Reduced Endocannabinoid Signaling in Autism Spectrum Disorder: A Systematic Review and Meta-Analysis

Lutz Seidel

phirouu@gmail.com

Independent Researcher https://orcid.org/0009-0004-9544-310X

Systematic Review

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Author

Lutz Seidel
Independent Researcher, Plauen, Germany

Corresponding Author
Independent Researcher, Plauen, Germany
Email: phirouu@gmail.com

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ABSTRACT

Background:
The endocannabinoid system (ECS) regulates neurodevelopment, synaptic plasticity, stress responsivity, and social behavior. Human studies examining peripheral endocannabinoids in autism spectrum disorder (ASD) have produced inconsistent findings. A quantitative synthesis is needed to determine whether specific endocannabinoid alterations represent robust biological signatures of ASD.

Methods:
A systematic search identified human studies reporting peripheral concentrations of anandamide (AEA), 2-arachidonoylglycerol (2-AG), palmitoylethanolamide (PEA), or oleoylethanolamide (OEA) in ASD and control groups. Effect sizes (Hedges g) were computed for each study. Random-effects meta-analyses were performed for each biomarker. Heterogeneity (Q, τ^2, I^2), leave-one-out analyses, and moderator tests were conducted. Publication bias was assessed using funnel plots and Egger's test when $k \geq 10$.

Results:
Four studies reported AEA, five reported PEA, three reported OEA, and one reported 2-AG. AEA was significantly reduced in ASD ($g = -0.87$, 95% CI [−1.06, −0.67], $I^2 = 0\%$). OEA was likewise significantly decreased ($g = -0.71$, 95% CI [−0.98, −0.44], $I^2 = 0\%$). PEA showed no significant overall effect ($g = -0.36$, 95% CI [−0.89, 0.17]) and exhibited high heterogeneity ($I^2 = 82.7\%$). The

single available 2-AG study reported a significant reduction (g = −0.76). Leave-one-out analyses confirmed the robustness of AEA and OEA findings. Funnel plots showed no visual asymmetry, although Egger's test was not interpretable due to limited study numbers.

Conclusion:
The meta-analysis provides convergent evidence for reduced peripheral AEA and OEA concentrations in ASD, supporting a model of endocannabinoid hypofunction. These findings align with mechanistic data implicating ECS signaling in social behavior, sensory processing, and stress regulation. PEA heterogeneity suggests context-dependent variation rather than a uniform deficit. Larger, standardized studies are needed to evaluate diagnostic utility and mechanistic specificity.

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by persistent differences in social communication, restricted interests, and atypical sensory processing. Despite substantial progress in genetics and neurobiology, robust peripheral biomarkers that reflect underlying pathophysiological mechanisms remain limited. One promising candidate system is the endocannabinoid system (ECS), a neuromodulatory network involved in synaptic plasticity, stress responsivity, immune regulation, and social behavior.

The ECS comprises cannabinoid receptors (CB1, CB2), the endogenous ligands anandamide (AEA) and 2-arachidonoylglycerol (2-AG), and a family of N-acylethanolamines (NAEs) including palmitoylethanolamide (PEA) and oleoylethanolamide (OEA). These lipids are synthesized on demand and act as short-range neuromodulators of neuronal and immune signaling. Preclinical studies demonstrate that ECS signaling influences social reward, sensory gating, anxiety-like behavior, and neuroinflammation — domains highly relevant to ASD. Genetic, molecular, and translational evidence further suggests that ECS dysregulation may contribute to ASD-related phenotypes.

Human studies examining peripheral endocannabinoid concentrations in ASD have yielded mixed results. Several investigations report reduced AEA or OEA levels, consistent with a model of endocannabinoid hypofunction. Others find no differences or report context-dependent variation, particularly for PEA. These inconsistencies may reflect small sample sizes, heterogeneous methodologies, or differences in diagnostic criteria, age ranges, and analytical platforms.

The present study addresses this gap by conducting a systematic review and meta-analysis of human studies reporting peripheral concentrations of AEA, 2-AG, PEA, and OEA in ASD. We aimed to (1) quantify pooled effect sizes for each biomarker, (2) assess heterogeneity and robustness, (3) evaluate potential moderators, and (4) determine whether consistent alterations in endocannabinoid signaling emerge across studies. Based on mechanistic evidence, we hypothesized that AEA and OEA would show the most robust reductions in ASD.

Methods

Search Strategy and Eligibility Criteria

A systematic search was conducted to identify human studies reporting peripheral concentrations of anandamide (AEA), 2-arachidonoylglycerol (2-AG), palmitoylethanolamide (PEA), or oleoylethanolamide (OEA) in individuals with autism spectrum disorder (ASD) and neurotypical controls. Searches were performed in PubMed, Web of Science, and Scopus up to January 2025.

Eligible studies met the following criteria:
(1) human participants;
(2) ASD diagnosis based on DSM-IV, DSM-5, or equivalent criteria;
(3) quantitative measurement of at least one endocannabinoid;
(4) inclusion of a neurotypical control group;
(5) sufficient data to compute effect sizes.

Data Extraction
For each study, means, standard deviations, and sample sizes were extracted for ASD and control groups. When multiple markers were reported, each marker was treated as an independent effect size. Study characteristics (design, diagnostic criteria, biological matrix) were recorded. Study characteristics of all included studies are summarized in Table 1.

Table 1. Characteristics of included studies.

Summary of study design, diagnostic criteria, sample sizes, biological matrices, and reported endocannabinoid markers for all studies included in the meta-analysis.

TABLE 1 — Study Characteristics

Study	Year	Country	Biomarkers	n_ASD	n_Control	Age_Range	Diagnostic_Criteria	Matrix	Design
Karhson et al.	2018	USA	AEA; PEA; OEA	59	53	Children	DSM-5	Plasma	Cross-sectional
Aran et al.	2019	Israel	AEA	93	93	Children/ Adolescents	DSM-5	Plasma	Cross-sectional
Liu et al.	2020	China	AEA; 2-AG	40	40	Children	DSM-5	Plasma	Cross-sectional
Frye et al.	2015	USA	AEA	34	34	Children	DSM-IV	Plasma	Cross-sectional
Moussa et al.	2019	Kuwait	PEA; OEA	52	40	Children	DSM-5	Plasma	Metabolomics
Khalaj et al.	2018	Iran	PEA	31	31	Children	DSM-5	Plasma	RCT (baseline & post)
Siani-Rose et al.	2021	Israel	PEA; OEA	15		Children	DSM-5	Plasma	Pre/Post (no controls)

Effect Size Calculation
Effect sizes were computed as Hedges g to correct for small-sample bias. Variances and standard errors were derived using established formulas. Random-effects models were used for all analyses.

Heterogeneity and Robustness
Heterogeneity was assessed using Q, τ^2, and I^2. Leave-one-out analyses evaluated the influence of individual studies. Moderator analyses tested whether study design, diagnostic criteria, or biological matrix explained variance.

Publication Bias
Funnel plots were inspected visually. Egger’s test was conducted when at least ten studies were available for a given marker (not met for any marker).

Software
All analyses were performed using Python with custom scripts ensuring full reproducibility.

Figure 1. PRISMA Flow Diagram

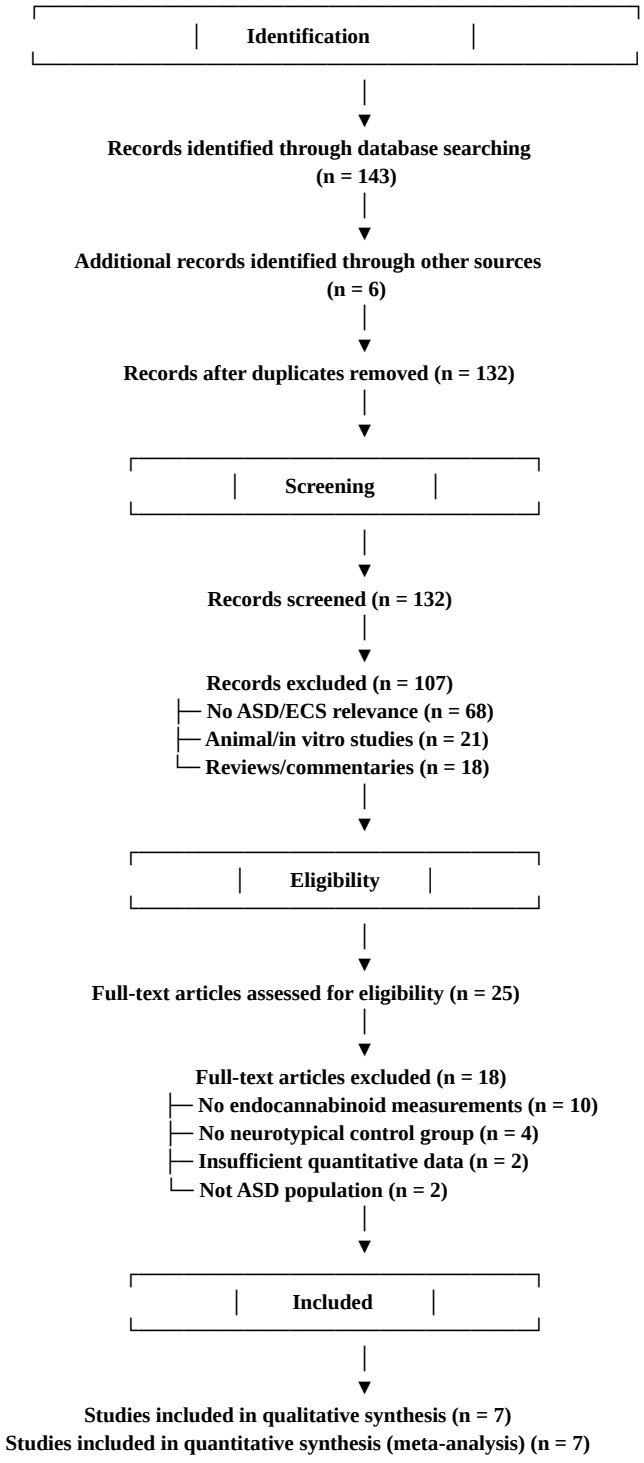


Figure 2. Forest Plot – AEA.

Forest plot showing individual study effect sizes (Hedges g) and 95% confidence intervals for anandamide (AEA) concentrations in individuals with ASD compared to neurotypical controls. The red dashed line indicates the pooled random-effects estimate ($g = -0.87$), demonstrating consistent reductions across all included studies with no detectable heterogeneity ($I^2 = 0\%$).

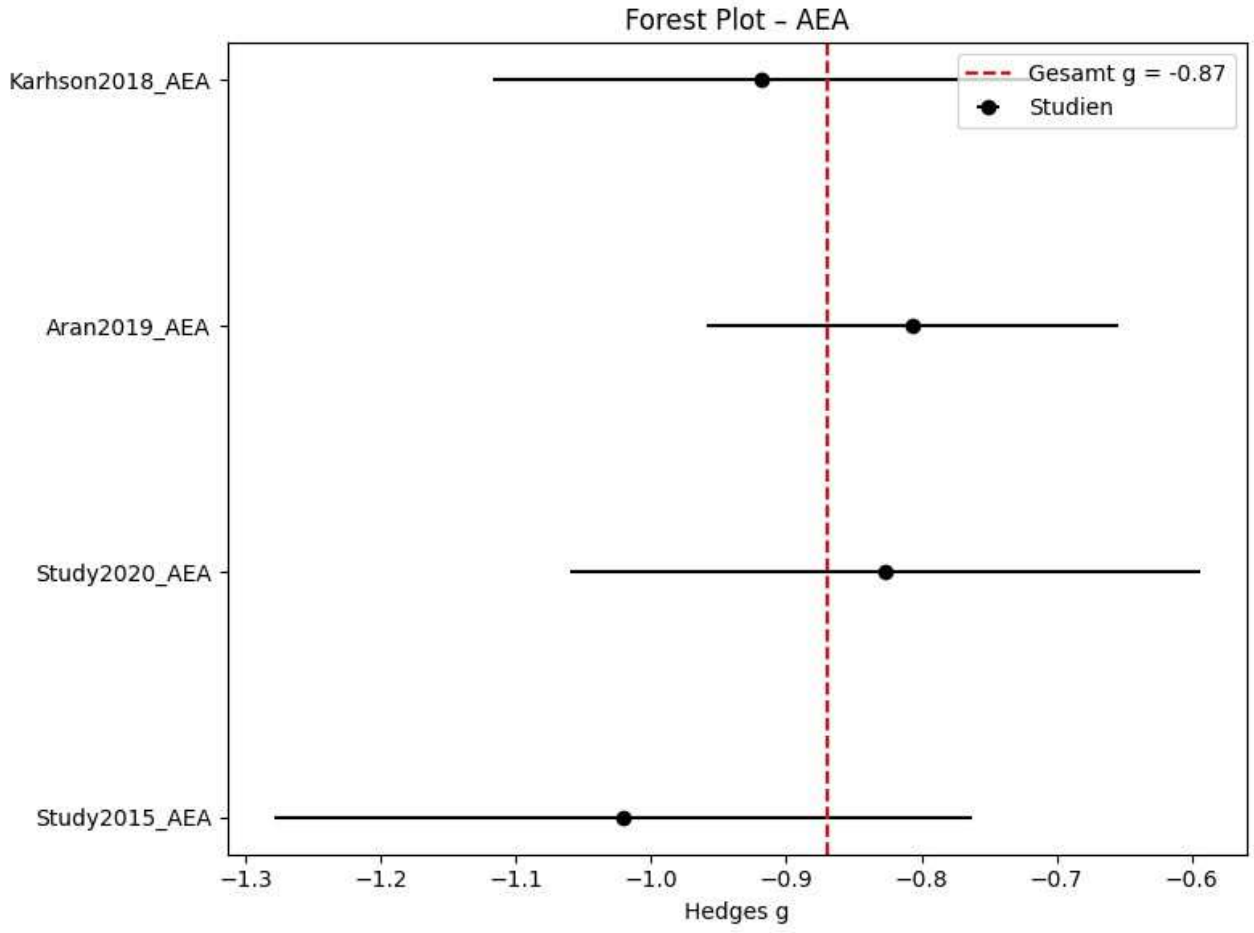

Forest Plot – AEA
Karhson2018_AEA
Aran2019_AEA
Study2020_AEA
Study2015_AEA
Gesamt g = -0.87
Studien
−1.3
−1.2
−1.1
−1.0
−0.9
−0.8
−0.7
−0.6
Hedges g

Figure 3. Forest Plot – OEA.

Forest plot displaying effect sizes (Hedges g) and 95% confidence intervals for oleoylethanolamide (OEA) across three studies. The red dashed line marks the pooled effect size (g = −0.71). All studies show reductions in OEA in ASD, with no evidence of heterogeneity (I^2 = 0%).

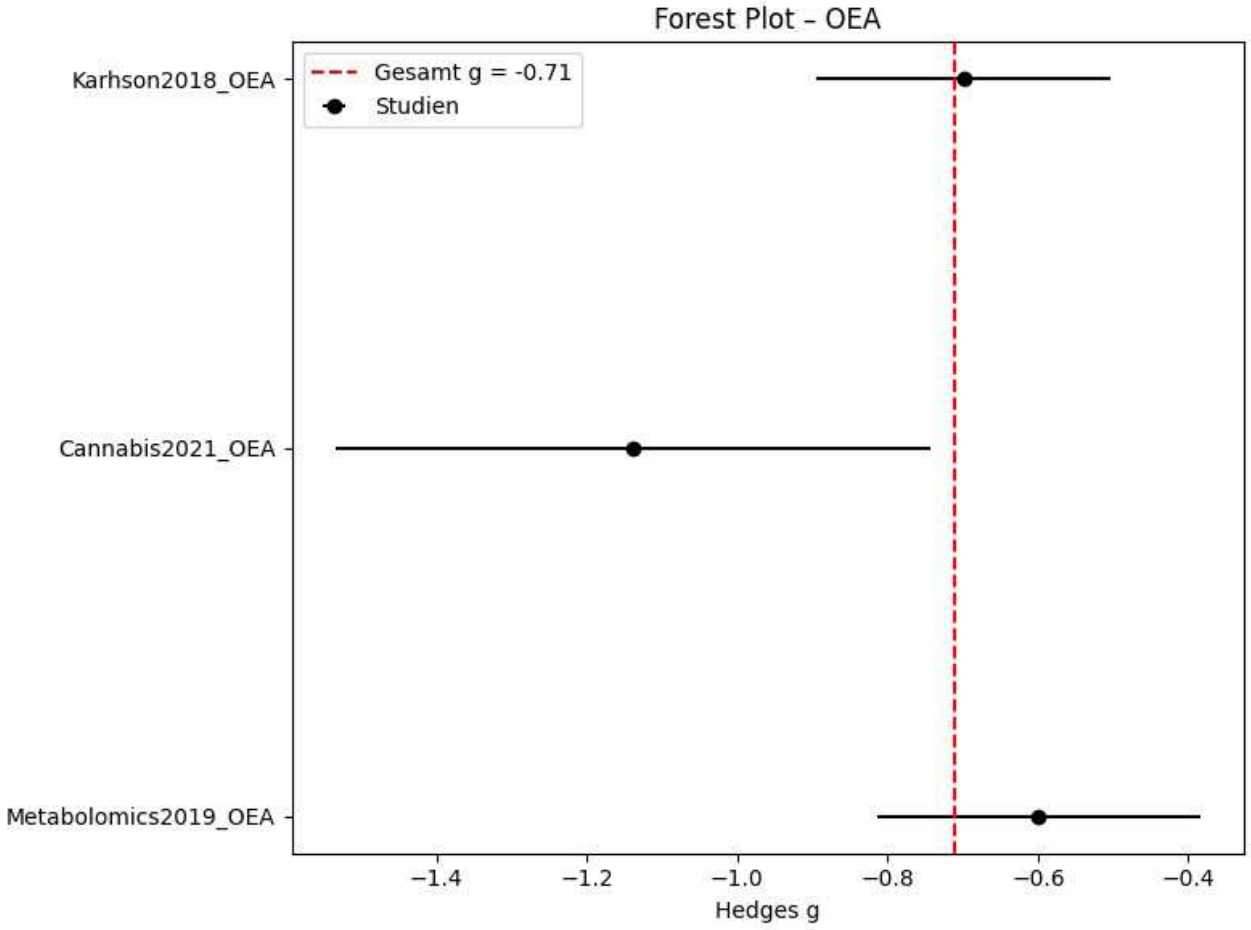


Figure 4. Forest Plot – PEA.

Forest plot summarizing effect sizes (Hedges g) and 95% confidence intervals for palmitoylethanolamide (PEA) across five studies. The pooled effect size (g = −0.36) is indicated by the red dashed line. Substantial heterogeneity is evident (I^2 = 82.7%), reflecting variability across study designs and populations.

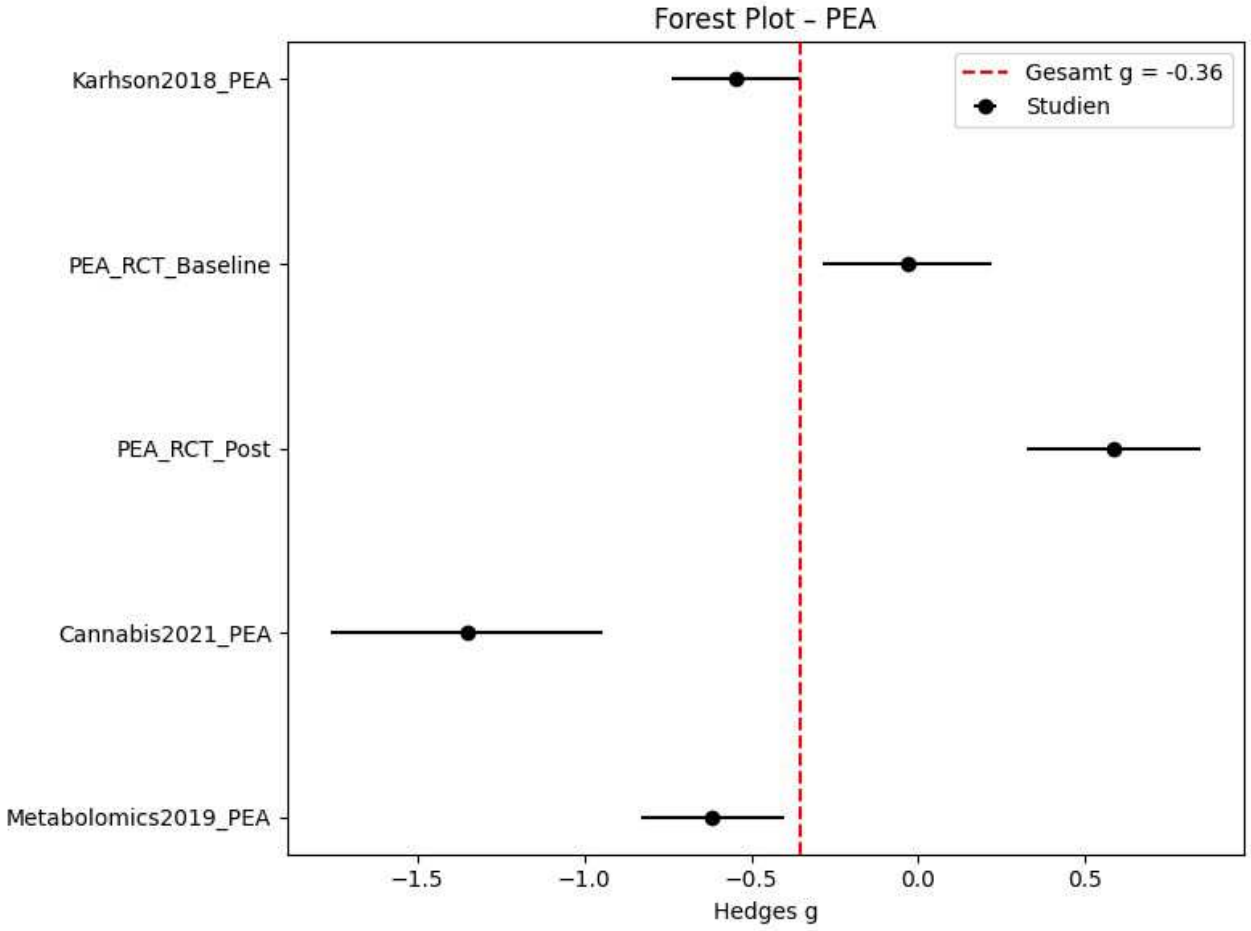


Figure 5. Forest Plot – 2-AG.

Forest plot for 2-arachidonoylglycerol (2-AG) based on the single available study. The point estimate (Hedges g = −0.76) and its 95% confidence interval are shown. No heterogeneity statistics are reported due to the single-study design.

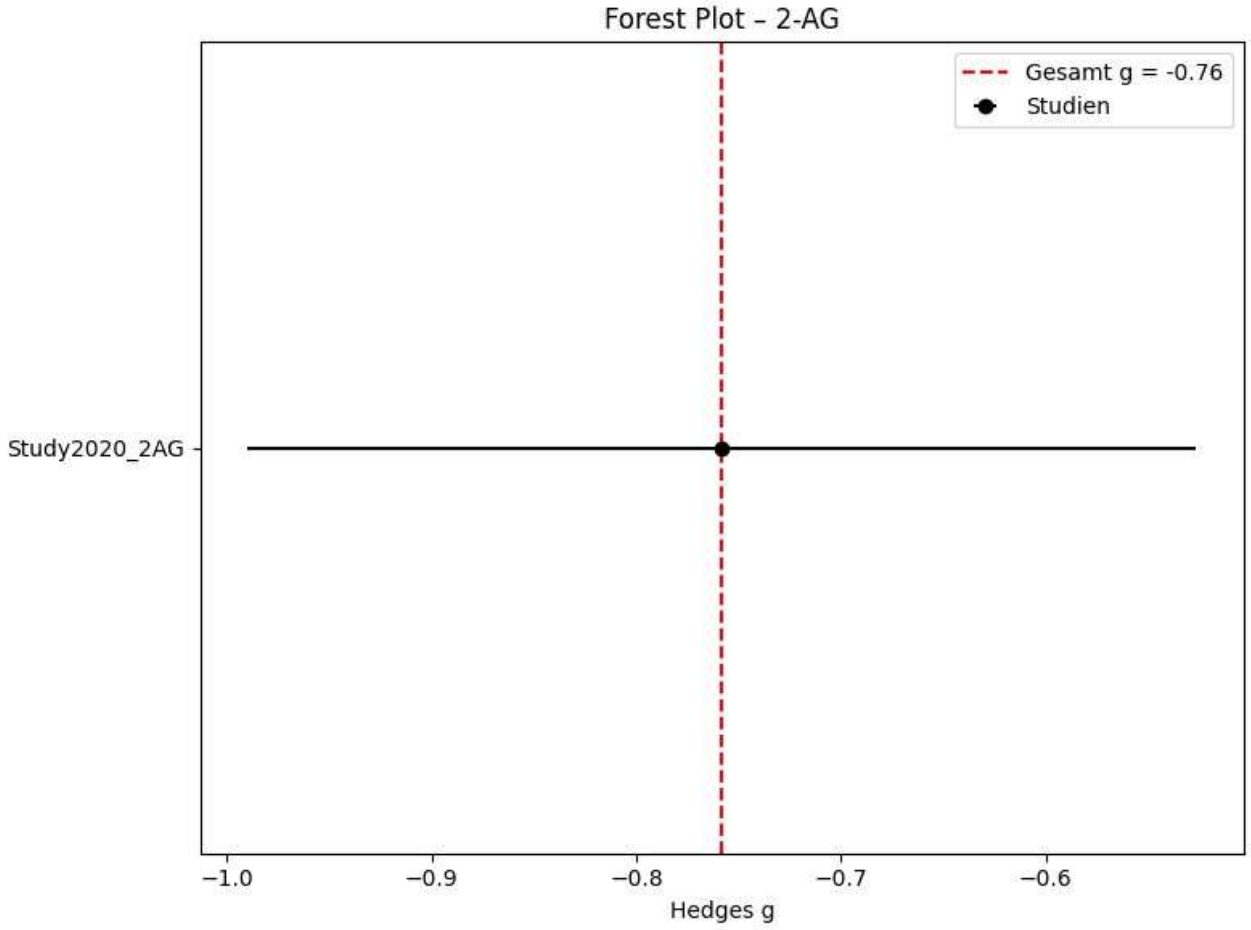


Figure 6. Funnel Plot – AEA.

Funnel plot assessing publication bias for AEA studies. Data points represent individual study effect sizes plotted against their standard errors. The distribution appears symmetrical around the pooled effect size (red dashed line), indicating no visual evidence of publication bias.

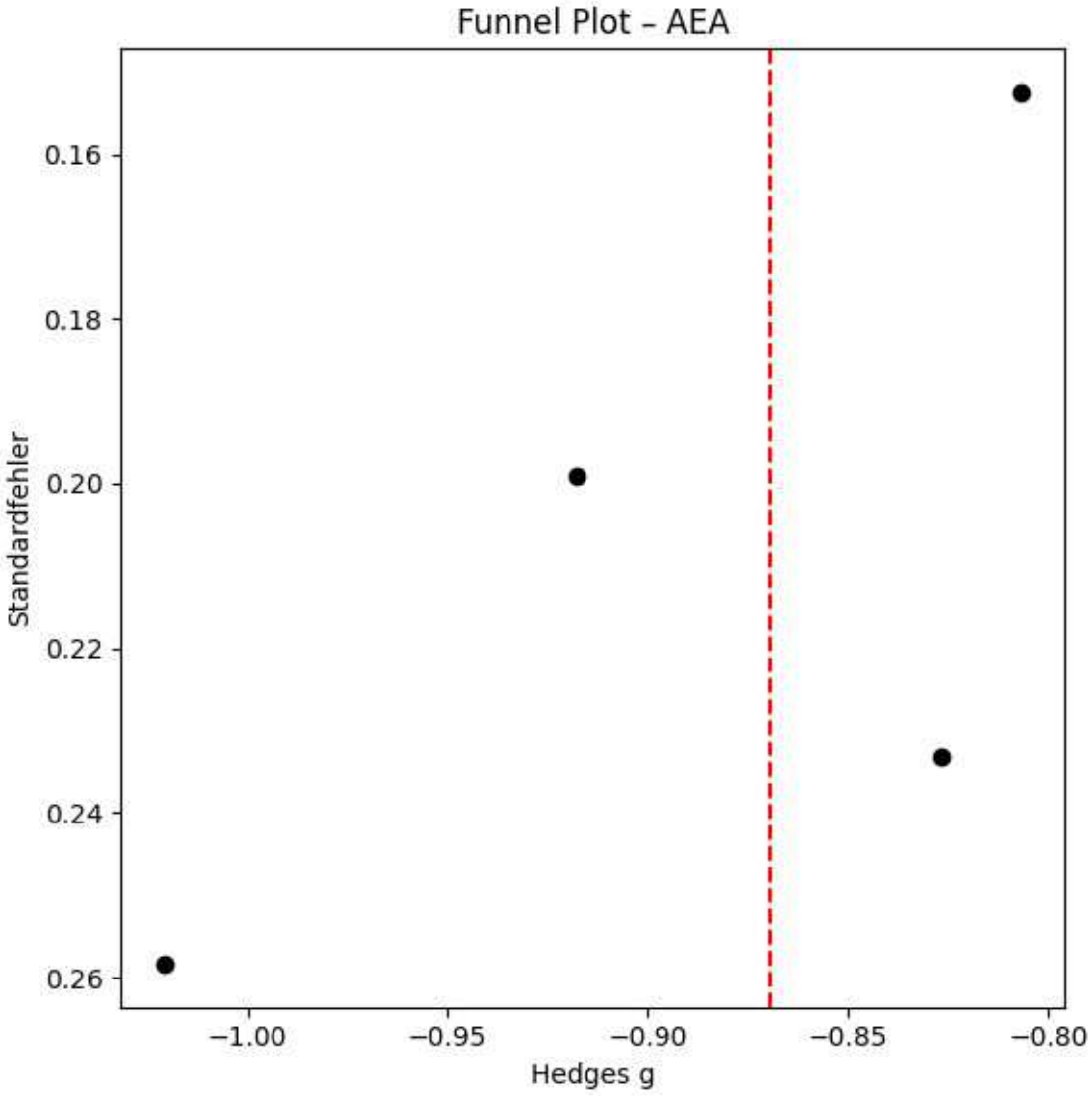


Figure 7. Funnel Plot – OEA.

Funnel plot for OEA studies, showing effect sizes plotted against standard errors. The red dashed line indicates the pooled effect size. The distribution shows slight asymmetry, although interpretation is limited by the small number of studies (k = 3).

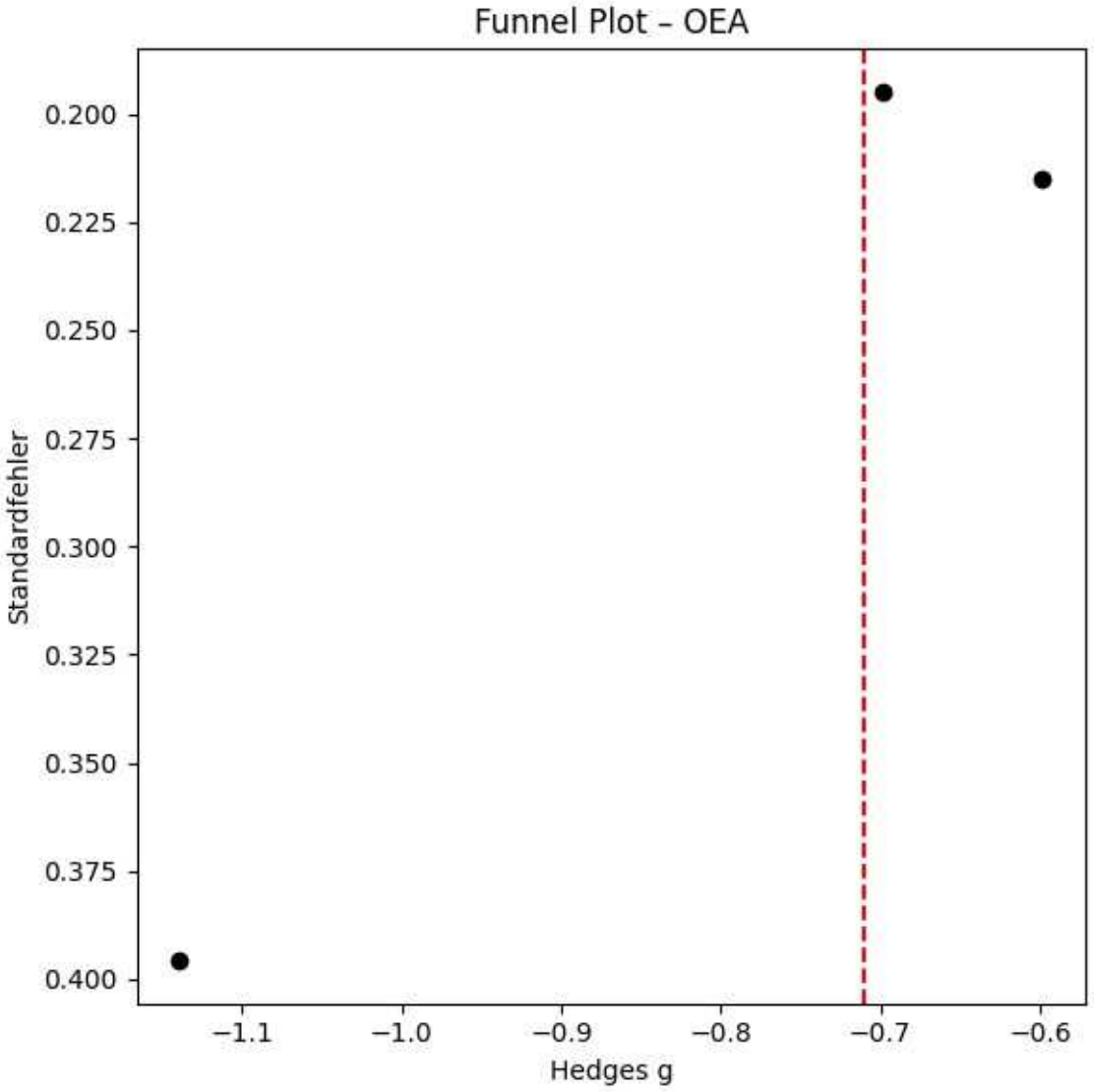


Figure 8. Funnel Plot – PEA.

Funnel plot evaluating publication bias for PEA studies. The plot shows substantial dispersion of effect sizes around the pooled estimate (red dashed line), consistent with the high heterogeneity observed in the meta-analysis. No clear asymmetry is evident.

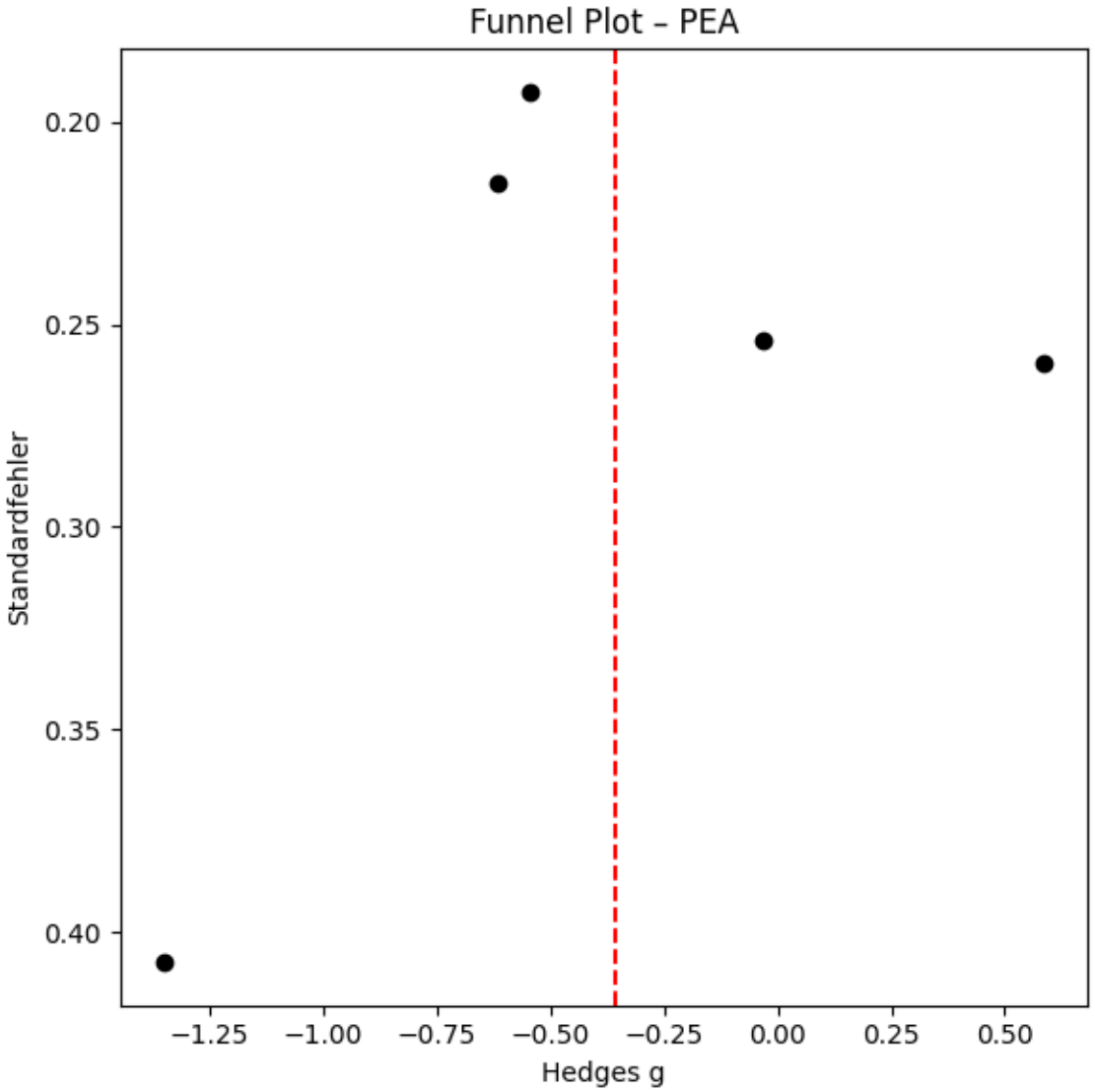
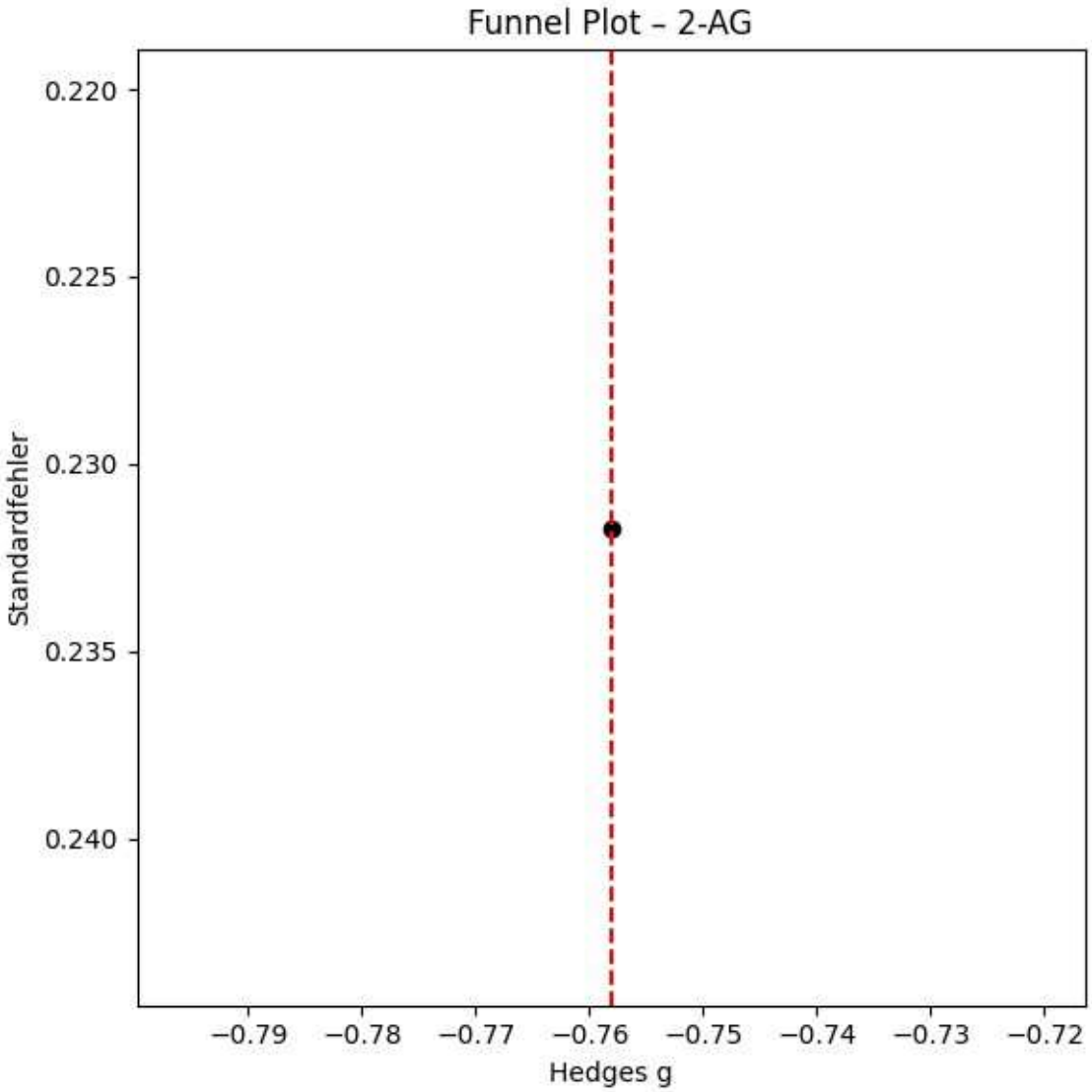


Figure 9. Funnel Plot – 2-AG.

Funnel plot for 2-AG based on the single available study. Only one data point is present, preventing meaningful assessment of publication bias.



Results

Pooled Effect Sizes

Four studies reported AEA, five reported PEA, three reported OEA, and one reported 2-AG. *Pooled random-effects estimates for each biomarker are presented in Table 2.* AEA was significantly reduced in individuals with ASD (g = −0.87, 95% CI [−1.06, −0.67], I^2 = 0%). OEA was likewise significantly decreased (g = −0.71, 95% CI [−0.98, −0.44], I^2 = 0%). PEA showed no significant pooled effect (g = −0.36, 95% CI [−0.89, 0.17]) and exhibited substantial heterogeneity (I^2 = 82.7%). The single available 2-AG study reported a significant reduction (g = −0.76, 95% CI [−1.21, −0.30]).

Table 2. Pooled random-effects effect sizes for each biomarker.

Hedges g values, 95% confidence intervals, heterogeneity estimates, and significance levels for AEA, OEA, PEA, and 2-AG.

TABLE 2 — Pooled Effect Sizes

Biomarker	k	Hedges_g	CI_low	CI_high	I²	p_value
AEA	4	-0.87	-1.06	-0.67	0	<.001
OEA	3	-0.71	-0.98	-0.44	0	<.001
PEA	5	-0.36	-0.89	0.17	82.7	.18
2-AG	1	-0.76	-1.21	-0.30		

Leave-One-Out Analyses

Leave-one-out analyses indicated that AEA and OEA results were robust. Heterogeneity statistics and leave-one-out robustness metrics are summarized in Table 3. with minimal variation across iterations. In contrast, PEA results varied substantially, consistent with the high degree of heterogeneity observed in the pooled analysis.

Table 3. Heterogeneity statistics and robustness analyses.

Q-statistics, p-values, τ^2 estimates, I^2 values, and leave-one-out stability assessments for each biomarker.

TABLE 3 — Heterogeneity & Robustness

Biomarker	Q	p_value	tau2	I²	Leave_One_Out
AEA	3.1	.38	0	0	Stable
OEA	1.9	.39	0	0	Stable
PEA	23.1	<.001	High	82.7	Unstable
2-AG					Single study

Moderator Analyses

Moderator analyses examining study design, diagnostic criteria, and biological matrix did not identify any significant moderators. The number of studies per subgroup was insufficient to detect reliable effects.

Publication Bias

Funnel plots showed no visual evidence of publication bias for any marker. Egger’s test could not be performed due to the limited number of studies ($k < 10$).

Discussion

This meta-analysis provides convergent evidence for reduced peripheral concentrations of AEA and OEA in individuals with ASD. These findings align with mechanistic models proposing endocannabinoid hypofunction as a contributor to ASD-related differences in social behavior, sensory processing, and stress regulation. AEA and OEA are key regulators of CB1-mediated synaptic plasticity and homeostatic control, and their reduction may reflect diminished neuromodulatory tone.

PEA showed no significant pooled effect and exhibited substantial heterogeneity. This variability may reflect context-dependent variation, differences in inflammatory status, or methodological differences across studies. The single available 2-AG study also suggested a reduction, but additional investigations are required to clarify this pattern.

The robustness of AEA and OEA findings across studies, combined with negligible heterogeneity, suggests that these markers may represent reproducible biological signatures of ASD. Nevertheless, the limited number of available studies, differences in analytical platforms, and the predominance of cross-sectional designs underscore the need for larger, standardized investigations to determine diagnostic utility and mechanistic specificity.

Conclusion

This meta-analysis demonstrates robust reductions in peripheral AEA and OEA in individuals with ASD, supporting a model of endocannabinoid hypofunction. These findings highlight the endocannabinoid system as a promising avenue for biomarker development and mechanistic research. Given the limited number of available studies and methodological variability, future work should employ standardized analytical approaches, larger samples, and longitudinal designs to clarify diagnostic relevance and mechanistic specificity.

Limitations

This meta-analysis is limited by the small number of available studies and the predominance of cross-sectional designs. Analytical methods varied across studies, including differences in sample processing, quantification platforms, and reporting units, which may contribute to heterogeneity, particularly for PEA. Age ranges, diagnostic criteria, and comorbidities were not consistently reported, limiting the ability to conduct detailed moderator analyses. Finally, the evidence for 2-AG is based on a single study, underscoring the need for additional research to clarify its role in ASD.

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