Received: 21 September 2023 | Accepted: 19 November 2023

DOI: 10.1002/epi4.12871

Epilepsia Open® Open Access

SHORT RESEARCH ARTICLE

Identification of metabolic biomarkers of chronic vagus nerve stimulation (VNS) in subjects with drug-resistant epilepsy (DRE)

Claudia Manca[1] | **Roberta Coa**[2] | **Elisabetta Murru**[1] | **Gianfranca Carta**[1] | **Giovanni Pinna**[3] | **Roberto Sanfilippo**[4] | **Lorenzo Polizzi**[2] | **Marco Pistis**[5,6,7] | **Paolo Follesa**[8] | **Monica Puligheddu**[2,9] | **Sebastiano Banni**[1]

[1]Department of Biomedical Sciences, Division of Physiology, University of Cagliari, Cagliari, Italy

[2]Center for the Diagnosis and Treatment of Adult Epilepsy, Neurology Unit, AOU Cagliari, Cagliari, Italy

[3]SC Neurosurgery, Neuroscience and Rehabilitation Department, San Michele Hospital, ARNAS G. Brotzu, Cagliari, Italy

[4]SC Vascular Surgery, AOU Cagliari, Cagliari, Italy

[5]Department of Biomedical Sciences, Division of Neuroscience and Clinical Pharmacology, University of Cagliari, Cagliari, Italy

[6]Neuroscience Institute, National Research Council of Italy (CNR), Section of Cagliari, Cagliari, Italy

[7]Clinical Pharmacology Unit, AOU Cagliari, Cagliari, Italy

[8]Department of Life and Environmental Sciences, Section of Neuroscience and Anthropology, University of Cagliari, Cagliari, Italy

[9]Department of Medical Sciences and Public Health, University of Cagliari, Cagliari, Italy

Correspondence
Monica Puligheddu, Center for the Diagnosis and Treatment of Adult Epilepsy, Neurology Unit, AOU Cagliari, Cagliari 09100, Italy.
Email: puligheddu@unica.it

Claudia Manca, Department of Biomedical Sciences, Division of Physiology, University of Cagliari, 09042 Monserrato, Cagliari, Italy.
Email: claumanca@unica.it

Abstract

Neuromodulation by means of vagus nerve stimulation (VNS) therapy, reduces seizure frequency and improves quality of life in subjects with drug-resistant epilepsy (DRE), yet its molecular mechanism remains unclear. This study investigates the impact of chronic VNS on lipid bioactive metabolites and fatty acids (FA) in the plasma and red blood cells of seven subjects with DRE. By measuring expression levels of peroxisome proliferator-activated receptor α (*PPARα*) and sirtuin1 (*SIRT1*) genes—key regulators in energy and lipid metabolism—and lipid profiles before and after various stages of VNS, this study identifies potential mechanisms by which VNS may reduce seizure frequency. Blood samples collected before VNS device implantation, after acute VNS stimulus, and following gradual intensity increments up to therapeutic levels revealed that VNS increases *SIRT1* and *PPARα* expression and erythrocyte concentrations of PPARα ligands. Additionally, we observe reduced de novo lipogenesis biomarkers in erythrocytes, indicating that VNS may influence systemic lipid and energy metabolism. Our findings suggest

Claudia Manca and Roberta Coa are the cofirst authors.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.
© 2023 The Authors. *Epilepsia Open* published by Wiley Periodicals LLC on behalf of International League Against Epilepsy.

that VNS could enhance neuronal function by modulating energy metabolism, thus potentially reducing seizure frequency in subjects with DRE. Future research targeting SIRT1 and PPARα may provide innovative therapeutic strategies for managing DRE.

Plain Language Summary: The exact mechanism of VNS is still unknown. This study investigated the effects of VNS Therapy on energetic metabolism, suggesting possible novel biomarkers for DRE subjects and neuromodulation therapies.

KEYWORDS

bioactive molecules, energy and lipid metabolism, fatty acids, neuromodulation, *PPARα*, *SIRT1*

1 | INTRODUCTION

Vagus nerve stimulation (VNS) is the most widely used neurostimulation therapy approved by Food and Drug Administration since 1997 for the treatment of subjects with drug-resistant epilepsy (DRE) not surgically treatable or refractory to it. Over 25 years of studies have demonstrated the clinical efficacy of VNS in DRE, both in controlling refractory seizures and in stabilizing mood disturbances and cognitive impairment.[1] Furthermore, chronic stimulation induces molecular, cellular, and electrophysiological changes within the vagal afferent network at three main levels (brainstem, limbic system, and cortex).[2] Notwithstanding, some crucial pieces are still missing.

Recent findings have stated a significant association between epilepsy and metabolism, with some researchers defining epilepsy as a "metabolic disease."[3] Uncontrolled seizures would alter major energetic pathways[3,4] and several known metabolic perturbations as mutations are sufficient to initiate acquired and certain genetic epilepsies.[3] Consistent with these epilepsy-related metabolic perturbations, beneficial effects of chronic VNS on energy balance have been demonstrated. Weight loss and an increase in whole-body energy expenditure have been observed in subjects with epilepsy treated with VNS,[5,6] but the effect of this therapy on body weight has remained unclear.

By using an experimental animal model, we first demonstrated that long-term VNS treatment alters the levels of lipid mediators that are part of the endocannabinoidome (eCBome), a complex lipid signaling system that is present in all mammalian tissues and plays important functions in the central nervous system and in the context of metabolic disorders.[6,7] Notably, we observed increased levels of the bioactive mediator *N*-palmitoylethanolamine (PEA), an endogenous ligand of the transcription factor peroxisome proliferator-activated receptor alpha (PPARα) involved in the modulation of energy metabolism both at central and peripheral level.[6,8] Pparα hepatic abundance was also increased following VNS[6]. These results led us hypothesize that in subjects with DRE treated with VNS, a metabolic improvement induced by PPARα activation at a central level as well as the activation of another regulator of energy metabolism, sirtuin1 (SIRT1), may contribute to reducing the frequency of epileptic seizures by enhancing fatty acids (FA) oxidation and ketogenesis, which have been widely shown to ameliorate neuron function.[9,10]

So far, to the best of our knowledge, no study has been conducted in humans to investigate the metabolic-related molecular mechanisms beneath VNS therapy. In this preliminary study, we, therefore, assessed, in seven subjects with DRE treated with chronic VNS, the plasma and red blood cells FA and eCBome-related molecules profiles, as well as leucocyte *PPARα* and *SIRT1* mRNA expression, in order to identify possible circulating metabolic biomarkers of VNS which could represent most likely candidates for innovative therapeutic treatments.

2 | METHODOLOGIES

2.1 | Population and recruitment

Candidates for VNS implantation ($n = 7$, Table 1), were recruited at the Center for Diagnosis and Treatment of Adult Epilepsy—AOU Cagliari (Ethical Committee approval Prot. PG. 2020/2970). Inclusion criteria were: diagnosis of focal or generalized DRE according to ILAE definition,[11] not feasibility of surgical treatment. Exclusion criteria were pregnancy, refusal to sign the informed consent, subjects younger than 18, and psychiatric comorbidities. After receiving an explanation of the study protocol and signing the informed consent form, the selected patients underwent a vagus nerve stimulator implantation Sentiva Model M1000, except for patient #3 (Demipulse 104 Duo).

TABLE 1 Clinical and registry characteristics, and VNS parameters of enrolled patients.

	Anagraphics		Clinical information					VNS parameters					
#	Sex	Age	Diagnosis	Comorbidity	ASM	Seizure f pre-VNS (per month)	Seizure f post-VNS (per month)	Implantation date	Max output current (mA)	Pattern	Frequency P. Width	Magnet parameters	Autostim parameters
1	M	42	Focal E.(T)	—	LCS, CLB, PB, TPM	45	9	11 Sep 2020	2.00	30″ on 5′ off	20 Hz 250 μs	2.25 mA 500 μs 60″	1.875 mA 250 μs 60″
2	F	51	Focal E.(NT)	Peutz-Jeghers S., obesity	CBZ, TPM, PHT	45	25	13 Aug 2020	2.00	30″ on 5′ off	20 Hz 250 μs	2.25 mA 500 μs 60″	2.00 mA 250 μs 60″
3	F	61	Focal E.(NT)	Osteoporosis	OXC, TPM, PB	12	10	17 Dec 2020	2.00	30″ on 5′ off	30 Hz 250 μs	2.25 mA 500 μs 60″	—
4	M	34	Focal E.(NT)	Blindness (sella turcica meningioma)	LCS, BRV, TPM, CLB	60	58	27 Oct 2021	2.00	21″ on 3′ off	20 Hz 250 μs	2.25 mA 250 μs 60″	2.00 mA 250 μs 30″
5	F	31	Generalized E.	—	VPA, CLB, PER	6	4	27 May 2021	2.00	21″ on 3′ off	20 Hz 250 μs	2.5 mA 500 μs 60″	2.25 mA 250 μs 60″
6	M	26	LGS	Cognitive impairment	VPA, CBZ, CLB	75	40	27 Oct 2021	2.00	21″ on 3′ off	20 Hz 250 μs	2.25 mA 250 μs 60″	1.75 mA 250 μs 30″
7	F	42	Focal E.(T)	–	LTG, LCS	30	8	30 Jun 2022	2.00	30″ on 5′ off	20 Hz 250 μs	2.25 mA 250 μs 60”	2.00 mA 250 μs 30″

Note: Preimplantation seizure frequency as number of seizures per month, considering the average of the previous 3 months; postimplantation frequency is calculated on the period between the last sampling at the end of titration and the annual sampling.

Abbreviations: ASM, antiseizure medications; BRV, Brivaracetam, VPA, Valproic Acid; CBZ, Carbamazepine; CLB, Clobazam; LCS, Lacosamide; LGS, Lennox–Gastaut Syndrome; LTG, Lamotrigine; NT, nontemporal; OXC, Oxcarbazepine; PB, Phenobarbital; PER, Perampanel; PHT, Phenytoin; T, temporal; TPM, Topiramate.

2.2 | Vagus nerve stimulator implantation and setup of the following increasing stimulus

Before the implantation, we drew blood samples (T0, baseline). Prior to initiating chronic stimulation, a single stimulus was delivered for impedance checking; 30 min after this acute stimulus, a blood sample was drawn and the stimulator was activated. The output current was gradually increased in increments of 0.25 mA, at intervals of at least 2 weeks, until reaching the therapeutic intensity of 1.5–2 mA, according to subject tolerance; prior to each increment, another blood sample was drawn. Sampling at maximum output was repeated after a period of chronic stimulation (10–14 months after implantation).

2.3 | Blood sampling, mRNA expression, and lipidomic analyses

Venous blood samples from the antecubital vein were collected into anticoagulant-coated vacutainers (K3-EDTA) and processed for gene expression and lipidomic analysis as previously described.[12]

PPARα and *SIRT1* gene expression was evaluated using specific primers (Bio-Rad) on a Rotorgene-Q System (Bio-Rad) using PowerUp SYBR Green qPCR master mix (Thermo Fisher Scientific) in duplicate reactions. TATA box binding protein (*TBP*) was used as housekeeping gene. Gene expression levels were evaluated by the $2^{-\Delta\Delta Ct}$ method.

FA and eCBome-related molecules were analyzed by LC–MS/MS as previously described.[13]

2.4 | Statistical analysis

Data are expressed as percent of baseline (T0) considered 100%. The values obtained from each subject prior to VNS device implantation were set as the T0 and considered 100%. For the subsequent timepoints, each result has been normalized with respect to the corresponding T0.

Statistical analysis was conducted using GraphPad Prism 8.0.1 software (La Jolla, CA, USA). The ROUT method and Shapiro–Wilk normality test were employed to identify and remove any outliers and to assess the normal distribution of the data, respectively. As the data did not follow normal distribution, nonparametric Mann–Whitney tests were performed to evaluate differences between each group and the T0. The statistical analysis performed in this study is univariate in nature, meaning it does not account for the influence of various covariates, including factors such as diagnosis, the impact of antiseizure medications (ASM), age, sex, and seizure frequency before the VNS placement.

Results are presented as mean ± SD, and a significance level of $P \leq 0.05$ was used to determine statistical significance among the groups.

3 | RESULTS

3.1 | Blood FA and eCBome-related mediators' profiles

Main changes in FA profile in red blood cells are reported in Figure 1. No alterations were detected in the plasma FA profile. No significant differences were also found in the eCBome profile in both red blood cells and plasma.

Particularly, there was a gradual increase in FA docosahexaenoic acid (DHA) concentration that reached significance after the stimulus was increased at 2 mA (2mA_1). Importantly, the significant increase was maintained after several months of 2 mA intensity (2mA_2) (Figure 1A). We also noticed a gradual increase in the concentration of the unusual FA conjugated linoleic acid (CLA), which reached significance at 2 mA. However, after 2 mA intensity was maintained for several months, CLA levels displayed a trend toward decrease (Figure 1B).

As for the erythrocyte levels of total saturated FA (SFA), we observed a gradual decrease which was significant at 0.25, 1.5, 1.75, and 2 mA (Figure 1C), with respect to baseline. This decrease was mainly due to two SFA, that is, myristic acid (MA, 14:0) and palmitic acid (PA, 16:0), whose levels were reduced at 1.5 and 2 mA, and at all timepoints with the only exception of 0.5 mA, respectively (Figure 1D,E).

3.2 | Gene expression analysis of genes involved in the regulation of energy metabolism

To verify our hypothesis that VNS treatment in subjects with DRE might contribute to reducing the seizures frequency by inducing the activations of modulators of energy metabolism thus enhancing FA oxidation and ketogenesis, and since DHA and CLA are PPARα ligands,[12] we also evaluated mRNA relative expression levels of the *PPARα* and *SIRT1* in leucocytes. We observed a gradual increase of *PPARα* gene expression which reached significance when the stimulus was set at 2 mA (38% of increase with respect to baseline). Peculiarly, as for CLA, after 2 mA intensity was maintained for several months, *PPARα* relative levels displayed a trend toward decrease (Figure 2A). As for *SIRT1* expression levels, we observed a gradual increase which was significant at 1.25 mA (Figure 2B).

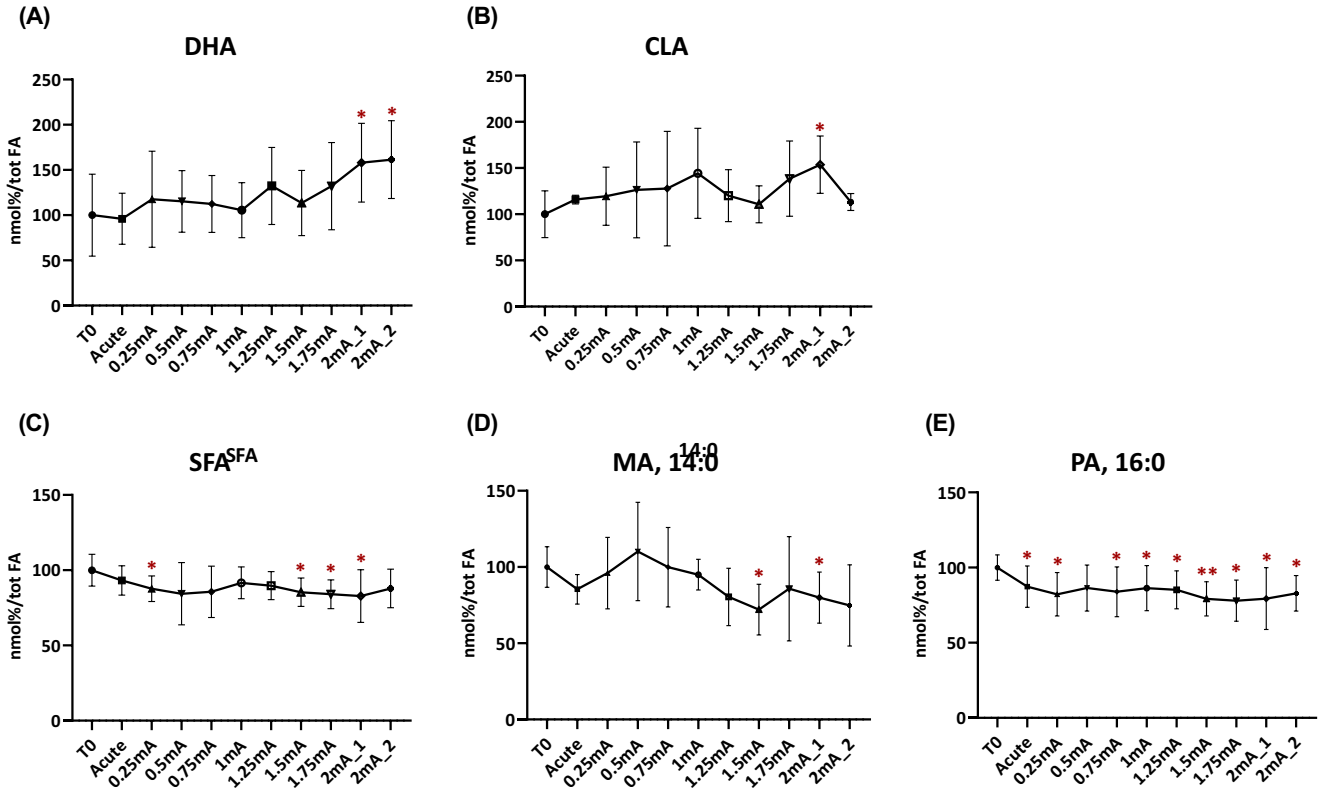

FIGURE 1 Red blood cells levels, expressed as nmol%/total FA, of: (A) docosahexaenoic acid (DHA), (B) conjugated linoleic acid (CLA), (C) total saturated fatty acids (SFA), (D) myristic acid (MA, 14:0) and (E) palmitic acid (PA, 16:0), before VNS device implantation (T0), after acute VNS stimulus (Acute), and following gradual intensity increments (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 mA). 2 mA_1: the stimulus was maintained for 2–3 weeks; 2 mA_2: the stimulus was maintained for several months. Statistical significance between each group and T0 was assessed by nonparametric Mann–Whitney tests. Error bars represent SEM ($n = 7$) *$P < 0.05$; **$P < 0.01$.

4 | DISCUSSION

VNS research focuses predominantly on its potential benefits in mitigating refractory seizures and stabilizing mood and cognitive impairments common among subjects with DRE. However, comprehensive data regarding the molecular mechanism of action and reliable biomarkers in humans is scarce, hindering precise assessment of therapeutic efficacy.

Our previous study conducted in rat models demonstrated that chronic VNS therapy modifies FA and eCBome-related lipid mediator levels, as well as Pparα hepatic abundance.[6] We hypothesized that PPARα, a transcription factor implicated in energy and lipid metabolism regulation,[6] could attenuate epileptic seizure frequency by promoting FA oxidation and ketogenesis, mechanisms known to enhance neuronal function.[10]

In the current investigation, VNS neuromodulation at the highest intensity increased *PPARα* gene expression in leukocytes, and intriguingly, we documented a concomitant gradual increase in erythrocyte DHA concentration. DHA biosynthesis necessitates a peroxisomal β-oxidation step sustained by PPARα,[14] and we have also shown that increased *PPARα* gene expression was associated with DHA biosynthesis in humans.[12] Interestingly, we noted a similar trend for CLA, yet the cause of this increase remains uncertain. The two potential sources of CLA in humans are dietary, primarily dairy products, or endogenously via microbiota.[15,16] The observed dose–response trend rules out the dietary source as a contributor and suggests that VNS may stimulate CLA biosynthesis through microbiota modification.[16] Moreover, as a PPARα ligand, CLA may stimulate PPARα activation, thereby fostering DHA formation. These three molecules may act cooperatively via a positive feedback loop.

Changes in the levels of the three molecules were statistically significant when VNS intensity reached 2 mA. However, unlike DHA, CLA and *PPARα* showed a trend toward a decrease when the 2 mA intensity was sustained over an extended period. This led us to postulate that chronic VNS activation of PPARα may result in an "adaptive response", leading to a decrease in its levels, suggesting that periodic adjustments in therapeutic intensity may be necessary to maintain elevated PPARα levels.

The progressive increase in *PPARα* relative expression, compared to baseline levels, coincided with the

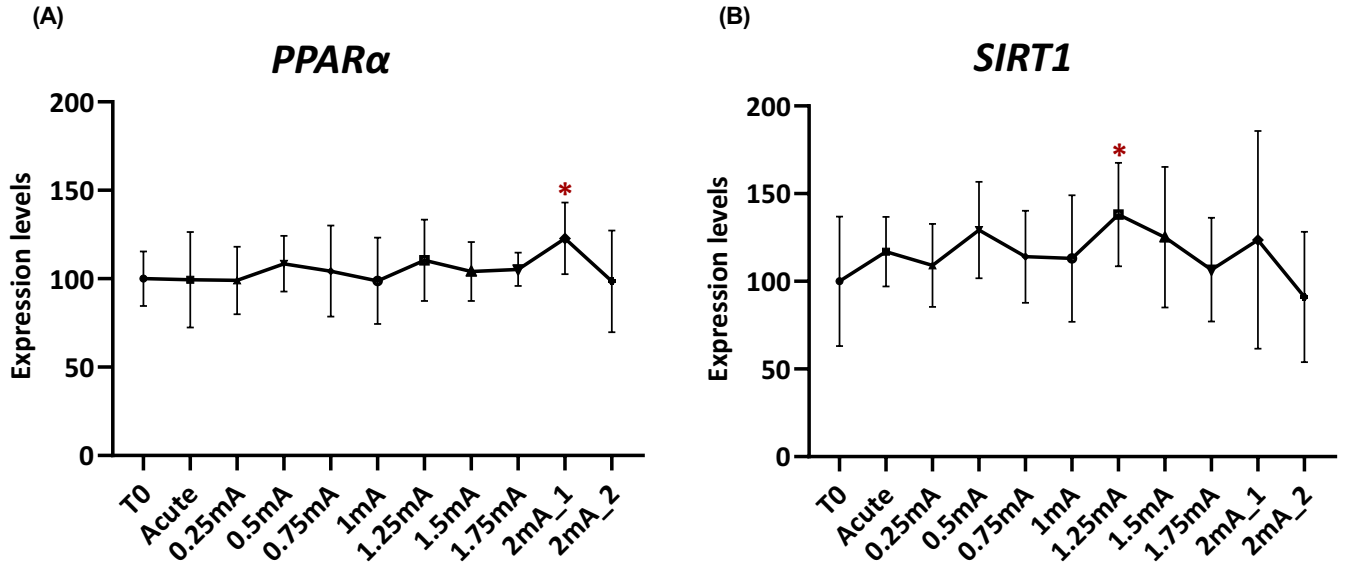

FIGURE 2 Leucocytes mRNA expression levels of key regulators in energy and lipid metabolism: (A) peroxisome proliferator-activated receptor α (*PPARα*) and (B) sirtuin1 (*SIRT1*), before VNS device implantation (T0), after acute VNS stimulus (Acute), and following gradual intensity increments (0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2 mA). 2 mA_1: the stimulus was maintained for 2–3 weeks; 2 mA_2: the stimulus was maintained for several months. Statistical significance between each group and T0 was assessed by nonparametric Mann–Whitney tests. Error bars represent SEM ($n = 7$) *$P < 0.05$.

observed surge in *SIRT1*, a NAD-dependent deacetylase that modulates cellular energy metabolism by deacetylating transcription factors and cofactors involved in energy metabolism.[9] The substantial *SIRT1* level augmentation at 1.25 mA, which preceded the significant *PPARα* level increase at 2 mA, infers that SIRT1 might regulate lipid metabolism via PPARα deacetylation. This unveils a potential mechanism by which SIRT1 and PPARα could synergistically modulate energy metabolism, attenuating seizure frequency in individuals with DRE subjected to VNS.[10]

The VNS-induced *SIRT1* increase could also preserve mitochondrial function and bestow neuroprotective effects through FA metabolism modulation.[17] The concurrent activation of SIRT1 and PPARα might inhibit de novo lipogenesis, a process converting carbohydrates into lipids[18] leading to a decrease in circulating levels of SFA. Indeed, our study detected a significant reduction in SFA levels, specifically MA and PA, in red blood cells post-VNS, suggesting a potential mechanism by which VNS impacts lipid metabolism and modulates FA profiles.

To investigate FA metabolism in humans, we often resort to limited specimen sources, predominantly plasma and red blood cells. While plasma is sensitive to numerous variables such as dietary intake and fasting, and exhibits differential uptake based on varied pathophysiological conditions, red blood cell FA profiles can provide more stable long-term biomarkers for metabolic modifications, as evidenced in our VNS chronic treatment findings.

Our study has unveiled a novel potential mechanism for VNS treatment in subjects with DRE through energy metabolism modulation. Additionally, CLA and the activation of PPARα may have antineuroinflammatory effects, as illustrated in the prevention of neurodegenerative disorders.[19] We acknowledge that the limited number of participants in this study is a notable constraint. To substantiate our findings and to investigate the molecular differences between individuals categorized as "responders" and "nonresponders," it is imperative that future investigations involve larger cohorts of individuals undergoing DRE. Nonetheless, our findings have illuminated previously obscure mechanisms within the enigmatic "VNS black box", providing a roadmap for future research in the identification of promising candidates for innovative molecular treatments for DRE.

AUTHOR CONTRIBUTIONS

C.M: writing—original draft preparation, conceptualization (supporting), investigation, visualization. R.C.—writing—original draft preparation, conceptualization (supporting), resources, visualization. E.M and G.C.—review and editing, investigation. G.P. and R.S.—resources (surgical procedure), review & editing. L.P.—resources, review & editing. M.P. and P.F.—review & editing. M.PU.—conceptualization (lead), review & editing, supervision, resources. S.B.—conceptualization (lead), review & editing, supervision. All authors critically revised the manuscript for important intellectual content and approved the final manuscript. The authors confirm that they we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ACKNOWLEDGMENTS

This work was supported by internal funds through the University of Cagliari.

CONFLICT OF INTEREST STATEMENT

The author Roberta Coa (R.C.), regarding the past 3 years, received support for congress attendance from a distributor of VNS therapy; the author Monica Puligheddu (M.PU.) had a consulting contract with Livanova, USA. The remaining authors have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data are contained within this article. The data presented in the current study are available from the corresponding author upon request: contact Monica Puligheddu (M.PU), Center for the Diagnosis and Treatment of Adult Epilepsy, Neurology Unit, AOU Cagliari, 09100 Cagliari, Italy, ph. +390 706 754 952, puligheddu@unica.it; Claudia Manca (C.M.), Department of Biomedical Sciences, Division of Physiology, University of Cagliari, 09042 Monserrato, Cagliari, Italy, ph. +390 706 754 959, claumanca@unica.it.

ETHICS STATEMENT

The authors confirm that have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

ORCID

Claudia Manca https://orcid.org/0000-0002-4081-9550
Roberta Coa https://orcid.org/0000-0001-9709-9585

REFERENCES

1. Pérez-Carbonell L, Faulkner H, Higgins S, Koutroumanidis M, Leschziner G. Vagus nerve stimulation for drug-resistant epilepsy. Pract Neurol. 2020;20(3):189–98.
2. Carron R, Roncon P, Lagarde S, Dibué M, Zanello M, Bartolomei F. Latest views on the mechanisms of action of surgically implanted cervical vagal nerve stimulation in epilepsy. Neuromodulation. 2023;26(3):498–506.
3. Patel M. A metabolic paradigm for epilepsy. Epilepsy Curr. 2018;18(5):318–22.
4. Murgia F, Muroni A, Puligheddu M, Polizzi L, Barberini L, Orofino G, et al. Metabolomics As a tool for the characterization of drug-resistant epilepsy. Front Neurol. 2017;8:459.
5. Vijgen GHEJ, Bouvy ND, Leenen L, Rijkers K, Cornips E, Majoie M, et al. Vagus nerve stimulation increases energy expenditure: relation to Brown adipose tissue activity. PLoS ONE. 2013;8(10):e77221.
6. Banni S, Carta G, Murru E, Cordeddu L, Giordano E, Marrosu F, et al. Vagus nerve stimulation reduces body weight and fat mass in rats. PLoS ONE. 2012;7(9):e44813.
7. Di Marzo V, Wang J. Endocannabinoidome: the world of endocannabinoids and related mediators. London: Academic Press, an imprint of Elsevier; 2015.
8. Petrosino S, Di Marzo V. The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations: Palmitoylethanolamide and its new formulations. Br J Pharmacol. 2017;174(11):1349–65.
9. Kosgei VJ, Coelho D, Guéant-Rodriguez R-M, Guéant J-L. Sirt1-PPARS cross-talk in complex metabolic diseases and inherited disorders of the one carbon metabolism. Cell. 2020;9(8):1882.
10. D'Andrea Meira I, Romão TT, Pires Do Prado HJ, Krüger LT, Pires MEP, Da Conceição PO. Ketogenic diet and epilepsy: what we know so far. Front Neurosci. 2019;13:5.
11. Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, et al. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. Epilepsia. 2010;51(6):1069–77.
12. Murru E, Carta G, Cordeddu L, Melis M, Desogus E, Ansar H, et al. Dietary conjugated linoleic acid-enriched cheeses influence the levels of circulating n-3 highly unsaturated fatty acids in humans. IJMS. 2018;19(6):1730.
13. Murru E, Lopes PA, Carta G, Manca C, Abolghasemi A, Guil-Guerrero JL, et al. Different dietary N-3 polyunsaturated fatty acid formulations distinctively modify tissue fatty acid and N-acylethanolamine profiles. Nutrients. 2021;13(2):625.
14. Ferdinandusse S, Denis S, Dacremont G, Wanders RJA. Studies on the metabolic fate of n-3 polyunsaturated fatty acids. J Lipid Res. 2003;44(10):1992–7.
15. Benjamin S, Spener F. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutr Metab (Lond). 2009;6(1):36.
16. Druart C, Neyrinck AM, Vlaeminck B, Fievez V, Cani PD, Delzenne NM. Role of the lower and upper intestine in the production and absorption of gut microbiota-derived PUFA metabolites. PLoS ONE. 2014;9(1):e87560.
17. Chuang Y-C, Chen S-D, Jou S-B, Lin T-K, Chen S-F, Chen N-C, et al. Sirtuin 1 regulates mitochondrial biogenesis and provides an endogenous neuroprotective mechanism against seizure-induced neuronal cell death in the hippocampus following status epilepticus. IJMS. 2019;20(14):3588.
18. Carta G, Murru E, Banni S, Manca C. Palmitic acid: physiological role. Metabolism and Nutritional Implications Front Physiol. 2017;8:902.
19. Esmaeili MA, Yadav S, Gupta RKR, Waggoner GR, Deloach A, Calingasan NY, et al. Preferential PPAR-α activation reduces neuroinflammation, and blocks neurodegeneration *in vivo*. Hum Mol Genet. 2016;25(2):317–27.

How to cite this article: Manca C, Coa R, Murru E, Carta G, Pinna G, Sanfilippo R, et al. Identification of metabolic biomarkers of chronic vagus nerve stimulation (VNS) in subjects with drug-resistant epilepsy (DRE). Epilepsia Open. 2024;9:432–438. https://doi.org/10.1002/epi4.12871