REVIEW ARTICLE

Journal of Pre-Clinical and Clinical Research
www.jpccr.eu

Ratio-based cannabinoid signalling – limitations of absolute endocannabinoid levels in predicting clinical states

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A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Dawson DA. Ratio-based cannabinoid signalling – limitations of absolute endocannabinoid levels in predicting clinical states. J Pre-Clin Clin Res. doi:10.26444/jpccr/221732

Abstract

Introduction and Objective. The endocannabinoid system (ECS) plays a central role in metabolic regulation, immunomodulation, and energy homeostasis. Although absolute circulating endocannabinoid concentrations are commonly used as biomarkers, clinical heterogeneity often persists despite similar absolute levels. The review aims to examine the limitations of absolute-value interpretation and propose a ratio-based framework for understanding cannabinoid signalling dynamics.

Review Methods. A narrative review of peer-reviewed pre-clinical and clinical literature was conducted, focusing on studies examining endocannabinoid signalling, metabolic regulation, insulin sensitivity, and inflammatory tone. Emphasis was placed on studies reporting multiple endocannabinoids concurrently, enabling a comparative or ratio-based interpretation.

Brief description of the state of knowledge. Current evidence supports the ECS as a coordinated modulatory network rather than a collection of independent signalling molecules. Relative relationships among endocannabinoids appear to better reflect system-level signalling behaviour than single-molecule concentrations alone, although this perspective remains inconsistently applied in clinical interpretation.

Summary. The review highlights the interpretive limitations of absolute endocannabinoid concentrations and underscores the explanatory value of ratio-based signalling frameworks. The analysis is conservative in scope, synthesizes existing evidence only, and does not propose therapeutic interventions or policy implications.

Key words

endocannabinoid system, anandamide, CB1 receptor, 2-arachidonoylglycerol, biomarker interpretation, ratio-based signalling, FAAH, MAGL

INTRODUCTION

The discovery of endogenous cannabinoids transformed understanding of neuromodulatory signalling by revealing a lipid-based system operating in parallel with classical neurotransmitters [1]. Since the identification of anandamide (AEA) and the 2-arachidonoylglycerol (2-AG), endocannabinoid system (ECS), research has expanded rapidly, linking dysregulated signalling to a wide range of neuropsychiatric, inflammatory, and metabolic conditions [2, 3]. Despite this progress, ECS biomarker research has struggled to translate into reliable clinical diagnostics [4, 5]. Studies frequently report overlapping endocannabinoid concentrations between healthy and diseased populations, inconsistent directionality of changes, and limited reproducibility across cohorts [6]. These inconsistencies raise a fundamental question: are absolute endocannabinoid concentrations the correct unit of interpretation?

This review argues that absolute endocannabinoid concentrations are insufficient when considered in isolation. Instead, ECS signalling should be conceptualized as a ratio-dependent regulatory system, in which relative balances between endocannabinoids shape receptor activation patterns, downstream signalling bias, and physiological outcomes.

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Received: 23.01.2026; accepted: 05.05.2026; first published: 20.05.2026

The author does not propose replacing absolute endocannabinoid quantification, but rather argues that isolated concentrations are insufficient when interpreted without relational context. The ratio-based framework presented here is intended to complement existing measurement approaches and improve interpretive resolution.

LIMITATIONS OF ABSOLUTE ENDOCANNABINOID QUANTIFICATION

High inter-individual variability. Absolute circulating levels of AEA and 2-AG vary substantially across individuals due to genetic polymorphisms, diet, circadian rhythm, stress exposure, and metabolic state. This variability often exceeds disease-associated differences, obscuring clinically meaningful signals [7].

Context-dependent signalling. Endocannabinoids function locally and transiently, with rapid synthesis and degradation at synaptic and paracrine sites [8,9]. Peripheral plasma measurements, therefore, represent an indirect and temporally averaged snapshot of a highly dynamic system, limiting their interpretive value when considered in isolation.

Contradictory clinical associations. Both elevated and reduced AEA levels have been reported in conditions such as PTSD, depression, and chronic pain, depending on cohort

characteristics and study design [10]. These contradictions suggest that directionality alone is insufficient and that a broader signalling context must be considered.

Endocannabinoid signalling is increasingly understood as a dynamic, context-dependent regulatory system in which ligand interactions, receptor efficacy, and enzymatic control determine functional output. Within this framework, relative ligand balance may provide greater biological insight into functional signalling states than absolute concentration alone. The concentration data referenced throughout this framework are derived from previously published studies that employed liquid chromatography–tandem mass spectrometry (LC-MS/MS) to quantify circulating anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in human plasma. These measurements provide the empirical basis for considering relative ligand relationships. However, the present review does not introduce new experimental data, instead it synthesizes existing findings into a conceptual framework for interpretation.

THE CASE FOR RATIO-BASED ECS INTERPRETATION

AEA and 2-AG as complementary signals. AEA and 2-AG differ markedly in abundance, receptor efficacy, and temporal dynamics (Tab. 1). AEA is generally a lower-efficacy (partial) agonist at CB1 receptors, whereas 2-AG typically behaves as a higher-efficacy (often a full) agonist, and is widely considered the principal mediator of rapid retrograde endocannabinoid signalling at many central synapses. The relative proportion of these ligands determines whether CB1 signalling is biased toward tonic modulation or phasic excitation. An elevated AEA/2-AG ratio may dampen excessive signalling, while a reduced ratio may favour heightened synaptic suppression [11–13].

Table 1. Comparative properties of anandamide (AEA) and 2-arachidonoylglycerol (2-AG)

Feature	Anandamide (AEA)	2-Arachidonoylglycerol (2-AG)
Relative abundance	Low; present at nanomolar concentrations	High; present at micromolar concentrations
Primary receptor targets	CB1 (primary), weak CB2; TRPV1	CB1 and CB2
CB1 receptor efficacy	Lower efficacy; partial agonist	Higher efficacy; often behaves as a full agonist
Primary physiological role	Tonic modulation of ECS tone	Phasic, activity-dependent retrograde signalling
Temporal dynamics	Slower, modulatory, context-dependent	Rapid, transient, synapse-specific
Dominant degradative enzyme	Fatty acid amide hydrolase (FAAH)	Monoacylglycerol lipase (MAGL)
Functional effect on neurotransmission	Fine-tuning and stabilization of synaptic activity	Robust suppression of neurotransmitter release
Interpretive significance	May dampen excessive CB1 signalling	Drives strong CB1-mediated synaptic suppression
Implication for ratio interpretation	Stabilizing/tonic regulatory bias	Phasic dominance/ strong synaptic suppression

Functional implications of AEA/2-AG balance. Rather than competing signals, AEA and 2-AG form a regulatory continuum (Fig. 1). Shifts in their ratio can alter:

- CB1 receptor occupancy and signalling intensity.
- G-protein coupling efficiency.
- Downstream modulation of neurotransmitter release.
- Stress and reward circuit responsivity.

Importantly, these effects may occur even when absolute concentrations remain within 'normal' ranges [9, 13, 14].

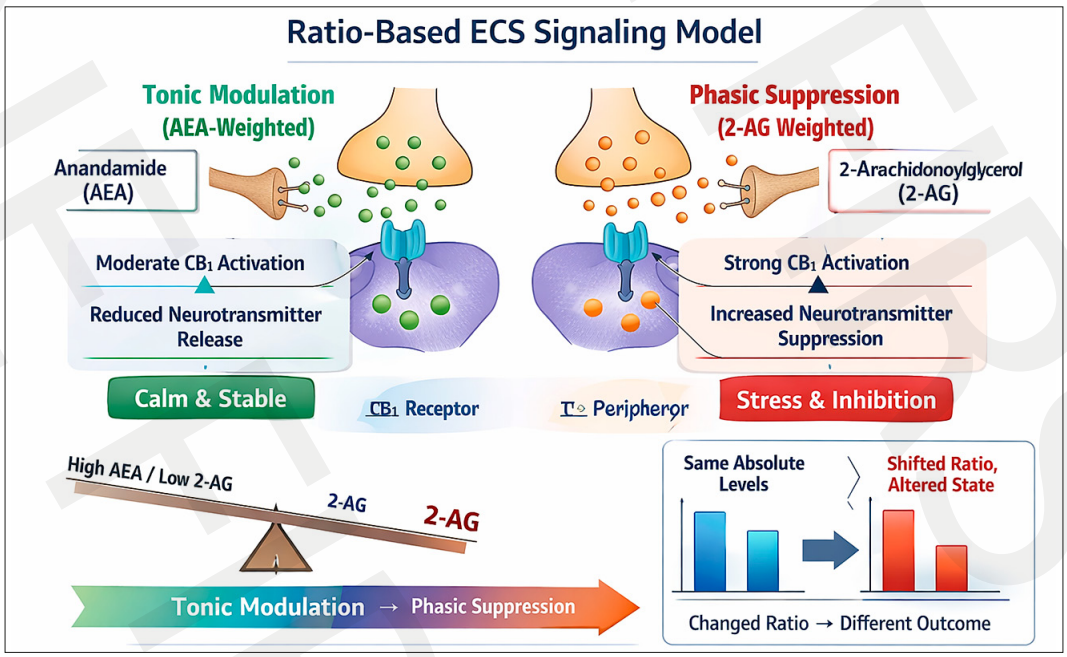

Figure 1. Ratio-based endocannabinoid system signalling model. Schematic representation of how shifts in the relative balance of anandamide (AEA) and 2-arachidonoylglycerol (2-AG) bias CB1 receptor signalling toward tonic modulation or phasic retrograde suppression. An AEA-weighted profile is associated with lower-efficacy, tonic CB1 activation and stable neuromodulatory tone, whereas a 2-AG-weighted profile promotes higher-efficacy CB1 activation, and rapid synaptic suppression of neurotransmitter release. Importantly, these distinct functional states may arise despite similar absolute endocannabinoid concentrations, illustrating how ratio-dependent interpretation can reveal biologically meaningful signalling differences that are obscured by absolute levels alone

Proposed quantitative framework for ratio-based ECS interpretation. To facilitate empirical testing and standardization of ratio-based interpretation of the endocannabinoid system, a simple quantitative framework can be defined based on the relative abundance of anandamide (AEA) and 2-arachidonoylglycerol (2-AG). The primary ratio of interest may be expressed as:

$$R = [AEA] / [2\text{-}AG]$$

where [AEA] and [2-AG] represent circulating or tissue concentrations measured under consistent analytical conditions.

Given the substantial difference in baseline concentrations between AEA (typically nanomolar range) and 2-AG (typically micromolar range), logarithmic transformation may improve interpretability and statistical stability:

$$R_{log} = \log_{10}([AEA] / [2\text{-}AG])$$

This transformation reduces scale disparity and allows proportional shifts in ligand balance to be more readily compared across individuals and study populations. From a functional perspective, this ratio reflects the relative contribution of partial versus higher-efficacy CB1 receptor agonism. Higher R values (AEA-weighted profiles) may correspond to tonic, modulatory signalling states characterized by reduced neurotransmitter suppression and greater system stability. Conversely, lower R values (2-AG-weighted profiles) may indicate phasic, high-efficacy signalling associated with stronger CB1-mediated suppression of neurotransmitter release. Importantly, this framework does not replace absolute concentration measurements; rather, it complements them by providing relational context. Two individuals with similar absolute endocannabinoid levels may exhibit substantially different R values, corresponding to distinct functional signalling states.

Future studies should report both absolute concentrations and derived ratios, and evaluate their independent and combined associations with physiological, behavioural, and clinical outcomes. Establishing reference distributions and clinically relevant thresholds for R and R_{log} will be essential for translating ratio-based ECS interpretation into diagnostic and research applications.

Receptor-level dynamics and partial agonism. CB1 receptor signalling is not binary. Partial agonism introduces graded control over receptor activation, enabling fine-tuning rather than maximal stimulation. AEA's partial agonist profile allows it to function as both an activator and functional antagonist depending on receptor occupancy and competing ligand presence [7, 11, 16]. In this context, the ratio of partial to full agonists becomes more informative than the absolute level of either ligand. High 2-AG dominance may produce hyperactive signalling states, whereas balanced or AEA-weighted profiles may promote stability and resilience.

ENZYMATIC REGULATION AS A RATIO MODULATOR

FAAH and MAGL as independent control nodes. Fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL) regulate AEA and 2-AG degradation, respectively. Differential expression or activity of these enzymes can selectively skew endocannabinoid ratios without proportionally altering total ligand burden [17, 18].

Pathophysiological implications. Altered FAAH/MAGL balance has been implicated in stress-related disorders, neuroinflammation, and metabolic dysfunction. From a ratio-based perspective, enzymatic dysregulation represents a mechanism for signalling imbalance, rather than simple deficiency or excess [19, 20].

Parallels with endocrine ratio models. Clinical endocrinology routinely interprets hormonal ratios – such as cortisol/DHEA, estrogen/progesterone, or T3/T4 – rather than relying solely on absolute hormone levels. These ratios often better predict physiological states, stress resilience, and disease risk [21–24].

The ECS shares key features with endocrine systems, including:

- diffuse signalling;
- feedback regulation;
- context-dependent effects.

Applying ratio-based logic to ECS biomarkers aligns cannabinoid science with established diagnostic paradigms rather than positioning it as an outlier [2, 3, 24].

CLINICAL AND RESEARCH IMPLICATIONS

Diagnostic refinement. Incorporating endocannabinoid ratios into biomarker panels may improve sensitivity and specificity for ECS-related disorders, particularly those characterized by regulatory imbalance rather than outright deficiency [7, 24].

Study design considerations. Future ECS studies should report relative ligand ratios alongside absolute concentrations and explore their associations with symptom clusters, phenotypes, and treatment response [23].

Therapeutic design. Interventions targeting ECS tone – whether pharmacologic or nutraceutical – may be more effective when designed to rebalance signalling ratios rather than globally increase or suppress endocannabinoid levels [7, 24]. At present, the strength of associations between specific AEA/2-AG ratios and defined physiological or pathophysiological states remains to be systematically validated, and the framework presented here is intended to guide future investigation rather than serve as a definitive diagnostic tool.

CONCLUSION

The failure of absolute endocannabinoid levels to consistently predict clinical states reflects a conceptual mismatch between measurement strategy and biological reality. The ECS operates as a balance-driven regulatory network, in which relative signalling ratios determine functional outcomes. A ratio-based framework offers a parsimonious explanation for contradictory findings in the literature, and aligns ECS

interpretation with established models in systems biology and endocrinology. Embracing this paradigm shift may unlock more reliable diagnostics and more precise therapeutic strategies for ECS-related disorders.

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