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MDPI

Study Protocol

The Supplementation Therapy in Autism and Response to Treatment (START) Study: An Open-Label Feasibility Trial of Ultramicronized Palmitoylethanolamide Potential to Alleviate Psychic Distress among Autistic Adults

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Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental condition characterized by impaired social communication and restricted or repetitive behavior and interests. Psychic distress is common among individuals with ASD, especially in its milder form (level 1), with anxiety and depression being the most common types. Recent research has identified neuroinflammation and gut dysbiosis as potential neurobiological mechanisms underlying ASD. Palmitoylethanolamide (PEA), an endocannabinoid (eCB)-like compound, has shown promise in modulating such mechanisms and may thus have therapeutic implications for ASD. To date, no clinical trial has evaluated the efficacy of PEA in adults with ASD. This 12-week open-label study will assess the feasibility, tolerability, safety, and efficacy of ultramicronized PEA (um-PEA) in treating symptoms of psychic distress, such as anxiety and depression, in adults with level 1 ASD. Secondary research endpoints will include um-PEA's effects on levels of personal autonomy and neurocognitive and interpersonal function. From a biological point of view, this study will assess um-PEA's effects on inflammatory markers, the metabolic profile, eCB system modulation, and microbial composition as potential mechanisms of action for its therapeutic effect. In conclusion, this study will investigate a novel approach to the treatment of adults presenting with psychic distress in the context of level 1 ASD. The results may provide valuable insight into the use of um-PEA as a treatment option for ASD adults, addressing a significant unmet clinical need.

Keywords: Asperger's syndrome; pervasive developmental disorder; mood disorder; cannabidiol; peroxisome proliferator-activated receptor α; glutamate signaling; microbiome

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1. Introduction

1.1. Background

Autism spectrum disorders (ASDs) are a group of complex, multifactorial, early-onset neurodevelopmental conditions that manifest phenotypically with varying degrees of severity [1]. ASD is defined by the presence of core symptoms in two areas—impaired social communication and restricted or repetitive behavior and interests—that allow for diagnosis on a behavioral basis [1,2]. The global prevalence of ASD is approximately 1% [3], and recent estimates have risen to 1.9% in specific subgroups [4,5]. This upward trend primarily involves autistic individuals without comorbid intellectual disability [6] and an extension of the diagnostic focus on adults with ASD [4,7].

Over the past decade, there has been a growing interest in exploring comorbid psychiatric disorders among autistic individuals, which appear to be more common than in the general population [8]. Indeed, it is estimated that up to 70% of people with ASD have at least one comorbid psychiatric disorder from childhood or adolescence [9,10] which persists into adulthood, resulting in impaired quality of life [11], reduced personal autonomy, a greater need for social and healthcare support, and worse general outcomes [10,12]. Psychiatric comorbidities appear to be more prevalent in adults with level 1 ASD, thus often requiring prioritization over ASD's core symptoms. Depression and anxiety are the most common psychiatric comorbidities in individuals with level 1 ASD, and such symptoms may be exacerbated during transitional periods, especially from adolescence to adulthood [12,13]. First-line treatment for ASD with co-occurring depression or anxiety symptoms is controversial, as there is no consensus on whether patients can benefit from interventions aimed at neurotypical individuals [10,14,15]. In this context, evidence on the efficacy, safety, and tolerability of mental health treatments remains scarce, with mixed results for either psychotherapeutic [16,17] or psychopharmacological [16,18] approaches.

Concurrently, accumulating evidence has converged toward aberrant activation of microglia and astrocytes, leading to altered peripheral and brain immune response, increased levels of inflammatory markers, oxidative damage, and glutamate excitotoxicity, as potential neurobiological underpinnings of ASD, paving the way for the investigation of new therapeutical targets [19]. In this regard, the endocannabinoid (eCB) system has attracted increasing interest in recent years, as it exerts a regulatory effect on synapses by acting on glutamatergic afferences with dopaminergic neurons in the central nervous system via cannabinoid type 1 (CB1) receptor agonism, and growing evidence identifies the eCB system as a modulator of neuroinflammation [20,21]. In addition, ASD has been associated with gut dysbiosis (e.g., reduced Bacteroidetes/Firmicutes ratio compared with neurotypical individuals), possibly causing metabolic unbalance, altered gut inflammatory response, and activation of mediators impacting neurological function via the gut-brain axis [19]. It is noteworthy that the endocannabinoidome (eCBome) and the gut microbiome seem to play a role in the pathophysiology of neuroinflammatory diseases and ASDs by mutually affecting one another [22–24].

Palmitoylethanolamide (PEA) is a saturated fatty acid N-acylethanolamine (NAE) originally found in egg yolk, peanut oil, and soybean and later in human tissues and fluids. It is referred to as an eCB-like compound due to its structural features shared with the major eCB anandamide (AEA). PEA is a well-known autacoid due to its ability to regulate the activity of mast cells, microglia, and astrocytes across the peripheral and central neuroimmune systems via multiple and synergistic mechanisms [25]. Primarily, it directly activates peroxisome proliferator-activated receptor alpha (PPAR-α) and G protein-coupled receptor 55 (GPR55), and it allosterically modulates transient receptor potential vanilloid 1 (TRPV-1). Also, PEA inhibits the expression of fatty acid amide hydrolase (FAAH) and enhances the activity of N-acylethanolamine acid amide hydrolase (NAAA), thus increasing AEA and 2-arachidonoylglycerol (2-AG) levels and potentiating their effects at CB1, the cannabinoid type 2 (CB2) receptor, and TRPV-1 [25–27]. PEA is over-produced by cells and tissues in response to actual or potential damage circumstances to restore homeostasis in illness conditions. Whenever an inflammatory response lingers or intensifies, PEA may become

depleted, thus justifying the rationale for its external supplementation [25]. According to a partly similar mechanism of action, PEA is considered the endogenous counterpart of cannabidiol (CBD) [25,26,28–30], albeit showing a safer profile and a closer biological proximity to its therapeutic targets. To this extent, in 2017, PEA was classified by the European Commission as a dietary supplement, being safe and effective for inflammatory disorders, neuropathic pain, and epilepsy management [25,31–33]. In particular, ultramicronized (um)- and micronized (m)- PEA forms are characterized by increased dissolution, absorption, and bioavailability and hence better penetration of the nervous system, exhibiting more marked neuroprotective effects [25] with possible therapeutic implications for neuropsychiatric conditions, including affective disorders [34,35], psychosis [36,37], and cognitive decline [38,39]. Findings regarding the biobehavioral role of PEA among autistic children and adolescents pointed to the potential of PEA supplementation as both monotherapy and an add-on to treatment as usual (TAU) in improving autism core symptoms and modulating the inflammatory response [40–43]. Also, PEA and other eCB or NAE blood levels in individuals with ASD are lower than those in in neurotypical subjects, independent of their sociodemographic and clinical characteristics [40,44]. Finally, PEA supplementation in different rodent models of autism has been shown to improve social and nonsocial behaviors, also possibly through the modulation of microbiota diversity and activity as well as several other neurobiological processes [24,40]. Though this research field is expanding, to date, no clinical trials have assessed the efficacy and tolerability of PEA monotherapy in an adult population with ASD. Also, to our knowledge, no trials have systematically addressed the effects of PEA supplementation on inflammatory response, the eCBome, or microbiome modulation among autistic individuals, despite these systems appearing to be altered early in such a condition as well as closely intertwined to the biobehavioral role of PEA in ASD [40,45].

1.2. Objectives

Our study aims to evaluate (1) the feasibility of identifying and gaining consent from adult level 1 ASD volunteers into a trial with um-PEA; (2) the effect and safety of um-PEA treatment for symptoms of psychic distress (e.g., subtle depressive or anxiety symptoms) among adult level 1 ASD volunteers; and (3) the biological basis of um-PEA's effect in the study population.

2. Methods

2.1. Trial Design

This is a 12-week, open-label, investigator-initiated, proof-of-concept, single-arm study (phase 2 pilot study) assessing the effect of 600 mg/day of um-PEA on the symptoms of psychic distress in adults with level 1 ASD. Within the first 12 months from the beginning of the study project, we will assess the feasibility of enrolling a minimum of 20 participants and whether at least 80% of the participants will have completed the 12-week follow-up. After completing the initial 12-week phase, the participants will be invited to take part in a 24-week extension phase of the study to evaluate the clinical stability of the treatment. During this phase, um-PEA may be titrated up to 1200 mg/day, based on clinical judgement and participant consent. Each participant will undergo um-PEA treatment for a maximum of 36 weeks. The study's flowchart is reported in Table 1.

This trial was approved by the Department of Medicine (DMED) at the University of Udine (Institutional Review Board: 188/2023) in September 2023 (https://osf.io/mhyg4, accessed on 18 May 2024) and subsequently registered at Clinicaltrials.gov with identification code NCT06187090.

Table 1. Study flowchart.

Procedures	Screening Phase	Feasibility Phase			Extension Phase	
	Screening (Day 7)	**Baseline (Day 0)**	**T1 (4 Weeks ± 7 Days)**	**T2 (12 Weeks ± 14 Days)**	**T3 (24 Weeks ± 14 Days)**	**T4 (36 Weeks ± 14 Days)**
Informed consent	X					
Medical history	X					
Physical examination	X	X		X		X
Electrocardiogram	X					
Urinalysis	X	X		X		X
WAIS-IV [46], RAADS-R [47], AQ [48], EQ [49], CAT-Q [50], ENB-2 battery [51], SCID-CV [52], SCID-PD [53], MMPI [54]	X					
Pregnancy test	X	X	X	X	X	
Hematology, biochemistry	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X
Exclusion criteria	X	X				
UKU-SERS [55]		X	X	X	X	X
Um-PEA dispensing		X	X	X	X	
Compliance assessment			X	X	X	X
SCL-90 [56], HADS [57], WHODAS 2.0 (36 items, interviewer-administered) [58]		X		X		X
Inflammation biomarkers, SERS, eCBome, microbiome assessments (blood and stool)		X	X	X	X	X

T1 = Timepoint 1; T2 = Timepoint 2; T3 = Timepoint 3; T4 = Timepoint 4; WAIS-IV = Wechsler Adult Intelligence Scale—Fourth Edition; RAADS-R = Ritvo Autism Asperger Diagnostic Scale-Revised; AQ = autism quotient; EQ = empathy quotient; CAT-Q = Camouflaging Autistic Traits Questionnaire; ENB = Esame Neuropsicologico Breve (Brief Neuropsychological Examination); SCID-CV = Structured Clinical Interview for DSM-5 Disorders, Clinician Version; SCID-PD = Structured Clinical Interview for DSM-5 Disorders, Personality Disorders Version; MMPI = Minnesota Multiphasic Personality Inventory; UKU-SERS = UKU Side Effect Rating Scale; Um-PEA = ultramicronized palmitoylethanolamide; SCL-90 = Symptom Checklist-90; HADS = Hospital Anxiety and Depression Scale; WHODAS 2.0 = World Health Organization Disability Assessment Schedule 2.0; SERS = surface-enhanced Raman scattering; eCBome = endocannabinoidome.

2.2. Participants

2.2.1. Settings and Recruitment

The trial will take place at the Unit of Psychiatry of the University Hospital of Udine, a clinical and research facility in Italy. Help-seeking individuals presenting upon self-referral, referral from general practitioners (GPs), or other mental healthcare professionals from the Udine catchment area and identified by the clinical team as individuals with level 1 ASD, according to the Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition (DSM-5), will be considered for enrolment according to inclusion and exclusion criteria. Eligible patients expressing interest in participating in the study will be approached by trained investigators, introduced to the trial, and provided with a comprehensive patient information sheet (PIS) and consent form (https://osf.io/vukht, accessed on 18 May 2024). Qualified physicians will then discuss the trial with the patients based on the information in the PIS, allowing sufficient time for participants to understand the details. Written informed consent will be obtained from those who wish to participate in the trial.

Those who agree to take part in the study will be invited to a screening visit. During the screening visit, sociodemographic and clinical information for each participant will be recorded in the study case report forms (CRFs; https://osf.io/csdqf, accessed on 18 May 2024). In addition, a physical examination, electrocardiogram, urinalysis, pregnancy test,

and blood tests will be performed to ascertain the good health condition of each participant before starting um-PEA treatment.

The autism baseline characteristics will be defined using the Wechsler Adult Intelligence Scale—Fourth Edition (WAIS-IV) [46], the Ritvo Autism Asperger Diagnostic Scale-Revised (RAADS-R) [47], the Autism Quotient (AQ) [48], the Empathy Quotient (EQ) [49], and the Camouflaging Autistic Traits Questionnaire (CAT-Q) [50]. Further baseline evaluations to assess comorbid neuropsychiatric conditions will be carried out with the Esame Neuropsicologico Breve (ENB, or Brief Neuropsychological Examination)-2 [51], the Structured Clinical Interview for DSM-5 Disorders, Clinician Version (SCID-CV) [52], the Structured Clinical Interview for DSM-5 Disorders, Personality Disorders Version (SCID-PD) [53], and the Minnesota Multiphasic Personality Inventory (MMPI) [54]. The collection of routine blood samples, urine samples, fecal samples, and genotyping will be specifically addressed through the collection of ad hoc consent for biological materials (https://osf.io/vukht, accessed on 18 May 2024).

2.2.2. Eligibility Criteria

The inclusion criteria for the study will be as follows: (1) diagnosis of level 1 ASD according to the DSM-5 criteria [1], where ASD can either be diagnosed for the first time by the medical team at the research site or diagnosed previously by other clinicians; (In the latter case, researchers will confirm the diagnosis during the screening visit.) (2) being 18–35 years of age; (3) the ability to speak and understand Italian; and (4) the ability to give informed consent. Patients will be excluded if (1) presenting with a diagnosis of level 2 or 3 ASD according to the DSM-5 [1]; (2) presenting with active suicidal ideation at the time of the screening visit or previous major suicide attempt(s); (3) presenting with ongoing acute psychiatric symptoms requiring psychopharmacological approach at the time of the screening visit; (4) reporting lifetime neurological disorders (e.g., epilepsy, except for febrile convulsions) or severe intercurrent physical illnesses; (5) being treated with psychotropic medication(s); (6) having an intellectual disability (intelligence quotient (IQ) < 70]; (7) women who are pregnant, breastfeeding, or who may become pregnant and are not using effective contraception; or (8) taking part in another pharmacological clinical trial.

2.3. Intervention

2.3.1. Study Medication and Drug Accountability

Participants will undergo 12 weeks of daily treatment with oral um-PEA (600 mg, tablet form, Normast®) taken near mealtimes. During the optional 24-week extension phase of the study, the medication will be taken from once daily up to twice daily (600–1200 mg per day) based on clinical judgment of the improvement achieved to date. Um-PEA will be obtained from a pharmaceutical company operating under good manufacturing practice (GMP) conditions [59] with appropriate certification. The information on the labels of um-PEA will comply with applicable national and local regulations.

Um-PEA can be purchased from pharmacies as a dietary supplement without a doctor's prescription [30]. While unknown risks cannot be excluded, serious adverse events, including overdoses, have not been documented [25,30]. Um-PEA will be stored at room temperature (<25 °C) in a secure area away from other treatments and clearly marked for this study.

Only qualified physicians clearly designated by the principal investigator (PI) can obtain, prescribe, and dispense the study medication supplied for this trial. Full accountability records will be completed, including the batch, expiry date, persons dispensing or checking the prescription, quantity, date of drug returns, and empty packaging. Nothing will be destroyed without the approval of the PI.

2.3.2. Withdrawal of Subjects

According to the Declaration of Helsinki [60,61], participants can withdraw from the study at any time without providing any reason, and this will not affect their future medical care. This will be explained before consent is obtained. Withdrawal may result in discontinuation of treatment but continuation of follow-up visits. Complete withdrawal will be respected, and although efforts will be made to understand the reason for it, participants are not obliged to give explanations. Already-collected data will still be used in the final analysis. Investigators may also withdraw participants for reasons such as protocol violations, illness, adverse events, or if participation affects their wellbeing. Withdrawn participants will continue follow-up assessments until the end of the 12-week period or until they develop a psychiatric disorder. Those who progress to a full psychiatric disorder will be classified as treatment failures and evaluated only for safety outcomes until the end of the follow-up period.

2.4. Outcomes

2.4.1. Feasibility Endpoints

We will document the count of participants consenting to take part in the trial and the percentage of participants who complete the initial 12-week follow-up.

Participants will be considered compliant if their pill count exceeds 50% of the expected number taken. Those failing to comply with medication will be classified as protocol deviators.

2.4.2. Research Endpoints

We will primarily address whether um-PEA improves symptoms of psychic distress related to autism using the Global Score Index (GSI) of the Symptom Checklist-90 (SCL-90) [56]. Secondary research endpoints will be (1) the effects of um-PEA on anxiety and depressive symptoms associated with autism, as measured with the Hospital Anxiety and Depression Scale (HADS) [57] and the somatization, anxiety, and depression subscales of the SCL-90 [56], (2) the effects of um-PEA on the levels of personal autonomy, as measured with the total score of the World Health Organization Disability Assessment Schedule 2.0 (WHODAS 2.0; 36 items, interviewer-administered) [58], and (3) the effects of um-PEA on neurocognitive and interpersonal functioning, as measured with the cognition and getting along domains of the WHODAS 2.0 (36 items, interviewer-administered) [58].

2.4.3. Safety Endpoints

We will assess the tolerability of sustained um-PEA treatment over at least a 12-week duration, aiming for minimal side effects. The occurrence of adverse effects throughout the study will be quantified utilizing the UKU Side Effect Rating Scale [55].

2.4.4. Biological Measures

Blood analyses, including hematological (e.g., full blood count and hemoglobin), biochemical (e.g., urea and electrolytes, liver function tests, and lipid profiles), and inflammatory profile assessments will be conducted at the clinical research recruitment hub following their established protocols. Following collection at the research recruitment hub, blood samples acquired for hematology, biochemistry, and inflammatory marker assessment will be transported by the study research team to the local laboratory for analysis. Serum samples will be collected to perform "metabolic fingerprinting" analysis using surface-enhanced Raman scattering (SERS) spectroscopy, a vibrational technique based on the inelastic scattering effect of light from a sample placed on a nanostructured metal surface (the SERS substrate) and illuminated with a low-power laser. SERS spectra can be easily obtained from solutions and offer information regarding the chemical composition of a sample [62]. Blood and fecal samples will also be gathered to measure the eCBome mediators' levels using liquid chromatography-mass spectrometry (LC/MS) techniques [23,63] and the microbiome mediators' levels using next-generation sequencing (NGS) of 16S rRNA and shotgun metagenomics methods [23]. Blood and fecal samples gathered for

"metabolic fingerprinting", evaluation of the eCBome mediators' levels, and determination of microbial composition will be stored in local facilities under appropriate conditions (−80 °C) before being dispatched to specialized counseling centers for further analysis.

2.5. Sample Size Calculation

Aiming for a power rating of 80% and statistical significance of 5%, one-way within-subject analysis of variance with three timepoints (i.e., baseline and two follow-ups for the feasibility phase) requires 16 observations for a large-sized effect (i.e., partial-η2 $\geq$ 0.140) in the case of a small-to-medium correlation between measures over time (r = $\pm$0.300), which decreases further for higher stability of measures. This could be sufficient to detect clinically significant effects on the main outcome, with this being a feasibility study. The proposed sample size of 20 participants also allows us to accept a high rate of dropouts (up to 20%). When, hopefully, the sample will be preserved, a correction for multiple independent comparisons can be used to evaluate the secondary outcomes (i.e., three aspects of interest, with a corrected α of 0.016), with a required sample size of 18 [64].

2.6. Statistical Methods

2.6.1. Data Verification, Statistical Monitoring, and Analysis

At the start of the trial, a structured data verification plan will be developed and agreed upon by the research team's chief statistician (M.G.). The quality of the data will be routinely checked in terms of consistency across paper- and electronic-based entry systems. Statistical monitoring will include the patient severity level, eventual withdrawals, baseline data, FUP visit data, and adverse effects. The research team will approve a statistical analysis plan within the early stages of the study and before the head statistician summarizes any data (i.e., with univariate and repeated measures analysis, including preliminary assessment and management of any outliers and assessment of the metric qualities of measurements, such as normality and sphericity, where necessary). Proportions will be presented for each feasibility outcome. Interim analyses are not planned. The head statistician will both carry out and interpret statistical analysis. The main outcome for the feasibility phase will be analyzed with one-way within-subjects analysis of variance for time effects on the GSI scale of the SCL-90, confirming results with Friedman's test in case of assumption violations. In case problematic outliers are observed (i.e., extreme outliers below 3 interquartile ranges from the 25th percentile or above 3 from the 75th percentile), the analyses will be checked using robust estimation of confidence intervals in the main univariate analyses (e.g., possibly using the non-parametric bootstrapping method). To reduce issues related to violation of the assumption of sphericity, an appropriate correction will be used (i.e., following evaluation by Mauchly's method, the Greenhouse–Geisser's correction will preferably be adopted). Linear planned comparisons will be preferred to evaluate differences between the baseline and follow-ups and between the first and second follow-up (i.e., orthogonal contrasts: +2, −1, −1; 0, +1, and −1). Also, the moderation effects of a possible confounder will be analyzed (e.g., introducing in the model general sociodemographic or ASD-associated clinical measures). A similar approach will be used for secondary outcomes (i.e., anxiety or depression scales from HADS and SCL-90, total WHODAS 2.0 score, and cognitive and social WHODAS 2.0 scores). For such more specific aspects, a multivariate approach will be used when possible to evaluate the overall effects on emotional problems or functioning. For the 24-week extension phase, the analyses will be supplemented with the introduction of a between-subjects predictor to compare the group of those who will continue the assumption of PEA with those willing to discontinue PEA and continue with TAU. Possible adverse effects will be described, reporting frequencies measured with the UKU scale. When there are drop-outs, these will be detailed and their reasons described as much as possible.

2.6.2. Missing Data

Analyses will be conducted on all participants, with at least the baseline assessment and one follow-up in the feasibility phase. Possible missing data will be treated by comparing the results with list-wise selection with those obtained after imputation (i.e., as appropriate, substitution with a moving average, a random forest algorithm, or the multiple imputation method). We consider the occurrence of withdrawal of consent rare, and it will be dealt with on an ad hoc basis if it occurs. In the case of dropouts, the reasons for it will be asked of from the participant whenever feasible.

2.7. Data Management and Confidentiality

Study-related information will be stored securely at the study site. All participants' information will be stored in locked filing cabinets in areas with limited access. All laboratory samples, reports, and data collection, procedural, and administrative forms will be identified with a coded identification (ID) number to ensure participant confidentiality, and they will be stored separately from records containing names or other personal identifiers. Local databases will be backed by password-protected access systems. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers with other identifying information will be kept in a separate locked file in an area with limited access. Participants' study information will not be released without the written consent of the participants.

3. Discussion

Psychiatric comorbidity is a significant burden for people with ASD and a major challenge for clinicians, as available treatments aimed at neurotypical individuals showed mixed results in this population [10–13,16–18]. A promising area of research in this field involves the role of the eCB system in modulating neuroinflammation and regulating glutamate and dopamine neurotransmission [19–21].

We presented a proof-of-concept phase 2 study proposing the use of a nutraceutical, namely the fatty acid amide PEA in its ultramicronized form [25], to reduce symptoms of psychic distress in adults with level 1 ASD. Patients will receive treatment for 12 weeks, with an optional 24-week extension phase, and they will be asked to undergo clinical and biological monitoring at three different timepoints (plus two during the extension phase). Psychiatric symptoms will be assessed using gold-standard psychometric tools, with a focus on anxiety and depression, and their impact on functioning will also be explored.

As already mentioned, previous research in the field mainly explored the potential of PEA treatment to halt ASD core symptoms among autistic children and adolescents with limited evidence regarding its biological effects [41–43], using similar but not overlapping methodologies in terms of study design (randomized controlled trial [41] and case report or series [42,43]), PEA formulation (native PEA [41], um-PEA [42], and co-ultramicronized-PEA-Luteolin [43]), PEA dosage (600 mg/bid [41,42], 300 mg/bid [42], 600 mg/day [42], and 700 mg/bid [43]), PEA use as an add-on to TAU [41] or as monotherapy [42,43], and treatment duration (10 weeks [41], 4 weeks [42], 12 weeks [42], and 12–14 months [43]).

This is the first clinical trial designed to evaluate the efficacy and safety of um-PEA monotherapy in adults with ASD. The study offers an extended treatment option for help-seeking individuals who may not benefit from TAU and are often concerned regarding the adverse effects associated with psychotropic medications. Indeed, um-PEA is a well-established supplementary food for long-term therapy in several clinical conditions [32,65–67], having almost no known or potential adverse effects [25,30] and well-documented safety and tolerability at doses of 300–1200 mg per day, with negligible acute and repeated dose toxicity [25,30]. Interestingly, PEA treatment has also been proven to provide relief to several other physical and neuropsychiatric unpleasant conditions which often affect the health status of autistic individuals (e.g., irritable bowel disease, dentary problems, headaches, and sleep problems) [32,66,68–73]. Additionally, blood and stool samples will be obtained at each timepoint for analysis of the inflammatory response, metabolic

profile, eCB system, and microbial composition. This comprehensive approach aims to provide a deeper insight into the biological mechanisms behind the effects of um-PEA, facilitating a better understanding of its potential not only as a symptomatic treatment for autism but also as a disease-modifying agent.

Finally, the feasibility of this proof-of-concept study may justify its continuation as a larger phase 3 study to investigate the efficacy, tolerability, and acceptability of um-PEA in adults with ASD.

One drawback of this study arises from its open-label design, highlighting the need for randomization and blinding in subsequent investigations to minimize potential bias. However, open-label trials are appropriate for early-stage trials to show safety and explore within-subject changes due to treatment, especially when the molecule is marketed for another disease, the condition of interest presents with significant symptom heterogeneity, and it would be unethical to have patients on a placebo in the absence of any valid therapeutic option [74]. Furthermore, given the single-center nature of this study, caution should be exercised in generalizing the results to the broader population. Future studies involving more rigorous methodologies may also be extended to a wider age spectrum, thereby providing a comprehensive representation of the whole adult autistic community. Also, it may be of interest to investigate um-PEA efficacy in addressing ASD's core symptoms and autism-related gastrointestinal issues among autistic adults, considering the established effectiveness of different PEA formulations in managing such disturbances in other patient cohorts [41,66,75].

In conclusion, this open-label, single-arm clinical trial may help to break new ground in the treatment of psychiatric comorbidities associated with ASD, which still represent an important unmet clinical need.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The trial has been registered at Clinicaltrials.gov with identification code NCT06187090. The patient information sheet and consent form are openly available at https://osf.io/vukht (accessed on 18 May 2024). The study case report form (CRF) is openly available at https://osf.io/csdqf (accessed on 18 May 2024).

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