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Ultramicronized *N*-palmitoylethanolamine associated with analgesics: Effects against persistent pain

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ABSTRACT

Current epidemiological data estimate that one in five people suffers from chronic pain with considerable impairment of health-related quality of life. The pharmacological treatment is based on first- and second-line analgesic drugs, including COX-2 selective and nonselective nonsteroidal anti-inflammatory drugs, paracetamol, antidepressants, anti-seizure drugs and opioids, that are characterized by important side effects.

N-palmitoylethanolamine (PEA) is a body's own fatty-acid ethanolamide belonging to the family of autacoid local injury antagonist amides. The anti-inflammatory and pain-relieving properties of PEA have been recognized for decades and prompted to depict its role in the endogenous mechanisms of pain control. Together with its relative abundance in food sources, this opened the way to the use of PEA as a pain-relieving nutritional intervention.

Naïve PEA is a large particle size lipid molecule with low solubility and bioavailability. Reducing particle size is a useful method to increase surface area, thereby improving dissolution rate and bioavailability accordingly. Micron-size formulations of PEA (e.g., ultramicronized and co-(ultra)micronized) have shown higher oral efficacy compared to naïve PEA. In particular, ultramicronized PEA has been shown to efficiently cross the intestinal wall and, more importantly, the blood-brain and blood-spinal cord barrier. Several preclinical and clinical studies have shown the efficacy, safety and tolerability of ultramicronized PEA.

This narrative review summarizes the available pharmacokinetic/pharmacodynamic data on ultramicronized PEA and focuses to its contribution to pain control, in particular as 'add-on' nutritional intervention. Data showing the ability of ultramicronized PEA to limit opioid side effects, including the development of tolerance, have also been reviewed.

Abbreviations: 2-AG, 2-arachidonoyl-glycerol; ABHD4, α/β-hydrolase domain-containing protein 4; AEA, anandamide; ALIA, autacoid local inflammation antagonist; ALIAmides, autacoid local injury antagonist amides; c-Fos, Fos proto-oncogene; AP-1, AP-1 transcription factor subunit (official symbol JUN); CAR, carrageenin; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; CCI, chronic constriction injury; CCL2, C-C motif chemokine ligand 2; CCRCT, Cochrane Central Register of Controlled Trials; CD30, cluster of differentiation 30 (official symbol: TNFRSF8); C_{max}, maximum concentration; CMC, carboxymethylcellulose; COX-2, cyclooxygenase 2 (official symbol: MT-CO2); COX-2/PGE2, COX-2-dependent prostaglandin E2; DMSO, dimethyl sulfoxide; EAAT2, excitatory amino acid transporter (official symbol: SLC1A2); EC_{50}, half-maximal effective concentration; EQ-5D, EuroQol five-dimensions; FAAH-2, fatty acid amide hydrolase-2; FAAH, fatty acid amide hydrolase; FAE, fatty acid ethanolamide; GD2, disialoganglioside; GDE, glycerophosphodiester phosphodiesterase; GDE1, glycerophosphodiester phosphodiesterase 1; GDE4, glycerophosphodiester phosphodiesterase domain containing 1 (official symbol: GDPD1); GDE7, glycerophosphodiester phosphodiesterase domain containing 3 (official symbol: GDPD3); GFAP, glial fibrillary acidic protein; GP-NAE, glycerophospho-*N*-palmitoyl ethanolamine; GPR119, G protein-coupled receptor 119; GPR55, G protein-coupled receptor 55; IASP, International Association for the Study of Pain; Iba-1, ionized calcium binding adaptor molecule 1; ICD, International Classification of Diseases; IL-1β, interleukin 1 beta; IL-6, interleukin 6; iNOS, nitric oxide synthase 2 (official symbol NOS2); IκB-α, Nfkb inhibitor alpha (official symbol Nfkbia); i.p., intraperitoneal; L4/L6, spinal cord L4/L6; lysoNAPE, lysophosphatidylethanolamine; N-acyl-lysoPE, N-acyl-lysophosphatidylethanolamine; LEA, linoleoylethanolamide; MnSOD, manganese superoxide dismutase; NAAA, *N*-acylethanolamine acid amidase; NAPE-PLD, *N*-acyl-phosphatidyl-ethanolamine phospholipase D; NF-κB p65, v-rel reticuloendotheliosis viral oncogene homolog A (avian) (official symbol: Rela); NF-κB, nuclear factor kappa B subunit 1 (official symbol: NF-κB1); NGF, nerve growth factor; NHS-HRA, National Health System Health Research Authority; NNT, number needed to treat; NPSI, Neuropatic Pain Symptom Inventory; NRS, Numeric Rating Scale; NSAIDs, nonsteroidal anti-inflammatory drugs; p.o., per os; OEA, oleoylethanolamide; PEA, *N*-palmitoylethanolamine; PPAR, peroxisome proliferator-activated receptor; PPARα, peroxisome proliferator-activated receptor alpha (official symbol: PPARA); PPARγ, peroxisome proliferator-activated receptor gamma (official symbol: PPARG); PPARδ, peroxisome proliferator-activated receptor delta (official symbol: PPARD); PTPN22, protein tyrosine phosphatase non-receptor type 22; QoL, quality of life; RBL-2H3, basophilic leukemia cell line (*Rattus norvegicus*); s.c., subcutaneous; SEA, stearoylethanolamide; Serpina3n, serine (or cysteine) peptidase inhibitor, clade A, member 3 N; SHIP1, inositol polyphosphate-5-phosphatase D (official symbol: INPP5D); SNRIs, serotonin and norepinephrine reuptake inhibitors; $sPLA_2$, secretory phospholipase A2; TNF-α, tumor necrosis factor (official symbol: TNF); TRPV1, Transient Receptor Potential cation channel subfamily V member 1; UK, United Kingdom; umPEA, ultramicronized PEA; VAS, Visual Analogue Scale; WHO, World Health Organization.

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Contents

1. Introduction

According to the International Association for the Study of Pain (IASP), chronic pain is defined as pain that persists or recurs for >3 months (Treede et al., 2019). Overall, the prevalence of chronic pain is estimated to be about 1 in 5 people (Breivik, Collett, Ventafridda, Cohen, & Gallacher, 2006).

The associated disease burden represented by functional impairment and disability (Breivik et al., 2006; Froud et al., 2014), emotional distress (Häuser, Schmutzer, Henningsen, & Brähler, 2014; Wang, Pu, Ghose, & Tang, 2018) and direct and indirect societal costs (Häuser, Marschall, L'Hoest, Komossa, & Henningsen, 2013; James et al., 2018), relevantly impacts on patients and society. The WHO International Classification of Diseases (ICD)-11 implemented the classification of chronic pain (developed by an international task force of IASP) in chronic primary pain which is conceived as a disease itself and is associated with significant emotional distress or functional disability, and chronic secondary pain which is regarded as a symptom of an underlying disease, like cancer-related pain, chronic postsurgical or post-traumatic pain, chronic secondary musculoskeletal pain, chronic secondary visceral pain, chronic neuropathic pain, chronic secondary headache or orofacial pain (Table 1) (ICD-11 WHO, 2019/2021; Treede et al., 2019). More recently, a third category of pain, i.e., nociplastic pain, has been introduced. It is different from nociceptive and neuropathic pain being represented by a chronic non-specific pain related to a central nervous system sensitization (Fitzcharles et al., 2021). The altered nociception occurs, in fact, without clear evidence of tissue or nerve damage (IASP, 2023).

Standard treatment of chronic pain is represented by a wide series of analgesic drugs, including COX-2 selective and nonselective nonsteroidal anti-inflammatory drugs (NSAIDs), paracetamol (also known as acetaminophen), antidepressants, anti-seizure agents and opioids. NSAIDs are commonly used to treat mild-to-moderate pain mainly due to arthritis and muscle sprains, with COX-2 inhibitors being preferred due to their highly selective targeting and safer toxicological profile. Paracetamol represents the first-line treatment for mild-to-moderate pain, although it is sometimes indicated in combination with opioids to reduce their dosage. Antidepressants (i.e., tricyclic antidepressants and serotonin and norepinephrine reuptake inhibitors, SNRIs) are mainly used to treat neuropathic pain, chronic headaches, fibromyalgia, while anti-seizure agents, including gabapentin and pregabalin, are preferred for postherpetic neuralgia, diabetic neuropathy, and fibromyalgia (Cohen, Vase, & Hooten, 2021; Marcianò et al., 2023). Although characterized by the development of tolerance, the prolonged use of opioids is still widespread due to their potent analgesic activity, especially in chronic pain due to cancer (Cohen et al., 2021; Marcianò et al., 2023).

A less neuronocentric view of chronic pain is rapidly evolving as increasing evidence now indicates that non-neuronal cells (e.g., mast cells and microglia) play key roles in the development of pathological pain (Vanderwall & Milligan, 2019). Most of the current analgesic drugs do not address the neuroinflammatory component of chronic pain. Safe interventions targeting the non-neuronal contribution are thus desirable for a successful management of chronic pain (Skaper, Facci, Zusso, & Giusti, 2018).

Table 1
International classification of disease – chronic pain.

Classification	Description
MG30 Chronic pain	Pain that persists or recurs for longer than 3 months.
MG30.0 Chronic primary pain	Chronic pain in one or more anatomical regions that is characterized by significant emotional distress (anxiety, anger/frustration or depressed mood) or functional disability (interference in daily life activities and reduced participation in social roles). Chronic primary pain is multifactorial: biological psychological and social factors contribute to the pain syndrome.
MG30.1 Chronic cancer related pain	Pain caused by the primary cancer itself or metastases (chronic cancer pain) or its treatment (chronic post-cancer treatment pain).
MG30.2 Chronic postsurgical or post traumatic pain	Pain developing or increasing in intensity after a surgical procedure or a tissue injury (involving any trauma including burns) and persisting beyond the healing process, i.e. at least 3 months after surgery or tissue trauma.
MG30.3 Chronic secondary musculoskeletal pain	Chronic pain arising from bone(s), joint(s), muscle(s), vertebral column, tendon(s)or related soft tissue(s).
MG30.4 Chronic secondary visceral pain	Persistent or recurrent pain originating from internal organs of the head/neck region and of the thoracic, abdominal and pelvic cavities.
MG30.5 Chronic neuropathic pain	Chronic pain caused by a lesion or disease of the somatosensory nervous system.
MG30.6 Chronic secondary headache or orofacial pain	It comprises all headache and orofacial pain disorders that have underlying causes and occur on at least 50% of the days during at least three months. The duration of pain per day is at least 2 h.

From ICD-11-WHO 2019/2021.

N-palmitoylethanolamine (PEA) is a naturally occurring fatty acid ethanolamide (FAE) endowed with several physiological properties (e.g., anti-inflammatory, neuroprotective, immunomodulating, anti-hyperalgesic functions), mainly due to its ability to down-modulate hyperactive non-neuronal cells (Skaper, Facci, & Giusti, 2013). PEA, that belongs to the family of autacoid local injury antagonist amides (ALIAmides), is one of the most studied members of the recently termed "paracannabinoid system", i.e., a family of naturally occurring lipid mediators known as endocannabinoid-like lipids, that are structurally related to the endocannabinoids, share similar biosynthetic and degradative pathways, but exert distinct effects and target different receptors (Piomelli & Mabou Tagne, 2022). Other endocannabinoid-like lipids are linoleoylethanolamide (LEA), oleoylethanolamide (OEA), and stearoylethanolamide (SEA). Similar to PEA, these compounds perform ancillary functions with respect to endocannabinoid mediators, i.e., anandamide (arachidonoylethanolamide, AEA) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Mechoulam et al., 1995; Sugiura et al., 1995), that were discovered about 30 years ago by the seminal work of Mechoulam's and Sugiura's independent groups. In contrast to endocannabinoids, the endocannabinoid-like lipids do not bind directly to the canonical cannabinoid receptors type 1 (CB1) and type 2 (CB2) (Fezza et al., 2014; Kleberg, Hassing, & Hansen, 2014).

Variable concentrations of PEA have been detected in several tissues including brain, liver, heart, intestine and adipose tissue (Balvers, Verhoeckx, Meijerink, Wortelboer, & Witkamp, 2013). Since 1950′, several physiological properties (e.g., anti-inflammatory, neuroprotective, immunomodulating, anti-hyperalgesic functions) have been recognized to PEA and the Nobel Laureate Rita Levi Montalcini importantly contributed to research advancement in the ALIAmide field (Levi-Montalcini, Skaper, Dal Toso, Petrelli, & Leon, 1996). Based on its protective properties, the use of PEA as a nutritional intervention for human and animal health has been explored, with this being greatly encouraged by the natural occurrence of this lipid amide not only in the animal and human body, but also in several food sources, as recently reviewed by Petrosino and colleagues (Petrosino & Schiano Moriello, 2020).

In order to overcome the bioavailability problems due to the lipid nature and the large particle size of PEA powder, micron-size formulations (i.e., micronized, ultramicronized and co(ultra)-micronized) have been developed and shown to have superior oral efficacy compared to unprocessed naïve PEA in several preclinical models (Impellizzeri et al., 2014; Petrosino et al., 2018), as further discussed in the next paragraphs.

In addition to preclinical studies - in which the analgesic properties of PEA and its complex mechanism(s) of action have been characterized - a relevant number of clinical trials have been performed on the efficacy and tolerability of ultramicronized PEA in humans, as reviewed in recent meta-analyses (Lang-Illievich et al., 2023; Paladini et al., 2016; Scuteri et al., 2022).

Through the years, several groups, including ours, have widely investigated the contribution of PEA in the control of pain with particular reference to its ability in co-adjuvating the action of other analgesics. This narrative review is aimed at discussing current knowledge on the pain-relieving properties of PEA with a focus on ultramicronized PEA as an add-on to the standard analgesic therapy.

2. Search strategy

The literature search was initially performed by PubMed using the following single terms "*N*-palmitoylethanolamine AND pharmacokinetics" (i.e. 32 results); "*N*-palmitoylethanolamine AND pharmacodynamics AND chronic pain" (i.e. 52 results); "*N*-palmitoylethanolamine AND preclinical studies" (i.e. 25 results); "*N*-palmitoylethanolamine AND animal studies" (i.e. 238 results); "*N*-palmitoylethanolamine AND chronic pain" (article type: clinical trials) (i.e. 11 results); "*N*-palmitoylethanolamine AND pain" (article type: clinical trials) (i.e. 26 results); "ultramicronized PEA AND pain" (i.e. 41 results); "ultramicronized palmitoilethanolamide and pain" (i.e. 26 results); "ultramicronized PEA AND pain" (article type: clinical trials) (i.e. 41 results). Due to the narrative approach of this review, the obtained results have been further investigated with more stringent terms (e.g., by adding "ultramicronized") or by allowing a more extensive search (e.g., "pain" instead of "chronic pain"). All the publications have been screened for consistence with the topic and for their relevance to the aims of this review. To cover most of the pertinent available literature, and better refine relevant information, a direct search on Google and the consultation of references from published papers have also been carried out. Paragraphs "History", "Biosynthesis and chemical synthesis", "Endogenous PEA: body's own levels and biological roles" and "Physical/chemical characteristics of PEA formulations" have been written by consulting major and fundamental publications on these topics. The search was performed from May to September 2023. No date or language restrictions were selected.

3. The emergence of PEA as an ALIAmide

The pioneering findings by Coburn and Moore (Coburn, Moore, & York, 1943) in rheumatic children at the Pelham Home of New York, pointed out the relevant health role played by some essential factors contained in the egg yolk, thus paving the way for the discovery of the naturally occurring FAEs. Indeed, it was in the late 1950s that it was first discovered that the anti-allergic and anti-inflammatory activities exerted by dietary supplementation with egg yolk, peanut oil or soybean lecithin (Coburn, Grahamt, & Haninger, 1954; Long & Martin, 1956) were due to the PEA content in such foods (Ganley, Graessle, & Robinson, 1958; Kuehl Jr, Jacob, Ganley, Ormond, & Meisinger, 1957). In particular, it was shown that PEA low doses were effective in the guinea pig joint anaphylaxis assay (Ganley et al., 1958), and protected against fatal anaphylactic shock in mice, with the effect being similar to 100 mg/kg hydrocortisone (Ganley & Robinson, 1959). Some years later, a series of studies performed at the Czechoslovak Academy of Sciences in Prague, showed the ability of PEA in increasing the resistance of mice to bacterial toxins (i.e. *Shigella dysenteriae* toxin and Streptolysin O) (Rasková, Masek, & Linèt, 1972). In addition, PEA pre-treatment was also shown to increase the tolerance to traumatic shock induced by a Noble-Collip drum in mice (Rasková et al., 1972). The same research group also highlighted the immunomodulatory properties of PEA in guinea pigs (Perlík, Krejcí, Elis, Pekárek, & Svejcar, 1973; Perlík, Rasková, & Elis, 1971).

Then, PEA was shown to exert protective properties in several types of cells and tissues, initially based on the evidence obtained in liver mitochondria of mice (Obermajerová, Masek, Seifert, Buchar, & Havlík, 1973) and in infarcted myocardium of dogs (Epps, Natarajan, Schmid, & Schmid, 1980).

Moreover, protective effects against the side effects of cytotoxic chemotherapy were shown in a rat leukemia model (Svec, Béderová, & Svec, 1975).

In most recent years, other studies have further shown the protective role of PEA in animal models, for instance in case of renal ischemia (Di Paola et al., 2012) or in case of nervous tissues damages (e.g. brain injury (Ahmad et al., 2012); Alzheimer's disease (D'Aloia et al., 2020). Today, we know that PEA is present not only in mammalians (Bachur, Masek, R, & Udenfriend, 1965), but also in plants (Kilaru et al., 2007; Long & Martin, 1956; Schuel et al., 2002) and marine sources (Sepe, De Petrocellis, Montanaro, Cimino, & Di Marzo, 1998).

From a historical point of view, to get insights on the mechanism of action of PEA, it was necessary to wait for the 1990s. Based on the studies by Prof. Rita Levi-Montalcini and her group on the effects of *N*-acylated lipids on mast cell activation, PEA was classified as an ALIAmide and its mechanism of action in inflammation was initially investigated in the seminal paper published in Agents Action on 1993 (Aloe, Leon, & Levi-Montalcini, 1993). This study showed that the systematic administration of particular *N*-acylethanolamines (including PEA) was able to

reduce mast cell degranulation induced by substance P injection in the rat ear pinna, thus contributing to the hypothesis that endogenous *N*-acylethanolamines were part of an endogenous/paracrine response for the negative feedback control of mast cells to agonistic signals.

It was later discovered that PEA was able to protect cerebellar granule cells from BALB/c mice against glutamate toxicity (Skaper et al., 1996) and the acronym ALIA was broadened to Autacoid Local Injury Antagonism (Levi-Montalcini et al., 1996; Skaper et al., 1996). In particular, it was speculated that PEA might accumulate in tissues following injury in order to exert a local, autacoid, anti-injury function, thus reducing tissue inflammation, decreasing hyperalgesia and exerting a neuroprotective function (Levi-Montalcini et al., 1996). In agreement, body's own PEA was shown to be involved in the intrinsic control of pain initiation (Calignano, La Rana, Giuffrida, & Piomelli, 1998) and the analgesic effects of endogenous and/or exogenous PEA were successfully investigated in *in vivo* pain models (Calignano, La Rana, & Piomelli, 2001; Re, Barbero, Miolo, & Di Marzo, 2007).

Since then, the functions of endogenous PEA in pain control and the pain-relieving effects of PEA administration via different routes and formulations have been extensively investigated worldwide by several research groups including ours.

4. Biosynthesis and degradation of PEA

By a reaction catalyzed by the *N*-acyl-phosphatidyl-ethanolamine-selective phospholipase D (NAPE-PLD) enzyme, *N*-palmitoyl-phosphatidyl-ethanolamine, the phospholipid precursor of PEA, is hydrolyzed to PEA (Okamoto, Morishita, Tsuboi, Tonai, & Ueda, 2004). NAPE-PLD independent pathways have also been identified. An example is represented by the multi-step reaction from NAPE to NAEs. Two consecutive *O*-deacetylation reactions (i.e. from NAPE to *N*-acyl-lysoPE (lysoNAPE) and from lysoNAPE to glycerophospho-NAE (GP-NAE) are followed by a hydrolysis by glycerophosphodiesterase 1 and 4 (GDE1, GDE4) that leads to NAEs production (Simon & Cravatt, 2008; Tsuboi et al., 2015). The hydrolysis of NAPE to lysoNAPE has been suggested to be mediated by group IB, IIA, and V secretory phospholipases A2 ($sPLA_2$s) (Sun et al., 2004), whereas a serine hydrolase α/β-hydrolase domain-containing protein 4 (ABHD4) has been shown to catalyze the hydrolytic reactions in the sequential deacylation of NAPE to GP-NAE (Simon & Cravatt, 2006). Alternatively, lysoNAPE may be hydrolyzed to NAEs by other GDEs, such as GDE4 and GDE7 (Rahman et al., 2016; Tsuboi et al., 2015). A further pathway occurs through the action of phospholipase C and phosphatase. In particular, tyrosine phosphatase PTPN22 and inositol 5′-phosphatase SHIP1 have been shown to exert this phosphatase activity (Liu et al., 2006; Liu et al., 2008).

Two enzymes contribute to the endogenous PEA degradation, i.e., a PEA selective enzyme, the amidase of *N*-acylethanolamine-hydrolyzing acid (NAAA) (Ueda, Yamanaka, & Yamamoto, 2001) and the fatty acid amide hydrolase (FAAH) that hydrolyzes various NAEs including PEA (Cravatt et al., 1996). Both enzymes hydrolyze PEA to palmitic acid and ethanolamine. A subsequent study identified a second human FAAH (i.e., FAAH-2) that shares about 20% sequence identity with the FAAH and that, however, is less active than FAAH in hydrolyzing PEA (Wei, Mikkelsen, McKinney, Lander, & Cravatt, 2006).

A marked cross-kingdom evolutionary conservation has been shown in the biosynthesis and degradation of PEA in mammals and plants (Kilaru et al., 2007). In fact, the involvement of the same routes and similar enzymes, especially in the degradative metabolism, has been widely reported (Blancaflor et al., 2014), suggesting the functional importance of PEA in homeostatic regulation.

5. Endogenous PEA: body's own levels and biological roles

As reviewed by Skaper and colleagues (Skaper, Facci, Giusti et al., 2014), there is a general consensus that changes in PEA levels are either suggestive of a loss of protection against inflammation/pain (i.e., decreased levels) or a compensatory synthesis in the attempt to limit the disease severity (i.e., increased levels). Accordingly, the decrease of PEA endogenous level is viewed as a contributing factor to the deleterious effect of a disease condition (Rinne et al., 2018; Roviezzo et al., 2017; Skaper et al., 2015), while the exogenous administration of PEA is considered as a promising approach to increase the body's own supply of this lipid amide (Della Rocca & Re, 2022; Skaper, Facci, Fusco, et al., 2014; Skaper, Facci, & Giusti, 2014).

Noteworthy, the maintenance of adequate endogenous PEA levels by the inhibition of PEA degradative enzymes (Alhouayek & Muccioli, 2014; Bottemanne, Muccioli, & Alhouayek, 2018; Piomelli et al., 2020) or by exogenous administration, allows to mimick and support its natural analgesic function (Gugliandolo, Peritore, Piras, Cuzzocrea, & Crupi, 2020; Petrosino & Di Marzo, 2017), thus contributing to support the hypothesis proposed in the late 1990s by Calignano and colleagues (Calignano et al., 1998), i.e. PEA is responsible for innate pain control.

This complex view is supported by observations reported in preclinical and clinical settings. Petrosino et al., (Petrosino et al., 2007) showed the decrease of PEA levels in the spinal cord and in dorsal rafe/rostral ventral medulla after 3 and 7 days, respectively, from chronic constriction injury (CCI) of the sciatic nerve in rats. A subsequent study performed in rats undergoing spinal cord injury, reported an early (i.e. one day after the lesion) accumulation of PEA in samples obtained by the adjacent rostral part of the spinal cord and by the epicentre of the lesion (Garcia-Ovejero et al., 2009). Levels of PEA, substantially comparable to those observed in sham animals, were observed at days 7 and 28 from the injury (Garcia-Ovejero et al., 2009). A further study, performed in a rat model of chronic granulomatous inflammation, showed that the granuloma formation was associated to a significant decrease in PEA levels and that the administration of PEA, dose-dependently reduced inflammatory hallmarks (De Filippis et al., 2010).

Through the years, several inhibitors of the degradative metabolism of endogenous ethanolamides, including PEA (i.e. NAAA and FAAH inhibitors) have been investigated. Focusing on endogenous PEA, the expectations from these inhibitors are the increase of PEA levels with a consequent reduction of inflammation and pain. A number of structurally unrelated selective inhibitors of NAAA (e.g. ARN077 (Sasso et al., 2013; Sasso et al., 2018), ARN726 (Ribeiro et al., 2015), F96 (Yang et al., 2015), F215 (Zhou et al., 2019), ARN19702 (Fotio et al., 2021; Fotio, Sasso, Ciccocioppo, & Piomelli, 2021) or FAAH (e.g. URB597 (Danandeh et al., 2018; Nasirinezhad, Jergova, Pearson, & Sagen, 2015), PF-3845 (Nasirinezhad et al., 2015), ASP8477 (Kiso, Watabiki, & Sekizawa, 2020)) have been tested in inflammation and/or pain animal models.

Diurnal variations (i.e., 24 h) of endogenous PEA levels have been detected in the brain of healthy rats, with increasing (cerebrospinal fluid) or decreasing levels (pons, hippocampus, and hypothalamus) during the light-on period while the opposite was observed during the light-off period (Murillo-Rodriguez, Désarnaud, & Prospéro-García, 2006).

To date, several studies accurately measured the endogenous levels of PEA in human tissues and body fluids, either in case of health or pain conditions (Aydin et al., 2023; Balvers, Verhoeckx, & Witkamp, 2009; Barry et al., 2018; Correia-Sá et al., 2020; Darmani et al., 2005; De Icco et al., 2021; Fanelli et al., 2012; Gachet, Rhyn, Bosch, Quednow, & Gertsch, 2015; Jumpertz, Guijarro, Pratley, Piomelli, & Krakoff, 2011; Koethe et al., 2019; Lam, Marczylo, & Konje, 2010; Quercioli et al., 2017; Schreiber et al., 2007; Wood et al., 2008) (Table 2). As observed in the preclinical setting, increases or decreases in the tissue or plasma levels of PEA have been found in patients affected by different pathologies compared to healthy individuals (Fichna et al., 2013; Richardson et al., 2008; Sarnelli et al., 2021; Stensson et al., 2018).

Based on the current evidence, endogenous PEA level variations related to the control of pain are being increasingly clarified. Nonetheless, *ad hoc* well-designed case-control clinical studies in painful diseases or

Table 2
Levels of endogenous PEA in cerebrospinal fluid and plasma.

Reference	Subjects	Fluid	Method	Concentration/amount
Aydin et al., 2023	118 (CNS infections)	CSF	UHPLC-ESI-MS/MS	0.0927 (0.0556–0.1321) pmol/mL
Quercioli et al., 2017	30 (healthy volunteers)	plasma	5500 QTrap® triple quadrupole/ linear ion trap (QqQLIT) mass spectrometer	8.75 (7.88–10.0) pmol/mL
Balvers et al., 2009	23 (healthy post-menopausal women)	plasma	LC–MS/MS	4.64 ± 1.2 pmol/mL
Jumpertz et al., 2011	27 (healthy volunteers)	CSF	LC-MS	2.12 ± 0.72 pmol/mL
		plasma		7.63 ± 1.91 pmol/mL
Darmani et al., 2005	10 (chronic low back pain)	plasma	LC-MS	14.46 ± 1.53 pmol/mL
De Icco et al., 2021	24 (migraine)	plasma	LC-MS	2.01 ± 1.47 pmol/mL
	19 (controls)	plasma	LC-MS	2.74 ± 2.02 pmol/mL
Koethe et al., 2019	16 (8 healthy twin pairs)	plasma	LC-MS	2.85 pmol/mL
Correia-Sá et al., 2020	50 (patients who underwent routine body-countoring surgery)	plasma	LC-MS	9566.26 ± 500.85 pmol/mL
Fanelli et al., 2012	121 (healthy volunteers)	plasma	Two dimensional-LC/MS/MS	7.34–28.0 pmol/mL
Gachet et al., 2015	32 (healthy volunteers)	plasma	LC-MS/MS	23.0 ± 5.2 pmol/mL
Barry et al., 2018	8 (healthy volunteers)	plasma	LC-MS/MS	10.55 ± 0.63 pmol/mL
	7 (burning mouth syndrome)	plasma		12.89 ± 0.73 pmol/mL
Lam et al., 2010	9 (healthy volunteers)	plasma	UPLC-MS/MS	16.91 ± 4.23 pmol/mL
Schreiber et al., 2007	8 (healthy volunteers)	serum	LC-ESI-MS/MS	28.44 ± 5.91 pmol/mL
Wood et al., 2008	9 (healthy volunteers)	plasma	LC-MS	6.01–7.01 pmol/mL

CNS, central nervous system; CSF, cerebrospinal fluid; LC-ESI-MS-MS, liquid chromatography coupled to electrospray ionization-tandem mass spectrometry; LC-MS, liquid chromatography–mass spectrometry; LC-MS/MS, liquid chromatography with tandem mass spectrometry; UHPLC-ESI-MS/MS, ultrahigh performance liquid chromatography–electrospray ionization tandem mass spectrometry; UPLC-MS/MS, liquid chromatography-tandem mass spectrometry.

conditions, could contribute to a better understanding of PEA changes and help to elucidate the best timing of PEA supplementation.

6. Physical/chemical characteristics of PEA formulations

Several synthetic procedures for the preparation of PEA have been described, with all of them starting from palmitic acid.

Overall, most of the synthetic procedures involve the use of coupling agents (Dang et al., 2011; Nagao et al., 1980; Ottria, Casati, & Ciuffreda, 2012), ferric and copper chloride (Miyasaka & Noguchi, 1985), or enzymes (Gunawan, Suhendra, NuansaWindari, & Kurniawati, 2019).

The major disadvantage of these methods is represented by residual impurities or by-products (e.g., reagents, catalysts, metals), which impair chemical purity of the final product, represent a potential health risk for patients, and negatively impact crystallization and subsequent manufacturing process (Abdin, Yeboah, & Jacob, 2020; Urwin, Yerdelen, Houson, & ter Horst, 2021). Conversely, a solvent- and catalyst-free synthetic procedure for PEA has been described (Roe, Scanlan, & Swern, 1949). It consists in reacting under reflux pure palmitic acid with ethanolamine under nitrogen atmosphere, as detailed (and completed with a subsequent crystallization technique) in a patent (Della Valle, Lorenzi, Samson, & Della Valle, 1999). Indeed, this synthetic procedure was shown to yield a crystalline PEA powder with greater purity compared to PEA powders obtained with different chemical synthesis processes (Impellizzeri et al., 2014).

Once PEA has been synthesized, a further issue needs to be considered, i.e., lipophilicity. In fact, PEA is a lipid compound, highly insoluble in water and poorly soluble in several other aqueous solvents as indicated by an octanol-water partition coefficient (log P) higher than 5 (Lambert, Vandevoorde, Jonsson, & Fowler, 2002). The partition coefficient represents the ratio of unionized PEA distributed between the organic phase (octanol) and aqueous phases (water) at equilibrium. Accordingly, unprocessed PEA is ~100.000-fold more soluble in octanol than water. Since aqueous solubility is critical for oral absorption and subsequent bioavailability (Arnott & Planey, 2012), unprocessed PEA is expected to have poor oral bioavailability (Clayton, Subah, Venkatesh, Hill, & Bogoda, 2023). This is a challenging issue, since oral delivery remains the most common route of administration in clinical practice for a number of reasons, mainly related to simplicity of administration, and patient compliance.

Among the formulation strategies to improve solubility of lipophilic compounds (e.g., inclusion in cyclodextrins, solid dispersion, use of surfactants), particle size reduction is still considered one of the most reliable techniques, both in the pharmaceutical and food field (Dhiman & Prabhakar, 2021; Rasenack & Müller, 2004). Decreasing particle size, in fact, allows to increase the surface area, which is directly proportional to dissolution, thereby increasing absorption and bioavailability of lipophilic compounds (Khadka et al., 2014; Olusanmi et al., 2014; Rasenack & Müller, 2004). Nowadays, two main formulations of PEA powder have been produced, i.e., the micronized and ultramicronized PEA, with the former being characterized by particles in the 2–10 μm range (80% <6 μm; 96% <10 μm) and the latter having particles ranging from 0.8 to 6 μm (99.9% <6 μm; 59.6% <2 μm). Both particle size ranges are significantly smaller compared to unprocessed (naïve) PEA (100–700 μm or more) (Skaper, Facci, Giusti et al., 2014; Impellizzeri et al., 2014; Petrosino et al., 2018).

Micron-size PEA formulations are obtained through a mechanical comminution process known as jet mill.

Briefly, a coarse powder of unprocessed PEA is slowly fed into a jet-mill equipment endowed with a chamber of 300 mm in diameter operating by pressurized air jet "spiral technology" (compressed air at 10 to 12 bars). As detailed in a patent (Della Valle, Marcolongo, & Della Valle, 2009), the fluid threads generated within the micronization chamber accelerate the powder particles to high speeds, yielding to a huge number of collisions with each other and the micronization chamber walls, finally resulting in micron-sized crystals (Paterniti, Impellizzeri, Di Paola et al., 2013; Impellizzeri et al., 2014). Depending on grinding conditions, a final particle size range can be selected.

A particular micronization procedure, performed by supercritical fluid technology (Dhiman & Prabhakar, 2021), has been recently applied to PEA (Campardelli, Oleandro, Scognamiglio, Della Porta, & Reverchon, 2017). Briefly, PEA was dissolved in an organic solvent, added to the dispersing phase (i.e., an antisolvent containing a surfactant) and delivered to a packed column to obtain a continuous counter-current contact with supercritical carbon dioxide. Finally, the suspension was washed to remove the surfactant and PEA particles were recovered by filtration using a 0.1 μm pore size membrane. This technique allowed to obtain micronized crystals with controlled and regular dimensions of about 0.1 μm (Campardelli et al., 2017). The supercritical extraction process has been performed at 120 bar, 44 °C and at a liquid to gas ratio of 0.05 (Campardelli et al., 2017).

A further technique to increase PEA bioavailability has been developed by an Australian company and is based on a delivery system aimed at increasing PEA dispersion in aqueous environments

(Lipisperse). The system consists in a mixture of coconut oil (fractionated), polyglycerol polyricinoleate, citrus oil, olive oil, lecithin, dl-alpha tocopheryl acetate, and silicon dioxide (Mallard, Briskey, Richards, Mills, & Rao, 2020).

While treatment of human patients is usually performed by oral administration of a solid dosage form (e.g., tablets, granules), cell exposure to treatment and oral administration to experimental animals (generally via gavage) require liquid forms and suitable vehicles accordingly (Singh, Dwivedi, & Chaturvedi, 2012).

Due to the lipophilic nature of PEA, when added to cell cultures (few μm in size), it is commonly dissolved in ethanol or dimethyl sulfoxide (DMSO). Both solvents at final concentrations ranging from 0.2% to 1% have been shown to be suitable for PEA to be added in cell cultures without interfering with cell responses (Facci et al., 1995; Cerrato, Brazis, della Valle, Miolo, & Puigdemont, 2010; Toti et al., 2023).

In such *in vitro* conditions, PEA purity rather than particle size is a critical issue, and PEA produced by the patented solvent- and catalyst-free synthetic procedure followed by crystallization and micronization (corresponding to (co)ultramicronized PEA) is one of the used alternatives (Facchinetti et al., 2022; Facci, Barbierato, Fusco, Giusti, & Zusso, 2021; Toti et al., 2023).

A different issue is represented by the oral administration of PEA to experimental animals that is generally performed through gastric gavage. In these settings, dispersion of PEA in 0.5%–1.5% solutions of carboxymethylcellulose (CMC) is the most frequently used method and allows for an accurate dosing of PEA. Overall, in studies investigating the antinociceptive and/or anti-inflammatory activities of ultramicronized PEA, control arms represented (when appropriate) by sham groups treated with CMC alone showed no interfering activity of the solvent (Borrelli et al., 2015; Cristiano et al., 2022; Micheli et al., 2024; Peritore et al., 2020; Petrosino et al., 2018).

7. Pharmacokinetics of PEA

To date few studies investigated PEA pharmacokinetics. Available information has been mainly obtained in animals. Actually, when dealing with pharmacokinetics of a body's own compound like PEA, the possible re-arrangement of exogenously administered and endogenous pool of PEA, due to the natural occurrence of biosynthetic/degradative enzymes (see paragraph 4) has to be considered.

This paragraph discusses the major findings on the ADME process of PEA in animals and humans, in health and in disease settings, by highlighting – when available - differences among the three formulations (i.e. naïve, micronized, ultramicronized PEA). To the best of our knowledge, only one pharmacokinetic study compared PEA plasma levels of ultramicronized and naïve PEA (Petrosino et al., 2018). A synthetic and 99% isotopically pure $[^{13}C]_4$-PEA, suitable to discriminate between endogenous and orally administered PEA was used. The oral administration of ultramicronized $[^{13}C]_4$-PEA (30 mg/kg) to healthy rats resulted in a significant peak plasma concentration of $[^{13}C]_4$-PEA after 5 min (5.4 ± 1.87 pmol/mL, $p < 0.0001$) and a second, although lower, peak at 60 min (2.7 ± 0.6 pmol/mL, $p = 0.0006$), while no significant peak was observed after naïve $[^{13}C]_4$-PEA administration at the corresponding time points (i.e., 1.1 ± 0.35 pmol/mL; $p = 0.0078$ and < 0.5 pmol/mL, respectively). Thus, plasma levels of ultramicronized $[^{13}C]_4$-PEA at 5 and 60 min in healthy rats, resulted in 4.9- and >5-fold variations, respectively, compared to naïve $[^{13}C]_4$-PEA (Petrosino et al., 2018).

Similarly, a high variation in PEA plasma levels was reported in a previous study (Vacondio et al., 2015) in which a higher oral dose of PEA (100 mg/kg) was administered via gavage to healthy rats after being suspended in corn oil and subjected to ultrasonication/vortexing. This procedure generates a formulation more similar to ultramicronized than to naïve PEA in terms of particle size. The PEA maximum plasma concentration (C_{max}) was observed after 15 min. Compared with the basal PEA plasma levels a 20-fold variation was achieved. In this case, PEA plasma levels decreased to values approximating the basal ones 2 h after the administration (Vacondio et al., 2015).

More recently, blood absorption of ultramicronized PEA was investigated in mice up to 4 h following a single oral dose (100 mg/kg) (Beggiato, Tomasini, & Ferraro, 2019). PEA C_{max} was detected 1 h after administration (about 9-fold the baseline), with values decreasing to basal ones 3 h later (Beggiato et al., 2019).

Interesting findings have been provided in a further study by Petrosino et al. (Petrosino et al., 2016) who investigated PEA plasma levels after oral administration of micronized and ultramicronized PEA to human volunteers and Beagle dogs, respectively. Two hours after a single oral administration of micronized PEA 300 mg to 10 healthy volunteers, PEA plasma levels were increased up to two-fold compared to baseline and decreased to the baseline at the fourth hour (Petrosino et al., 2016). On the other hand, 1 and 2 h after administering ultramicronized PEA 30 mg/kg to spontaneously *Ascaris suum* hypersensitive dogs, PEA plasma levels were increased up to five-fold compared to baseline; even in this case, after 4 h from PEA administration, PEA plasma levels approximated baseline ones (Petrosino et al., 2016).

To the best of our knowledge, only another study on the pharmacokinetics of PEA in humans is available (Briskey, Mallard, & Rao, 2020). This study compared the bioavailability of a single 300 mg dose of a property formulation of unprocessed PEA with the same formulation spiked with a delivery complex (i.e., a mixture of surfactants, polar lipids and solvents), that was designed to increase the dispersion of PEA in water, thus preventing the agglomeration of PEA crystals (Briskey et al., 2020). Although the C_{max} of the unprocessed and spiked formulation was observed at different times (i.e., 125 and 105 min from PEA administration, respectively), no statistically significant difference was observed between the two formulations. Thus, two hours after administration (i.e., the peak time observed in the study of Petrosino and colleagues (Petrosino et al., 2016)), no difference was observed between the two formulations, whereas higher plasma concentrations of PEA were observed after 3 and 4 h in subjects who consumed the spiked formulation.

Overall, these data suggest that the different formulations of PEA, in health condition, are characterized by a distinct pharmacokinetic behavior. This may be due to differences among species and doses, although a correlation between particle size and extent of absorption cannot be ruled out (Briskey et al., 2020; Petrosino et al., 2016).

Interestingly, attention has also been paid to investigating whether the absorption of orally administered PEA could be affected by the disease state. This is a key issue, since the compound has been developed as a nutritional intervention for inflammatory and pain conditions, as it will be discussed in more detail in the next paragraphs. In particular, Petrosino et al. (Petrosino et al., 2018) compared plasma levels of PEA after oral administration of ^{13}C labeled PEA, either in naïve or ultramicronized form, to healthy rats and rats subjected to sub-plantar carrageenin (CAR) injection, i.e., a well-known model of inflammation. A significantly higher plasma level of $[^{13}C]_4$-PEA was observed after administration of ultramicronized compared to naïve PEA in CAR-injected animals. Moreover, the blood absorption of ultramicronized PEA (but not naïve PEA) was significantly higher in CAR-injected compared to healthy animals, with plasma levels being about 20-fold and 6-fold higher at 30 and 60 min from the administration, respectively (Petrosino et al., 2018).

Together with absorption, tissue distribution after oral administration is a critical issue for treatment outcome, especially when dealing with chronic pain, that may originate from and radiate to different areas of the body. The pioneering experiments carried out at the Palladin Institute of Biochemistry of National Academy of Sciences of Ukraine in the late nineties revealed the following tissue distribution of radioactivity after intraperitoneal (i.p.) administration of radiolabeled PEA in rats: adrenal > > diaphragm > spleen > kidney > testis > lung > liver > heart > brain > plasma > erythrocytes (Zhukov, 1999). Since radioactivity did not specifically identify the whole

amide, the possibility that PEA metabolites were actually detected cannot be ruled out. More recently, increased levels of PEA were observed in several tissues of mice (including retina, heart, blood serum and brain), following one subcutaneous (s.c.) depot injection of PEA emulsified in sterile corn oil (10 mg/kg) (Grillo, Keereetaweep, Grillo, Chapman, & Koulen, 2013). In terms of particle size this formulation may be more similar to ultramicronized than naïve PEA (i.e., PEA was grinded and emulsified by vortexing/ultrasonification).

The specific distribution of PEA in the central nervous system was first studied in rats by the same above-mentioned group of Ukrainian biochemists. PEA accumulated primarily in the hypothalamus, pituitary (and adrenal gland) following a single ^{3}H-PEA oral dose (Artamonov et al., 2005). This study represents the first demonstration that PEA can cross the blood brain barrier as later confirmed by Siracusa et al. (Siracusa et al., 2017) who detected brain accumulation of [^{13}C]$_4$-PEA 15 min after oral administration of ultramicronized [^{13}C]$_4$-PEA to healthy rats. Similar results were found by Petrosino and colleagues (Petrosino et al., 2018), with relevant levels of [^{13}C]$_4$-PEA in both the spinal cord and brain of healthy rats being observed following a single oral administration of ultramicronized [^{13}C]$_4$-PEA. The latter study also investigated whether disease conditions could impact tissue distribution of PEA after a single oral dose (10 mg/kg) of ultramicronized [^{13}C]$_4$-PEA. Healthy and CAR-injected animals were studied at different time points up to 360 min. Distribution of [^{13}C]$_4$-PEA in the paw and spinal cord (but not brain) was significantly higher in CAR-injected compared to healthy animals at most of the study time points (Petrosino et al., 2018). Importantly, this finding confirmed that ultramicronized PEA may reach body sites involved in central sensitization, a condition typically occurring during persistent pain.

Since, as mentioned in the paragraph "Biosynthesis and chemical synthesis", endogenous PEA is rapidly hydrolysed by FAAH and NAAA and the metabolism of exogenous PEA mediated by FAAH may be partially responsible for the limited exposure of oral PEA (Desarnaud, Cadas, & Piomelli, 1995), some efforts have been performed in synthesizing PEA derivatives less prone to hepatic inactivation. For instance, a series of pro-drugs of PEA (i.e., carbonates, esters and carbamates at the hydroxyl group) were prepared, but only some ester derivatives - in particular those obtained by conjugating PEA with L-valine and D-valine - showed a promising conversion rate to PEA in plasma and reduced hepatic clearance (Vacondio et al., 2015). However, at the longest times the levels of PEA released from these prodrugs were comparable with those obtained by administering equimolar PEA and no pharmacokinetic advantage emerged (Vacondio et al., 2015). A further example came from a study by D'Aloia et al. (D'Aloia et al., 2020), in which one PEA analogue, named RePEA, was selected from a small library of PEA analogues as the best PEA prodrug candidate, since in contrast to PEA, it was not hydrolyzed by FAAH ex vivo.

Finally, no data concerning the excretion of exogenous PEA in animals or humans are available. It is desirable that further information on human pharmacokinetics of PEA will come from two ongoing clinical trials listed in the Cochrane Central Register of Controlled Trials (CCRCT, 2023) and in the UK National Health System (NHS) Health Research Authority (HRA) register (NHS HRA, 2023), respectively. The Cochrane study will establish the main pharmacokinetic parameters in six cohorts of healthy volunteers orally treated with ultramicronized PEA in fed or fasted conditions at single ascending doses ranging from 600 mg to 2400 mg (cohorts 1–4) and multiple ascending doses of 600 mg or 1200 mg (cohorts 5 and 6) *vs* matching placebo (CCRCT, 2023). Lower doses will be administered in the study of the NHS HRA through three treatment periods in which ultramicronized PEA will be administered as a single dose of 300 mg in the fasted state, as a single dose of 300 mg in the fed state and as a single dose of 600 mg in either the fed or fasted state (NHS HRA, 2023).

Overall, the limited available pharmacokinetic data of PEA do not allow to draw conclusive results, especially in relation to potential differences among the three oral formulations (i.e. naïve, micronized and ultramicronized PEA). However, in the presence of comparative data between naïve and/or micronized and/or ultramicronized PEA, the latter seems to show a more favorable pharmacokinetic profile.

8. Pharmacodynamics of PEA

A great deal of evidence shows that PEA may control the behavior of mast cells, but also several other non-neuronal cells (including but not limited to microglia) (Bettoni, Comelli, Colombo, Bonfanti, & Costa, 2013; Petrosino et al., 2019; Skaper et al., 2013). Even neurons themselves have been shown to be cellular targets of PEA, since PEA may control the spontaneous GABAergic synaptic activity in striatal neurons (Musella et al., 2017), modulate dorsal root ganglion neurons (Khasabova, Xiong, Coicou, Piomelli, & Seybold, 2012) and desensitize neuronal pain receptor channels (Ambrosino, Soldovieri, Russo, & Taglialatela, 2013). In particular, the neuronal and non-neuronal cell modulation depends on the ability of PEA to interact with a number of receptors and ion channels, through multitarget and highly redundant mechanisms. Indeed, several direct and indirect molecular targets of PEA have been described, such as the transcription factor peroxisome proliferator-activated receptor alpha (PPARα) (Lo Verme, Astarita, La Rana et al., 2005; Lo Verme, La Rana, Russo, Calignano, & Piomelli, 2005), other members of the PPAR family (Costa, Comelli, Bettoni, Colleoni, & Giagnoni, 2008; Paterniti, Impellizzeri, Di Paola et al., 2013) and the orphan receptor GPR55 (Pertwee, 2007). In addition, it is today well recognized that PEA exerts indirect receptor-mediated actions on CB1 and CB2 (reviewed in Petrosino & Di Marzo, 2017) as well as on the Transient Receptor Potential cation channel subfamily V member 1 (TRPV1) (Ambrosino et al., 2013; Costa et al., 2008) by the so-called "entourage" effect, i.e., the ability to increase the level or the receptor affinity of the endocannabinoids anandamide (AEA) and 2-arachidonoyl-glycerol (2-AG) (De Petrocellis, Davis, & Di Marzo, 2001; Ho, Barrett, & Randall, 2008; Petrosino et al., 2019; Smart, Jonsson, Vandevoorde, Lambert, & Fowler, 2002) **(**Fig. 1**).**

8.1. Direct molecular targets

PPAR family that belongs to the steroid hormone receptor superfamily, includes various PPAR isoforms that have been suggested to mediate the anti-inflammatory and analgesic effects of PEA. *PPARα* gene codifies for the nuclear transcription factor PPARα subtype that modulates the expression of target genes involved in immune and inflammatory responses. *PPARα* is highly expressed in kidney, gastro-intestinal tract, heart but also in liver and brain (Fagerberg et al., 2014) as well as in immune cells (Glass & Ogawa, 2006). PPARα activation results in repressing pro-inflammatory transcription factors such as NFκB, responsible for upregulating inflammatory cytokines (e.g., tumor necrosis factor α, interleukin 1, interleukin 6), cyclooxygenase 2 (D'Agostino et al., 2009).

PEA is a PPARα agonist (EC_{50} 3.1 ± − 0.4 microM) as elegantly shown by Lo Verme et al. (Lo Verme, Astarita, La Rana et al., 2005) about twenty years ago. These authors showed that PEA administration was able to decrease inflammation in *PPARα* wild-type mice whereas no effect was observed in *PPARα* deficient mice (Lo Verme, Astarita, La Rana et al., 2005). Several studies, including ours, have later confirmed that the beneficial effects of PEA are counteracted by antagonism or genetic blockade of PPARα (Di Cesare Mannelli et al., 2013; Paterniti, Impellizzeri, Crupi, et al., 2013; Paterniti, Impellizzeri, Di Paola, et al., 2013).

Although knockout studies can present false phenotypes because of genetic compensation (El-Brolosy & Stainier, 2017), results from different experiments using PPARα antagonists (e.g., MK886, GW6471) have also provided supportive evidence to the need of this receptor in order for PEA to exert pain relief (Alsalem et al., 2019; Cristiano et al., 2022; Déciga-Campos et al., 2023).

In addition to PPARα, other PPAR isoforms such as PPARγ and PPARδ have been shown to contribute to the anti-inflammatory and neuroprotective effects of PEA (Paterniti, Impellizzeri, Crupi et al., 2005). PPARγ

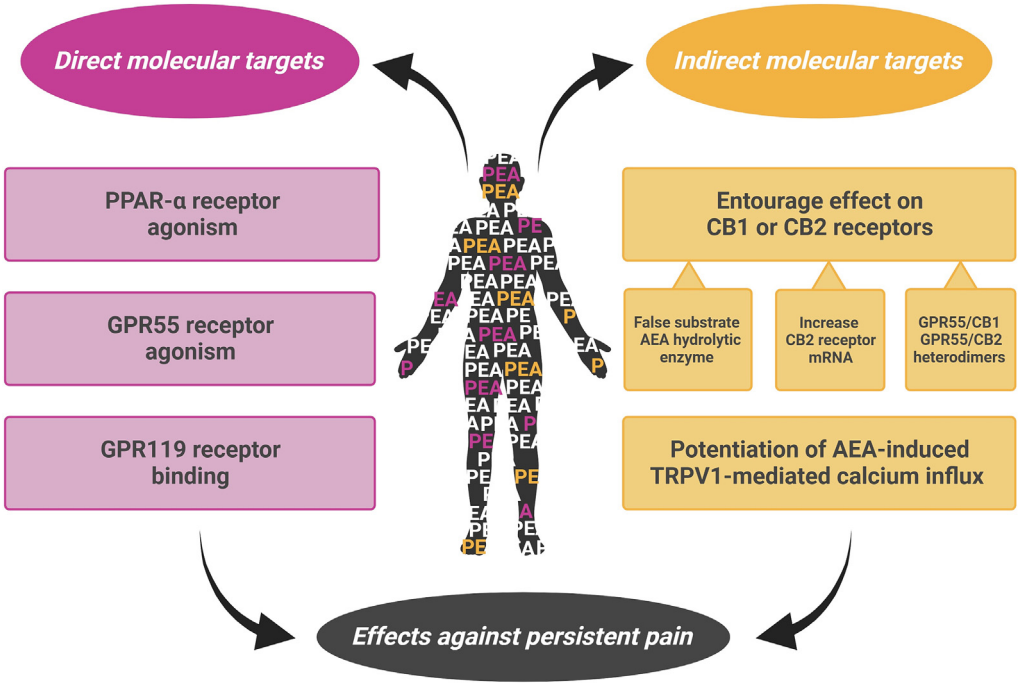

Fig. 1. Pharmacodynamics of PEA. Main established mechanisms of action are shown.

and PPARδ are widely expressed in adipose tissue and nervous system, respectively, and both exert anti-inflammatory effects in various tissues and organs (Iannotti & Vitale, 2021). Paterniti et al. (Paterniti et al., 2013) evaluated the involvement of PPARγ and PPARδ in mediating the anti-inflammatory and neuroprotective activities of PEA in a mouse model of spinal cord trauma. In this animal model, the anti-inflammatory activity of PEA was reduced either in PPARα knockout mice or in the presence of PPARγ or PPARδ antagonists (i.e. GW9662 and GSK0660, respectively), in PPARα WT mice. Overall, these results confirm the role of PPARα in the mechanism of action of PEA and suggest a compensatory mechanism exerted by PPARγ and PPARδ, as well evidenced in PPARα knockout mice treated with PEA. However, if PPARα is considered a direct target of PEA, current evidence suggests that the action of PEA related to PPARγ or PPARδ is substantially mediated by CB1-dependent changes in PPAR expression (Paterniti et al., 2013) (see paragraph 8.2).

G protein-coupled receptor 55 (GPR55) was cloned in 1999 (Sawzdargo et al., 1999) and belongs to the G-protein-coupled receptor superfamily. Formerly considered to be a cannabinoid receptor (Moriconi, Cerbara, Maccarrone, & Topai, 2010; Ryberg et al., 2007; Sharir et al., 2012; Yang, Zhou, & Lehmann, 2016), it is now known that GPR55 and cannabinoid receptors share limited similarities in their sequences (Baker, Pryce, Davies, & Hiley, 2006) and GPR55 does not possess the classical endocannabinoid binding pocket (Moriconi et al., 2010). PEA has been suggested to be an *in vitro* agonist of GPR55 at low concentrations (EC_{50} 4 nM) (Cantarella et al., 2011; Ryberg et al., 2007) and several effects of PEA are supposed to depend on GPR55 binding (Borrelli et al., 2015; Rinne et al., 2018), although further investigation is warranted.

In addition to GPR55, PEA showed affinity for a further orphan receptor, i.e., GPR119 (Overton et al., 2006), that codifies for a member of the rhodopsin subfamily of G-protein-coupled receptors, mainly expressed in pancreas and in the gastrointestinal tract. However, the role of GPR119 in the action of PEA has still to be clarified.

8.2. Indirect molecular targets

It is today well recognized that PEA has no affinity for CB1 or CB2 receptors, although it can indirectly activate them by the entourage effect. In fact, PEA may act as a false substrate for AEA hydrolytic enzyme leading to an increased cannabinoid receptor-mediated signaling (Petrosino et al., 2016; Petrosino & Di Marzo, 2017). Indeed, the administration to mice of the CB1 receptor antagonist, SR141716, partially counteracted the anti-hyperalgesic effects of PEA in a chronic constriction injury model of neuropathic pain (Costa et al., 2008).

PEA may also increase CB2 receptor mRNA and protein levels following PPARα activation (Guida et al., 2017), and modulate CB1- and/or CB2-mediated signaling by targeting the GPR55 protomer in the GPR55/CB1 or GPR55/CB2 heterodimers (Balenga et al., 2014; Martínez-Pinilla et al., 2014; Martínez-Pinilla et al., 2019).

Finally, PEA may indirectly activate the TRPV1 channel, as shown by its ability to potentiate the AEA-induced TRPV1-mediated calcium influx in the HEK293 cell line (De Petrocellis et al., 2001) and by the ability of the specific TRPV1 antagonist capsazepine to partially counteract the anti-hyperalgesic effects of PEA in a murine model of neuropathic pain (Costa et al., 2008).

9. Preclinical studies on pain control

To date, several preclinical studies have been performed in different animal models, in order to investigate the protective effects of PEA, with special reference to pain control. Three main formulations have been investigated, i.e., naïve, micronized and ultramicronized PEA.

In this setting, PEA has been widely investigated in combination with other drugs (mainly analgesics) since this represents the most reliable clinical strategy to be exploited in chronic pain. However, a relevant number of studies in which PEA has been investigated as single agent is also available. This approach is instrumental to explore the protective effects of PEA net of the effects of other drugs, as well as to identify dosages and schedules to be modulated in the drug combination studies.

With a focus on ultramicronized PEA, the main studies are summarized in Table 3 and briefly discussed in the following paragraphs.

9.1. Ultramicronized PEA as a single agent

The study of Impellizzeri et al. (Impellizzeri et al., 2014) pointed out the ability of oral micronized or ultramicronized PEA (10 mg/kg for both

Table 3
Ultramicronized PEA (umPEA) in preclinical studies.

Authors	Model	Treatment*	PEA effects
PEA single agent			
Impellizzeri et al., 2014	CAR-induced paw edema rats.	Non micronized, micronized, umPEA: 10 mg/kg p.o. or i.p., single dose 30 min before CAR injection.	*p.o. administration:* reduction of the accumulation of infiltrating inflammatory cells and reduction of the increased myeloperoxidase activity in micronized and umPEA treated animals. *i.p administration:* all the formulations were able to improve both parameters.
Petrosino et al., 2018	CAR-induced paw edema rats.	umPEA (10 mg/kg p.o., single dose 30 min before CAR injection) vs no treatment.	Improvement of paw inflammation, thermal hyperalgesia and tissue damage in umPEA treated animals.
Fusco et al., 2017	Distal tibia fractured CD-1 mice (i.e. CRPS-I).	Micronized (300 mg/kg) and umPEA (600 mg/kg) p.o. daily, 1 h after surgery for 28 days.	Improved healing process, fracture recovery and fibrosis score, decreased mast cell density, nerve growth factor, matrix metalloproteinase 9 and cytokine expression in umPEA treated animals.
PEA in association with standard analgesic drugs			
Di Cesare Mannelli et al., 2018	Healthy male Sprague-Dawley rats.	*Morphine tolerance study:* Pre-emptive umPEA (30 mg/kg p.o. daily (in the evening) from day −8 to day 0 and from day 1 to the end of the experiment. Morphine (10 mg/kg, s.c.) from day 1 until the development of tolerance) in the morning, 16 h after PEA.	Increase of the responsiveness to morphine by pretreatment with umPEA. On day 7, rats pretreated with PEA and morphine showed a delay in the onset of tolerance until day 12. The extension of morphine response was promoted by umPEA also in the absence of the pretreatment although pretreatment was needed to increase the efficacy of morphine during the first days.
		Potentiation of morphine analgesia: Pre-emptive umPEA as in the Morphine tolerance study. Increasing dose of morphine (5–100 mg/kg s.c.) and variable doses of umPEA (30–120 mg/kg p.o.) from day 1 to the end of the experiment.	The same pain threshold increase was achieved when preemptive PEA was associated to a combinatorial acute treatment with morphine and PEA. As a representative data: day 17: the magnitude of analgesia induced by 100 mg/kg morphine was obtained by combining 13 mg/kg of morphine with 120 mg/kg of PEA.
Micheli et al., 2024	Chronic constriction injury (CCI) model of neuropathic pain in rats.	*Morphine tolerance study:* pre-emptive umPEA 30 mg/kg p.o. (day −8 to day 15) + morphine 10 mg/kg s.c. (day 1–15).	Rats showed a delayed onset of tolerance till day 11.
		Potentiation of morphine analgesia: pre-emptive umPEA 30 mg/kg p.o. (from day −8 to day 23); increasing morphine 5–7 mg/kg s.c. (day 1–23); increasing acute PEA 30–60 mg/kg p.o. (day 8–23).	At, day 8, rats treated with the PEA and morphine showed a higher pain threshold compared to rats treated with morphine alone, despite the 30% lower dose of morphine used in combination treatment). The oral administration of PEA, according to the different schedules used, was able to reduce both GFAP and Iba-1 immunoreactivity observed when animals were untreated or treated with morphine alone. The number of mast cells was significantly reduced in animals treated with PEA alone (high dose) or in combination with morphine.
		Reduction of CCI-induced hyperalgesia: pre-emptive umPEA 30 mg/kg p.o. (from day −8 to day 4) and 60 mg kg-1 p.o. (days 5–23) + increasing acute PEA 30–120 mg/kg p.o. (day 3–23).	Preemptive PEA improved the weight tolerated by the animals on the ipsilateral paw compared with untreated animals from day 1 to the end of the experiment.
Micheli et al., 2022	Health male Sprague-Dawley rats.	*Morphine tolerance study:* pre-emptive umPEA (30 mg/kg p.o. daily (in the evening) from day −8 to day 29; increasing acute PEA 60–120 mg/kg p.o. (day 6–29); tramadol 20 mg/kg s.c. (day 1–29) or oxycodone 0.5 mg/kg s.c. (day 1–29).	Pre-emptive and continuative treatment with umPEA delayed the onset of opioid tolerance and enhanced opioid analgesia when it was acutely administered in association with tramadol or oxycodone. A decrease in astrocyte activation in the dorsal horn of the spinal cord and a modulation of IL-6 and serpin-A3 mRNA expression levels were also observed.
		Potentiation of morphine analgesia: Pre-emptive umPEA (30 mg/kg p.o. daily (in the evening) from day −8 to day 31; increasing acute PEA 39–90 mg/kg (day 16–31); increasing tramadol 15–50 mg/kg s.c. (day 1–31); increasing oxycodone 0.3–1 mg/kg s.c. (day 1–31); increasing acute PEA 30–90 mg/kg p.o. (day −8-31 or day 16–31).	The preemptive and co-administration of umPEA with oxycodone or tramadol significantly reduced the doses of both drugs useful to maintain the planned pain threshold (i.e. 90 +/− 10 g). As a representative data: day 4: oxycodone 0,3 mg/kg when associated to PEA vs 0.5 mg/kg when single agent; tramadol 15 mg/kg when associated to PEA *vs* 18 mg/kg when single agent.
Peritore et al., 2020	Sciatic Nerve Injury (SNI) model.	umPEA 5 mg/kg p.o. (day 1–14); paracetamol 30 mg/kg p.o. (day 1–14).	umPEA + paracetamol significantly reduced thermal hyperalgesia compared with both drugs administered as single agents. Reduction in mast cell activation, c-Fos and NGF expression, neural histological damage, cytokine release, and apoptosis was also observed with the combination treatment.
PEA as revertant of neuropathy induced by anticancer agents			
Di Cesare Mannelli, Pacini, et al., 2015	OHP-induced CIPN (male Sprague-Dawley rats, 2.4 mg kg-1 OHP administered i.p. for 5 consecutive days q week for 3 weeks).	umPEA 10 or 30 mg/kg acutely i.p. on day 21 or daily starting from the first day of OHP administration up to day 20.	umPEA 30 mg/ kg, single administration, reduced OHP-dependent pain. umPEA 30 mg/kg daily i.p. for 21 days prevented lowering of pain threshold and increased pain on suprathreshold stimulation. The normalization of the electrophysiological activity of the spinal nociceptive neurons was reported.
Cristiano et al., 2022	PTX-induced CIPN (CD1 male mice, 8 mg/kg PTX administered i.p. days 1,3,5 and 7 for one week).	umPEA 30 mg/kg p.o. for 7 days from one hour after the last administration of PTX.	umPEA reduced the development of hypersensitivity by reduction of levels of spinal and hippocampal pro-inflammatory cytokines.

CAR, carrageenan; CIPN, chemotherapy-induced peripheral neurotoxicity; CRPS-1, Complex regional pain syndrome type 1; i.p., intraperitoneal; NGF, nerve growtelh factor; MPO, myeloperoxidase; p.o., per os; s.c., subcutaneous; OHP, oxaliplatin; PTX, paclitaxel.

* Only doses and posology of investigated agents are reported. For detailed information on controls and specific treatment groups, refer to the text and/or to the original articles.

formulations) to significantly down-modulate inflammation in response to subplantar CAR injection into the rat paw. No significant effect was observed following oral administration of naïve PEA (10 mg/kg). On the contrary, the three formulations showed the substantial same efficacy in decreasing the effect of subplantar CAR injection when the intraperitoneal route was used. Overall, the findings highlighted the advantage provided by smaller particle size with regard to oral administration.

A subsequent study (Petrosino et al., 2018) confirmed and further investigated the effect of oral ultramicronized PEA in the paw CAR-injected rat model. Ultramicronized PEA (10 mg/kg) was shown to counteract CAR-induced signs of inflammation (paw edema) and pain (thermal hyperalgesia) within the first two hours from PEA administration. The histological damage and the neutrophil/mast cell infiltration in the paw were also reduced. In addition, ultramicronized PEA decreased the CAR-induced increase in cytokine release, nitrotyrosine formation, iNOS and COX-2 expression, IκBα degradation and NFκB p65 nuclear translocation in paw tissues. COX-2, manganese superoxide dismutase (MnSOD) and iNOS expression were also evaluated in the spinal cord. Ultramicronized PEA significantly counteracted both the CAR-induced decrease of MnSOD and upregulation of COX-2 and iNOS expression at the spinal level. Moreover, the decrease of spinal IκBα and the nuclear translocation of the NFκB p65 at L4/L6 level were prevented by the oral administration of ultramicronized PEA.

In a murine model of post-fracture complex regional pain syndrome type 1, a four-week daily oral administration of micronized or ultramicronized PEA (10 mg/kg) was shown to control pain (as measured by mechanical allodynia and thermal hyperalgesia), decrease early inflammatory responses (i.e., mast cell hyperplasia and NGF expression in the inflamed tissues) and improve fracture healing as well as bone remodeling during the late phase (Fusco et al., 2017).

The ability of ultramicronized PEA (10 mg/kg/day, i.p. for 15 days) to mitigate pain-induced sensory and cognitive impairments was recently investigated in a mouse model of neuropathic pain obtained by spared nerve injury (Boccella, Cristiano, Romano et al., 2019). Improvement of pain behavior (as measured by mechanical allodynia and thermal hyperalgesia) and pain-related memory deficit was observed and found to rely on PPARα-mediated restoration of glutamatergic transmission homeostasis. Interestingly, the analgesic effect of PEA was obtained even when it was administered 15 days after the spared nerve injury, thus in a condition that may be comparable to an advanced stage of human neuropathy.

Indeed, a further study by the same group investigated and confirmed the role of metabotropic glutamate receptors 5 and 8 in mediating the effects of ultramicronized PEA on pain-associated cognitive decline (Boccella, Morabese, Iannotta et al., 2019) in the neuropathic rat model previously described and treated accordingly (Boccella, Cristiano, Romano et al., 2019). Despite their opposite roles (i.e., excitatory/inhibitory) these receptor subtypes were found to be required in order to promote the neuroprotective effect of ultramicronized PEA in neuropathic pain (Boccella, Marabese, et al., 2019).

Overall, these studies provide relevant information on the *in vivo* efficacy of ultramicronized PEA monotherapy in counteracting inflammation and pain in animal models. In addition, these studies are also highly informative about the dynamic events following the PEA administration. Altogether, these findings represent an important and solid basis for more complex and advanced preclinical as well as clinical studies.

9.2. Ultramicronized PEA in association with standard analgesic drugs

As it will be more deeply discussed in the clinical section of this review, first- and second-line treatment of chronic pain is based on a variety of analgesic drugs. Although belonging to different classes, all of them are characterized by relevant side effects, ranging from sedation and somnolence (typical of antidepressants or anticonvulsants) to tolerance and dependance (mainly associated to opioid overdose). A nutritional intervention based on bioavailable PEA formulations to be used in conjunction with these drugs holds the promise to reduce dosages and consequently side effects of standard analgesics, while maintaining their efficacy. Several studies addressed this issue in the preclinical setting and will be briefly discussed below.

The long-term use of opioids in the treatment of chronic pain leads to the decrease of the analgesic effects due to the development of tolerance. Therefore, increasing doses are needed to maintain the antinociceptive efficacy. Our group widely investigated the potential role of PEA micron-size formulations in increasing the antinociceptive effect of morphine while reducing the development of tolerance by lowering the effective dose.

We have initially shown that the daily subcutaneous administration of micronized PEA (30 mg/kg) in healthy rats combined with intraperitoneal morphine (10 mg/kg) successfully delayed the onset of morphine tolerance compared to controls (Di Cesare Mannelli, Corti, Micheli, Zanardelli, & Ghelardini, 2015). Subsequently, the preemptive (i.e., from day -8 to day 0) and concomitant (from day 0 to the end of the experiments) oral administration of 30 mg/kg ultramicronized PEA increased the acute antinociceptive efficacy of subcutaneously administered morphine (10 mg/kg) and delayed the development of morphine tolerance in healthy male Sprague-Dawley rats (Di Cesare Mannelli, Micheli, Lucarini, & Ghelardini, 2018). In fact, rats treated with morphine alone, needed increasing doses of morphine (from 5 to 100 mg/kg over 17 days of daily treatment) to maintain a significant analgesia, while rats treated with ultramicronized PEA and morphine achieved the same extent of analgesia when preemptive PEA (30 mg/kg, daily) and subsequent acute treatment with morphine (5–20 mg/kg, s.c.) together with PEA (30–120 mg/kg, p.o.) was used (Di Cesare Mannelli et al., 2018).

The above data were obtained in healthy rats. Interestingly, the preemptive and morphine concomitant administration of ultramicronized PEA decreased the effective dose of morphine, delayed the onset of morphine tolerance and improved the analgesic efficacy of the opioid in a chronic constriction injury (CCI) model of neuropathic pain in rats (Micheli et al., 2024). Morphine and ultramicronized PEA were administered according to fixed (i.e., 10 mg/kg s.c. and 30 mg/kg p.o., respectively) or increasing doses (i.e., 5–35 mg/kg s.c. and 30–120 mg/kg p.o.), in relation to the different schedules. To characterize the underlying mechanisms, a series of biomolecular investigations were performed. GFAP and Iba-1 were studied by immunofluorescence to evaluate the activation of spinal microglia and astrocytes. Both markers were increased in CCI untreated or morphine treated animals in a PEA-sensitive manner. Also, the observed increased density of endoneural mast cells within the sciatic nerve of morphine-treated and untreated CCI rats was significantly reduced by the administration of ultramicronized PEA. The decrease of mast cell degranulation, evaluated by studying plasma levels of histamine and *N*-methylhistamine metabolite, was mainly observed at intermediate-high doses of ultramicronized PEA, with or without morphine (Micheli et al., 2024).

Based on these findings, we investigated the mechanism(s) by which ultramicronized PEA could delay the opioid tolerance in vitro (Toti et al., 2023). The hypothesis was that PEA could control the crosstalk between mast cells and glial cells, the latter known to be involved in opioid tolerance. Using a validated model of mast cells (i.e., the rat basophilic leukemia cells RBL—2H3), the pre-treatment with PEA (100 μM, 18 h) was shown to prevent the increase in mast cell degranulation induced by morphine (30 μM for 30 min). The non-toxic concentrations of PEA and morphine (100 μM and 30 μM, respectively) were selected according to preliminary tests. Results showed that ultramicronized PEA was able to long-lasting down-modulate mast cell degranulation, leading to a significant decrease in released histamine, as well as a reduction in the expression of cytokines (such as CCL2) and chemokines (such as TNF-α) mRNA when compared to the control group. A number of genes expressed by astrocytes (i.e., GFAP,

EAAT2, Serpina3n, IL-1β, IL-6, CCL2) were studied to evaluate the effect (s) of ultramicronized PEA pre-treated mast-cell secretome. Morphine treatment did not alter the expression of these genes on astrocytes, whereas control astrocytes incubated with control mast cell medium showed a significant increase in the expression levels of the study genes, with the exception of EAAT2. However, pre-treatment of mast cells with ultramicronized PEA reversed the increased expression levels of all the investigated genes, with the exception of GFAP that instead further increased. When astrocytes were treated with morphine and incubated with control mast cell media, a significant increase in the expression of all the study genes was observed. The incubation of astrocytes with cell medium from PEA pre-treated mast cells significantly reversed gene expression except for GFAP. Overall, this study showed that ultramicronized PEA was able to significantly down-modulate both morphine-induced mast cell degranulation and the expression of inflammatory and pain-related genes from astrocytes challenged with mast cell medium, suggesting that PEA may delay morphine tolerance through the regulation of mast cell-astrocyte crosstalk (Toti et al., 2023).

The possible beneficial effect of co-administering PEA with other opioids was also evaluated. In particular, the ability of ultramicronized PEA in controlling analgesia and tolerance induced by oxycodone and tramadol was investigated in healthy male Sprague-Dawley rats (Micheli et al., 2022). The treatment schedule was similar to that performed with the ultramicronized PEA and morphine combination described in Micheli et al. (Micheli et al., 2024). The oral daily preemptive and continuative treatment with ultramicronized PEA (30 mg/kg) delayed the onset of opioid tolerance and enhanced opioid analgesia when it was acutely administered in association with daily subcutaneous tramadol (20 mg/kg) or oxycodone (0.5 mg/kg) (Micheli et al., 2022). In addition, ultramicronized PEA showed antinociceptive effects on tolerant rats, thus suggesting the potential association of PEA with opioids to obtain a stable and long-lasting analgesia. Interestingly, in the groups treated with oxycodone or tramadol alone, it was necessary to increase the dose from 0.3 mg/kg up to 1 mg/kg and from 15 mg/kg up to 50 mg/kg, respectively, from day 1 to day 31. On the contrary, the acute oral co-treatment with ultramicronized PEA (120 mg/kg) allowed to obtain the same degree of analgesia without the need to increase the dose of oxycodone or tramadol (Micheli et al., 2022). A biomolecular investigation performed at the end of the behavioral experiments and when tolerance to the antinociceptive effect of opioids was still present (i.e., day 29), showed that the combined use of ultramicronized PEA and opioids led to a decreased astrocyte activation in the dorsal horn of the spinal cord (i.e., an index of the development of opioid tolerance), as indicated by the reduced GFAP fluorescence (Micheli et al., 2022). Moreover, ultramicronized PEA administration was able to reduce the tramadol- and oxycodone-induced increase of mRNA expression level for IL-6 and Serpin-A3, respectively (Micheli et al., 2022). A study by the group of Cuzzocrea (Peritore et al., 2020) investigated the effects of ultramicronized PEA in combination with paracetamol in a rat model of sciatic nerve injury. Daily low doses of ultramicronized PEA and paracetamol (i.e., 5 mg/kg and 30 mg/kg, respectively), were orally administered for 14 days after the sciatic nerve injury surgery. The drug combination showed promising results by reducing hyperalgesia, mast cell number, c-Fos and NGF expression, degree of histological nerve damage, proinflammatory cytokine level, and apoptosis. The authors also suggested that ultramicronized PEA exerted a synergistic effect with regard to the paracetamol-induced reduction of two markers of astrocyte and microglial activation, i.e., GFAP and Iba-1 (Peritore et al., 2020). Moreover, the mechanisms of the anti-inflammatory effects of this drug combination were also investigated through the effect on the NFκB pathway. The degradation of IkBα and the nuclear translocation of NFκB were counteracted only when ultramicronized PEA was added to paracetamol. In addition, the drug combination led to a decrease of COX-2-dependent prostaglandin E2 (COX-2/PGE2) release (Peritore et al., 2020).

Overall, these data suggest an additive and stable antinociceptive effect of combining ultramicronized PEA with opioids or paracetamol. Interestingly, when combined with the studied opioids (i.e. morphine, tramadol, oxycodone), ultramicronized PEA also allow for tolerance delay. The preemptive administration of ultramicronized PEA (i.e. 8 days starting from the day of the CCI surgery or from the first day of the paw pressure test in health rats) followed by the acute association of opioids and ultramicronized PEA, emerged as a key factor and might suggest the clinical advantage of pretreatment (and combined) administration regimen in the clinical setting.

9.3. Ultramicronized PEA protects against anticancer drug-induced neuropathic pain

A number of anticancer agents, traditionally coordination complexes of platinum, taxanes, Vinca alkaloids, bortezomib, but also more recently approved drugs, such as immune checkpoint inhibitors (Farina, Villagrán-García, & Honnorat, 2023), the anti-CD30 antibody-drug conjugate brentuximab vedotin (Velasco, Domingo-Domenech, & Sureda, 2021), or the anti-GD2 monoclonal antibody dinutuximab (Mastrangelo et al., 2021), induce chronic peripheral neuropathy as a side effect. This occurrence strongly impairs the quality of life of patients, especially of those who are substantially cured by anticancer treatment and whose expectation of life is similar to that of the healthy population (Burgess et al., 2021). To date, despite many efforts, no therapeutic strategy has been shown to successfully reverse this side effect and thus no drug has been approved by international drug regulatory agencies. Due to the neuroprotective and pain-relieving functions of PEA, some research groups, including ours, have investigated the potential ability of ultramicronized PEA to counteract this side effect (Table 3).

In an oxaliplatin-induced neuropathic pain rat model, ultramicronized PEA (10 mg/kg or 30 mg/kg) was administered acutely i.p. on day 21 or daily for 20 days starting from the oxaliplatin first administration (Di Cesare Mannelli, Pacini, Corti et al., 2019). The neuropathic pain model was obtained by administering oxaliplatin 2.4 mg/kg daily for 5 consecutive days a week for 3 weeks. This dose corresponds to the human dosage, based on the Km factor 37 for the conversion of animal doses to the Human Equivalent Dose (Freireich, Gehan, Rall, Schmidt, & Skipper, 1966). After daily administration, oxaliplatin reached a cumulative dose of 36 mg/kg, i.e., 1332 mg/m^2, that corresponds to the clinical cumulative oxaliplatin dose causing chronic neuropathy. On day 21, a single administration of 30 mg/kg ultramicronized PEA reduced pain induced by mechanical and thermal stimuli. Moreover, the repeated treatment with PEA for 20 days significantly reduced oxaliplatin-induced hypersensitivity and significantly relieved motor alterations. The neuroprotective effects of PEA were evidenced by the *ex vivo* evaluations of dorsal root ganglia, peripheral nerves and spinal cord. The prevention of glia-activation was also observed after repeated administrations of ultramicronized PEA (Di Cesare Mannelli, Pacini, et al., 2015).

More recently, Cristiano et al. (Cristiano et al., 2022) investigated the ability of ultramicronized PEA to reduce paclitaxel-induced peripheral neuropathy (8 mg/kg i.p. daily for 7 days) and associated mood disorders in rats. Ultramicronized PEA (30 mg/kg) was administered p.o. one hour after the last paclitaxel administration. Results showed that PEA was able to reduce the development of pain hypersensitivity and this effect was associated with the reduction of pro-inflammatory cytokines in spinal and hippocampal regions as well as antidepressive and anxiolytic effects.

The mitigation by PEA of the painful and quality of life limiting neuropathy due to neurotoxic anticancer agents is also supported by mechanistic data that have been collected with naïve PEA (Donvito, Wilkerson, Damaj, & Lichtman, 2016; Elfarnawany & Dehghani, 2022). Although data on this potential use of PEA are still preliminary, the rationale appears solid, and it can be reasonably considered successful.

10. Clinical studies

Due to the growing number of preclinical studies demonstrating the pain-relieving properties of PEA, interest in the clinical application of micron-sized PEA formulations has grown accordingly. Several double-blind, open label and N-of-1 randomized trials have been performed in the last 10–15 years, with different formulations of PEA being administered either as a single or an add-on nutritional intervention.

However, no clinical trial has been designed to investigate if one formulation (i.e. naïve, micronized, ultramicronized) is clinically superior on the other. Although pharmacokinetic findings argue in favor of the superior bioavailability of micron-size formulations, the current lack of comparative clinical studies does not provide a solid base to assess differences in terms of efficacy and/or side effects among the available PEA formulations.

An interesting piece of information about the clinical effect of PEA emerged from the *post-hoc* analysis performed by Cruccu et al. (Cruccu, Stefano, Marchettini, & Truini, 2019) on a previously published placebo-controlled study (Guida et al., 2010). The analysis allowed to estimate the "number needed to treat" (NNT), i.e., a measure depicting the effectiveness of an intervention. The percentage of patients who manifested at least 50% pain relief in response to the daily supplementation with micronized PEA was calculated and NNT was found to be 1.7 (Cruccu et al., 2019). Interestingly, higher NNTs, i.e., 3.6, 6.3, 6.4 and 7.7 for tricyclic antidepressants, gabapentin, serotonin-norepinephrine reuptake inhibitors and pregabalin, respectively have been calculated (Finnerup et al., 2015).

In the available clinical trials, pain intensity was most commonly evaluated by the 10 cm Visual Analogue Scale (VAS) and 0–10 Numeric Rating Scale for Pain (NRS Pain) (Hawker, Mian, Kendzerska, & French, 2011), with other evaluation tools being also used, like the Neuropathic Pain Symptom Inventory (NPSI) (Padua et al., 2009), the Health questionnaire five dimensions (EQ-5D) (Rabin, Gudex, Selai, & Herdman, 2014) and the 12-Item Short-Form Health Survey (SF-12) (Ware, Kosinski, & Keller, 1996).

The most representative clinical trials investigating the clinical benefit and safety profile of dietary interventions with ultramicronized PEA, even by meta-analyses, are presented below.

10.1. Efficacy

Overall, the available clinical studies have been mainly designed to evaluate if supplementing PEA improved the efficacy of standard analgesics or otherwise allowed for the reduction of their frequency, duration or dose of administration. In the following sections, the main uncontrolled and controlled trials, as well as meta-analyses investigating the effect of ultramicronized PEA on chronic pain, mainly as an add-on regimen, will be briefly reviewed (Tables 4 and 5).

10.1.1. Uncontrolled clinical trials

A relevant number of uncontrolled, observational, retrospective or prospective clinical trials have been performed with ultramicronized PEA being administered as a supplementation to different analgesics. Overall, three of the reviewed studies were retrospective trials (Chirchiglia, Chirchiglia, & Signorelli, 2018; Marini et al., 2018; Schweiger et al., 2019) (Table 4).

The first studies that investigated the effect of ultramicronized PEA as a co-adjuvant to analgesic therapies date back to 2010–2011 and were performed in patients with chronic pain of different etiology (Desio, 2010; Desio, 2011; Desio, Bonadiman, Fusco, & Cenacchi, 2010). Ultramicronized PEA 1200 mg/day in two doses was administered for 30 days in combination with increasing doses of oxycodone (i.e., 5 mg daily, days 1–5 and 5 mg twice daily, days 6–30) in 20 patients with **low back pain** (Desio, 2011). The same daily fixed dose was administered for 45 days, either in combination with increasing doses of pregabalin (from 150 mg/die to 400 mg/day) in 30 patients affected by **diabetic neuropathy or post-herpetic neuralgia** (Desio, 2010) or in combination with carbamazepine (3 × 100 mg days 1–5, 3 × 200 mg days 6–10, 3 × 400 mg days 11–40) in 31 patients affected by **trigeminal neuralgia** (Desio et al., 2010). A significant reduction of disability and pain intensity as scored on VAS, together with an improvement in quality of life were shown. Importantly, the effects were observed even if the analgesic drugs were used at lower than effective doses, suggesting that ultramicronized PEA helped controlling pain and associated symptoms. Although these trials did not include mechanistic studies, the authors suggested that such potential additive or synergistic effect could be due to the activity of PEA on mast cells and microglia.

A large cohort of patients ($n = 610$) suffering from **uncontrolled chronic pain of different etiology** was enrolled in an observational prospective study evaluating the effects of ultramicronized PEA used in addition to standard analgesic treatment (i.e., anticonvulsants plus opioids or anticonvulsants plus rescue drugs) (84% of cases) or as single agent (the remaining 16% of patients) (Gatti et al., 2012). The administration of ultramicronized PEA alone in this small percentage of patients was due to the discontinuation of standard therapy because of important side effects or treatment refusal. Ultramicronized PEA 1200 mg/day was administered for 3 weeks followed by 600 mg/day for 4 weeks. Results showed a marked decrease in the mean score of pain intensity from baseline to the end of treatment. No relationship was found between the effects of ultramicronized PEA and associated analgesic treatments. Interestingly, ultramicronized PEA used as a single agent was shown to be as effective as its combined use with analgesics.

A relevant number of studies was performed in patients with chronic back pain due to different pathological causes (Chirchiglia, Paventi, et al., 2018; Scaturro et al., 2020) including failed back surgery (Paladini et al., 2017).

Ultramicronized PEA has been evaluated in a prospective single-blind trial performed in patients with **low back pain related to nonsurgical lumbar radiculopathy** (Chirchiglia, Paventi, et al., 2018). Patients received a daily fixed combination of paracetamol/codeine (500 mg + 30 mg) for 7 days, followed by ultramicronized PEA (1200 mg/day) alone for 30 days. Patients who did not experience an improvement in pain or disability started a second 30-day cycle with ultramicronized PEA (600 mg/day) followed by a further 30-day treatment with paracetamol/codeine. A follow-up of 24 months was performed. Overall, 155 patients were evaluated, and results showed an improvement in pain and disability in those with mild to moderate pain. At the end of treatment, 74 % of those who underwent the second cycle of ultramicronized PEA and standard analgesic treatment experienced an improvement of disability even in case of severe pain (VAS > 7). These effects were maintained also at the two planned follow-up visits (12 and 24 months).

A prospective ($n = 30$)/retrospective ($n = 25$) trial investigated the effect of 6-month administration of ultramicronized PEA (600 mg twice daily) as an add-on nutritional intervention to tapentadol (100–500 mg according to the patient needs; prospective arm), compared to tapentadol alone (retrospective arm) in patients suffering from **chronic low back pain** (Passavanti et al., 2017). Paracetamol 1000 mg was used as rescue drug in case of pain exacerbation. Tapentadol is a centrally-acting synthetic analgesic whose exact mechanism of action is still partially unknown. Preclinical studies indicate tapentadol as a μ-opioid receptor agonist and a norepinephrine reuptake inhibitor (Tzschentke et al., 2007) and its analgesic effects in animal models are due to both these mechanisms. Results of the study by Passavanti (Passavanti et al., 2017) showed that combining ultramicronized PEA with tapentadol allowed for a superior effect on pain relief and improvement of disability. Interestingly, a reduction of the needed tapentadol dose, compared to tapentadol alone, was also observed.

Patients with **chronic low back pain** caused by intervertebral disc herniation were enrolled in a prospective observational study

Table 4
Efficacy of oral ultramicronized PEA (umPEA) in uncontrolled clinical trials.

Authors	Study design	Pain etiology	No. of patients	Treatment arm	PEA effects	Side effects
Desio et al., 2010	Open label	Trigeminal neuralgia	31	umPEA 1200 mg/day for 45 days in add-on to carbamazepine	Significant reduction in pain intensity, improvement of functional ability and quality of the sleep	Not reported
Desio, 2010	Open label	Diabetic neuropathy/ Postherpetic neuralgia	30	umPEA 1200 mg/day for 45 days in add-on to pregabalin	Significant reduction of pain intensity, improvement of the functional ability, with a better quality of the sleep	Not reported
Desio, 2011	Open-label	Low back pain	20	umPEA 1200 mg/day for 30 days in add-on to increasing doses of oxycodone (5 mg/day for 5 days and 10 mg/day for 25 days)	Significant reduction of pain intensity and improvement of functional ability. A better quality of sleep was reported by 80% of patients	Not reported
Gatti et al., 2012	Open-label	Chronic pain of different etiology	610	umPEA 1200 mg/day for 3 weeks, then 600 mg/day for 4 weeks in add-on to eventual standard analgesic treatment (antidepressants, anticonvulsants, opioids, non-steroidal anti-inflammatory drugs)	Significant decrease of pain intensity, maintained 6 months after discontinuation of umPEA treatment	Patients who completed the study did not report any treatment-related adverse events or serious adverse events.
Cocito et al., 2014	Prospective, Open-label	Diabetic neuropathy/ Traumatic neuropathy	30	umPEA 1200 mg/day for 40 days in add-on to pregabalin, gabapentin and/or tramadol	Significant improvement of pain, neuropathic pain intensity and quality of life	Not reported
Del Giorno et al., 2015	Observational (prospective vs retrospective)	Fibromyalgia	80	Prospective arm: umPEA 1200 mg/day for 1-month, followed by mPEA 600 mg/day for 2 months, in add-on to the existing therapeutic regimen with duloxetine and pregabalin ($n = 35$) Retrospective arm: duloxetine and pregabalin for 6 months ($n = 45$)	Significant improvement of pain symptoms, with a further reduction in the number of positive tender points compared to patients treated with duloxetine/pregabalin	None of the patients experienced side effects
Putzu, 2016	Open-label	Charcot-Marie-Tooth Neuropathy	22	umPEA 1200 mg/die for 80 days (20 days sublingual microgranules and 60 days tablets)	umPEA significantly improved the clinical symptoms of CMT neuropathy (pain, fatigue and painful cramps)	There were no adverse events related to treatment at any time during the course of the study
Dalla Volta, 2016	Open-label	Migraine with aura	50	umPEA 1200 mg/day for 3 months in add-on to analgesic treatment	Significant reduction in frequency, duration and intensity of migraine attacks and analgesics consumption. Thermographic patterns showed a reduction of hypothermia as well as of the response to trigger factors	1 woman withdrew after 1 month of treatment due to migraine worsening. None of the other patients reported treatment-related adverse events
Paladini et al., 2017	Prospective, Observational	Failed Back Surgery Syndrome	35	umPEA 1200 mg/day for 1 month followed by umPEA 600 mg/day for another month, in add-on to tapentadol (150 mg/day) and pregabalin (300 mg/day) therapy (started one month before umPEA introduction)	Further and significant decrease of pain intensity after umPEA treatment introduction	None of the patients experienced adverse events after um-PEA add-on to the standard treatment
Passavanti et al., 2017	Pilot, Observational	Low back pain	55	Prospective arm: umPEA 1200 mg/day in add-on to tapentadol (100–500 mg/day) for 6 months, plus paracetamol 1000 mg in case of pain exacerbation (n = 30) Retrospective arm: tapentadol (100–500 mg/day) for 6 months, plus paracetamol 1000 mg in case of pain exacerbation ($n = 25$)	Significantly higher reduction in pain intensity, in its neuropathic component and the degree of disability in umPEA group. umPEA allowed a reduction in tapentadol dose	Episodes of diarrhoea in 15% of patients in the prospective arm
Marini et al., 2018	Retrospective, Open-label	Temporo-Mandibular Joint (TMJ) arthralgia (in osteoarthritis patients)	12	umPEA 600 mg/day in add-on to Celecoxib 200 mg 2/day (morning and evening) for 4 days, followed by umPEA 600 mg/day as single agent for 2 weeks	Pain progressively decreased over time, with a significant reduction after the first 4 days and no significant pain at the end of treatment. Maximum mouth opening also improved	None of the participants reported any side effects related to treatment
Chirchiglia, Chirchiglia, & Signorelli, 2018	Retrospective, Observational	Nonsurgical lumbar radiculopathies	100	I cycle: umPEA microgranules 1200 mg/day for 10 days, followed by umPEA tablets 1200 mg/day for 20 days in add-on to paracetamol 500 mg plus codeine 30 mg/day for 4 days, and then as needed for 1 month total; II cycle: umPEA tablets 600 mg/day in add-on to paracetamol 500 mg	Significant decrease of average pain intensity after one month of therapy with a further improvement after the second cycle. Total success percentage was of about 80%, with a greater effect on patients with mild and moderate grade of pain	During the two cycles of therapy nobody reported any adverse events

(continued on next page)

Table 4 (*continued*)

Authors	Study design	Pain etiology	No. of patients	Treatment arm	PEA effects	Side effects
				plus codeine 30 mg/day for 4 days, and then as needed, for 30 days		
Chirchiglia, Paventi, Seminara, Cione and Gallelli, 2018	Prospective, Single-blind	Nonsurgical lumbar radiculopathies	155	I cycle: paracetamol/codeine 500/30 mg/day (1000–60 mg/day for patients with severe pain) for 7 days followed by umPEA 1200 mg/day for 30 days. II cycle: patients who experienced a persistence of pain or disability started again umPEA 600 mg/day for 30 days followed by paracetamol/codeine for further 30 days	umPEA treatment administered after paracetamol/codeine at low dosage was able to reduce pain and disability in all treated patients, albeit patients with severe pain showed an incomplete resolution of the latter. These effects were maintained at each follow 12 and 24 months after the beginning of the study.	Authors did not record any side effects or drug-drug interactions.
Schweiger et al., 2019	Retrospective, Observational	Fibromyalgia	407	umPEA 1800 mg/day for 10 days, 1200 mg/day for 20 days and 600 mg/day for 15 months as maintenance therapy, in add-on to concomitant pharmacological therapy	Significant improvement of pain intensity and quality of life	Mainly gastrointestinal side effects in 13.7% of patients (diarrhoea, dyspepsia, bloating, constipation, vomiting)
Stochino Loi et al., 2019	Pilot, Open-label, Single-arm, non-Randomized	Endometriosis	30	umPEA microgranules 1200 mg/day for 10 days, followed by PEAPol 400 mg + 40 mg 2/day for 80 days. Ketoprofen lysine salt sachet 80 mg 2/day was the only NSAID allowed	Significant improvement in chronic pelvic pain, deep dyspareunia, dysmenorrhea, dyschezia, as well as in quality of life and psychological well-being; significant reduction in the use of ketoprofen; chronic pelvic pain and dysmenorrhea maintained a statistically significant difference compared to baseline 30 days after treatment discontinuation	Only 2 patients withdrew from the study, 1 due to pelvic inflammatory disease and 1 to non-compliance. Treatment was well tolerated and adverse events were not observed
Papetti et al., 2020	Pilot, Open-label	Migraine without aura (pediatric population)	70	umPEA 600 mg/day for 12 weeks. NSAIDs (i.e. ibuprofen, paracetamol, diclofenac sodium, ketorolac) were used as needed, during acute attack	Significant reduction in the number and intensity of monthly attacks after 12 weeks of umPEA treatment and significant reduction of monthly assumption of rescue drugs	One patient developed nausea and bloating
Scaturro et al., 2020	Prospective, Observational	Low back pain	120	umPEA 1200 mg/day in add-on to daily functional rehabilitation session and a decontracting massage for 20 days, followed by umPEA 600 mg/day for 40 days in add-on to standard therapy performed (e.g. gabapentinoids or opioids)	Significant decrease in pain intensity; improvement of the quality of life (i.e., increased physical and mental components); decreased pain-dependent disability	Not reported

investigating the effect of ultramicronized PEA in combination with a rehabilitative therapy (Scaturro et al., 2020). Ultramicronized PEA 600 mg twice a day *plus* standard analgesic therapy (mainly gabapentinoids or opioids) was associated to a daily functional rehabilitation session for 20 days, followed by ultramicronized PEA 600 mg/day for 40 days. Overall, 120 patients were evaluated, and their average pain intensity scores decreased significantly with a concomitant improvement in both physical and mental components of the quality of life as well as decreased pain-dependent disability.

An observational study further evaluated the efficacy of ultramicronized PEA, used as an add-on therapy for the management of chronic pain in pain-resistant patients affected by **failed back surgery syndrome** (Paladini et al., 2017). After an interval from surgery in which pain persisted, patients were treated with tapentadol (150 mg/day) and pregabalin (300 mg/day). One month after standard treatment had started, ultramicronized PEA was added (1200 mg/day subdivided in two doses during the first month and 600 mg/day in the following month). The 35 patients enrolled had already been treated with tapentadol and pregabalin in the month before surgery. Pain was evaluated by VAS at baseline and monthly during the three months of treatment. Results showed that after one month of treatment with standard analgesics only, VAS score decreased significantly although no significant subjective improvement in pain symptoms was recorded. The addition of ultramicronized PEA in the subsequent two months contributed to a further and significant decrease in pain intensity.

A prospective open-label clinical trial investigated the efficacy of ultramicronized PEA as an add-on treatment in patients with **diabetic or traumatic neuropathic pain** (Cocito, Peci, Ciaramitaro, Merola, & Lopiano, 2014). Overall, 30 patients were orally administered with 1200 mg/day ultramicronized PEA as a supplementation to other analgesic drugs (pregabalin, gabapentin, and/or tramadol), whose dose was maintained stable during the whole study period. The mean pain score on VAS significantly improved within the first 10 days of treatment and further decreased after 40 days of PEA dietary intervention. In addition, NPSI total score and EQ-5D improved from baseline to the end of treatment.

Ultramicronized PEA was also investigated in two trials performed in adult (Dalla Volta, 2016) and pediatric (Papetti et al., 2020) patients affected by **migraine without aura**.

A total of 50 patients were included in an open label pilot study and received sublingual ultramicronized PEA 600 mg, twice daily for 3 months (Dalla Volta, 2016). A significant reduction of the monthly frequency of migraine attacks was observed in 69.3% of patients. Moreover, a reduction of migraine intensity and intake of analgesics was observed in 71.4% and 59.1% of patients, respectively. Also, 72.3% of patients experienced a less severe complexity of the migraine accompanying symptoms as well as a reduction of the response to trigger factors.

Similarly, 70 pediatric patients were recently included in a prospective trial to evaluate the efficacy and tolerability of ultramicronized PEA (600 mg/day in two doses for three months) as a prophylactic nutritional intervention for **migraine without aura** (Papetti et al., 2020). In

Table 5
Efficacy of oral ultramicronized PEA (umPEA) in controlled clinical trials.

Authors	Study design	Pain etiology	No. of patients	Treatment arm	Comparator arm	PEA effects	Side effects / Adverse events
Andresen et al., 2016	Double-blind, randomized	Spinal cord injury	73	umPEA 1200 mg/day for 12 weeks in add-on to standard therapy ($n = 36$).	Placebo twice/daily for 12 weeks in add-on to standard therapy ($n = 37$)	No difference between treatment arms	Adverse events in 9.6% of patients (paralytic ileus, cholecystolithiasis, and erysipelas, fungus infection); umPEA was not associated with more adverse effects than placebo
Chirchiglia, Cione, Caroleo et al., 2018	Prospective, Single-blind	Migraine with aura	40	umPEA 1200 mg/day for 90 days and NSAIDs as needed (ibuprofen, diclofenac sodium, or nimesulide) during acute attack (n = 20)	NSAIDs as needed (ibuprofen, diclofenac sodium, or nimesulide) during acute attack for 90 days ($n = 20$)	Significant and time-dependent pain relief evident at 60 days and until the end of the study	No side effects or drug interaction adverse events
Evangelista et al., 2018	Open-label, Randomized	Carpal Tunnel Syndrome	42	Pre-operative phase: umPEA microgranules 1200 mg/day for 10 days, followed by umPEA tablets 1200 mg/day for 50 days; Post-operative phase: umPEA tablets 1200 mg/day for 60 days, followed by umPEA 600 mg/day for 30 days. Rescue drug were permitted ($n = 22$)	No treatment, apart from surgery, plus rescue drug as needed (n = 20)	At the end of the pre-surgery period there was a highly significant improvement in overall sleep quality with an increase of continuous sleep time and a reduction of sleep latency and disturbances, as well as a significant mitigation of painful symptoms in favor of the treated group	All patients completed the observational pre- and post-surgery periods without reporting any treatment-associated side effects
Ottaviani et al., 2019	Double blind, Randomized	Burning mouth syndrome	35	umPEA 1200 mg/day for 60 days ($n = 17$). (Concomitant medications allowed)	Placebo 2/day for 60 days ($n = 18$)	Statistically significant reduction of burning mouth sensation at the end of treatment in the umPEA group	No side effects

this case, the headache frequency was reduced by >50% per month in 63.9% of patients and a significant reduction in the number of monthly attacks was reported. Also, the mean intensity of the attacks and the percentage of patients with severe attacks significantly decreased. Interestingly, the monthly use of drugs for the attacks, was also significantly reduced. Overall, the authors found that patients who had at least a 50% of attack frequency reduction after treatment were supplemented with ultramicronized PEA at 40 mg/kg dose (Papetti et al., 2020).

An observational retrospective/prospective trial was performed to evaluate the efficacy of the add-on supplementation of micronized and ultramicronized PEA to duloxetine (i.e., a selective serotonin and norepinephrine reuptake inhibitor antidepressant) combined with the anticonvulsant pregabalin in patients with **fibromyalgia syndrome** (Del Giorno, Skaper, Paladini, Varrassi, & Coaccioli, 2015). Patients treated with duloxetine and pregabalin for 6 months ($n = 45$) were retrospectively selected, while patients in the PEA group ($n = 35$) were prospectively enrolled and treated for 3 months with a mean daily dose of duloxetine and pregabalin of 36 mg and 49.2 mg, respectively, followed by ultramicronized PEA 600 mg/twice a day for one month and micronized PEA 300 mg/twice a day for two further months. The add-on administration of ultramicronized and micronized PEA resulted in a significant decrease of pain and number of tender points compared to duloxetine and pregabalin only.

A different approach was used in patients with chronic pelvic pain associated with **endometriosis**, a well-known estrogen-dependent chronic benign inflammatory disease with difficult management. In an open-label pilot study, ultramicronized PEA (600 mg/twice a day for 10 days) followed by co-micronized PEA/polydatin (400 mg + 40 mg twice a day for 80 days) was administered in 30 symptomatic women (Stochino Loi et al., 2019). Ketoprofen 80 mg was allowed maximum twice a day. A statistically significant decrease between baseline and the end of treatment was observed in the mean score of chronic pelvic pain, as well as in the severity of dysmenorrhea, dyspareunia, and dyschezia. Interestingly, it emerged that although one month after the end of ultramicronized/co-micronized PEA supplementation the mean scores of the study parameters slightly increased, chronic pelvic pain and dysmenorrhea mean scores were still significantly lower compared to baseline. A significant reduction in the use of ketoprofen at the end of the study was also reported.

Finally, an open label trial was performed in 22 patients affected by **Charcot-Marie-Tooth disease** (Putzu, 2016). Overall, patients belonging to four families, received sublingual ultramicronized PEA 600 mg twice daily (20 days) followed by ultramicronized PEA tablets 600 mg twice daily (2 months). A reduction of pain, fatigue and painful cramps was observed already after the first 20 days and a further improvement was reported at the end of treatment according to VAS score.

Although informative, these studies did not provide adequate evidence on the activity of ultramicronized PEA alone or as add-on to standard analgesic treatments. The main factor relates to their uncontrolled design. In fact, the lack of a control group (only two studies included a retrospective control group) does not allow to establish the real contribution of PEA in the reduction of pain intensity. In addition, some of these studies are retrospective and this further decreases their level of evidence (Oxford Centre for Evidence Based Medicine, 2024).

A further aspect that has to be taken into consideration is the heterogeneity of pain conditions and treatment duration. In fact, although all the discussed studies concern diseases that are recognized to cause persistent pathological pain (i.e. nociplastic, e.g. fibromyalgia, back pain, temporomandibular disorder; neuropathic, e.g. radiculopathies, diabetic neuropathy; nociceptive, e.g. spinal pain), such diseases may not provoke the same pain intensity as well as patients may not be affected by chronic pain equally (Cohen et al., 2021). These aspects may find an explanation in the variable duration of PEA treatment among these studies (range 19–180 days, mean 66.6 days) at the substantially same dose (i.e. 1200 mg/day in 14 out of 17 described studies).

However, it cannot be disregarded the high number of patients who have been enrolled in the above-mentioned studies (about 1800) that allow to consider PEA widely safe waiting for more rigorous efficacy trials.

10.1.2. Controlled clinical trials

Ultramicronized PEA has been investigated in a limited number of randomized or non-randomized controlled trials, most of which have been conducted in chronic pain of different etiology (Table 5).

Interestingly, one study is represented by N-of-1 randomized trial in a geriatric population affected by **chronic pain of different etiology** (i.e., degenerative due to osteoarthritis/spondylosis/radiculopathy; diabetic neuropathy; post-herpetic neuralgia; restless leg syndrome; post-trauma) (Germini et al., 2017). Patients were randomized to receive ultramicronized PEA (1200 mg daily, two periods of 3 weeks each with a 2-week washout interval) or placebo. The concomitant use of analgesics, when needed, was allowed. Seven out of the 11 patients completed the study. Overall, a statistically significant effect on pain intensity and functional impairment was observed in 3 out of 7 patients who completed the study.

Chirchiglia and colleagues (Chirchiglia, Cione, Caroleo et al., 2018) performed a single blind, controlled study in patients suffering from **migraine with aura** who experienced ≥2 attacks/month in the past year, according to ICHD-3 criteria. Forty patients were enrolled in two groups (20 patients each) and studied for up to 90 days. The treatment group received daily supplementation with ultramicronized PEA (1200 mg) in combination with NSAIDs (e.g., ibuprofen, diclofenac, nimesulide), while the control group received NSAIDs alone. Regardless the treatment group, the NSAID administration was limited to the acute attacks. The add-on supplementation with ultramicronized PEA resulted in a statistically significant and time-dependent pain relief, mainly observed at 60 days and until the end of the study. A decrease in the number of migraine attacks was also observed. Conversely, the treatment with NSAIDs alone, although able to induce a significant decrease in pain severity during the attacks, failed to modify pain intensity during the recurrence or the number of attacks per month (Chirchiglia, Cione, Caroleo et al., 2018).

As far as randomized trials are concerned, the effect of ultramicronized PEA on chronic pain was also compared to placebo (Andresen et al., 2016; Ottaviani et al., 2019) or no treatment (Evangelista, Cilli De Vitis, Militerno, & Fanfani, 2018).

Andresen et al. (Andresen et al., 2016) performed a randomized, double-blind, controlled multicentric study to investigate the effect of ultramicronized PEA (sublingual 600 mg twice a day for 12 weeks) as an add-on therapy *vs* placebo in patients with neuropathic pain due to **spinal cord injury**. Concomitant treatment with spasmolytics and analgesic drugs was allowed. The primary outcome was the change in mean neuropathic pain intensity from baseline to the study end on NRS. Overall, 68 patients were included in the primary analysis. No difference in the mean pain intensity between the experimental and placebo arms was observed and no effect of the add-on intervention was either shown on spasticity, insomnia, or psychological functioning. The study by Ottaviani et al. (Ottaviani et al., 2019) evaluated the effect of administering sublingual ultramicronized PEA in patients suffering from **burning mouth syndrome** with symptom intensity score >4 on NRS. Thirty-five out of 40 enrolled patients were eligible and thus randomized to receive ultramicronized PEA or placebo 600 mg twice daily for 60 days. Concomitant medications, including analgesic drugs, were allowed. A significant reduction of burning mouth sensation was reported at the end of the treatment in the supplemented group compared to the placebo.

Finally, the study by Evangelista et al. (Evangelista et al., 2018) compared the effect of ultramicronized PEA 600 mg twice daily administered peri-operatively ($n = 22$) *vs* no treatment ($n = 20$) in patients with **carpal tunnel syndrome**. Results showed a significant mitigation of pain and a significant improvement in the quality of sleep in patients treated with ultramicronized PEA compared to no treatment.

Three out of 4 of the above-described controlled trials were randomized and the design appear rigorous. Except for the study of Evangelista et al. (Evangelista et al., 2018) in which the administration of analgesic rescue drugs was permitted but not planned, the PEA treatment was always associated with standard analgesics in the experimental arm. As observed for the uncontrolled trials, also in this case, the source of pain was different among the studies, including spinal cord injury, migraine with aura, burning mouth syndrome and carpal tunnel syndrome surgery. Overall, PEA treatment lasted from 60 to 150 days with a mean of 97.5 days and the dose was 1200 mg/day. Andresen et al. (Andresen et al., 2016) did not show an advantage in the experimental arm compared with the control arm, although in the other three studies, improvements in terms of reduction of pain intensity and/or symptoms in relation to the different pain conditions was reported. Although including a substantial low number of patients, these trials suggest that ultramicronized PEA may represent a conceivable strategy as add-on to analgesics in the control of chronic pain.

10.1.3. Meta-analyses

Four meta-analyses focusing on trials evaluating the effect of PEA on chronic pain, regardless the exact nature of PEA formulations being used (Artukoglu, Beyer, Zuloff-Shani, Brener, & Bloch, 2017; Lang-Illievich et al., 2023; Paladini et al., 2016; Scuteri et al., 2022) were identified by the PubMed search. Overall, the results confirmed that oral supplementation with PEA exerted pain-relieving effects. Only a small overlap between the clinical studies included in the four meta-analyses was observed (Fig. 2) with this probably being due both to the different years in which the meta-analyses were performed and the different search strategies used by each meta-analysis. In agreement with the aim of this review, the meta-analysis of Artukoglu et al. (Artukoglu et al., 2017) will not be discussed since it does not include clinical trials performed with ultramicronized PEA.

Paladini and colleagues (Paladini et al., 2016) originally identified 26 studies, including some unpublished trials, and meta-analyzed the pooled raw data of 12 of them. Dietary supplementation with micronized and ultramicronized PEA to patients with chronic pain of different origin (e.g., lumbosciatalgia, carpal tunnel syndrome, radiculopathy, osteoarthritis, Herpes Zoster, as well as diabetic, post-traumatic and chemotherapy-induced neuropathies) was commonly associated with NSAIDs, opioids, or anticonvulsants. Three out of 12 studies were double-blinded, however, only one was published as a full paper

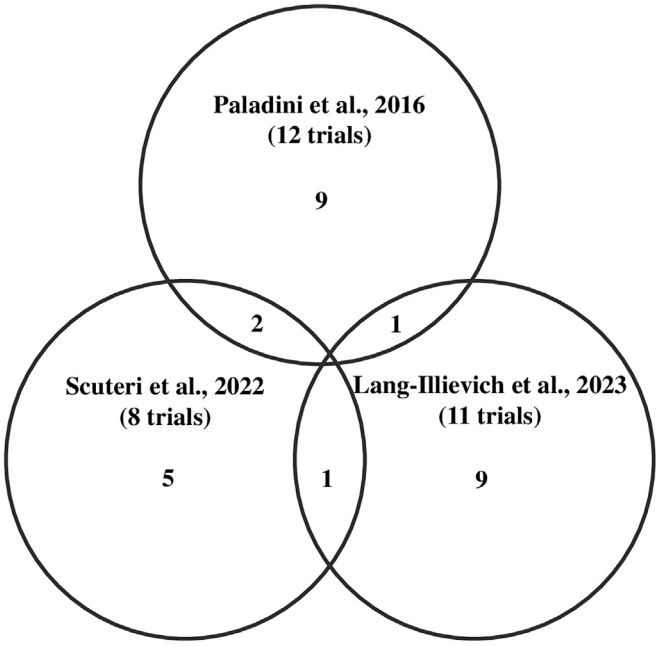

Fig. 2. Venn diagram displaying the number of overlapping clinical trials among three meta-analyses evaluating the contribution of PEA in chronic pain control. Overall, numbers take into account only the meta-analyzed trials.

(Guida et al., 2010), with the others being either published as abstract (Montella, Carotenuto, Orefice, & Orefice, 2014) or unpublished at the time of the analysis. The design of three further studies was open-label controlled randomized versus no treatment (an abstract by Assini et al., 2010 and an unpublished study) or physiotherapy (an abstract by Parabita et al., 2011). The remaining 6 studies were open-label trials, with two being unpublished and four published as full-papers (reviewed in the previous paragraphs: Gatti et al., 2012; Desio, 2011; Desio, 2010; Cocito et al., 2014). Ten out of twelve studies were performed with ultramicronized PEA, with the remaining investigating micronized PEA. Overall, PEA dose ranged from 300 mg/day to 1200 mg/day and duration of treatment varied from 21 to 365 days. The presence of abstracts and unpublished data represents a relevant limitation of this meta-analysis, despite the statistical approach (i.e., analysis of the whole pooled raw data with Generalized Linear Mixed Model) was adequate. Moreover, to take into account the different contribution of PEA in studies with different design, "double-blind" was included as a covariate in the model, thus pain reduction was net of the placebo effect. Overall, 1484 patients were included, 80% treated with micron-sized PEA formulations and 20% controls. PEA treatment led to a significantly greater reduction in pain severity compared to control patients (1.04 points every 2 weeks with a 35% response variance explained by the linear model in the PEA group *vs* 0.20 points every 2 weeks with 1% response variance in the control group). Also, a pain score ≤ 3 was reported in 81% and 40.9% of patients after 2 months with or without PEA supplementation, respectively. Finally, the benefit of micron-size PEA supplementation was shown to be independent from either demographical feature (i.e., age and gender) or type of chronic pain.

A subsequent meta-analysis performed by Scuteri et al. (Scuteri et al., 2022) considered clinical trials on nociceptive, musculoskeletal and neuropathic pain. Ten trials were identified among the 2022 originally retrieved results and only 8 were eligible for quantitative analysis (Andresen et al., 2016; Cocito et al., 2014; Faig-Martí & Martínez-Catassús, 2017; Gatti et al., 2012; Paladini et al., 2017; Parisi, Ditto, Borrelli, & Fusaro, 2021; Passavanti et al., 2017; Scaturro et al., 2020). Two trials had been included in the meta-analysis by Paladini (Paladini et al., 2016) (Cocito et al., 2014; Gatti et al., 2012). Overall, a total of 933 patients were evaluated and the primary outcome was pain reduction on NRS and VAS. Two out of 8 studies (Andresen et al., 2016; Faig-Martí & Martínez-Catassús, 2017) were randomized double-blind trials and compared ultramicronized and unprocessed PEA, respectively, with placebo. The others were open label (Cocito et al., 2014) or observational (Gatti et al., 2012; Paladini et al., 2017; Parisi et al., 2021; Passavanti et al., 2017; Scaturro et al., 2020) trials. PEA (daily dose range 600 mg - 1200 mg) was administered as a stand-alone nutritional intervention or add-on to standard analgesics. In 6 out of 8 studies, ultramicronized PEA was administered (Andresen et al., 2016; Cocito et al., 2014; Gatti et al., 2012; Paladini et al., 2017; Passavanti et al., 2017; Scaturro et al., 2020). Overall, results showed a significantly superior effect of PEA over the control, although the high heterogeneity of the studies ($I^2 = 99\%$) and asymmetry of the funnel plot were indicative of publication bias. No data stratification and therefore no analysis on different PEA formulations were performed.

The meta-analysis by Lang-Illievich and collaborators (Lang-Illievich et al., 2023) has been very recently published and is the most comprehensive in terms of analysis and stratification of data. It included only double-blind randomized controlled trials performed in patients with chronic pain of several origins. The accurate screening of a total of 316 papers, allowed to select 11 studies (Andresen et al., 2016; Cobellis et al., 2011; Cremon et al., 2017; Faig-Martí & Martínez-Catassús, 2017; Marini, Bartolucci, Bortolotti, Gatto, & Bonetti, 2012; Murina, Graziottin, Felice, Radici, & Tognocchi, 2013; Orefice et al., 2016; Ottaviani et al., 2019; Pickering, Steels, Steadman, Rao, & Vitetta, 2022; Steels, Venkatesh, Steels, Vitetta, & Vitetta, 2019; Tartaglia et al., 2015). The control arm was represented by placebo in 9 out of 11 studies (Andresen et al., 2016; Cremon et al., 2017; Faig-Martí & Martínez-Catassús, 2017; Murina et al., 2013; Orefice et al., 2016; Ottaviani et al., 2019; Pickering et al., 2022; Steels et al., 2019; Tartaglia et al., 2015), while the remaining two studies used either ibuprofen (Marini et al., 2012) or placebo and celecoxib (i.e., three arms (Cobellis et al., 2011)) as control. The primary outcome of this meta-analysis was pain reduction on quantitative pain scales. The overall sample size was 774 patients. The authors rigorously took into consideration a series of critical issues due to differences among the 11 selected studies, mainly in terms of the (i) origin of chronic pain, (ii) daily doses of PEA (i.e., from 300 mg to 1200 mg, although >600 mg was the most preferred dose), (iii) duration of treatment (i.e., from 10 days to 12 months, although 2–3 month-duration was most represented) and (iv) formulations of PEA. Three out of the 11 trials were performed with ultramicronized (Andresen et al., 2016; Orefice et al., 2016; Ottaviani et al., 2019), one with micronized PEA (Marini et al., 2012), four with PEA co-micronized with polydatin (Cobellis et al., 2011; Cremon et al., 2017; Murina et al., 2013; Tartaglia et al., 2015), one with PEA obtained according to Briskey et al. (2020) (Pickering et al., 2022) and the remaining two with unprocessed (i.e., naïve) PEA formulations (Faig-Martí & Martínez-Catassús, 2017; Steels et al., 2019). The available information did not allow the authors to establish if one formulation showed greater benefit than the other. Nonetheless, the effect of PEA resulted to be superior to placebo in 8 studies and a statistically significant reduction of pain intensity in a pooled estimate emerged with a standard mean difference of 1.68 (Lang-Illievich et al., 2023).

Data obtained by the meta-analytic approach are characterized by the highest level of evidence in medicine. Thus, the results of the three described meta-analyses suggest that PEA treatment improves chronic pain. However, as affirmed by the authors themselves, these publications present some bias (e.g. different trial design, chronic pain of different origin, variable PEA doses and treatment duration, different analgesic treatment in the control arms, when present). Also, results were not stratified according to the different PEA formulations, thus preventing from a definitive answer on the superiority of one formulation over the other(s).

10.2. Safety

Based on the available data, tolerability of ultramicronized PEA appears to be good, as very few side effects and adverse events were ever observed, as detailed in Tables 4 and 5. In particular, only one patient developed mild side effects (nausea and bloating) in the study of Papetti et al. (Papetti et al., 2020). Similar gastrointestinal side effects were observed in 13.7% of patients administered ultramicronized PEA together with analgesics (Schweiger et al., 2019) and 15% of patients combinedly treated with ultramicronized PEA and tapentadol (Passavanti et al., 2017). No side effects were reported by Desio (2010), Paladini et al. (2017) and Del Giorno et al. (2015) in patients administered ultramicronized PEA, respectively associated with pregabalin, carbamazepine, oxycodone, tapentadol and pregabalin, or duloxetine and pregabalin.

Among the controlled trials, only Andresen and colleagues (Andresen et al., 2016), reported that a small percentage of patients (7 out of 73, 9.6%) developed adverse events, with 5 being serious, irrespective from treatment group (urinary tract infection, paralytic ileus, cholecystolithiasis, and erysipelas, fungus infection causing hospitalization in 3 patients treated with ultramicronized PEA and 1 treated with placebo). In the ultramicronized PEA supplemented group, one patient committed suicide, but no relationship was found with the study intervention. One further patient in the ultramicronized PEA group experienced a fungus infection and 1 in the placebo group experienced blurred vision. All adverse events were consistent with the disease condition of the study population (i.e., spinal cord injury) and ultramicronized PEA was not associated with more adverse effects than placebo.

Altogether, these studies provide a very promising safety profile of ultramicronized PEA. In fact, data reported in the many discussed trials, may be considered adequately reliable, independently from the design of the study.

11. Conclusions

Chronic pain seriously affects the quality of life of patients. The available pharmacological interventions may be inadequate to reduce pain or may induce important side effects that further impair the life of patients, especially the frailest and oldest ones. The most analgesic drugs address the neuronal component of pain, generally neglecting the neuroinflammatory one. Thus, the search for substances targeting non-neuronal cells and thereby able to co-adjuvate standard analgesics (i.e., improving efficacy and reducing side effects) is of pivotal importance. The physiological protective functions of endogenous PEA - mainly in terms of pain control through non-neuronal cell downregulation - are mimicked or sustained by the exogenous administration of PEA, provided that bioavailable formulations are used, in particular for oral delivery. Accordingly, orally administered ultramicronized PEA has been investigated in the preclinical setting and the available data, although limited, have shown superior pharmacokinetic profile compared with unprocessed formulations. To date, a direct comparison in terms of efficacy between ultramicronized and naïve or micronized PEA formulations is still missing both at the preclinical and clinical level.

Results from the available controlled clinical trials suggest that patients combinedly treated with analgesics and ultramicronized PEA generally experienced superior benefits in terms of pain relief compared to those without PEA supplementation. In addition, the good tolerability profile of ultramicronized PEA allows it to be used safely, even in frail patients (i.e., aged, multidiseased or on polypharmacy patients). However, only the implementation of controlled clinical trials comparing the three PEA formulations in terms of efficacy and tolerability will definitely establish the clinical superiority of a formulation on the others. Finally, *in vitro* and *in vivo* studies characterizing the complex and multiple mechanisms sustaining the effects of PEA in pain control are also warranted.

CRediT authorship contribution statement

Stefania Nobili: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Laura Micheli:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Elena Lucarini:** Writing – review & editing, Writing – original draft, Investigation. **Alessandra Toti:** Writing – review & editing, Writing – original draft, Investigation. **Carla Ghelardini:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization. **Lorenzo Di Cesare Mannelli:** Writing – review & editing, Writing – original draft, Validation, Supervision, Conceptualization.

Declaration of competing interest

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None.

References

Abdin, A. Y., Yeboah, P., & Jacob, C. (2020). Chemical impurities: An epistemological riddle with serious side effects. *International Journal of Environmental Research and Public Health 17*(3), 1030. https://doi.org/10.3390/ijerph17031030.

Ahmad, A., Genovese, T., Impellizzeri, D., Crupi, R., Velardi, E., Marino, A., Esposito, E., & Cuzzocrea, S. (2012). Reduction of ischemic brain injury by administration of palmitoylethanolamide after transient middle cerebral artery occlusion in rats. *Brain Research 1477*, 45–58. https://doi.org/10.1016/j.brainres.2012.08.006.

Alhouayek, M., & Muccioli, G. G. (2014). Harnessing the anti-inflammatory potential of palmitoylethanolamide. *Drug Discovery Today 19*(10), 1632–1639. https://doi.org/10.1016/j.drudis.2014.06.007.

Aloe, L., Leon, A., & Levi-Montalcini, R. (1993). A proposed autacoid mechanism controlling mastocyte behaviour. *Agents and Actions 39*. https://doi.org/10.1007/BF01972748 spec. No. C145-7.

Alsalem, M., Haddad, M., Aldossary, S. A., Kalbouneh, H., Altarifi, A., Jaffal, S. M., Abbas, M. A., Aldaoud, N., & El-Salem, K. (2019). Role of cannabinoid receptor 1 and the peroxisome proliferator-activated receptor α in mediating anti-nociceptive effects of synthetic cannabinoids and a cannabinoid-like compound. *Inflammopharmacology 27*(6), 1131–1142. https://doi.org/10.1007/s10787-019-00584-7.

Ambrosino, P., Soldovieri, M. V., Russo, C., & Taglialatela, M. (2013). Activation and desensitization of TRPV1 channels in sensory neurons by the PPARalpha agonist palmitoylethanolamide. *British Journal of Pharmacology 168*(6), 1430–1444. https://doi.org/10.1111/bph.12029.

Andresen, S. R., Bing, J., Hansen, R. M., Biering-Sørensen, F., Johannesen, I. L., Hagen, E. M., ... Finnerup, N. B. (2016). Ultramicronized palmitoylethanolamide in spinal cord injury neuropathic pain: A randomized, double-blind, placebo-controlled trial. *Pain 157*(9), 2097–2103. https://doi.org/10.1097/j.pain.0000000000000623.

Arnott, J. A., & Planey, S. L. (2012). The influence of lipophilicity in drug discovery and design. *Expert Opinion in Drug Discovery 7*(10), 863–875. https://doi.org/10.1517/17460441.2012.714363.

Artamonov, M., Zhukov, O., Shuba, I., Storozhuk, L., Khmel, T., Klimashevsky, V., Mikosha, A., & Gula, N. (2005). Incorporation of labelled N-acylethanolamine (NAE) into rat brain regions in vivo and adaptive properties of saturated NAE under x-ray irradiation. *Ukrainskiĭ Biokhimicheskiĭ Zhurnal 77*(6), 51–62.

Artukoglu, B. B., Beyer, C., Zuloff-Shani, A., Brener, E., & Bloch, M. H. (2017). Efficacy of Palmitoylethanolamide for pain: A Meta-analysis. *Pain Physician 20*(5), 353–362.

Assini, A., Laricchia, D., Belletti, M., Pandolfini, L., Pizzo, R., & Ratto, S. (2010). The carpal tunnel syndrome in diabetes: Clinical and electrophysiological improvement after treatment with palmitoylethanolamide. EFNS. *European Journal of Neurology 17*, 295.

Aydin, E., Cebo, M., Mielnik, J., Richter, H., Schüle, R., Sievers-Engler, A., Młynarz, P., & Lämmerhofer, M. (2023). UHPLC-ESI-MS/MS assay for quantification of endocannabinoids in cerebrospinal fluid using surrogate calibrant and surrogate matrix approaches. *Journal of Pharmaceutical and Biomedical Analysis 222*. https://doi.org/10.1016/j.jpba.2022.115090.

Bachur, N. R., Masek, K., R, K. L., & Udenfriend, S. (1965). Fatty acid amides of ethanolamine in mammalian tissues. *The Journal of Biological Chemistry 240*, 1019–1024.

Baker, D., Pryce, G., Davies, W. L., & Hiley, C. R. (2006). In silico patent searching reveals a new cannabinoid receptor. *Trends in Pharmacological Sciences 27*(1), 1–4. https://doi.org/10.1016/j.tips.2005.11.003.

Balenga, N. A., Martínez-Pinilla, E., Kargl, J., Schröder, R., Peinhaupt, M., Platzer, W., ... Franco, R. (2014). Heteromerization of GPR55 and cannabinoid CB2 receptors modulates signalling. *British Journal of Pharmacology 171*(23), 5387–5406. https://doi.org/10.1111/bph.12850.

Balvers, M. G. J., Verhoeckx, K. C. M., Meijerink, J., Wortelboer, H. M., & Witkamp, R. F. (2013). Measurement of Palmitoylethanolamide and other N-Acylethanolamines during physiological and pathological conditions. *CNS & Neurological Disorders - Drug Targets 12*(1), 26–33. https://doi.org/10.2174/18715273113120100007.

Balvers, M. G. J., Verhoeckx, K. C. M., & Witkamp, R. F. (2009). Development and validation of a quantitative method for the determination of 12 endocannabinoids and related compounds in human plasma using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 877*(14–15), 1583–1590. https://doi.org/10.1016/j.jchromb.2009.04.010.

Barry, A., O'Halloran, K. D., McKenna, J. P., McCreary, C., Harhen, B., Kerr, D. M., ... Downer, E. J. (2018). Plasma N-acylethanolamine and endocannabinoid levels in burning mouth syndrome: Potential role in disease pathogenesis. *Journal of Oral Pathology and Medicine 47*(4), 440–442. https://doi.org/10.1111/jop.12692.

Beggiato, S., Tomasini, M. C., & Ferraro, L. (2019). Palmitoylethanolamide (PEA) as a potential therapeutic agent in Alzheimer's disease. *Frontiers in Pharmacology 10*, 821. https://doi.org/10.3389/fphar.2019.00821.

Bettoni, I., Comelli, F., Colombo, A., Bonfanti, P., & Costa, B. (2013). Non-neuronal cell modulation relieves neuropathic pain: Efficacy of the endogenous lipid palmitoylethanolamide. *CNS & Neurolological Disorders - Drug Targets 12*(1), 34–44. https://doi.org/10.2174/18715273113120100008.

Blancaflor, E. B., Kilaru, A., Keereetaweep, J., Khan, B. R., Faure, L., & Chapman, K. D. (2014). N-Acylethanolamines: Lipid metabolites with functions in plant growth and development. *Plant Journal 79*(4), 568–583. https://doi.org/10.1111/tpj.12427.

Boccella, S., Cristiano, C., Romano, R., Iannotta, M., Belardo, C., Farina, A., Guida, F., Piscitelli, F., Palazzo, E., Mazzitelli, M., Imperatore, R., Tunisi, L., de Novellis, V., Cristino, L., Di Marzo, V., Calignano, A., Maione, S., & Luongo, L. (2019). Ultra-micronized palmitoylethanolamide rescues the cognitive decline-associated loss of neural plasticity in the neuropathic mouse entorhinal cortex-dentate gyrus pathway. *Neurobiology of Disease 121*, 106–119. https://doi.org/10.1016/j.nbd.2018.09.023.

Boccella, S., Marabese, I., Iannotta, M., Belardo, C., Neugebauer, V., Mazzitelli, M., Pieretti, G., Maione, S., & Palazzo, E. (2019). Metabotropic glutamate receptor 5 and 8 modulate the ameliorative effect of ultramicronized palmitoylethanolamide on cognitive decline associated with neuropathic pain. *International Journal of Molecular Sciences 20*(7). https://doi.org/10.3390/ijms20071757.

Borrelli, F., Romano, B., Petrosino, S., Pagano, E., Capasso, R., Coppola, D., ... Izzo, A. A. (2015). Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent. *British Journal of Pharmacology 172*(1), 142–158. https://doi.org/10.1111/bph.12907.

Bottemanne, P., Muccioli, G. G., & Alhouayek, M. (2018). N-acylethanolamine hydrolyzing acid amidase inhibition: Tools and potential therapeutic opportunities. *Drug Discovery Today 23*(8), 1520–1529. https://doi.org/10.1016/j.drudis.2018.03.007.

Breivik, H., Collett, B., Ventafridda, V., Cohen, R., & Gallacher, D. (2006). Survey of chronic pain in Europe: Prevalence, impact on daily life, and treatment. *European Journal of Pain 10*(4), 287. https://doi.org/10.1016/j.ejpain.2005.06.009.

Briskey, D., Mallard, A. R., & Rao, A. (2020). Increased absorption of palmitoylethanolamide using a novel dispersion technology system (LipiSperse®). *Journal Nutraceuticals and Food Science 5*(2:3), 1–6. https://doi.org/10.36648/nutraceuticals.5.2.3.

Burgess, J., Ferdousi, M., Gosal, D., Boon, C., Matsumoto, K., Marshall, A., ... Alam, U. (2021). Chemotherapy-induced peripheral neuropathy: Epidemiology, pathomechanisms and treatment. *Oncology and Therapy 9*(2), 385–450. https://doi.org/10.1007/s40487-021-00168-y.

Calignano, A., La Rana, G., Giuffrida, A., & Piomelli, D. (1998). Control of pain initiation by endogenous cannabinoids. *Nature 394*(6690), 277–281. https://doi.org/10.1038/28393.

Calignano, A., La Rana, G., & Piomelli, D. (2001). Antinociceptive activity of the endogenous fatty acid amide, palmitylethanolamide. *European Journal of Pharmacology 419*(2–3), 191–198. https://doi.org/10.1016/s0014-2999(01)00988-8.

Campardelli, R., Oleandro, E., Scognamiglio, M., Della Porta, G., & Reverchon, E. (2017). Palmitoylethanolamide sub-micronization using fast precipitation followed by supercritical fluids extraction. *Powder Technology 305*, 217–225. https://doi.org/10.1016/j.powtec.2016.09.084.

Cantarella, G., Scollo, M., Lempereur, L., Saccani-Jotti, G., Basile, F., & Bernardini, R. (2011). Endocannabinoids inhibit release of nerve growth factor by inflammation-activated mast cells. *Biochemical Pharmacology 82*(4), 380–388. https://doi.org/10.1016/j.bcp.2011.05.004.

Cerrato, S, Brazis, P, della Valle, MF, Miolo, A, & Puigdemont, A (2010). Effects of palmitoylethanolamide on immunologically induced histamine, PGD2 and TNFalpha release from canine skin mast cells. *Vet Immunol Immunopathol 133*(1), 9–15. https://doi.org/10.1016/j.vetimm.2009.06.011.

Chirchiglia, D., Chirchiglia, P., & Signorelli, F. (2018). Nonsurgical lumbar radiculopathies treated with ultramicronized palmitoylethanolamide (umPEA): A series of 100 cases. *Neurologia i Neurochirurgia Polska 52*(1), 44–47. https://doi.org/10.1016/j.pjnns.2017.11.002 (a).

Chirchiglia, D., Cione, E., Caroleo, M. C., Wang, M., Di Mizio, G., Faedda, N., ... Gallelli, L. (2018). Effects of add-on ultramicronized N-palmitol ethanol amide in patients suffering of migraine with aura: A pilot study. *Frontiers in Neurology 9*, 674. https://doi.org/10.3389/fneur.2018.00674 (c).

Chirchiglia, D., Paventi, S., Seminara, P., Cione, E., & Gallelli, L. (2018). N-Palmitoyl ethanol amide pharmacological treatment in patients with nonsurgical lumbar radiculopathy. *Journal of Clinical Pharmacology 58*(6), 733–739. https://doi.org/10.1002/jcph.1070 (b).

Clayton, P., Subah, S., Venkatesh, R., Hill, M., & Bogoda, N. (2023). Palmitoylethanolamide: A Potential Alternative to Cannabidiol. *Journal of Dietary Supplements 20*(3), 505–530. https://doi.org/10.1080/19390211.2021.2005733.

Cobellis, L., Castaldi, M. A., Giordano, V., Trabucco, E., De Franciscis, P., Torella, M., & Colacurci, N. (2011). Effectiveness of the association micronized N-Palmitoylethanolamine (PEA)-transpolydatin in the treatment of chronic pelvic pain related to endometriosis after laparoscopic assessment: A pilot study. *European Journal of Obstetrics, Gynecology, and Reproductive Biology 158*(1), 82–86. https://doi.org/10.1016/j.ejogrb.2011.04.011.

Coburn, A. F., Grahamt, C. E., & Haninger, J. (1954). The effect of egg yolk in diets on anaphylactiv arthritis (passive Arthus phenomenon) in the guinea pig. *Journal of Experimental Medicine 100*(5), 425–435. http://rupress.org/jem/article-pdf/100/5/425/1078370/425.pdf.

Coburn, A. F., Moore, L. V., & York, N. (1943). Nutrition as a conditioning factor in the rheumatic state. *American Journal of Diseases of Children 65*, 744–756. http://archpedi.jamanetwork.com/.

Cochrane Central Register of Controlled Trials (CCRCT) (2023). A Phase 1, randomized, Double-Blind, Placebo-Controlled study to evaluate the Safety, Tolerability and Pharmacokinetics of single and multiple ascending doses of Ultramicronized PEA in normal healthy volunteers-ACTRN12620000213943. https://trialsearch.who.int/Trial2.aspx?TrialID=ACTRN12620000213943 last accessed September 26, 2023.

Cocito, D., Peci, E., Ciaramitaro, P., Merola, A., & Lopiano, L. (2014). Short-term efficacy of ultramicronized palmitoylethanolamide in peripheral neuropathic pain. *Pain Research and Treatment 2014*. https://doi.org/10.1155/2014/854560.

Cohen, S. P., Vase, L., & Hooten, W. M. (2021). Chronic pain 1 chronic pain: An update on burden, best practices, and new advances. *The Lancet 397*(10289), 2082–2097. https://doi.org/10.1016/S0140-6736(21)00393-7.

Correia-Sá, I. B., Carvalho, C. M., Serrão, P. V., Loureiro, A. I., Fernandes-Lopes, C., Marques, M., & Vieira-Coelho, M. A. (2020). A new role for anandamide: Defective link between the systemic and skin endocannabinoid systems in hypertrophic human wound healing. *Scientific Reports 10*(1). https://doi.org/10.1038/s41598-020-68058-3.

Costa, B., Comelli, F., Bettoni, I., Colleoni, M., & Giagnoni, G. (2008). The endogenous fatty acid amide, palmitoylethanolamide, has anti-allodynic and anti-hyperalgesic effects in a murine model of neuropathic pain: Involvement of CB1, TRPV1 and PPARγ receptors and neurotrophic factors. *Pain 139*(3), 541–550. https://doi.org/10.1016/j.pain.2008.06.003.

Cravatt, B. F., Giang, D. K., Mayfield, S. P., Boger, D. L., Lerner, R. A., & Gilula, N. B. (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature 384*(6604), 83–87. https://doi.org/10.1038/384083a0.

Cremon, C., Stanghellini, V., Barbaro, M. R., Cogliandro, R. F., Bellacosa, L., Santos, J., ... Barbara, G. (2017). Randomised clinical trial: The analgesic properties of dietary supplementation with palmitoylethanolamide and polydatin in irritable bowel syndrome. *Alimentary Pharmacology and Therapeutics 45*(7), 909–922. https://doi.org/10.1111/apt.13958.

Cristiano, C., Avagliano, C., Cuozzo, M., Liguori, F. M., Calignano, A., & Russo, R. (2022). The beneficial effects of ultramicronized palmitoylethanolamide in the management of neuropathic pain and associated mood disorders induced by paclitaxel in mice. *Biomolecules 12*(8). https://doi.org/10.3390/biom12081155.

Cruccu, G., Stefano, G. D., Marchettini, P., & Truini, A. (2019). Micronized palmitoylethanolamide: A post hoc analysis of a controlled study in patients with low back pain - Sciatica. *CNS & Neurolological Disorders - Drug Targets 18*(6), 491–495. https://doi.org/10.2174/1871527318666190703110036.

D'Agostino, G., La Rana, G., Russo, R., Sasso, O., Iacono, A., Esposito, E., ... Calignano, A. (2009). Central administration of palmitoylethanolamide reduces hyperalgesia in mice via inhibition of NF-κB nuclear signalling in dorsal root ganglia. *European Journal of Pharmacology 613*(1–3), 54–59. https://doi.org/10.1016/j.ejphar.2009.04.022.

Dalla Volta, G. (2016). Ultramicronized palmitoylethanolamide reduces frequency and pain intensity in migraine. A pilot study. *International Journal of Neurology and Brain Disorders 3*(2), 1–5. https://doi.org/10.15436/2377-1348.16.019.

D'Aloia, A., Arrigoni, F., Tisi, R., Palmioli, A., Ceriani, M., Artusa, V., ... Cipolla, L. (2020). Synthesis, molecular modeling and biological evaluation of metabolically stable analogues of the endogenous fatty acid amide palmitoylethanolamide. *International Journal of Molecular Sciences 21*(23), 1–25. https://doi.org/10.3390/ijms21239074.

Danandeh, A., Vozella, V., Lim, J., Oveisi, F., Ramirez, G. L., Mears, D., ... Piomelli, D. (2018). Effects of fatty acid amide hydrolase inhibitor URB597 in a rat model of trauma-induced long-term anxiety. *Psychopharmacology 235*(11), 3211–3221. https://doi.org/10.1007/s00213-018-5020-7.

Dang, H. T., Kang, G. J., Yoo, E. S., Hong, J., Choi, J. S., Kim, H. S., ... Jung, J. H. (2011). Evaluation of endogenous fatty acid amides and their synthetic analogues as potential anti-inflammatory leads. *Bioorganic and Medicinal Chemistry 19*(4), 1520–1527. https://doi.org/10.1016/j.bmc.2010.12.046.

Darmani, N. A., Izzo, A. A., Degenhardt, B., Valenti, M., Scaglione, G., Capasso, R., ... Di Marzo, V. (2005). Involvement of the cannabimimetic compound, N-palmitoyl-ethanolamine, in inflammatory and neuropathic conditions: Review of the available preclinical data, and first human studies. *Neuropharmacology 48*(8), 1154–1163. https://doi.org/10.1016/j.neuropharm.2005.01.001.

De Filippis, D., D'Amico, A., Cipriano, M., Petrosino, S., Orlando, P., Di Marzo, V., Iuvone, T., & Endocannabinoid Research Group (2010). Levels of endocannabinoids and palmitoylethanolamide and their pharmacological manipulation in chronic granulomatous inflammation in rats. *Pharmacological Research 61*(4), 321–328. https://doi.org/10.1016/j.phrs.2009.11.005.

De Icco, R., Greco, R., Demartini, C., Vergobbi, P., Zanaboni, A., Tumelero, E., Reggiani, A., Realini, N., Sances, G., Grillo, V., Allena, M., & Tassorelli, C. (2021). Spinal nociceptive sensitization and plasma palmitoylethanolamide levels during experimentally induced migraine attacks. *Pain 162*(9), 2376–2385. https://doi.org/10.1097/j.pain.0000000000002223.

De Petrocellis, L., Davis, J. B., & Di Marzo, V. (2001). Palmitoylethanolamide enhances anandamide stimulation of human vanilloid VR1 receptors. *FEBS Letters 506*(3), 253–256. https://doi.org/10.1016/S0014-5793(01)02934-9.

Déciga-Campos, M., Jaramillo-Morales, O. A., Espinosa-Juárez, J. V., Aguilera-Martínez, M. E., Ventura-Martínez, R., & López-Muñoz, F. J. (2023). N-palmitoylethanolamide synergizes the antinociception of morphine and gabapentin in the formalin test in mice. *Journal of Pharmacy and Pharmacology 75*(9), 1154–1162. https://doi.org/10.1093/jpp/rgad004.

Del Giorno, R., Skaper, S., Paladini, A., Varrassi, G., & Coaccioli, S. (2015). Palmitoylethanolamide in fibromyalgia: Results from prospective and retrospective observational studies. *Pain and therapy 4*(2), 169–178. https://doi.org/10.1007/s40122-015-0038-6.

Della Rocca, G., & Re, G. (2022). Palmitoylethanolamide and related ALIAmides for small animal health: State of the art. *Biomolecules 12*(9). https://doi.org/10.3390/biom1209118.

Della Valle, F., Lorenzi, S., Samson, J., & Della Valle, F. (1999). *Use of N-acyl derivatives of aminoalcohols for the manufacture of a medicament for the treatment of pathologies involving mast cells.* EP 0 550 006.

Della Valle, F., Marcolongo, G., & Della Valle, F. (2009). *Composition containing ultramicronized palmitoyl-ethanolamide.* EP 2 475 352.

Desarnaud, F., Cadas, J. H., & Piomelli, D. (1995). Anandamide Amidohydrolase activity in rat brain Microsomes Identificatyion and partial characterization. *The Journal of Biological Chemistry 270*(11), 6030–6035. https://doi.org/10.1074/jbc.270.11.6030.

Desio, P. (2010). Combination of pregabalin and palmitoylethanolamide (PEA) for neuropathic pain treatment. *Pathos 7*, 9–14.

Desio, P. (2011). Combination of oxycodone and palmitoylethanolamide for low back pain treatment. *Anestesia e Medicina critica 1*, 63–71.

Desio, P., Bonadiman, L., Fusco, M., & Cenacchi, T. (2010). Combination of carbamazepine and palmitoylethanolamide for trigeminal neuralgia. *PeriMed 4*, 4.

Devane, W. A., Hanus, L., Breuer, A., Pertwee, R. G., Stevenson, L. A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., & Mechoulam, R. (1992). Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science 258*(5090), 1946–1949. https://doi.org/10.1126/science.1470919.

Dhiman, A., & Prabhakar, P. K. (2021). Micronization in food processing: A comprehensive review of mechanistic approach, physicochemical, functional properties and self-stability of micronized food materials. *Journal of Food Engineering 292*, Article 110248. https://doi.org/10.1016/j.jfoodeng.2020.110248.

Di Cesare Mannelli, L., Corti, F., Micheli, L., Zanardelli, M., & Ghelardini, C. (2015). Delay of morphine tolerance by palmitoylethanolamide. *BioMed Research International*, 894732. https://doi.org/10.1155/2015/894732.

Di Cesare Mannelli, L., D'Agostino, G., Pacini, A., Russo, R., Zanardelli, M., Ghelardini, C., & Calignano, A. (2013). Palmitoylethanolamide is a disease-modifying agent in peripheral neuropathy: Pain relief and neuroprotection share a PPAR-alpha-mediated mechanism. *Mediators of Inflammation 2013*, Article 328797. https://doi.org/10.1155/2013/328797.

Di Cesare Mannelli, L., Micheli, L., Lucarini, E., & Ghelardini, C. (2018). N-palmitoylethanolamine supplementation for long-lasting, low-dosed morphine antinociception. *Frontiers in Pharmacology 9*, 473. https://doi.org/10.3389/fphar.2018.00473.

Di Cesare Mannelli, L., Pacini, A., Corti, F., Boccella, S., Luongo, L., Esposito, E., ... Ghelardini, C. (2015). Antineuropathic profile of N-Palmitoylethanolamine in a rat model of oxaliplatin-induced neurotoxicity. *PLoS One 10*(6). https://doi.org/10.1371/journal.pone.0128080.

Di Paola, R., Impellizzeri, D., Mondello, P., Velardi, E., Aloisi, C., Cappellani, A., Esposito, E., & Cuzzocrea, S. (2012). Palmitoylethanolamide reduces early renal dysfunction and injury caused by experimental ischemia and reperfusion in mice. *Shock 38*(4), 356–366. https://doi.org/10.1097/SHK.0b013e318267bbb9.

Donvito, G., Wilkerson, J. L., Damaj, M. I., & Lichtman, A. H. (2016). Palmitoylethanolamide reverses paclitaxel-induced allodynia in mice. *Journal of Pharmacology and Experimental Therapeutics 359*(2), 310–318. https://doi.org/10.1124/jpet.116.236182.

El-Brolosy, M. A., & Stainier, D. Y. R. (2017). Genetic compensation: A phenomenon in search of mechanisms. *PLoS Genetics 13*(7), Article e1006780. https://doi.org/10.1371/journal.pgen.1006780.

Elfarnawany, A., & Dehghani, F. (2022). Palmitoylethanolamide mitigates paclitaxel toxicity in primary dorsal root ganglion neurons. *Biomolecules 12*(12), 1873. https://doi.org/10.3390/biom12121873.

Epps, D. E., Natarajan, V., Schmid, P. C., & Schmid, H. O. (1980). Accumulation of N-acylethanolamine glycerophospholipids in infarcted myocardium. *Biochimica et Biophysica Acta 618*(3), 420–430. https://doi.org/10.1016/0005-2760(80)90260-x.

Evangelista, M., Cilli De Vitis, R., Militerno, A., & Fanfani, F. (2018). Ultra-micronized Palmitoylethanolamide effects on sleep-wake rhythm and neuropathic pain phenotypes in patients with carpal tunnel syndrome: An open-label, randomized controlled study. *CNS & Neurological Disorders - Drug Targets 17*(4), 291–298. https://doi.org/10.2174/1871527317666180420143830.

Facchinetti, R., Valenza, M., Gomiero, C., Mancini, G. F., Steardo, L., Campolongo, P., & Scuderi, C. (2022). Co-ultramicronized palmitoylethanolamide/luteolin restores oligodendrocyte homeostasis via peroxisome proliferator-activated receptor-α in an in vitro model of Alzheimer's disease. *Biomedicines 10*(6), 1236. https://doi.org/10.3390/biomedicines10061236.

Facci, L., Barbierato, M., Fusco, M., Giusti, P., & Zusso, M. (2021). Co-Ultramicronized palmitoylethanolamide/luteolin-induced oligodendrocyte precursor cell differentiation is associated with Tyro3 receptor upregulation. *Frontiers in Pharmacology 12*, Article 698133. https://doi.org/10.3389/fphar.2021.698133.

Facci, L., Dal Toso, R., Romanello, S., Buriani, A., Skaper, S. D., & Leon, A. (1995). Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proceedings of the National Academy of Sciences USA 92*(8), 3376–3380. https://doi.org/10.1073/pnas.92.8.3376.

Fagerberg, L., Hallstrom, B. M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J., ... Uhlen, M. (2014). Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Molecular and Cellular Proteomics 13*(2), 397–406. https://doi.org/10.1074/mcp.M113.035600.

Faig-Martí, J., & Martínez-Catassús, A. (2017). Use of palmitoylethanolamide in carpal tunnel syndrome: A prospective randomized study. *Journal of Orthopaedics and Traumatology 18*(4), 451–455. https://doi.org/10.1007/s10195-017-0453-z.

Fanelli, F., Di Lallo, V. D., Belluomo, I., De Iasio, R., Baccini, M., Casadio, E., ... Pagotto, U. (2012). Estimation of reference intervals of five endocannabinoids and endocannabinoid related compounds in human plasma by two dimensional-LC/MS/MS. *Journal of Lipid Research 53*(3), 481–493. https://doi.org/10.1194/jlr.M021378.

Farina, A., Villagrán-García, M., & Honnorat, J. (2023). Neurological adverse events of immune checkpoint inhibitors: An update of clinical presentations, diagnosis, and management. *Revue Neurologique (Paris) 179*(5), 506–515. https://doi.org/10.1016/j.neurol.2023.03.003.

Fezza, F., Bari, M., Florio, R., Talamonti, E., Feole, M., & Maccarrone, M. (2014). Endocannabinoids, related compounds and their metabolic routes. *Molecules 19*(11), 17078–17106. https://doi.org/10.3390/molecules191117078.

Fichna, J., Wood, J. A. T., Papanastasiou, M., Vadivel, S. K., Oprocha, P., Sałaga, M., ... Storr, M. A. (2013). Endocannabinoid and cannabinoid-like fatty acid amide levels correlate with pain-related symptoms in patients with IBS-D and IBS-C: A pilot study. *PLoS One 8*(12). https://doi.org/10.1371/journal.pone.0085073.

Finnerup, N. B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R. H., ... Wallace, M. (2015). Pharmacotherapy for neuropathic pain in adults: A systematic review and meta-analysis. *The Lancet Neurology 14*(2), 162–173. https://doi.org/10.1016/S1474-4422(14)70251-0.

Fitzcharles, M. A., Cohen, S. P., Clauw, D. J., Littlejohn, G., Usui, C., & Häuser, W. (2021). Nociplastic pain: Towards an understanding of prevalent pain conditions. *The Lancet 397*(10289), 2098–2110. https://doi.org/10.1016/S0140-6736(21)00392-5.

Fotio, Y., Jung, K. M., Palese, F., Obenaus, A., Tagne, A. M., Lin, L., Rashid, T. I., Pacheco, R., Jullienne, A., Ramirez, J., Mor, M., Spadoni, G., Jang, C., Hohmann, A. G., & Piomelli, D. (2021). NAAA-regulated lipid signaling governs the transition from acute to chronic pain. *Science Advances 7*(43), eabi8834. https://doi.org/10.1126/sciadv.abi8834.

Fotio, Y., Sasso, O., Ciccocioppo, R., & Piomelli, D. (2021). Antinociceptive profile of ARN19702, (2-Ethylsulfonylphenyl)-[(2S)-4-(6-fluoro-1,3-benzothiazol-2-yl)-2-methylpiperazin-1-yl]methanone, a novel orally active N-acylethanolamine acid amidase inhibitor, in animal models. *Journal of Pharmacology and Experimental Therapeutics 378*(2), 70–76. https://doi.org/10.1124/jpet.121.000674.

Freireich, E. J., Gehan, E. A., Rall, D. P., Schmidt, L. H., & Skipper, H. E. (1966). Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemotherapy Reports 50*, 219–244.

Froud, R., Patterson, S., Eldridge, S., Seale, C., Pincus, T., Rajendran, D., ... Underwood, M. (2014). A systematic review and meta-synthesis of the impact of low back pain on people's lives. *BMC Musculoskeletal Disorders 15*(1). https://doi.org/10.1186/1471-2474-15-50.

Fusco, R., Gugliandolo, E., Campolo, M., Evangelista, M., Di Paola, R., & Cuzzocrea, S. (2017). Effect of a new formulation of micronized and ultramicronized N-palmitoylethanolamine in a tibia fracture mouse model of complex regional pain syndrome. *PLoS One 12*(6). https://doi.org/10.1371/journal.pone.0178553.

Gachet, M. S., Rhyn, P., Bosch, O. G., Quednow, B. B., & Gertsch, J. (2015). A quantitiative LC-MS/MS method for the measurement of arachidonic acid, prostanoids, endocannabinoids, N-acylethanolamines and steroids in human plasma. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences 976–977*, 6–18. https://doi.org/10.1016/j.jchromb.2014.11.001.

Ganley, O. H., Graessle, O. E., & Robinson, H. J. (1958). Anti-inflammatory activity on compounds obtained from egg yolk, peanut oil, and soybean lecithin. *Journal of Laboratory and Clinical Medicine 51*(5), 709–714.

Ganley, O. H., & Robinson, H. J. (1959). Antianaphylactic and antiserotonin activity of a compound obtained from egg yolk, peanut oil and soybean lecithin. *The Journal of Allergy 30*, 415–419.

Garcia-Ovejero, D., Arevalo-Martin, A., Petrosino, S., Docagne, F., Hagen, C., Bisogno, T., Watanabe, M., Guaza, C., Di Marzo, V., & Molina-Holgado, E. (2009). The endocannabinoid system is modulated in response to spinal cord injury in rats. *Neurobiology of Disease 33*(1), 57–71. https://doi.org/10.1016/j.nbd.2008.09.015.

Gatti, A., Lazzari, M., Gianfelice, V., Di Paolo, A., Sabato, E., Sabato, A. F., ... Vergata, T. (2012). Palmitoylethanolamide in the treatment of chronic pain caused by different etiopathogenesis. *Pain Medicine 13*(9), 1121–1130. https://doi.org/10.1111/j.1526-4637.2012.01432.x.

Germini, F., Coerezza, A., Andreinetti, L., Nobili, A., Rossi, P. D., Mari, D., ... Marcucci, M. (2017). N-of-1 randomized trials of ultra-micronized Palmitoylethanolamide in older patients with chronic pain. *Drugs and Aging 34*(12), 941–952. https://doi.org/10.1007/s40266-017-0506-2.

Glass, C. K., & Ogawa, S. (2006). Combinatorial roles of nuclear receptors in inflammation and immunity. *Nature Reviews Immunology 6*(1), 44–55. https://doi.org/10.1038/nri1748.

Grillo, S. L., Keereetaweep, J., Grillo, M. A., Chapman, K. D., & Koulen, P. (2013). N-Palmitoylethanolamine depot injection increased its tissue levels and those of other acylethanolamide lipids. *Drug Design, Development and Therapy 7*, 747–752. https://doi.org/10.2147/DDDT.S48324.

Gugliandolo, E., Peritore, A. F., Piras, C., Cuzzocrea, S., & Crupi, R. (2020). Palmitoylethanolamide and related ALIAmides: Prohomeostatic lipid compounds for animal health and wellbeing. *Veterinary Sciences 7*(2), 78. https://doi.org/10.3390/vetsci7020078.

Guida, F., Luongo, L., Boccella, S., Giordano, M. E., Romano, R., Bellini, G., ... Maione, S. (2017). Palmitoylethanolamide induces microglia changes associated with increased migration and phagocytic activity: Involvement of the CB2 receptor. *Scientific Reports 7*(1). https://doi.org/10.1038/s41598-017-00342-1.

Guida, G., De Martino, M., De Fabiani, A., Cantieri, L., Alexandre, A., Vassallo, G. M., ... Petrosino, S. (2010). Palmitoylethanolamide (Normast) in chronic neuropathic pain caused by compressive-type lumbar sciatica: A multicenter clinical trial. *Dolor 25*, 35–42.

Gunawan, E. R., Suhendra, D., NuansaWindari, B. A., & Kurniawati, L. (2019). Enzymatic synthesis of palmitoylethanolamide from ketapang kernel oil. *Journal of Physics: Conference Series 1321*, Article 022034. https://doi.org/10.1088/1742-6596/1321/2/022034.

Häuser, W., Marschall, U., L'Hoest, H., Komossa, K., & Henningsen, P. (2013). Administrative Prävalenz, Behandlung und Krankheitskosten der somatoformen Schmerzstörung: Analyse von Daten der BARMER GEK für die Jahre 2008-2010. *Schmerz 27*(4), 380–386. https://doi.org/10.1007/s00482-013-1340-z.

Häuser, W., Schmutzer, G., Henningsen, P., & Brähler, E. (2014). Chronic pain, pain disease, and satisfaction of patients with pain treatment in Germany: Results of a representative population survey. *Schmerz 28*(5), 483–492. https://doi.org/10.1007/s00482-014-1438-y.

Hawker, G. A., Mian, S., Kendzerska, T., & French, M. (2011). Measures of adult pain: Visual analog scale for pain (VAS pain), numeric rating scale for pain (NRS pain), McGill pain questionnaire (MPQ), short-form McGill pain questionnaire (SF-MPQ), chronic pain grade scale (CPGS), short Form-36 bodily pain scale (SF-36 BPS), and measure of intermittent and constant osteoarthritis pain (ICOAP). *Arthritis Care and Research 63*(Suppl. 11). https://doi.org/10.1002/acr.20543.

Ho, W. S. V., Barrett, D. A., & Randall, M. D. (2008). "Entourage" effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. *British Journal of Pharmacology 155*(6), 837–846. https://doi.org/10.1038/bjp.2008.324.

Iannotti, F. A., & Vitale, R. M. (2021). The endocannabinoid system and PPARs: Focus on their Signalling crosstalk, action and transcriptional regulation. *Cells 10*(3), 586. https://doi.org/10.3390/cells10030586.

Impellizzeri, D., Bruschetta, G., Cordaro, M., Crupi, R., Siracusa, R., Esposito, E., & Cuzzocrea, S. (2014). Micronized/ultramicronized palmitoylethanolamide displays superior oral efficacy compared to nonmicronized palmitoylethanolamide in a rat model of inflammatory pain. *Journal of Neuroinflammation 11*(1). https://doi.org/10.1186/s12974-014-0136-0.

International Association for the Study of Pain (IASP) (2023). Pain terms and definitions. https://www.iasp-pain.org/resources/terminology/ last accessed September 26, 2023.

International Classification of Diseases, Eleventh Revision (ICD-11), World Health Organization (WHO) (2019/2021). https://www.who.int/standards/classifications/classification-of-diseases.

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., ... Murray, C. J. L. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: A systematic analysis for the global burden of disease study 2017. *The Lancet 392*(10159), 1789–1858. https://doi.org/10.1016/S0140-6736(18)32279-7.

Jumpertz, R., Guijarro, A., Pratley, R. E., Piomelli, D., & Krakoff, J. (2011). Central and peripheral endocannabinoids and cognate acylethanolamides in humans: Association with race, adiposity, and energy expenditure. *Journal of Clinical Endocrinology and Metabolism 96*(3), 787–791. https://doi.org/10.1210/jc.2010-2028.

Khadka, P., Ro, J., Kim, H., Kim, I., Kim, J. T., Kim, H., Cho, J. M., Yun, G., & Lee, J. (2014). Pharmaceutical particle technologies: An approach to improve drug solubility, dissolution and bioavailability. *Asian Journal of Pharmaceutical Sciences 9*, 304–316. https://doi.org/10.1016/j.ajps.2014.05.005.

Khasabova, I. A., Xiong, Y., Coicou, L. G., Piomelli, D., & Seybold, V. (2012). Peroxisome proliferator-activated receptor α mediates acute effects of palmitoylethanolamide on sensory neurons. *Journal of Neuroscience 32*(37), 12735–12743. https://doi.org/10.1523/JNEUROSCI.0130-12.2012.

Kilaru, A., Blancaflor, E. B., Venables, B. J., Tripathy, S., Mysore, K. S., & Chapman, K. D. (2007). The N-acylethanolamine-mediated regulatory pathway in plants. *Chemistry and Biodiversity 4*(8), 1933–1955. https://doi.org/10.1002/cbdv.200790161.

Kiso, T., Watabiki, T., & Sekizawa, T. (2020). ASP8477, a fatty acid amide hydrolase inhibitor, exerts analgesic effects in rat models of neuropathic and dysfunctional pain. *European Journal of Pharmacology 881*, Article 173194. https://doi.org/10.1016/j.ejphar.2020.173194.

Kleberg, K., Hassing, H. A., & Hansen, H. S. (2014). Classical endocannabinoid-like compounds and their regulation by nutrients. *Biofactors 40*(4), 363–372. https://doi.org/10.1002/biof.1158.

Koethe, D., Pahlisch, F., Hellmich, M., Rohleder, C., Mueller, J. K., Meyer-Lindenberg, A., ... Leweke, F. M. (2019). Familial abnormalities of endocannabinoid signaling in schizophrenia. *World Journal of Biological Psychiatry 20*(2), 117–125. https://doi.org/10.1080/15622975.2018.1449966.

Kuehl, F. A., Jr., Jacob, T. A., Ganley, O. H., Ormond, R. E., & Meisinger, M. A. P. (1957). The identification of N-(2-hydroxyethyl)-palmitoile as a naturally occuring anti-inflammatory agent. *Journal of the American Chemical Society 79*(20), 5577–5578. https://doi.org/10.1021/ja01577a066.

Lam, P. M. W., Marczylo, T. H., & Konje, J. C. (2010). Simultaneous measurement of three N-acylethanolamides in human bio-matrices using ultra performance liquid chromatography-tandem mass spectrometry. *Analytical and Bioanalytical Chemistry 398*(5), 2089–2097. https://doi.org/10.1007/s00216-010-4103-z.

Lambert, D. M., Vandevoorde, S., Jonsson, K. -O., & Fowler, C. J. (2002). The Palmitoylethanolamide family: A new class of anti-inflammatory agents? *Current Medicinal Chemistry 9*(6), 663–674. https://doi.org/10.2174/0929867023370707.

Lang-Illievich, K., Klivinyi, C., Lasser, C., Brenna, C. T. A., Szilagyi, I. S., & Bornemann-Cimenti, H. (2023). Palmitoylethanolamide in the treatment of chronic pain: A systematic review and meta-analysis of double-blind randomized controlled trials. *Nutrients 15*(6), 1350. https://doi.org/10.3390/nu15061350.

Levi-Montalcini, R., Skaper, S. D., Dal Toso, R., Petrelli, L., & Leon, A. (1996). Nerve growth factor: From neurotrophin to neurokine. *Trends in Neurosciences 19*(11), 514–520. https://doi.org/10.1016/S0166-2236(96)10058-8.

Liu, J., Wang, L., Harvey-White, J., Huang, B. X., Kim, H. Y., Luquet, S., Palmiter, R. D., Krystal, G., Rai, R., Mahadevan, A., Razdan, R. K., & Kunos, G. (2008). Multiple pathways involved in the biosynthesis of anandamide. *Neuropharmacology 54*(1), 1–7. https://doi.org/10.1016/j.neuropharm.2007.05.020.

Liu, J., Wang, L., Harvey-White, J., Osei-Hyiaman, D., Razdan, R., Gong, Q., Chan, A. C., Zhou, Z., Huang, B. X., Kim, H. Y., & Kunos, G. (2006). A biosynthetic pathway for anandamide. *Proceedings of the National Academy of Sciences USA 103*(36), 13345–13350. https://doi.org/10.1073/pnas.0601832103.

Lo Verme, J., Fu, J., Astarita, G., La Rana, G., Russo, R., Calignano, A., & Piomelli, D. (2005). The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. *Molecular Pharmacology 67*(1), 15–19. https://doi.org/10.1124/mol.104.006353.

Lo Verme, J., La Rana, G., Russo, R., Calignano, A., & Piomelli, D. (2005). The search for the palmitoylethanolamide receptor. *Life Sciences 77*(14), 1685–1698. https://doi.org/10.1016/j.lfs.2005.05.012.

Long, D. A., & Martin, A. J. (1956). Factor in arachis oil depressing sensitivity to tuberculin in B.C.G.-infected guineapigs. *The Lancet 270*(6921), 464–466. https://doi.org/10.1016/s0140-6736(56)90529-3.

Mallard, A., Briskey, D., Richards, A., Mills, D., & Rao, A. (2020). The effect of orally dosed Levagen+TM (palmitoylethanolamide) on exercise recovery in healthy males-A double-blind, randomized, placebo-controlled study. *Nutrients 12*(3), 596. https://doi.org/10.3390/nu12030596.

Marcianò, G., Vocca, C., Evangelista, M., Palleria, C., Muraca, L., Galati, C., ... Gallelli, L. (2023). The pharmacological treatment of chronic pain: From guidelines to daily clinical practice. *Pharmaceutics 15*(4), 1165. https://doi.org/10.3390/pharmaceutics15041165.

Marini, I., Bartolucci, M. L., Bortolotti, F., Gatto, M. R., & Bonetti, G. A. (2012). Palmitoylethanolamide versus a nonsteroidal anti-inflammatory drug in the treatment of temporomandibular joint *inflammatory pain*. *Journal of Orofacial Pain 26*(2), 99–104.

Marini, I., Cavallaro, M., Bartolucci, M. L., Alessandri-Bonetti, A., Gatto, M. R., Cordaro, M., & Checchi, L. (2018). Can celecoxib enhance palmitoylethanolamide's effect in the treatment of temporo-mandibular arthralgia in osteoarthritis patients? *Journal of Translational Science 5*(2). https://doi.org/10.15761/jts.1000255.

Martínez-Pinilla, E., Aguinaga, D., Navarro, G., Rico, A. J., Oyarzábal, J., Sánchez-Arias, J. A., ... Franco, R. (2019). Targeting CB1 and GPR55 endocannabinoid receptors as a potential neuroprotective approach for Parkinson's disease. *Molecular Neurobiology 56*(8), 5900–5910. https://doi.org/10.1007/s12035-019-1495-4.

Martínez-Pinilla, E., Reyes-Resina, I., Oñatibia-Astibia, A., Zamarbide, M., Ricobaraza, A., Navarro, G., ... Franco, R. (2014). CB1 and GPR55 receptors are co-expressed and form heteromers in rat and monkey striatum. *Experimental Neurology 261*, 44–52. https://doi.org/10.1016/j.expneurol.2014.06.017.

Mastrangelo, S., Rivetti, S., Triarico, S., Romano, A., Attinà, G., Maurizi, P., & Ruggiero, A. (2021). Mechanisms, characteristics and treatment of neuropathic pain and peripheral neuropathy associated with dinutuximab in neuroblastoma patients. *International Journal of Molecular Sciences 22*(23), 12648. https://doi.org/10.3390/ijms222312648.

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N. E., Schatz, A. R., Gopher, A., Almog, S., Martin, B. R., Compton, D. R., Pertwee, R. G., Griffin, G., Bayewitch, M., Barg, J., & Vogel, Z. (1995). Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochemical Pharmacololgy 50*(1), 83–90. https://doi.org/10.1016/0006-2952(95)00109-d.

Micheli, L., Lucarini, E., Nobili, S., Bartolucci, G., Pallecchi, M., Toti, A., ... Di Cesare Mannelli, L. (2024). Ultramicronized N-palmitoylethanolamine contributes to morphine efficacy against neuropathic pain: Implication of mast cells and glia. *Current Neuropharmacology 22*(1), 88–106. https://doi.org/10.2174/1570159X21666221128091453.

Micheli, L., Lucarini, E., Toti, A., Ferrara, V., Ciampi, C., Parisio, C., ... Ghelardini, C. (2022). Effects of ultramicronized N-palmitoylethanolamine supplementation on tramadol and oxycodone analgesia and tolerance prevention. *Pharmaceutics 14*(2), 403. https://doi.org/10.3390/pharmaceutics14020403.

Miyasaka, T., & Noguchi, S. (1985). High chemoselective synthesis of carboxamides by using syn-phenylpyridyl-o-acyl oximes (PPKO). *Chemistry Letters 14*(701–704), 1985. https://doi.org/10.1246/cl.1985.701.

Montella, S., Carotenuto, A., Orefice, N. S., & Orefice, G. (2014). A double-blind, randomized, versus-placebo study of ultramicronized palmitoylethanolamide in subjects with relapsing-remitting multiple sclerosis: Preliminary results. EFNS-ENS. *European Journal of Neurology 21*, 676.

Moriconi, A., Cerbara, I., Maccarrone, M., & Topai, A. (2010). GPR55: Current knowledge and future perspectives of a purported "Type-3" cannabinoid receptor. *Current Medicinal Chemistry 17*(14), 1411–1429. https://doi.org/10.2174/092986710790980069.

Murillo-Rodriguez, E., Désarnaud, F., & Prospéro-García, O. (2006). Diurnal variation of arachidonoylethanolamine, palmitoylethanolamide and oleoylethanolamide in the brain of the rat. *Life Sciences 79*(1), 30–37. https://doi.org/10.1016/j.lfs.2005.12.028.

Murina, F., Graziottin, A., Felice, R., Radici, G., & Tognocchi, C. (2013). Vestibulodynia: Synergy between palmitoylethanolamide + transpolydatin and transcutaneous electrical nerve stimulation. *Journal of Lower Genital Tract Disease 17*(2), 111–116. https://doi.org/10.1097/LGT.0b013e3182652316.

Musella, A., Fresegna, D., Rizzo, F. R., Gentile, A., Bullitta, S., De Vito, F., Guadalupi, L., Centonze, D., & Mandolesi, G. (2017). A novel crosstalk within the endocannabinoid system controls GABA transmission in the striatum. *Scientific Reports 7*(1), 7363. https://doi.org/10.1038/s41598-017-07519-8.

Nagao, Y., Seno, K., Kawabata, K., Miyasaka, T., Takao, S., & Fujita, E. (1980). Monitored aminolysis of 3-acylthiazolidine-2-thione: A new convenient synthesis of amide. *Tetrahedron Letters 21*, 841–844. https://doi.org/10.1016/S0040-4039(00)71520-6.

Nasirinezhad, F., Jergova, S., Pearson, J. P., & Sagen, J. (2015). Attenuation of persistent pain-related behavior by fatty acid amide hydrolase (FAAH) inhibitors in a rat model of HIV sensory neuropathy. *Neuropharmacology 95*, 100–109. https://doi.org/10.1016/j.neuropharm.2014.11.024.

NHS Health Research Authority (HRA) (2023). A study to investigate the PK profile of palmitoylethanolamide (PEA) – IRAS ID 252210. https://www.hra.nhs.uk/ last accessed September 26, 2023.

Obermajerová, H., Masek, K., Seifert, J., Buchar, E., & Havlík, I. (1973). Structural and functional changes in liver mitochondria of mice fed palmitoylethanolamide (PEA). *Biochemical Pharmacology 22*(20), 2529–2536. https://doi.org/10.1016/0006-2952(73)90061-0.

Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T., & Ueda, N. (2004). Molecular characterization of a phospholipase D generating anandamide and its congeners. *Journal of Biological Chemistry 279*(7), 5298–5305. https://doi.org/10.1074/jbc.M306642200.

Olusanmi, D., Jayawickrama, D., Bu, D., McGeorge, G., Sailes, H., Kelleher, J., ... Tobyn, M. (2014). A control strategy for bioavailability enhancement by size reduction: Effect of micronization conditions on the bulk, surface and blending characteristics of an active pharmaceutical ingredient. *Powder Technology 258*, 222–233. https://doi.org/10.1016/j.powtec.2014.03.032.

Orefice, N. S., Alhouayek, M., Carotenuto, A., Montella, S., Barbato, F., Comelli, A., ... Orefice, G. (2016). Oral palmitoylethanolamide treatment is associated with reduced cutaneous adverse effects of interferon-β1a and circulating proinflammatory cytokines in relapsing–remitting multiple sclerosis. *Neurotherapeutics 13*(2), 428–438. https://doi.org/10.1007/s13311-016-0420-z.

Ottaviani, G., Rupel, K., Gobbo, M., Poropat, A., Zoi, V., Faraon, M., ... Biasotto, M. (2019). Efficacy of ultramicronized palmitoylethanolamide in burning mouth syndrome-affected patients: A preliminary randomized double-blind controlled trial. *Clinical Oral Investigations 23*(6), 2743–2750. https://doi.org/10.1007/s00784-018-2720-7.

Ottria, R., Casati, S., & Ciuffreda, P. (2012). Optimized synthesis and characterization of N-acylethanolamines and O-acylethanolamines, important family of lipid-signalling molecules. *Chemistry and Physics of Lipids 165*(7), 705–711. https://doi.org/10.1016/j.chemphyslip.2012.06.010.

Overton, H. A., Babbs, A. J., Doel, S. M., Fyfe, M. C. T., Gardner, L. S., Griffin, G., ... Reynet, C. (2006). Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents. *Cell Metabolism 3*(3), 167–175. https://doi.org/10.1016/j.cmet.2006.02.004.

Oxford Centre for Evidence Based Medicine (2024). http://www.cebm.net last accessed January 29, 2024.

Padua, L., Briani, C., Jann, S., Nobile-Orazio, E., Pazzaglia, C., Morini, A., ... Cruccu, G. (2009). Validation of the Italian version of the neuropathic pain symptom inventory in peripheral nervous system diseases. *Neurological Sciences 30*(2), 99–106. https://doi.org/10.1007/s10072-009-0025-y.

Paladini, A., Fusco, M., Cenacchi, T., Schievano, C., Piroli, A., & Varrassi, G. (2016). Palmitoylethanolamide, a special food for medical purposes, in the treatment of chronic pain: A pooled data Meta-analysis. *Pain Physician 19*(2), 11–24.

Paladini, A., Varrassi, G., Bentivegna, G., Carletti, S., Piroli, A., & Coaccioli, S. (2017). Palmitoylethanolamide in the treatment of failed Back surgery syndrome. *Pain Research and Treatment 2017*, 1486010. https://doi.org/10.1155/2017/1486010.

Papetti, L., Sforza, G., Tullo, G., Alaimo Di Loro, P., Moavero, R., Ursitti, F., ... Valeriani, M. (2020). Tolerability of Palmitoylethanolamide in a pediatric population suffering from migraine: A pilot study. *Pain Research and Management 2020*, 3938640. https://doi.org/10.1155/2020/3938640.

Parabita, M., D'Amore, R. M., Marinosci, F., Matera, M. G., Izzo, A., & Russo, G. A. (2011). Reduction of post stroke spasticity with palmitoylethanolamide treatment. *XIV Congress of the European Shock Society. Taormina, August 31, September 2, 2011.*

Parisi, S., Ditto, M. C., Borrelli, R., & Fusaro, E. (2021). Efficacy of a fixed combination of palmitoylethanolamide and acetyl-l-carnitine (PEA+ALC FC) in the treatment of neuropathies secondary to rheumatic diseases. *Minerva Medica 112*(4), 492–499. https://doi.org/10.23736/S0026-4806.21.07486-3.

Passavanti, M. B., Fiore, M., Sansone, P., Aurilio, C., Pota, V., Barbarisi, M., ... Pace, M. C. (2017). The beneficial use of ultramicronized palmitoylethanolamide as add-on therapy to Tapentadol in the treatment of low back pain: A pilot study comparing prospective and retrospective observational arms. *BMC Anesthesiology 17*(1), 171. https://doi.org/10.1186/s12871-017-0461-9.

Paterniti, I., Impellizzeri, D., Crupi, R., Morabito, R., Campolo, M., Esposito, E., & Cuzzocrea, S. (2013). Molecular evidence for the involvement of PPAR-δ and PPAR-γ in anti-inflammatory and neuroprotective activities of palmitoylethanolamide after spinal cord trauma. *Journal of Neuroinflammation 10*, 20. http://www.jneuroinflammation.com/content/10/1/20.

Paterniti, I., Impellizzeri, D., Di Paola, R., Navarra, M., Cuzzocrea, S., & Esposito, E. (2013). A new co-ultramicronized composite including palmitoylethanolamide and luteolin to prevent neuroinflammation in spinal cord injury. *Journal of Neuroinflammation 10*, 91. https://doi.org/10.1186/1742-2094-10-91.

Peritore, A. F., Siracusa, R., Fusco, R., Gugliandolo, E., D'amico, R., Cordaro, M., ... Di Paola, R. (2020). Ultramicronized palmitoylethanolamide and paracetamol, a new association to relieve hyperalgesia and pain in a sciatic nerve injury model in rat. *International Journal of Molecular Sciences 21*(10), 3509. https://doi.org/10.3390/ijms21103509.

Perlík, F., Krejcí, J., Elis, J., Pekárek, J., & Svejcar, J. (1973). The effect of N-(2-hydroxyethyl)-palmitamide on delayed hypersensitivity in guinea-pig. *Experientia 29*(1), 67–68. https://doi.org/10.1007/BF01913256.

Perlík, F., Rasková, H., & Elis, J. (1971). Anti-inflammatory properties of N(2-hydroxyethyl) palmitamide. *Acta Physiologica Academiae Scientiarum Hungaricae 39*(4), 395–400.

Pertwee, R. G. (2007). GPR55: A new member of the cannabinoid receptor clan? *British Journal of Pharmacology 152*(7), 984–986. https://doi.org/10.1038/sj.bjp.0707464.

Petrosino, S., Cordaro, M., Verde, R., Schiano Moriello, A., Marcolongo, G., Schievano, C., ... Di Marzo, V. (2018). Oral ultramicronized palmitoylethanolamide: Plasma and tissue levels and spinal anti-hyperalgesic effect. *Frontiers in Pharmacology 9*(249). https://doi.org/10.3389/fphar.2018.00249.

Petrosino, S., & Di Marzo, V. (2017). The pharmacology of palmitoylethanolamide and first data on the therapeutic efficacy of some of its new formulations. *British Journal of Pharmacology 174*, 1349–1365. https://doi.org/10.1111/bph.v174.11/issuetoc.

Petrosino, S., Palazzo, E., de Novellis, V., Bisogno, T., Rossi, F., Maione, S., & Di Marzo, V. (2007). Changes in spinal and supraspinal endocannabinoid levels in neuropathic rats. *Neuropharmacology 52*(2), 415–422. https://doi.org/10.1016/j.neuropharm.2006.08.011.

Petrosino, S., & Schiano Moriello, A. (2020). Palmitoylethanolamide: A nutritional approach to keep neuroinflammation within physiological boundaries—A systematic review. *International Journal of Molecular Sciences 21*(24), 1–25. https://doi.org/10.3390/ijms21249526.

Petrosino, S., Schiano Moriello, A., Cerrato, S., Fusco, M., Puigdemont, A., De Petrocellis, L., & Di Marzo, V. (2016). The anti-inflammatory mediator palmitoylethanolamide enhances the levels of 2-arachidonoyl-glycerol and potentiates its actions at TRPV1 cation channels. *British Journal of Pharmacology 173*(7), 1154–1162. https://doi.org/10.1111/bph.13084.

Petrosino, S., Schiano Moriello, A., Verde, R., Allarà, M., Imperatore, R., Ligresti, A., ... Di Marzo, V. (2019). Palmitoylethanolamide counteracts substance P-induced mast cell activation in vitro by stimulating diacylglycerol lipase activity. *Journal of Neuroinflammation 16*(1), 274. https://doi.org/10.1186/s12974-019-1671-5.

Pickering, E., Steels, E. L., Steadman, K. J., Rao, A., & Vitetta, L. (2022). A randomized controlled trial assessing the safety and efficacy of palmitoylethanolamide for treating diabetic-related peripheral neuropathic pain. *Inflammopharmacology 30*(6), 2063–2077. https://doi.org/10.1007/s10787-022-01033-8.

Piomelli, D., & Mabou Tagne, A. (2022). Endocannabinoid-based therapies. *Annual Review of Pharmacology and Toxicology 62*, 483–507. https://doi.org/10.1146/annurev-pharmtox-052220-021800.

Piomelli, D., Scalvini, L., Fotio, Y., Lodola, A., Spadoni, G., Tarzia, G., & Mor, M. (2020). N-Acylethanolamine acid amidase (NAAA): Structure, function, and inhibition. *Journal of Medicinal Chemistry 63*(14), 7475–7490. https://doi.org/10.1021/acs.jmedchem.0c00191.

Putzu, G. A. (2016). Efficacy of Ultramicronized Palmitoylethanolamide on the clinical symptoms of Charcot-Marie-tooth neuropathy. *Archives of Neurology and Neurosurgery 1*, 12–14.

Quercioli, A., Carbone, F., Bonaventura, A., Liberale, L., Pataky, Z., Thomas, A., ... Montecucco, F. (2017). Plasma palmitoylethanolamide (PEA) as a potential biomarker for impaired coronary function. *International Journal of Cardiology 231*, 1–5. https://doi.org/10.1016/j.ijcard.2016.12.023.

Rabin, R., Gudex, C., Selai, C., & Herdman, M. (2014). From translation to version management: A history and review of methods for the cultural adaptation of the euroqol five-dimensional questionnaire. *Value in Health 17*(1), 70–76. https://doi.org/10.1016/j.jval.2013.10.006.

Rahman, I. A., Tsuboi, K., Hussain, Z., Yamashita, R., Okamoto, Y., Uyama, T., Yamazaki, N., Tanaka, T., Tokumura, A., & Ueda, N. (2016). Calcium-dependent generation of N-acylethanolamines and lysophosphatidic acids by glycerophosphodiesterase GDE7. *Biochimica et Biophysica Acta 1861*(12 Pt A), 1881–1892. https://doi.org/10.1016/j.bbalip.2016.09.008.

Rasenack, N., & Müller, B. W. (2004). Micron-size drug particles: Common and novel micronization techniques. *Pharmaceutical Development and Technology 9*(1), 1–13. https://doi.org/10.1081/PDT-120027417.

Rasková, H., Masek, K., & Linèt, O. (1972). Non-specific resistance induced by palmitoylethanolamide. *Toxicon 10*(5), 485–490. https://doi.org/10.1016/0041-0101(72)90174-2.

Re, G., Barbero, R., Miolo, A., & Di Marzo, V. (2007). Palmitoylethanolamide, endocannabinoids and related cannabimimetic compounds in protection against tissue inflammation and pain: Potential use in companion animals. *The Veterinary Journal 173*(1), 21–30. https://doi.org/10.1016/j.tvjl.2005.10.003.

Ribeiro, A., Pontis, S., Mengatto, L., Armirotti, A., Chiurchiù, V., Capurro, V., ... Piomelli, D. (2015). A potent systemically active N-Acylethanolamine acid amidase inhibitor that suppresses inflammation and human macrophage activation. *ACS Chemical Biology 10*(8), 1838–1846. https://doi.org/10.1021/acschembio.5b00114.

Richardson, D., Pearson, R. G., Kurian, N., Latif, M. L., Garle, M. J., Barrett, D. A., ... Chapman, V. (2008). Characterisation of the cannabinoid receptor system in synovial tissue and fluid in patients with osteoarthritis and rheumatoid arthritis. *Arthritis Research and Therapy 10*(2), R43. https://doi.org/10.1186/ar2401.

Rinne, P., Guillamat-Prats, R., Rami, M., Bindila, L., Ring, L., Lyytikainen, L. P., ... Steffens, S. (2018). Palmitoylethanolamide promotes a proresolving macrophage phenotype and attenuates atherosclerotic plaque formation. *Arteriosclerosis, Thrombosis, and Vascular Biology 38*(11), 2562–2575. https://doi.org/10.1161/ATVBAHA.118.311185.

Roe, E. T., Scanlan, J. T., & Swern, D. (1949). Fatty acid amides. I. Preparation of amides of oleic and the 9,10-Dihydroxystearic acids. *Journal of the American Chemical Society 71*(6), 2215–2218.

Roviezzo, F., Rossi, A., Caiazzo, E., Orlando, P., Riemma, M. A., Iacono, V. M., ... Izzo, A. A. (2017). Palmitoylethanolamide supplementation during sensitization prevents airway allergic symptoms in the mouse. *Frontiers in Pharmacology 8*, 857. https://doi.org/10.3389/fphar.2017.00857.

Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N. O., Leonova, J., ... Greasley, P. J. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology 152*(7), 1092–1101. https://doi.org/10.1038/sj.bjp.0707460.

Sarnelli, G., Pesce, M., Seguella, L., Lu, J., Efficie, E., Tack, J., ... Esposito, G. (2021). Impaired duodenal Palmitoylethanolamide release underlies acid-induced mast cell activation in functional dyspepsia. *Cellular and Molecular Gastroenterology and Hepatology 11*(3), 841–855. https://doi.org/10.1016/j.jcmgh.2020.10.001.

Sasso, O., Moreno-Sanz, G., Martucci, C., Realini, N., Dionisi, M., Mengatto, L., Duranti, A., Tarozzo, G., Tarzia, G., Mor, M., Bertorelli, R., Reggiani, A., & Piomelli, D. (2013). Antinociceptive effects of the N-acylethanolamine acid amidase inhibitor ARN077 in rodent pain models. *Pain 154*(3), 350–360. https://doi.org/10.1016/j.pain.2012.10.018.

Sasso, O., Summa, M., Armirotti, A., Pontis, S., De Mei, C., & Piomelli, D. (2018). The N-Acylethanolamine acid amidase inhibitor ARN077 suppresses inflammation and pruritus in a mouse model of allergic dermatitis. *Journal of Investigative Dermatology 138*(3), 562–569. https://doi.org/10.1016/j.jid.2017.07.853.

Sawzdargo, M., Nguyen, T., Lee, D. K., Lynch, K. R., Cheng, R., Heng, H. H. Q., ... O'dowd, B.F. (1999). Identification and cloning of three novel human G protein-coupled receptor genes GPR52, CGPR53 and GPR55: GPR55 is extensively expressed in human brain 1. *Molecular Brain Research 64*(2), 193–198. https://doi.org/10.1016/s0169-328x(98)00277-0.

Scaturro, D., Asaro, C., Lauricella, L., Tomasello, S., Varrassi, G., & Mauro, G. L. (2020). Combination of rehabilitative therapy with Ultramicronized Palmitoylethanolamide for chronic low Back pain: An observational study. *Pain and therapy 9*(1), 319–326. https://doi.org/10.1007/s40122-019-00140-9.

Schreiber, D., Harlfinger, S., Nolden, B. M., Gerth, C. W., Jaehde, U., Schömig, E., ... Markus Leweke, F. (2007). Determination of anandamide and other fatty acyl ethanolamides in human serum by electrospray tandem mass spectrometry. *Analytical Biochemistry 361*(2), 162–168. https://doi.org/10.1016/j.ab.2006.11.027.

Schuel, H., Burkman, L. J., Lippes, J., Crickard, K., Forester, E., Piomelli, D., & Giuffrida, A. (2002). N-Acylethanolamines in human reproductive fluids. *Chemistry and Physics of Lipids 121*(1–2), 211–227. https://doi.org/10.1016/s0009-3084(02)00158-5.

Schweiger, V., Martini, A., Bellamoli, P., Donadello, K., Schievano, C., Balzo, G. D., ... Polati, E. (2019). Ultramicronized palmitoylethanolamide (um-PEA) as add-on treatment in fibromyalgia syndrome (FMS): Retrospective observational study on 407 patients. *CNS & Neurological Disorders - Drug Targets 18*(4), 326–333. https://doi.org/10.2174/1871527318666190227205359.

Scuteri, D., Guida, F., Boccella, S., Palazzo, E., Maione, S., Rodríguez-Landa, J. F., ... Corasaniti, M. T. (2022). Effects of Palmitoylethanolamide (PEA) on nociceptive, musculoskeletal

and neuropathic pain: Systematic review and Meta-analysis of clinical evidence. *Pharmaceutics 14*(8), 1672. https://doi.org/10.3390/pharmaceutics14081672.
Sepe, N., De Petrocellis, L., Montanaro, F., Cimino, G., & Di Marzo, V. (1998). Bioactive long chain N-acylethanolamines in five species of edible bivalve molluscs possible implications for mollusc physiology and sea food industry. *Biochimica et Biophysica Acta 1389* (2), 101–111. https://doi.org/10.1016/s0005-2760(97)00132-x.
Sharir, H., Console-Bram, L., Mundy, C., Popoff, S. N., Kapur, A., & Abood, M. E. (2012). The endocannabinoids anandamide and virodhamine modulate the activity of the candidate cannabinoid receptor GPR55. *Journal of Neuroimmune Pharmacology 7*(4), 856–865. https://doi.org/10.1007/s11481-012-9351-6.
Simon, G. M., & Cravatt, B. F. (2006). Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *Journal of Biological Chemistry 281*(36), 26465–26472. https://doi.org/10.1074/jbc.M604660200.
Simon, G. M., & Cravatt, B. F. (2008). Anandamide biosynthesis catalyzed by the phosphodiesterase GDE1 and detection of glycerophospho-N-acyl ethanolamine precursors in mouse brain. *Journal of Biological Chemistry 283*(14), 9341–9349. https://doi.org/10.1074/jbc.M707807200.
Singh, S., Dwivedi, R., & Chaturvedi, V. (2012). Influence of vehicles used for oral dosing of test molecules on the progression of Mycobacterium tuberculosis infection in mice. *Antimicrobial Agents and Chemotherapy 56*(11), 6026–6028. https://doi.org/10.1128/AAC.01702-12.
Siracusa, R., Impellizzeri, D., Cordaro, M., Crupi, R., Esposito, E., Petrosino, S., & Cuzzocrea, S. (2017). Anti-inflammatory and neuroprotective effects of co-ultraPEALut in a mouse model of vascular dementia. *Frontiers in Neurology 8*, 233. https://doi.org/10.3389/fneur.2017.00233.
Skaper, S. D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L., & Leon, A. (1996). The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons (N-methyl-D-aspartate/neurotoxicity/N-acylethanolamides/neuroprotection/receptor). *Proceedings of the National Academy of Sciences USA 93* (9), 3984–3989. https://doi.org/10.1073/pnas.93.9.3984.
Skaper, S. D., Facci, L., Barbierato, M., Zusso, M., Bruschetta, G., Impellizzeri, D., ... Giusti, P. (2015). N-palmitoylethanolamine and neuroinflammation: A novel therapeutic strategy of resolution. *Molecular Neurobiology 52*(2), 1034–1042. https://doi.org/10.1007/s12035-015-9253-8.
Skaper, S. D., Facci, L., Fusco, M., Della Valle, M. F., Zusso, M., Costa, B., & Giusti, P. (2014). Palmitoylethanolamide, a naturally occurring disease-modifying agent in neuropathic pain. *Inflammopharmacology 22*(2), 79–94. https://doi.org/10.1007/s10787-013-0191-7.
Skaper, S. D., Facci, L., & Giusti, P. (2013). Glia and mast cells as targets for palmitoylethanolamide, an anti-inflammatory and neuroprotective lipid mediator. *Molecular Neurobiology 48*(2), 340–352. https://doi.org/10.1007/s12035-013-8487-6.
Skaper, S. D., Facci, L., & Giusti, P. (2014). Mast cells, glia and neuroinflammation: Partners in crime? *Immunology 141*(3), 314–327. https://doi.org/10.1111/imm.12170.
Skaper, S. D., Facci, L., Zusso, M., & Giusti, P. (2018). An inflammation-centric view of neurological disease: Beyond the neuron. *Frontiers in Cellular Neuroscience 12*, 72. https://doi.org/10.3389/fncel.2018.00072 Erratum in: Frontiers in Cellular Neuroscience, 13, 578.
Smart, D., Jonsson, K. O., Vandevoorde, S., Lambert, D. M., & Fowler, C. J. (2002). "Entourage" effects of N-acyl ethanolamines at human vanilloid receptors. Comparison of effects upon anandamide-induced vanilloid receptor activation and upon anandamide metabolism. *British Journal of Pharmacology 136*(3), 452–458. https://doi.org/10.1038/sj.bjp.0704732.
Steels, E., Venkatesh, R., Steels, E., Vitetta, G., & Vitetta, L. (2019). A double-blind randomized placebo controlled study assessing safety, tolerability and efficacy of palmitoylethanolamide for symptoms of knee osteoarthritis. *Inflammopharmacology 27*(3), 475–485. https://doi.org/10.1007/s10787-019-00582-9.
Stensson, N., Ghafouri, N., Ernberg, M., Mannerkorpi, K., Kosek, E., Gerdle, B., & Ghafouri, B. (2018). The relationship of endocannabinoidome lipid mediators with pain and psychological stress in women with fibromyalgia: A case-control study. *Journal of Pain 19* (11), 1318–1328. https://doi.org/10.1016/j.jpain.2018.05.008.
Stochino Loi, E. S., Pontis, A., Cofelice, V., Pirarba, S., Fais, M. F., Daniilidis, A., ... Angioni, S. (2019). Effect of ultramicronized-palmitoylethanolamide and co-micronizedpalmitoylethanolamide/polydatin on chronic pelvic pain and quality of life in endometriosis patients: An open-label pilot study. *International Journal of Women's Health 11*, 443–449. https://doi.org/10.2147/IJWH.S204275.
Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., ... Waku, K. (1995). 2-Arachidonoylglycerol: A possible endogenous cannabinoid receptor ligand in brain. *Biochemical and Biophysical Research Communications 215*(1), 89–97. https://doi.org/10.1006/bbrc.1995.2437.
Sun, Y. X., Tsuboi, K., Okamoto, Y., Tonai, T., Murakami, M., Kudo, I., & Ueda, N. (2004). Biosynthesis of anandamide and N-palmitoylethanolamine by sequential actions of phospholipase A2 and lysophospholipase D. *Biochemical Journal 380*(Pt 3), 749–756. https://doi.org/10.1042/BJ20040031.
Svec, P., Béderová, E., & Svec, F. (1975). The effect of long-term administration of N-(2-hydroxyethyl)palmitamide on the chemotherapy of RBA rat leukemia. *Neoplasma 22*(6), 625–630.
Tartaglia, E., Armentano, M., Giugliano, B., Sena, T., Giuliano, P., Loffredo, C., & Mastrantonio, P. (2015). Effectiveness of the association N-palmitoylethanolamine and transpolydatin in the treatment of primary dysmenorrhea. *Journal of Pediatric and Adolescent Gynecology 28*(6), 447–450. https://doi.org/10.1016/j.jpag.2014.12.011.
Toti, A., Micheli, L., Lucarini, E., Ferrara, V., Ciampi, C., Margiotta, F., ... Di Cesare Mannelli, L. (2023). Ultramicronized N-palmitoylethanolamine regulates mast cell-astrocyte crosstalk: A new potential mechanism underlying the inhibition of morphine tolerance. *Biomolecules 13*(2), 233. https://doi.org/10.3390/biom13020233.
Treede, R. D., Rief, W., Barke, A., Aziz, Q., Bennett, M. I., Benoliel, R., ... Wang, S. J. (2019). Chronic pain as a symptom or a disease: The IASP classification of chronic pain for the international classification of diseases (ICD-11). *Pain 160*(1), 19–27. https://doi.org/10.1097/j.pain.0000000000001384.
Tsuboi, K., Okamoto, Y., Rahman, I. A., Uyama, T., Inoue, T., Tokumura, A., & Ueda, N. (2015). Glycerophosphodiesterase GDE4 as a novel lysophospholipase D: A possible involvement in bioactive N-acylethanolamine biosynthesis. *Biochimica et Biophysica Acta 1851*(5), 537–548. https://doi.org/10.1016/j.bbalip.2015.01.002.
Tzschentke, T. M., Christoph, T., Kögel, B., Schiene, K., Hennies, H. H., Englberger, W., ... De Vry, J. (2007). (−)-(1R,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride (Tapentadol HCl): A novel μ-opioid receptor agonist/norepinephrine reuptake inhibitor with broad-spectrum analgesic properties. *Journal of Pharmacology and Experimental Therapeutics 323*(1), 265–276. https://doi.org/10.1124/jpet.107.126052.
Ueda, N., Yamanaka, K., & Yamamoto, S. (2001). Purification and characterization of an acid amidase selective for N-palmitoylethanolamine, a putative endogenous anti-inflammatory substance. *Journal of Biological Chemistry 276*(38), 35552–35557. https://doi.org/10.1074/jbc.M106261200.
Urwin, S. J., Yerdelen, S., Houson, I., & ter Horst, J. H. (2021). Impact of impurities on crystallization and product quality: A case study with paracetamol. *Crystals 11*(11), 1344. https://doi.org/10.3390/cryst11111344.
Vacondio, F., Bassi, M., Silva, C., Castelli, R., Carmi, C., Scalvini, L., ... Rivara, S. (2015). Amino acid derivatives as palmitoylethanolamide prodrugs: Synthesis, in vitro metabolism and in vivo plasma profile in rats. *PLoS One 10*(6), Article e0128699. https://doi.org/10.1371/journal.pone.0128699.
Vanderwall, A. G., & Milligan, E. D. (2019). Cytokines in pain: Harnessing endogenous anti-inflammatory signaling for improved pain management. *Frontiers in Immunology 10*, 3009. https://doi.org/10.3389/fimmu.2019.03009.
Velasco, R., Domingo-Domenech, E., & Sureda, A. (2021). Brentuximab-induced peripheral neurotoxicity: A multidisciplinary approach to manage an emerging challenge in hodgkin lymphoma therapy. *Cancers 13*(23), 6125. https://doi.org/10.3390/cancers13236125.
Wang, C., Pu, R., Ghose, B., & Tang, S. (2018). Chronic musculoskeletal pain, self-reported health and quality of life among older populations in South Africa and Uganda. *International Journal of Environmental Research and Public Health 15*(12), 1–13. https://doi.org/10.3390/ijerph15122806.
Ware, J., Kosinski, M., & Keller, S. D. (1996). A 12-item short-form health survey: Construction of scales and preliminary tests of reliability and validity. *Medical Care 34* (3), 220–233. https://doi.org/10.1097/00005650-199603000-00003.
Wei, B. Q., Mikkelsen, T. S., McKinney, M. K., Lander, E. S., & Cravatt, B. F. (2006). A second fatty acid amide hydrolase with variable distribution among placental mammals. *Journal of Biological Chemistry 281*(48), 36569–36578. https://doi.org/10.1074/jbc.M606646200.
Wood, J. A. T., Williams, J. S., Pandarinathan, L., Courville, A., Keplinger, M. R., Janero, D. R., ... Lammi-Keefe, C. J. (2008). Comprehensive profiling of the human circulating endocannabinoid metabolome: Clinical sampling and sample storage parameters. *Clinical Chemistry and Laboratory Medicine 46*(9), 1289–1295. https://doi.org/10.1515/CCLM.2008.242.
Yang, H., Zhou, J., & Lehmann, C. (2016). GPR55 - A putative "type 3" cannabinoid receptor in inflammation. *Journal of Basic and Clinical Physiology and Pharmacology 27*(3), 297–302. https://doi.org/10.1515/jbcpp-2015-0080.
Yang, L., Li, L., Chen, L., Li, Y., Chen, H., Li, Y., Ji, G., Lin, D., Liu, Z., & Qiu, Y. (2015). Potential analgesic effects of a novel N-acylethanolamine acid amidase inhibitor F96 through PPAR-α. *Scientific Reports 5*, 13565. https://doi.org/10.1038/srep13565.
Zhou, P., Xiang, L., Yang, Y., Wu, Y., Hu, T., Liu, X., Lin, F., Xiu, Y., Wu, K., Lu, C., Ren, J., Qiu, Y., & Li, Y. (2019). N-Acylethanolamine acid amidase (NAAA) inhibitor F215 as a novel therapeutic agent for osteoarthritis. *Pharmacological Research 145*, Article 104264. https://doi.org/10.1016/j.phrs.2019.104264.
Zhukov, O. D. (1999). Rozpodil N-([1-14C]-pal'mitoïl)etanolaminu v tkanynakh shchuriv [distribution of N-([1-14C]-palmitoyl)]ethanolamine in rat tissues. *Ukrainskiĭ Biokhimicheskiĭ Zhurnal 71*(4), 124–125.