Naturally Informed:

Sleep, Stress, and Discomfort:

The Microbiome Connection

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Refocusing the Microbiome Paradigm

Over the past two decades, the rise of –biotics has shaped a microbiome first approach, largely viewing the microbiome as the starting point for health.

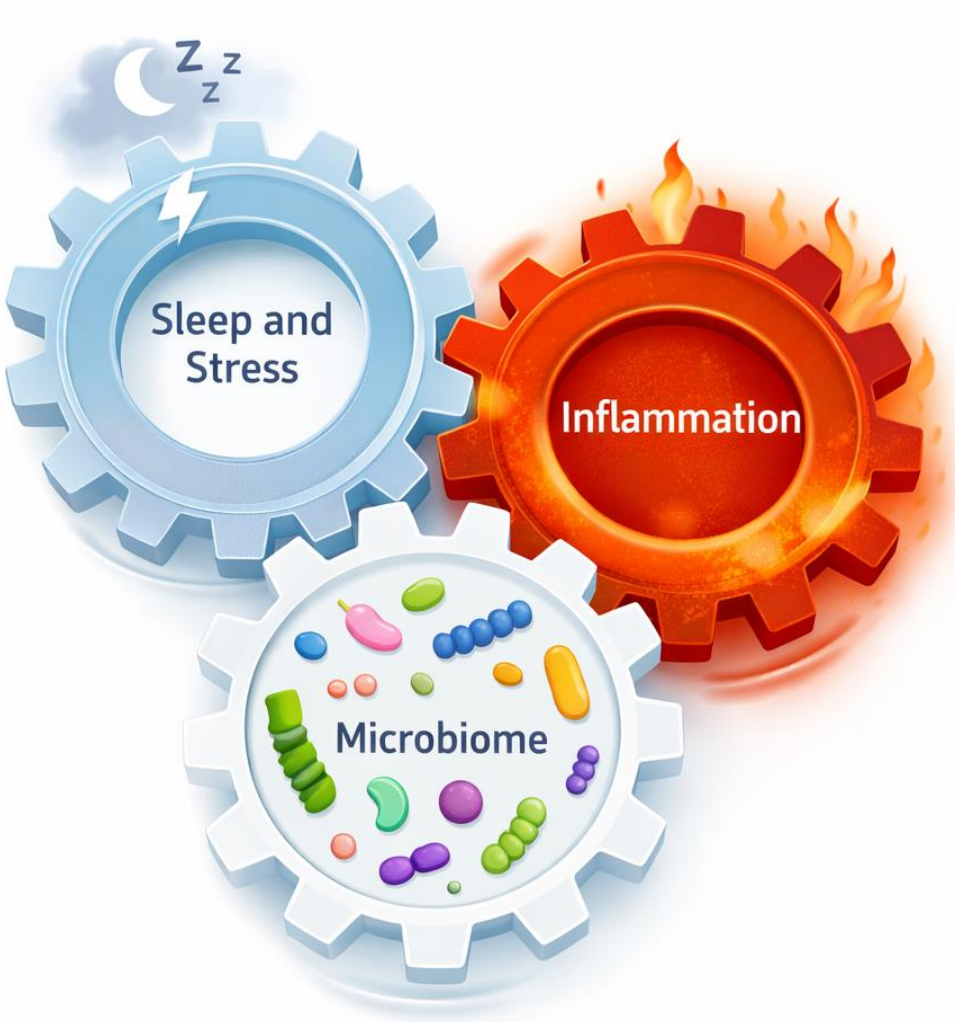
Nobody can deny that truth!

We've expanded our toolkit with enzymes, fibers, and postbiotics; each advancing our ability to modulate microbial composition.

This model has been a revolutionary leap, but is it the whole story?

The microbiome, inflammation, and stress physiology operate as interconnected, reinforcing systems — each influencing one another.

Inflammation and stress physiology do not just result from microbiome changes — they actively influence which microbes can survive and thrive.



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Back In Balance

- Dysbiosis and inflammation or stress can form a **self-reinforcing loop.**
- Dysbiosis → inflammatory signaling/stress dysregulation.
- Inflammation/stress → environment that drives further dysbiosis.
- **Intervention is possible at any point in the cycle.**
- Microbiome-targeted approaches address dysbiosis directly.
- Inflammation/stress-targeted approaches stabilize the host environment.
- **Both pathways can shift the system back toward balance.**

Faecalibacterium: A Key Microbe

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- **Key modulatory microbe:** *Faecalibacterium prausnitzii* is a major butyrate-producing bacterium that helps modulate pro-inflammatory pathways which are typically elevated in individuals with poor sleep as well as ongoing discomfort.

- **Butyrate links gut to brain:** The butyrate produced by *Faecalibacterium* supports gut barrier integrity and influences neuroinflammation and brain signaling—mechanisms that are increasingly tied to sleep quality and circadian regulation.

- **Reduced in poor sleepers:** Human data shows that lower abundance of *Faecalibacterium* is associated with worse sleep quality, suggesting a loss of inflammation modulation and gut-supportive functions.

- **Amplifies the sleep–inflammation cycle:** Declines in *Faecalibacterium* may contribute to increased gut permeability and endotoxin (LPS) exposure, fueling systemic inflammation that can further impair sleep—creating a reinforcing feedback loop.

https://doi.org/10.1016/j.neuroscience.2024.11.070
https://www.sciencedirect.com/science/article/abs/pii/S0306452224006894

Gut Microbiome & Chronic Discomfort

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Tieppo, A.M.; Tieppo, J.S.; Rivetti, L.A. Analysis of Intestinal Bacterial Microbiota in Individuals with and without Chronic Low Back Pain. *Curr. Issues Mol. Biol.* **2024**, *46*, 7339-7352.

This research seeks to evaluate the bacterial composition of the intestinal microbiota of two similar groups: one with chronic low back pain (PG) and the control group (CG).
Clinical data from 73 participants and bacterial genome sequencing data from stool samples were analyzed. There were 40 individuals in PG and 33 in CG, aged between 20 and 50 years and with a body mass index of up to 30 kg/m^2

Dysbiosis Pattern Observed:

- ↑ *Clostridium* spp. (incl. *C. difficile*)
- ↑ Pathobiont / Proteobacteria species
- ↓ *Faecalibacterium prausnitzii*
- ↓ *Roseburia* spp. (butyrate producers)
- **Functional Impact:**
- ↓ Short-chain fatty acids (SCFAs)
- ↑ Immune activation
- Disrupted gut barrier integrity

Clinical Implication:
→ Microbiome imbalance may contribute to **pain signaling and persistence**

What Do We Do?

We Do It All!

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Coincidence?

About 30% of Americans live in chronic discomfort.

About 25% use prescription medications.

Over 10% of Americans have ongoing insomnia, up to 40% have regular periodic issues.

About 20% take either a prescription or an OTC sleep aid.

About 30% of the US population suffer from functional gastrointestinal disorders.

https://nida.nih.gov/research-topics/pain

https://www.cdc.gov/nchs/products/databriefs

https://www.gastrojournal.org/article

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Address the Issues From All Angles

What is PEA?

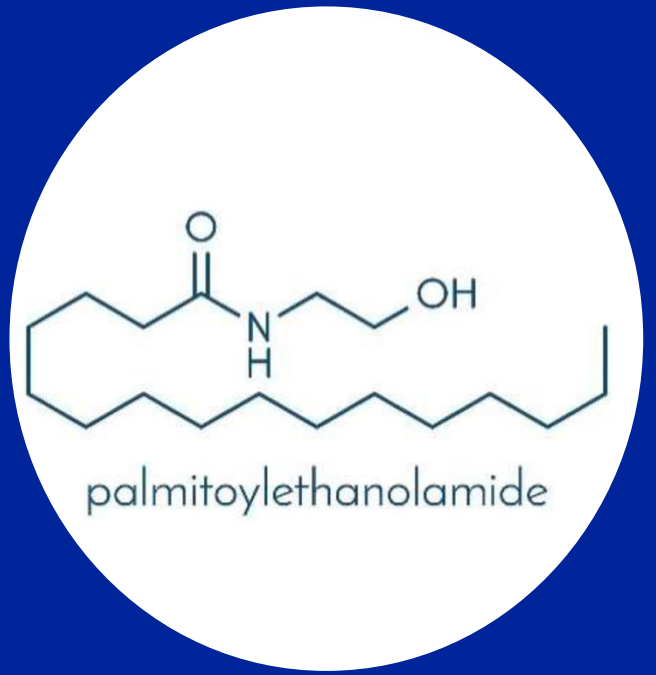
Palmitoylethanolamide (PEA) is an endogenous fatty acid produced by the body in response to stress, inflammation, and cellular irritation.

It functions as a local signaling molecule that helps regulate inflammatory pathways, stress responses, and nerve activity.

PEA is synthesized on demand and rapidly broken down, meaning tissue levels depend on continuous production during periods of physiological stress.

Its primary role is to help restore balance by modulating key systems involved in inflammation and stress physiology, including PPAR-α activation and downstream signaling pathways.

Because these pathways influence gut barrier function, immune signaling, and the intestinal environment, PEA acts as a regulator of the host conditions that shape the microbiome.



Mechanisms of PEA

1. Helps limit excessive inflammatory responses at the earliest stage.*

- •Stabilizes mast cells, preventing excessive activation, while supporting PPAR-a
- •Reduces release of histamine and pro-inflammatory cytokines
- •Helps limit initiation of inflammatory signaling cascades

2. Helps modulat production of inflammatory mediators.*

- •Helps reduce COX-2 and LOX activity
- •Helps limit chemical drivers of pain, swelling, and tissue irritation

3. Helps calm irritated nerves.*

- •Calms overactive nerve support (glial) cells
- •Helps reduce neuroinflammatory signaling in the nervous system
- •Stabilizes nerve firing and lowers hypersensitivity

4. Supports the body's own calming and recovery processes.*

- •Protects anandamide, the 'bliss molecule'
- •Slows enzymatic breakdown of endogenous calming signals
- •Indirectly supports endocannabinoids for mood, stress response, & sleep

*These statements have not been evaluated by the Food and Drug Administration.

Endocannabinoid Signaling & the Microbiome

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Anandamide (AEA) is an endogenous cannabinoid that regulates inflammation, gut barrier function, and immune signaling.
Clinical evidence shows AEA can shift the microbiome under inflammatory stress:

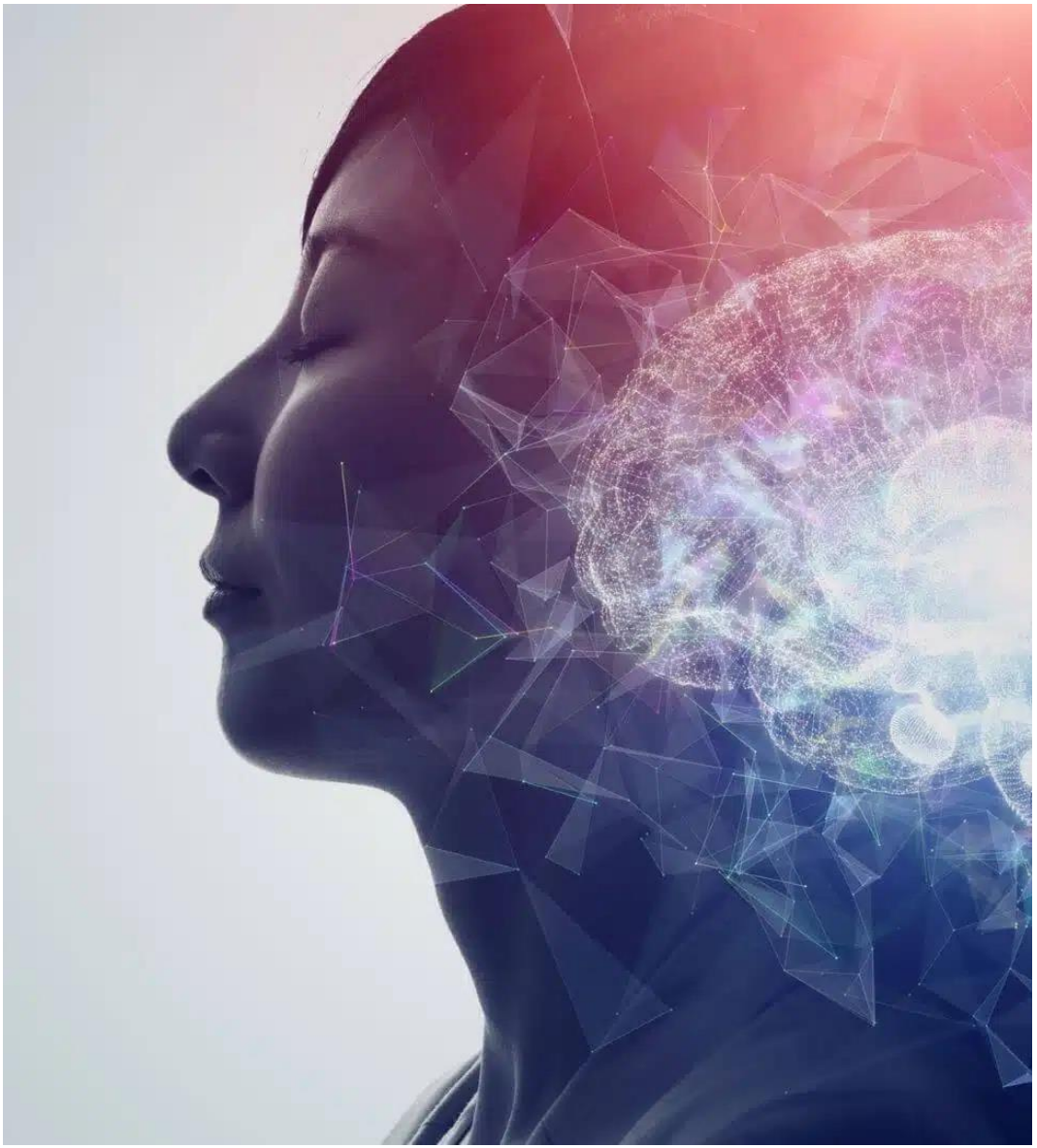
- ↑ Firmicutes and butyrate-producing bacteria
- ↑ short-chain fatty acid (SCFA) production
- ↓ Proteobacteria (inflammation-associated)

These changes are associated with:

- Improved barrier integrity
- Reduced inflammatory tone
- More stable microbial environment

Key Insight:
Endocannabinoid tone helps shape the gut environment and microbial balance.

Relevance to PEA:
PEA enhances anandamide signaling, positioning it as a modulator of the host environment that influences the microbiome.*



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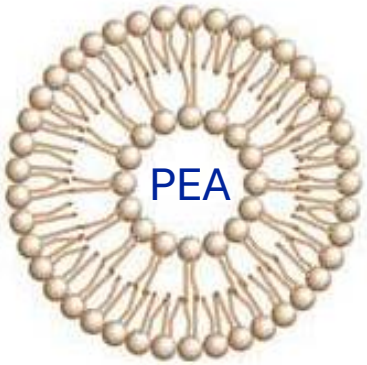
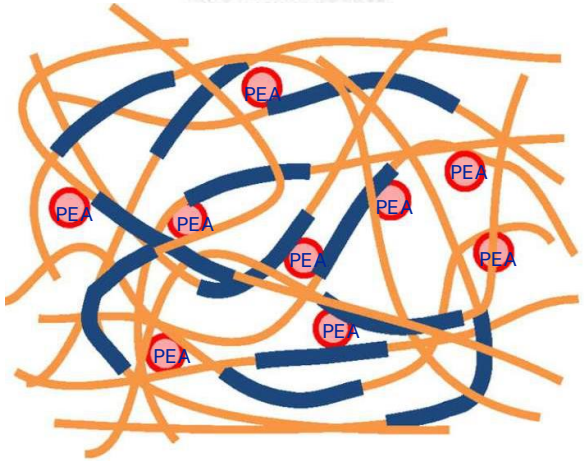
Challenges of PEA - Solved

- PEA is made on demand and rapidly degraded. Food is not a viable source of PEA.
- The large particle size of **native PEA** limits surface area exposure in the gut, slowing dissolution and reducing effective absorption.
- **Micronized and ultra-micronized** PEA improve absorption by reducing particle size, increasing surface area but not fundamentally changing membrane interaction.
- **Liposomes** encapsulate PEA in lipid vesicles, which can be variably destabilized by bile salts and digestive enzymes, leading to inconsistent release and absorption.
- **Phytosome technology** (Indena) forms a molecular complex between PEA and phospholipids (sunflower lecithin), enhancing compatibility with biological membranes.
- Unlike liposomes, **phytosomes do not rely on vesicle integrity**, making them less vulnerable to digestive disruption.

Phytosomes bind active material and phospholipids together into a solid dispersion, meaning the plant compound and the phospholipid behave as ***one functional unit.***





Joint & Muscle

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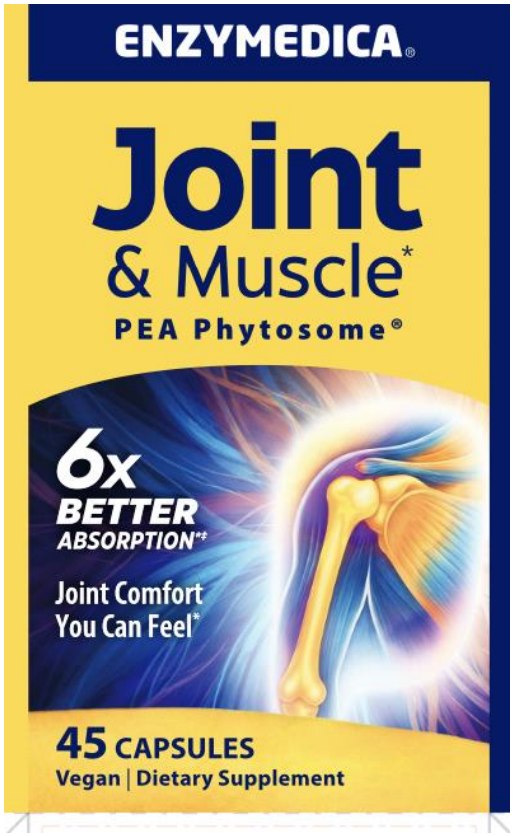
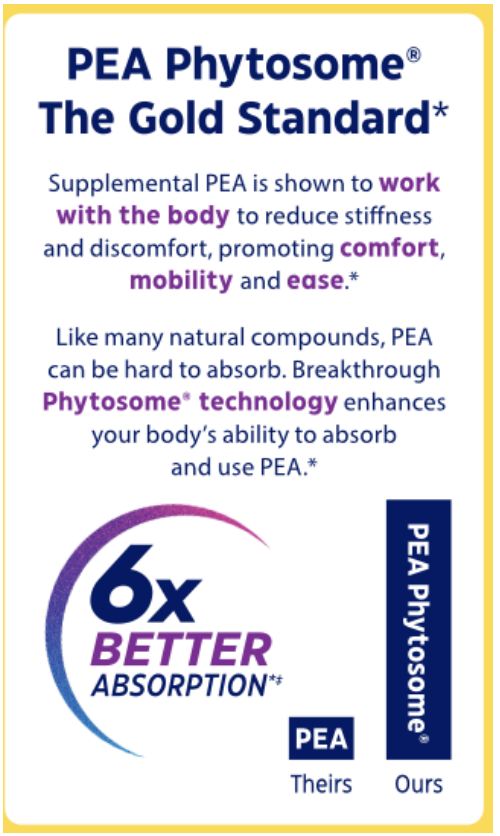
- PEA Phytosome is the solution to bioavailability challenges with PEA.
- Supports inflammation modulation and discomfort with human clinical studies.*
- Works through various pathways to reduce signaling for pain.*
- Helpful for pain that disrupts sleep.*
- Post exercise recovery*
- For individuals sensitive to NSAIDs or other options.*
- Directions: Take two capsules for five days, after 5 days take one capsule per day
- Vegan





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Human Clinical Study

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STUDY DESIGN:

Total population PP 120

Three-arm, randomized, placebo-controlled, double-blind,(double dummy) parallel-groups clinical study. Registered on clinical-trials.gov identified as no NCT06694337.

STUDY POPULATION:

n. 120 subjects, >18 and ≤ 80 years old of either gender, with **low back pain with neuropathic component for at least 3 months**, as confirmed by clinical evaluation and **DN4 screening questionnaire with score ≥4** and **pain intensity score ≥ 4 and ≤ 6** measured by NPRS (Numerical Pain Rating Scale) score

Low Back Discomfort*	Ongoing Ache*	Neuropathic Discomfort*	Sleep Quality & Disturbances*
Improvement vs. Placebo:	Improvement vs. Placebo:	Improvement vs. Placebo:	Improvement vs. Placebo:
Week 1: 44%	Week 1: 22%	Week 8: 90%	Week 4: 58%
Week 4: 76%	Week 4: 44%		Week 8: 63%
Week 8: 88%	Week 8: 85%		
Oswestry Disability Index	Numeric Pain Rating Scale	Douleur Neuropathique Score	Pittsburgh Sleep Quality Index Score

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Dysbiosis Driven Gut Inflammation

When inflammation is elevated in the body, it often shows up in the gut.

Inflammatory conditions

- *IBD (Crohn's, Ulcerative colitis)*
- *Gastritis, esophagitis, ulcers*
- *Celiac disease, micróscopic colitis*

Functional disorders

- *IBS & functional GI disorders*

Microbial imbalance / overgrowth

- *SIBO*
- *H. pylori, C. difficile, infections*

Immune-related responses

- *Food allergies*
- *Eosinophilic GI disorders*

Barrier dysfunction

- *"Leaky gut"*

*Takeaway: Different conditions — but often the **same underlying drivers: inflammation and dysbiosis.***

Putting it All Together

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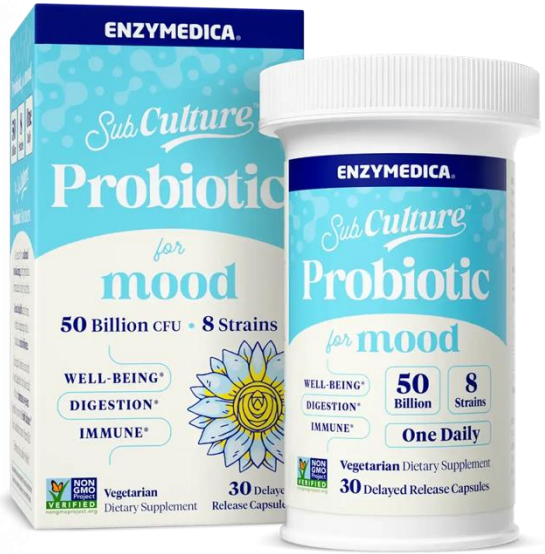
Complete digestion*
More nutrients*
Less dietary antigens*
Food Intolerances*
Enzymes as prebiotics

Bowel health*
Increase in *Akkermansia* and *Bifidobacteria*
Pre/Pro/Post Biotics



Diversity Support
Post-biotic *L. Gasseri CP2305*
L-Theanine

Inflammation modulation*
Better microbial balance*
Decreased discomfort*
Enhanced sleep*

Q&A

www.Enzymedica.com