proton leak were significantly reduced, while non-mitochondrial respiration was higher in AMN cells compared to normal human dermal fibroblasts cells. These observations strongly support the involvement of bioenergetics failure in ALD pathogenesis.

3.2 | Nervonic acid contributes to mitochondrial function improvement in AMN fibroblasts

Following the confirmation of mitochondrial dysfunction in AMN cells, we investigated the mitochondrial effects of nervonic acid in this *in vitro* model. Treatment with 50 μM nervonic acid for 5 days significantly improved key parameters, including basal respiration and ATP-linked respiration. Additionally, maximal respiration and spare

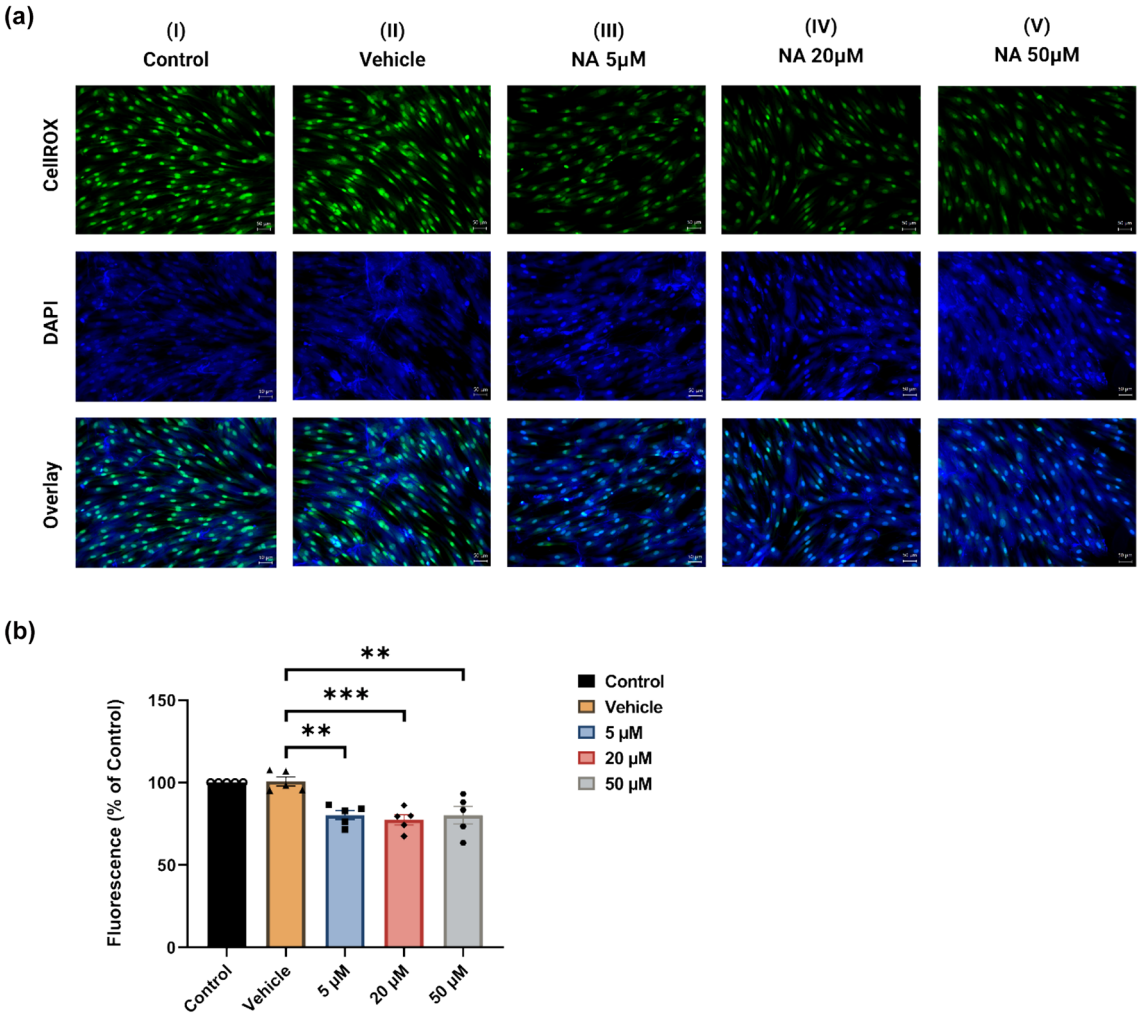

FIGURE 3 (a) Representative images of adrenomyeloneuropathy (AMN) fibroblasts after 5 days of treatment as follows: (I) control (cell culture medium only); (II) vehicle (0.1% ethanol); (III) 5 μM nervonic acid (NA); (IV) 20 μM NA; (V) 50 μM NA. Reactive oxygen species in cells were stained by CellROX™ in green as shown in the first row; nuclear DNA was stained by DAPI in blue as shown in the second row; overlay of the two fluorescent signals is shown in the third row. (b) Quantification of CellROX™ fluorescence intensity in AMN fibroblasts after 5 days of different treatments ($n = 5$). Data are presented as individual values and means ± SEM. Data were normalised with the control group, which was set to 100% fluorescence. Data from all groups were obtained from independent experiments performed on different days. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to compare the fluorescence intensity among groups and to correct for multiple comparisons. (**, $P < 0.01$; ***, $P < 0.001$).

respiratory capacity showed an increasing trend following nervonic acid treatment, although the changes were not statistically significant (Figure 2b–f). Notably, the parameter values increased to levels comparable to those observed in normal cells (compare parameters in Figure 2 versus Figure 1). However, proton leak and non-mitochondrial respiration did not show significant changes. We also observed a concentration-dependent effect across all three nervonic acid concentrations on most of the parameters following 5-day treatment. In contrast, nervonic acid treatment at the same concentrations for only 24 h did not show mitochondrial functional benefits, thus requiring longer-term exposure. Mitochondrial respiration parameters from the 24-h and 5-day nervonic acid treatment studies are summarised in Tables S1 and S2.

3.3 | Nervonic acid mitigates oxidative stress by decreasing the total cellular ROS

Given the close association between mitochondrial dysfunction and oxidative damage, we further assessed the impact of nervonic acid on cellular oxidative stress by quantifying the total ROS levels in AMN cells. After 5 days of nervonic acid treatment, a significant reduction in cellular ROS levels was observed across all nervonic acid treatment groups compared to the vehicle control (Figure 3). However, we did not observe a concentration-dependent effect of nervonic acid on ROS, as evidenced by the consistent reduction of up to 23% in ROS levels observed across the three concentrations of nervonic acid treated groups.

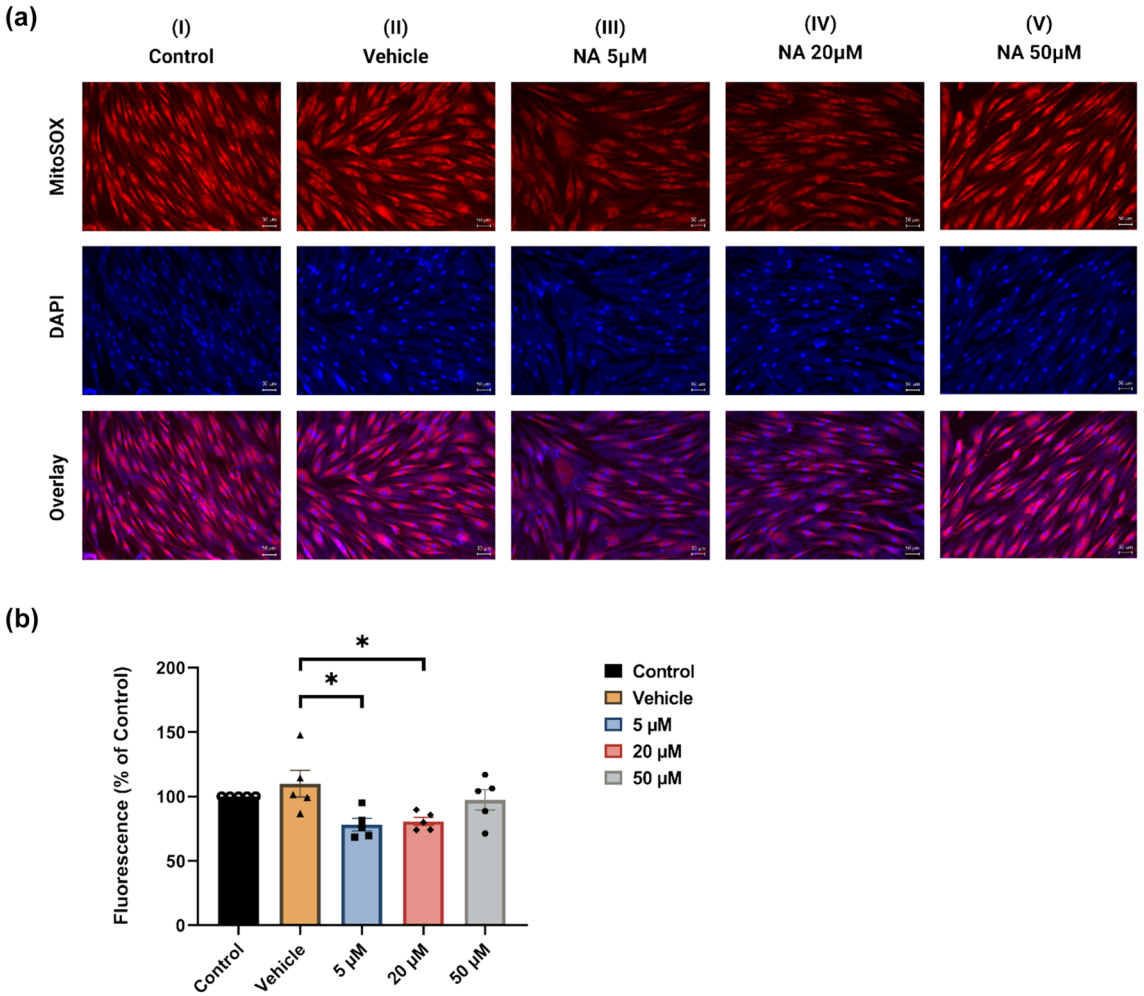

FIGURE 4 (a) Summarised representative images of adrenomyeloneuropathy (AMN) fibroblasts after 5 days of treatment as follows: (I) control (cell culture medium only); (II) vehicle (0.1% ethanol); (III) 5 μM nervonic acid (NA); (IV) 20 μM NA; (V) 50 μM NA. Superoxide generated in the mitochondria was stained by MitoSOX™ in red as shown in the first row; nuclear DNA was stained by DAPI in blue as shown in the second row; overlay of the two fluorescent signals is shown in the third row. (b) Quantification of MitoSOX™ fluorescence intensity in AMN fibroblasts after 5 days of different treatments ($n = 5$). Data are presented as individual values and means ± SEM. Data were normalised with the control group, which was set to 100% fluorescence. Data from all groups were obtained from independent experiments performed on different days. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to compare the fluorescence intensity among the groups and to correct for multiple comparisons. (*, $P < 0.05$).

Furthermore, the corresponding multiple comparisons conducted among the nervonic acid treatment groups revealed no statistically significant differences in ROS levels. Overall, this finding provides clear evidence of attenuated oxidative stress in AMN cells treated with nervonic acid.

3.4 | Nervonic acid significantly attenuates mitochondrial superoxide production

Building upon the effect of nervonic acid on cellular ROS levels, we proceeded to investigate its specific impact on superoxide generated

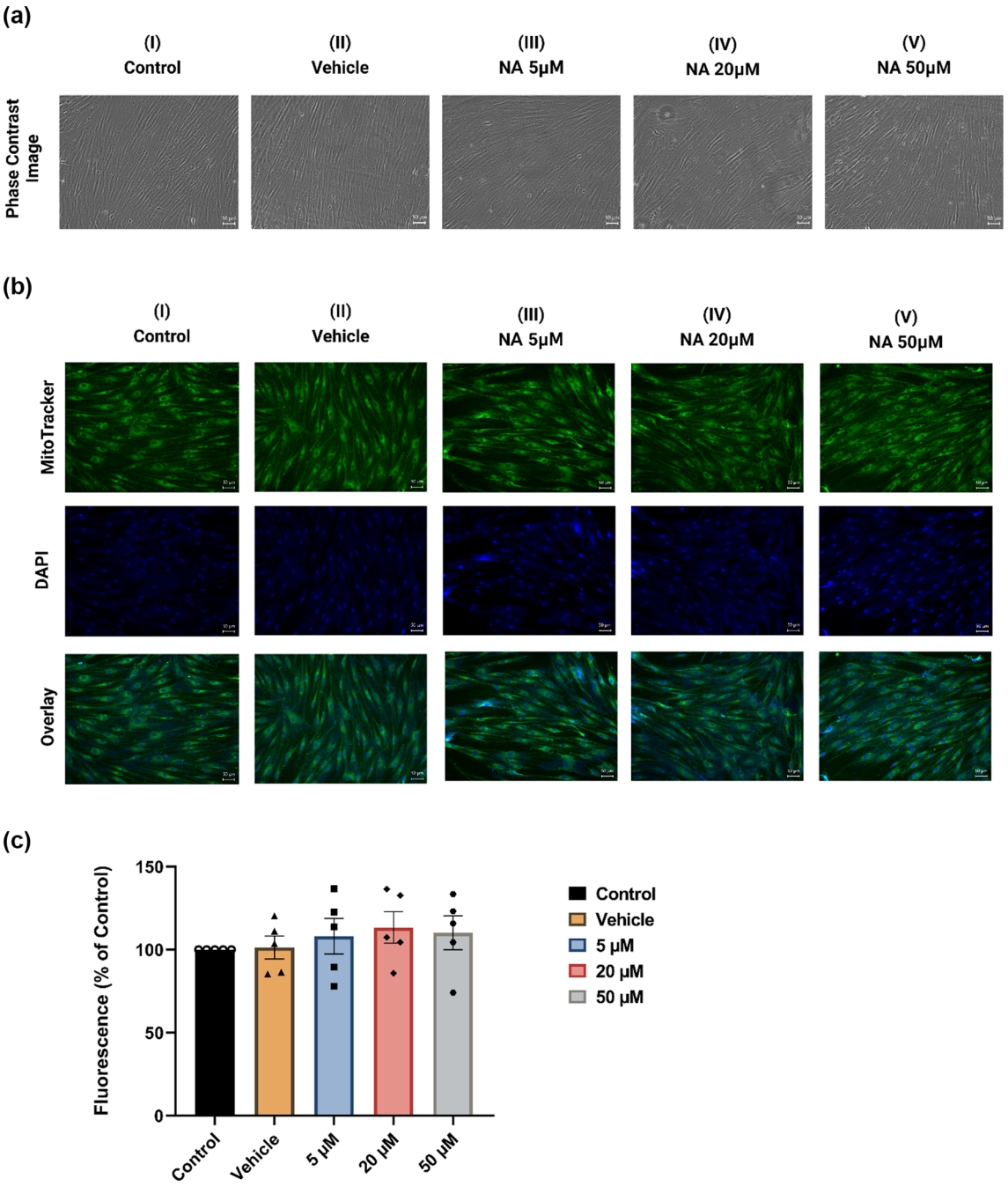

FIGURE 5 (a) Phase-contrast images and (b) summarised representative images of adrenomyeloneuropathy (AMN) fibroblasts after 5 days of treatment as follows: (I) control (cell culture medium only); (II) vehicle (0.1% ethanol); (III) 5 μM nervonic acid (NA); (IV) 20 μM NA; (V) 50 μM NA. Active mitochondria in cells were stained by MitoTracker™ in green as shown in the first row; nuclear DNA was stained by DAPI in blue as shown in the second row; overlay of the two fluorescent signals is shown in the third row. (c) Quantification of MitoTracker™ fluorescence intensity in AMN fibroblasts after 5 days of different treatments ($n = 5$). Data are presented as individual values and means ± SEM. During image acquisition, data were normalised with the control group, which was set to 100% fluorescence. Data from all groups were obtained from independent experiments performed on different days. One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was employed to compare the fluorescence intensity among groups and to correct for multiple comparisons.

within mitochondria. In line with the above results, we observed a significant decrease of up to 22% in mitochondrial superoxide levels following the treatment with 5 and 20 μM of nervonic acid, whereas treatment with 50 μM nervonic acid did not lead to an obvious reduction in the level of superoxides (Figure 4). Moreover, the effect of nervonic acid on mitochondrial superoxide was not found to be concentration dependent, as there was no statistically significant difference in superoxide levels between the 5 and 20 μM nervonic acid treatment groups. The findings from this study provide additional evidence supporting the beneficial effect of nervonic acid in mitigating oxidative stress by specifically targeting mitochondria. Furthermore, our results suggest that the concentration–response relationship of nervonic acid on total ROS and mitochondrial superoxide may follow a nonlinear pattern, with an optimal concentration likely falling within the range of 20 to 50 μM. However, further investigation is necessary to elucidate the underlying mechanisms driving this nonlinear response.

3.5 | Nervonic acid demonstrates no toxic effect on cellular morphology and mitochondrial content

Considering the impact of nervonic acid on mitochondrial function and cellular oxidative stress, it is essential to ensure that nervonic acid concentrations tested here do not lead to mitochondrial damage or disrupt the normal cellular structure. Phase-contrast imaging was performed to evaluate the morphology of cells subjected to the same treatments as described earlier. After 5 days of treatment, no significant differences in cell size and morphology were observed among the treatment groups (ranging from 5 to 50 μM), indicating that nervonic acid did not induce cellular toxicity (Figure 5a). The total content of mitochondria was then quantified and visualised using fluorescent imaging. Our results indicate that nervonic acid did not reduce or impair the total level of mitochondria in AMN fibroblasts (Figure 5b,c). Moreover, cells treated with nervonic acid exhibited a slightly increasing trend in mitochondrial quantity, implying its potential mitochondrial benefits.

4 | DISCUSSION AND CONCLUSIONS

Mitochondrial dysfunction and oxidative stress may play a causal role in neurodegenerative disease pathogenesis (Lin & Beal, 2006). Mitochondrial damage leads to increased ROS production, while oxidative stress can exacerbate the decline of mitochondrial function and ultimately cause neuronal death (Carrì et al., 2015). In adrenoleukodystrophy (ALD), oxidative damage has been observed in the brain and adrenal cortex obtained posthumously from patients (Powers et al., 2005). The metabolic dysfunction of very long-chain fatty acids (VLCFAs) triggers a cascade of pathological changes such as destabilisation of the myelin sheath and defective cellular functions that worsen the disease condition (Dubois-Dalcq et al., 1999; Ho et al., 1995; Kruska et al., 2015). The accumulated C26:0 has been found to specifically impair oxidative phosphorylation and mitochondrial function while promoting mitochondrial ROS generation in both the spinal cord of *Abcd1* mice and ALD fibroblasts (Fourcade et al., 2008; López-Erauskin et al., 2012, 2013; Singh & Pujol, 2010). Altogether, secondary pathologies in ALD, including mitochondrial damage, oxidative stress, proteostasis dysfunction and neuroinflammation, synergistically contribute to axonal degeneration and neuronal demise (Fourcade et al., 2015). The combined information unveils the potential therapeutic targets for ALD, focussing on mitochondrial failure and oxidative stress, thereby bringing more opportunities for the development of disease-modifying therapies.

To date, there is a lack of FDA-approved therapies to prevent or arrest the pathogenesis of AMN, the most common phenotype of ALD. The two approved therapies, haematopoietic stem cell transplantation and lentiviral-based gene therapy have only demonstrated favourable outcomes when administered during the early stages of active childhood cALD. Furthermore, allogeneic transplantation is typically accompanied by significant complications and carries a risk of infections, while gene therapy poses the potential risk of inducing hematologic malignancy (Gupta et al., 2022). Since 2016, ALD has been added to the Recommended Uniform Screening Panel for newborn screening in the United States, leading to an increased number of at-risk individuals (Moser et al., 2016). Several pharmacological candidates with various therapeutic goals are currently under development for ALD; however, none of them have demonstrated significant efficacy in arresting or delaying disease progression. Vitamin D supplementation has been developed to mitigate the risk of brain lesions and inflammation, but its efficacy has not been fully determined (Van Haren et al., 2023). **Pioglitazone**, an anti-diabetic thiazolidinedione, has been shown to reduce VLCFA levels and improve mitochondrial function in ALD cell and mouse models (Monternier et al., 2022). A phase 2 trial has been conducted to evaluate the effectiveness of Pioglitazone in adrenomyeloneuropathy (AMN) patients, but the results are still pending. Leriglitazone, a **peroxisome proliferator-activated receptor gamma (NR1C3)** agonist, has also been investigated for its potential in regulating oxidative stress and neuroinflammation, but the phases 2 and 3 trials failed to meet its primary endpoint in patients with AMN (Köhler et al., 2023). Taken together, there is an urgent need to develop a single, efficacious agent capable of targeting the multiple pathological mechanisms in ALD.

Nervonic acid stands out as one such therapeutic candidate with multiple mechanisms of action. As an indispensable component for myelination, nervonic acid has recently gained increasing attention across various diseases, including neurological disorders, diabetes and cancer (Phung et al., 2023). Furthermore, a decreased level of nervonic acid has been observed in sphingolipids from postmortem brains of patients with multiple sclerosis and ALD, indicating the significance of nervonic acid in demyelinating diseases (Sargent et al., 1994). Literature evidence indicates that nervonic acid exhibits antioxidant capacity, improves learning and memory ability, and alleviates inflammation in an Alzheimer's disease mouse model (Aihaiti et al., 2023). Similarly, nervonic acid treatment was found to activate the cellular antioxidant defence system, improve cell viability and upregulate the elimination

of ROS, highlighting its potential as a neuroprotective agent in the brain (Umemoto et al., 2021). Furthermore, nervonic acid has also been found to tackle inflammation in the intestine and liver by reducing the secretion of inflammatory factors (**tumour necrosis factor-α**, **interleukin-6 [IL-6**] and **interleukin-1β [IL-1β**]) and suppressing the nuclear factor kappa B signalling pathway (Hu et al., 2023; Wang et al., 2023; Yuan et al., 2023).

We demonstrated that nervonic acid treatment can increase ATP production and effectively improve total lipid profiles in ALD fibroblasts (Terluk et al., 2022). Here we present the protective effects of nervonic acid treatment on mitochondrial function and oxidative stress. Baseline comparison of AMN with healthy cells reveals impaired energy production, which is critical for cellular function and survival, and consistent with literature findings (Fourcade et al., 2014). The lower proton leak suggests reduced mitochondrial membrane permeability to protons, contributing to bioenergetics impairment. The decreased maximal respiration and spare respiratory capacity highlight the limited ability of these cells to meet increased energy demands as observed during chronic VLCFA toxicity. In AMN, the increased non-mitochondrial respiration and coupling efficiency when compared to control cells suggest compensatory mechanisms to keep energy homeostasis, but these are insufficient to overcome the overall bioenergetics failure. These findings contribute to the increasing evidence implicating mitochondrial dysfunction in the pathogenesis of ALD. An excess of C26:0 induces oxidation of mitochondrial DNA and impairs oxidative phosphorylation (OXPHOS), leading to increased production of mitochondrial ROS from electron transport chain complexes (López-Erauskin et al., 2013). This also highlights the cross-talk between mitochondria and peroxisomes in ALD pathogenesis. The diminished mitochondrial bioenergetics observed in fibroblasts derived from patients with ALD were also linked to decreased levels of AMP-activated protein kinase alpha1 (AMPKA1), which regulates mitochondrial biogenesis and function (O'Neill et al., 2011; Singh & Giri, 2014). In addition to the peroxisomal defect, examination of neural tissue from patients with AMN revealed lipidic inclusions within the mitochondria at the ultrastructural level. These inclusions may contribute to impaired mitochondrial function and subsequent axonal degeneration (Berger et al., 2014; Powers et al., 2001).

At the molecular level, the ROS reduction following nervonic acid treatment was associated with increased expression of Mn-SOD, Cu/Zn-SOD and γ-glutamylcysteine synthetase, which is responsible for **glutathione** synthesis (Umemoto et al., 2021). Mn-SOD, located in the mitochondria, and Cu/Zn-SOD, primarily found in the cytoplasm, play crucial roles in antioxidant defence. By boosting glutathione synthesis, which is essential for maintaining redox balance and reducing oxidative stress, nervonic acid may protect mitochondrial integrity and functionality in AMN cells. This can also explain the lack of nervonic acid effects on mitochondrial parameters following the 24 h treatment. Additionally, we have observed that pre-incubation with nervonic acid, at concentrations up to 50 μM for 24 h or 5 days, does not significantly reduce ROS production when AMN fibroblasts are exposed to high concentrations (25 to 75 μM) of the oxidising agent tert-Butyl hydroperoxide (TBHP). Thus, nervonic acid does not appear to act as a direct free radical scavenger. Further investigations are warranted to decipher the underlying mechanisms of how nervonic acid acts against oxidative damage in ALD. On the other hand, evidence from the literature supports that fatty acids are main source of acetyl-CoA that can be used in the citric acid cycle for energy production (de Carvalho & Caramujo, 2018). Polyunsaturated fatty acids can be incorporated into mitochondrial membranes, thereby potentially affecting the membrane structure and function (Sullivan et al., 2018). In addition, stearic acid has been identified as a regulator of mitochondrial function, with dietary stearic acid capable of promoting mitochondrial fusion, altering mitochondrial morphology and restoring mitochondrial bioenergetics (Senyilmaz et al., 2015; Senyilmaz-Tiebe et al., 2018). Similarly, another study reported that mono- and polyunsaturated fatty acids ameliorate mitochondrial morphological changes and improve mitochondrial respiration (Nisr et al., 2020). Therefore, we propose that the effects of nervonic acid on mitochondrial function are likely mediated through the modulation of antioxidant signalling pathways, alterations in mitochondrial membrane composition, regulation of mitochondrial morphology or the enhancement of fatty acid β-oxidation pathways. nervonic acid, being a monounsaturated omega-9 fatty acid, may thus modulate lipid metabolism leading to increased energy production. Overall, our study highlights the benefits of long-term exposure to nervonic acid in targeting bioenergetics failure and ameliorating redox imbalances, suggesting a promising therapeutic approach for AMN.

Dietary fatty acid supplements have been developed for the management of various diseases. Dietary supplementation of omega-3, a polyunsaturated fatty acid, can help reduce relapse rates and improve the quality of life in patients with multiple sclerosis (AlAmmar et al., 2021). Moreover, polyunsaturated fatty acids have also been found to be beneficial in cardiovascular diseases by preventing myocardial infarction, exerting antithrombotic activity and improving vascular function (Sokoła-Wysoczańska et al., 2018). Similarly, dietary intake of monounsaturated fatty acids is inversely associated with the risk of all-cause mortality, indicating its potential benefits for human health (Lotfi et al., 2021). This evidence indicates nervonic acid to be a safe and viable dietary option in AMN. This would also benefit women with the *ABCD1* gene mutation, who are classified as patients with AMN but who will develop symptoms in the later stages of life. As a dietary therapy that can boost energy production and maintain an optimal lipid profile, nervonic acid will be a promising intervention for all individuals with ALD variants.

Herein, we present a well-designed adequately controlled study demonstrating the therapeutic efficacy of nervonic acid on mitochondrial function and oxidative stress utilising live cell techniques. Our study provides robust evidence that enhances the current understanding of the mechanism of nervonic acid action on cellular bioenergetics, thereby laying the foundation for future evaluations in this area. Further assessment of nervonic acid in a cALD cell line is needed to gain a comprehensive understanding of its therapeutic benefits in

the aggressive form of ALD. Moreover, the underlying mechanisms by which nervonic acid improves mitochondrial function and metabolic pathways require further in-depth investigation. It is also crucial to investigate nervonic acid's impact on mitochondrial morphometry, particularly regarding fission/fusion events and cristae swelling, in future studies. The concentrations of nervonic acid used in this study were determined based on our previous findings, wherein the selected concentrations have shown biochemical effects in ALD cells (Terluk et al., 2022). However, additional research is necessary to translate these concentrations into those that can generate equivalent exposures in future preclinical and clinical studies. To be applicable for clinical use in conditions such as ALD and other neurological diseases, it is also crucial to thoroughly understand the pharmacokinetics and tissue disposition of nervonic acid, particularly in the brain. However, plasma nervonic acid levels were considered potential biomarkers for major depressive disorder and attention levels, suggesting the importance of safety assessment and dose determination in its development as a pharmacological treatment (de Seymour et al., 2022; Kageyama et al., 2017).

In summary, the multifaceted actions of nervonic acid, which include reversing biochemical abnormalities, enhancing mitochondrial function and reducing oxidative stress, position it as a promising therapeutic option for all patients with *ABCD1* gene variants. Having a single therapy with multitarget activity would markedly enhance efficacy, mitigate the risk of potential drug interactions and expedite the drug development process, which would be particularly crucial in rare disease research. Furthermore, the mitochondrial benefits and antioxidant/anti-inflammatory properties of nervonic acid, as demonstrated in our study and supported by literature, render it a possible candidate for treating various neurological disorders associated with mitochondrial dysfunction and inflammation.

AUTHOR CONTRIBUTIONS

C. Li: Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing—original draft (lead); writing—review and editing (lead). **M. R. Terluk:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); validation (equal); visualization (equal); writing—original draft (equal); writing—review and editing (equal). **R. V. Kartha:** Conceptualization (lead); data curation (lead); funding acquisition (lead); project administration (lead); resources (lead); supervision (lead); writing—review and editing (lead).

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

REFERENCES

Aihaiti, M., Shi, H., Liu, Y., Hou, C., Song, X., Li, M., & Li, J. (2023). Nervonic acid reduces the cognitive and neurological disturbances induced by combined doses of D-galactose/AlCl3 in mice. *Food Science & Nutrition*, *11*(10), 5989–5998. https://doi.org/10.1002/fsn3.3533

AlAmmar, W. A., Albeesh, F. H., Ibrahim, L. M., Algindan, Y. Y., Yamani, L. Z., & Khattab, R. Y. (2021). Effect of omega-3 fatty acids and fish oil supplementation on multiple sclerosis: A systematic review. *Nutritional Neuroscience*, *24*(7), 569–579. https://doi.org/10.1080/1028415X.2019.1659560

Alexander, S. P. H., Fabbro, D., Kelly, E., Mathie, A. A., Peters, J. A., Veale, E. L., Armstrong, J. F., Faccenda, E., Harding, S. D., Davies, J. A., Amarosi, L., Anderson, C. M. H., Beart, P. M., Broer, S., Dawson, P. A., Gyimesi, G., Hagenbuch, B., Hammond, J. R., Hancox, J. C., … Verri, T. (2023). The Concise Guide to PHARMACOLOGY 2023/24: Transporters. *British Journal of Pharmacology*, *180*(Suppl 2), S374–S469. https://doi.org/10.1111/bph.16182

Berger, J., Forss-Petter, S., & Eichler, F. S. (2014). Pathophysiology of X-linked adrenoleukodystrophy. *Biochimie*, *98*(100), 135–142. https://doi.org/10.1016/j.biochi.2013.11.023

Carrì, M. T., Valle, C., Bozzo, F., & Cozzolino, M. (2015). Oxidative stress and mitochondrial damage: Importance in non-SOD1 ALS. *Frontiers in Cellular Neuroscience*, *9*, 41. https://doi.org/10.3389/fncel.2015.00041

Curtis, M. J., Alexander, S. P. H., Cirino, G., George, C. H., Kendall, D. A., Insel, P. A., Izzo, A. A., Ji, Y., Panettieri, R. A., Patel, H. H., Sobey, C. G., Stanford, S. C., Stanley, P., Stefanska, B., Stephens, G. J., Teixeira, M. M., Vergnolle, N., & Ahluwalia, A. (2022). Planning experiments: Updated guidance on experimental design and analysis and their reporting III. *British Journal of Pharmacology*., *179*, 3907–3913. https://doi.org/10.1111/bph.15868

de Carvalho, C. C. C. R., & Caramujo, M. J. (2018). The various roles of fatty acids. *Molecules (Basel, Switzerland)*, *23*(10), 2583. https://doi.org/10.3390/molecules23102583

de Seymour, J. V., Beck, K. L., Conlon, C. A., von Hurst, P. R., Mumme, K. D., Haskell-Ramsay, C. F., & Jones, M. B. (2022). Plasma nervonic acid levels were negatively associated with attention levels in community-living older adults in New Zealand. *Metabolomics: Official Journal of the Metabolomic Society*, *18*(8), 54. https://doi.org/10.1007/s11306-022-01908-5

Dubois-Dalcq, M., Feigenbaum, V., Aubourg, P., Dubois-Dalcq, M., Feigenbaum, V., & Aubourg, P. (1999). The neurobiology of X-linked adrenoleukodystrophy, a demyelinating peroxisomal disorder. *Trends in Neurosciences*, *22*(1), 4–12. https://doi.org/10.1016/S0166-2236(98)01319-8

Engelen, M., Kemp, S., de Visser, M., van Geel, B. M., Wanders, R. J., Aubourg, P., & Poll-The, B. T. (2012). X-linked adrenoleukodystrophy (X-ALD): Clinical presentation and guidelines for diagnosis, follow-up and management. *Orphanet Journal of Rare Diseases*, *7*, 51. https://doi.org/10.1186/1750-1172-7-51

Ferrer, I., Aubourg, P., & Pujol, A. (2010). General aspects and neuropathology of X-linked adrenoleukodystrophy. *Brain Pathology*, *20*(4), 817–830. https://doi.org/10.1111/j.1750-3639.2010.00390.x

Fourcade, S., Ferrer, I., & Pujol, A. (2015). Oxidative stress, mitochondrial and proteostasis malfunction in adrenoleukodystrophy: A paradigm for axonal degeneration. *Free Radical Biology and Medicine*, *88*, 18–29. https://doi.org/10.1016/j.freeradbiomed.2015.05.041

Fourcade, S., López-Erauskin, J., Galino, J., Duval, C., Naudi, A., Jove, M., Kemp, S., Villarroya, F., Ferrer, I., Pamplona, R., Portero-Otin, M., & Pujol, A. (2008). Early oxidative damage underlying neurodegeneration in X-adrenoleukodystrophy. *Human Molecular Genetics*, *17*(12), 1762–1773. https://doi.org/10.1093/hmg/ddn085

Fourcade, S., López-Erauskin, J., Ruiz, M., Ferrer, I., & Pujol, A. (2014). Mitochondrial dysfunction and oxidative damage cooperatively fuel axonal degeneration in X-linked adrenoleukodystrophy. *Biochimie*, *98*, 143–149. https://doi.org/10.1016/j.biochi.2013.09.012

Gordon, H. B., Valdez, L., & Letsou, A. (2018). Etiology and treatment of adrenoleukodystrophy: New insights from Drosophila. *Disease Models & Mechanisms*, *11*(6), dmm031286. https://doi.org/10.1242/dmm.031286

Gupta, A. O., Raymond, G., Pierpont, E. I., Kemp, S., McIvor, R. S., Rayannavar, A., Miller, B., Lund, T. C., & Orchard, P. J. (2022). Treatment of cerebral adrenoleukodystrophy: Allogeneic transplantation and lentiviral gene therapy. *Expert Opinion on Biological Therapy*, *22*(9), 1151–1162. https://doi.org/10.1080/14712598.2022.2124857

Ho, J. K., Moser, H., Kishimoto, Y., & Hamilton, J. A. (1995). Interactions of a very long chain fatty acid with model membranes and serum albumin. Implications for the pathogenesis of adrenoleukodystrophy. *The Journal of Clinical Investigation*, *96*(3), 1455–1463. https://doi.org/10.1172/JCI118182

Hu, D., Cui, Y., & Zhang, J. (2023). Analysis of the improvement effect of nervonic acid extracted from Xanthoceras sorbifolium bunge oil on antioxidant response and inflammatory response in Parkinson's disease. *Journal of Integrative Neuroscience*, *22*(6), 6. https://doi.org/10.31083/j.jin2206161

Huffnagel, I. C., Dijkgraaf, M. G. W., Janssens, G. E., van Weeghel, M., van Geel, B. M., Poll-The, B. T., Kemp, S., & Engelen, M. (2019). Disease progression in women with X-linked adrenoleukodystrophy is slow. *Orphanet Journal of Rare Diseases*, *14*(1), 30. https://doi.org/10.1186/s13023-019-1008-6

Kageyama, Y., Kasahara, T., Nakamura, T., Hattori, K., Deguchi, Y., Tani, M., Kuroda, K., Yoshida, S., Goto, Y., Inoue, K., & Kato, T. (2017). Plasma Nervonic acid is a potential biomarker for major depressive disorder: A pilot study. *International Journal of Neuropsychopharmacology*, *21*(3), 207–215. https://doi.org/10.1093/ijnp/pyx089

Kemp, S., & Wanders, R. (2010). Biochemical aspects of X-linked adrenoleukodystrophy. *Brain Pathology*, *20*(4), 831–837. https://doi.org/10.1111/j.1750-3639.2010.00391.x

Köhler, W., Engelen, M., Eichler, F., Lachmann, R., Fatemi, A., Sampson, J., Salsano, E., Gamez, J., Molnar, M. J., Pascual, S., Rovira, M., Vilà, A., Pina, G., Martín-Ugarte, I., Mantilla, A., Pizcueta, P., Rodríguez-Pascau, L., Traver, E., Vilalta, A., & ADVANCE Study Group. (2023). Safety and efficacy of leriglitazone for preventing disease progression in men with adrenomyeloneuropathy (ADVANCE): A randomised, double-blind, multi-centre, placebo-controlled phase 2-3 trial. *The Lancet. Neurology*, *22*(2), 127–136. https://doi.org/10.1016/S1474-4422(22)00495-1

Kruska, N., Schönfeld, P., Pujol, A., & Reiser, G. (2015). Astrocytes and mitochondria from adrenoleukodystrophy protein (ABCD1)-deficient mice reveal that the adrenoleukodystrophy-associated very long-chain fatty acids target several cellular energy-dependent functions. *Biochimica et Biophysica Acta*, *1852*(5), 925–936. https://doi.org/10.1016/j.bbadis.2015.01.005

Lewkowicz, N., Piątek, P., Namiecińska, M., Domowicz, M., Bonikowski, R., Szemraj, J., Przygodzka, P., Stasiołek, M., & Lewkowicz, P. (2019). Naturally occurring nervonic acid ester improves myelin synthesis by human oligodendrocytes. *Cells*, *8*(8), 786. https://doi.org/10.3390/cells8080786

Li, Q., Chen, J., Yu, X., & Gao, J.-M. (2019). A mini review of nervonic acid: Source, production, and biological functions. *Food Chemistry*, *301*, 125286. https://doi.org/10.1016/j.foodchem.2019.125286

Lin, M. T., & Beal, M. F. (2006). Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature*, *443*(7113), 787–795. https://doi.org/10.1038/nature05292

López-Erauskin, J., Galino, J., Bianchi, P., Fourcade, S., Andreu, A. L., Ferrer, I., Muñoz-Pinedo, C., & Pujol, A. (2012). Oxidative stress modulates mitochondrial failure and cyclophilin D function in X-linked adrenoleukodystrophy. *Brain*, *135*(12), 3584–3598. https://doi.org/10.1093/brain/aws292

López-Erauskin, J., Galino, J., Ruiz, M., Cuezva, J. M., Fabregat, I., Cacabelos, D., Boada, J., Martínez, J., Ferrer, I., Pamplona, R., Villarroya, F., Portero-Otín, M., Fourcade, S., & Pujol, A. (2013). Impaired mitochondrial oxidative phosphorylation in the peroxisomal disease X-linked adrenoleukodystrophy. *Human Molecular Genetics*, *22*(16), 3296–3305. https://doi.org/10.1093/hmg/ddt186

Lotfi, K., Salari-Moghaddam, A., Yousefinia, M., Larijani, B., & Esmaillzadeh, A. (2021). Dietary intakes of monounsaturated fatty acids and risk of mortality from all causes, cardiovascular disease and cancer: A systematic review and dose-response meta-analysis of prospective cohort studies. *Ageing Research Reviews*, *72*, 101467. https://doi.org/10.1016/j.arr.2021.101467

Martínez, M., & Mougan, I. (1998). Fatty acid composition of human brain phospholipids during normal development. *Journal of Neurochemistry*, *71*(6), 2528–2533. https://doi.org/10.1046/j.1471-4159.1998.71062528.x

Monternier, P., Singh, J., Parasar, P., Theurey, P., DeWitt, S., Jacques, V., Klett, E., Kaur, N., Nagaraja, T. N., Moller, D. E., & Hallakou-Bozec, S. (2022). Therapeutic potential of deuterium-stabilized (R)-pioglitazone—PXL065—For X-linked adrenoleukodystrophy. *Journal of Inherited Metabolic Disease*, *45*(4), 832–847. https://doi.org/10.1002/jimd.12510

Moser, A. B., Jones, R. O., Hubbard, W. C., Tortorelli, S., Orsini, J. J., Caggana, M., Vogel, B. H., & Raymond, G. V. (2016). Newborn screening for X-linked adrenoleukodystrophy. *International Journal of Neonatal Screening*, *2*(4), 4. https://doi.org/10.3390/ijns2040015

Nisr, R. B., Shah, D. S., & Hundal, H. S. (2020). Mono- and polyunsaturated fatty acids counter palmitate-induced mitochondrial dysfunction in rat skeletal muscle cells. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, *54*(5), 975–993. https://doi.org/10.33594/000000282

O'Neill, H. M., Maarbjerg, S. J., Crane, J. D., et al. (2011). AMP-activated protein kinase (AMPK) beta1beta2 muscle null mice reveal an essential role for AMPK in maintaining mitochondrial content and glucose uptake during exercise. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(38), 16092–16097. https://doi.org/10.1073/pnas.1105062108

Phung, N. V., Rong, F., Xia, W. Y., Fan, Y., Li, X. Y., Wang, S. A., & Li, F. L. (2023). Nervonic acid and its sphingolipids: Biological functions and potential food applications. *Critical Reviews in Food Science and Nutrition*, *64*, 8766–8785. https://doi.org/10.1080/10408398.2023.2203753

Powers, J. M., DeCiero, D. P., Cox, C., Richfield, E. K., Ito, M., Moser, A. B., & Moser, H. W. (2001). The dorsal root ganglia in adrenomyeloneuropathy: Neuronal atrophy and abnormal mitochondria.

Journal of Neuropathology and Experimental Neurology, *60*(5), 493–501. https://doi.org/10.1093/jnen/60.5.493

Powers, J. M., Pei, Z., Heinzer, A. K., Deering, R., Moser, A. B., Moser, H. W., Watkins, P. A., & Smith, K. D. (2005). Adreno-leukodystrophy: Oxidative stress of mice and men. *Journal of Neuropathology & Experimental Neurology*, *64*(12), 1067–1079. https://doi.org/10.1097/01.jnen.0000190064.28559.a4

Ronghe, M. D., Barton, J., Jardine, P. E., Crowne, E. C., Webster, M. H., Armitage, M., Allen, J. T., & Steward, C. G. (2002). The importance of testing for adrenoleucodystrophy in males with idiopathic Addison's disease. *Archives of Disease in Childhood*, *86*(3), 185–189. https://doi.org/10.1136/adc.86.3.185

Santosh Rai, P. V., Suresh, B. V., Bhat, I. G., Sekhar, M., & Chakraborti, S. (2013). Childhood adrenoleukodystrophy – Classic and variant – Review of clinical manifestations and magnetic resonance imaging. *Journal of Pediatric Neurosciences*, *8*(3), 192. https://doi.org/10.4103/1817-1745.123661

Sargent, J. R., Coupland, K., & Wilson, R. (1994). Nervonic acid and demyelinating disease. *Medical Hypotheses*, *42*(4), 237–242. https://doi.org/10.1016/0306-9877(94)90122-8

Senyilmaz, D., Virtue, S., Xu, X., Tan, C. Y., Griffin, J. L., Miller, A. K., Vidal-Puig, A., & Teleman, A. A. (2015). Regulation of mitochondrial morphology and function by stearoylation of TFR1. *Nature*, *525*(7567), 124–128. https://doi.org/10.1038/nature14601

Senyilmaz-Tiebe, D., Pfaff, D. H., Virtue, S., Schwarz, K. V., Fleming, T., Altamura, S., Muckenthaler, M. U., Okun, J. G., Vidal-Puig, A., Nawroth, P., & Teleman, A. A. (2018). Dietary stearic acid regulates mitochondria in vivo in humans. *Nature Communications*, *9*(1), 3129. https://doi.org/10.1038/s41467-018-05614-6

Singh, I., & Pujol, A. (2010). Pathomechanisms underlying X-adrenoleukodystrophy: A three-hit hypothesis. *Brain Pathology*, *20*(4), 838–844. https://doi.org/10.1111/j.1750-3639.2010.00392.x

Singh, J., & Giri, S. (2014). Loss of AMP-activated protein kinase in X-linked adrenoleukodystrophy patient-derived fibroblasts and lymphocytes. *Biochemical and Biophysical Research Communications*, *445*(1), 126–131. https://doi.org/10.1016/j.bbrc.2014.01.126

Sokoła-Wysoczańska, E., Wysoczański, T., Wagner, J., Czyż, K., Bodkowski, R., Lochyński, S., & Patkowska-Sokoła, B. (2018). Polyunsaturated fatty acids and their potential therapeutic role in cardiovascular system disorders—A review. *Nutrients*, *10*(10), 1561. https://doi.org/10.3390/nu10101561

Song, W., Zhang, K., Xue, T., Han, J., Peng, F., Ding, C., Lin, F., Li, J., Sze, F. T. A., Gan, J., & Chen, X. (2022). Cognitive improvement effect of nervonic acid and essential fatty acids on rats ingesting *Acer truncatum* bunge seed oil revealed by lipidomics approach. *Food & Function*, *13*(5), 2475–2490. https://doi.org/10.1039/D1FO03671H

Sullivan, E. M., Pennington, E. R., Green, W. D., Beck, M. A., Brown, D. A., & Shaikh, S. R. (2018). Mechanisms by which dietary fatty acids regulate mitochondrial structure-function in health and disease. *Advances in Nutrition (Bethesda, Md.)*, *9*(3), 247–262. https://doi.org/10.1093/advances/nmy007

Terluk, M. R., Tieu, J., Sahasrabudhe, S. A., Moser, A., Watkins, P. A., Raymond, G. V., & Kartha, R. V. (2022). Nervonic acid attenuates accumulation of very long-chain fatty acids and is a potential therapy for adrenoleukodystrophy. *Neurotherapeutics*, *19*(3), 1007–1017. https://doi.org/10.1007/s13311-022-01226-7

Turk, B. R., Theda, C., Fatemi, A., & Moser, A. B. (2020). X-linked adrenoleukodystrophy: Pathology, pathophysiology, diagnostic testing, newborn screening and therapies. *International Journal of Developmental Neuroscience: The Official Journal of the International Society for Developmental Neuroscience*, *80*(1), 52–72. https://doi.org/10.1002/jdn.10003

Umemoto, H., Yasugi, S., Tsuda, S., Yoda, M., Ishiguro, T., Kaba, N., & Itoh, T. (2021). Protective effect of Nervonic acid against 6-hydroxydopamine-induced oxidative stress in PC-12 cells. *Journal of Oleo Science*, *70*(1), 95–102. https://doi.org/10.5650/jos.ess20262

Van Haren, K. P., Cunanan, K., Awani, A., Gu, M., Peña, D., Chromik, L. C., Považan, M., Rossi, N. C., Goodman, J., Sundaram, V., Winterbottom, J., Raymond, G. V., Cowan, T., Enns, G. M., Waubant, E., Steinman, L., Barker, P. B., Spielman, D., & Fatemi, A. (2023). A phase 1 study of oral vitamin D3 in boys and young men with X-linked adrenoleukodystrophy. *Neurology: Genetics*, *9*(2), e200061. https://doi.org/10.1212/NXG.0000000000200061

Wang, X., Liang, T., Mao, Y., Li, Z., Li, X., Zhu, X., Cao, F., & Zhang, J. (2023). Nervonic acid improves liver inflammation in a mouse model of Parkinson's disease by inhibiting proinflammatory signaling pathways and regulating metabolic pathways. *Phytomedicine*, *117*, 154911. https://doi.org/10.1016/j.phymed.2023.154911

Yu, J., Yuan, T., Zhang, X., Jin, Q., Wei, W., & Wang, X. (2019). Quantification of nervonic acid in human milk in the first 30 days of lactation: Influence of lactation stages and comparison with infant formulae. *Nutrients*, *11*(8), 1892. https://doi.org/10.3390/nu11081892

Yuan, S.-N., Wang, M., Han, J.-L., Feng, C.-Y., Wang, M., Wang, M., Sun, J.-Y., Li, N., Simal-Gandara, J., & Liu, C. (2023). Improved colonic inflammation by nervonic acid *via* inhibition of NF-κB signaling pathway of DSS-induced colitis mice. *Phytomedicine*, *112*, 154702. https://doi.org/10.1016/j.phymed.2023.154702

Zhang, X., Wu, D., & Yin, Z. (2024). Nervonic acid alleviates MPTP-induced Parkinson's disease via MEK/ERK pathway. *Cellular and Molecular Biology (Noisy-le-Grand, France)*, *70*(4), 100–106. https://doi.org/10.14715/cmb/2024.70.4.16

Zhao, J., Wang, X., Huo, Z., Chen, Y., Liu, J., Zhao, Z., Meng, F., Su, Q., Bao, W., Zhang, L., Wen, S., Wang, X., Liu, H., & Zhou, S. (2022). The impact of mitochondrial dysfunction in amyotrophic lateral sclerosis. *Cells*, *11*(13), 2049. https://doi.org/10.3390/cells11132049

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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