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A Systematic Review and Meta-Analysis on the Effects of Exercise on the Endocannabinoid System

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Abstract

Introduction: The endocannabinoid (eCB) system plays a key role in maintaining homeostasis, including the regulation of metabolism and stress responses. Chronic stress may blunt eCB signaling, and disruptions in eCB signaling have been linked to stress-related psychiatric disorders and physical health conditions, including anxiety, depression, post-traumatic stress disorder (PTSD), diabetes, and obesity. Pharmacological and nonpharmacological behavioral interventions (e.g., exercise) that target the eCB system may be promising therapeutic approaches for the prevention and treatment of stress-related diseases. In this study, we perform a systematic review and the first meta-analysis to examine the impact of exercise on circulating eCB concentrations.

Materials and Methods: We performed a review of the MEDLINE (PubMed) database for original articles examining the impact of exercise on eCBs in humans and animal models. A total of 262 articles were screened for initial inclusion.

Results: Thirty-three articles (reporting on 57 samples) were included in the systematic review and 10 were included in the meta-analysis. The majority of samples that measured anandamide (AEA) showed a significant increase in AEA concentrations following acute exercise (74.4%), whereas effects on 2-arachidonoylglycerol (2-AG) were inconsistent. The meta-analysis, however, revealed a consistent increase in both AEA and 2-AG following acute exercise across modalities (e.g., running, cycling), species (e.g., humans, mice), and in those with and without pre-existing health conditions (e.g., PTSD, depression). There was substantial heterogeneity in the magnitude of the effect across studies, which may relate to exercise intensity, physical fitness, timing of measurement, and/or fasted state. Effects of chronic exercise were inconsistent.

Conclusions: Potential interpretations and implications of exercise-induced mobilization of eCBs are discussed, including refilling of energy stores and mediating analgesic and mood elevating effects of exercise. We also offer recommendations for future work and discuss therapeutic implications for exercise in the prevention and treatment of stress-related psychopathology.

Keywords: anandamide; mental health; endocannabinoids; physical activity; running

Introduction

The endocannabinoid (eCB) system plays a key role in maintaining homeostasis throughout the brain and body, including regulating energy metabolism, cognition, sleep, inflammation, and stress responses.[1–3] eCB receptors, including cannabinoid type-1 (CB1Rs) are located throughout the brain and play a neuromodulatory role in various neurotransmitter systems.[4,5] Although they are widespread, CB1Rs are most densely located in limbic brain regions (e.g., including the prefrontal

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cortex, amygdala, and hippocampus)[6] implicated in stress and emotion regulation, reward processes, fear learning, and extinction. Cannabinoid type-2 receptors (CB2Rs) are found on immune tissues and play an immunomodulatory role in cytokine release.[7]

The two most well-studied eCBs (i.e., endogenous ligands for cannabinoid receptors) are anandamide (AEA) and 2-arachidonoylglycerol (2-AG). These eCBs are synthesized "on demand" and released into the synapse from the post-synaptic neuron to inhibit pre-synaptic activity in a retrograde manner.[8–10] Activity of AEA and 2-AG is primarily modulated by enzymes that synthesize (i.e., N-acylphosphatidylethanolamine phospholipase D [NAPE], diacylglycerol lipase [DAGL], respectively) or catabolize (i.e., fatty acid amide hydrolase [FAAH], monoacylglycerol lipase, respectively) eCBs.

In addition to regulating synaptic activity, eCBs can inhibit activity of the hypothalamo–pituitary–adrenocortical axis and the sympathetic nervous system.[11,12] eCB concentrations are modulated in response to both acute and chronic stress exposure,[13] and disruptions in eCB signaling are linked to several stress-related diseases, including anxiety, depression, post-traumatic stress disorder (PTSD), obesity, and diabetes.[14–19]

Given the critical role in regulating stress responses, the eCB system has emerged as a promising therapeutic target for the prevention and treatment of stress-related psychopathology.[16,20] For example, pharmacologic interventions that enhance AEA concentrations through

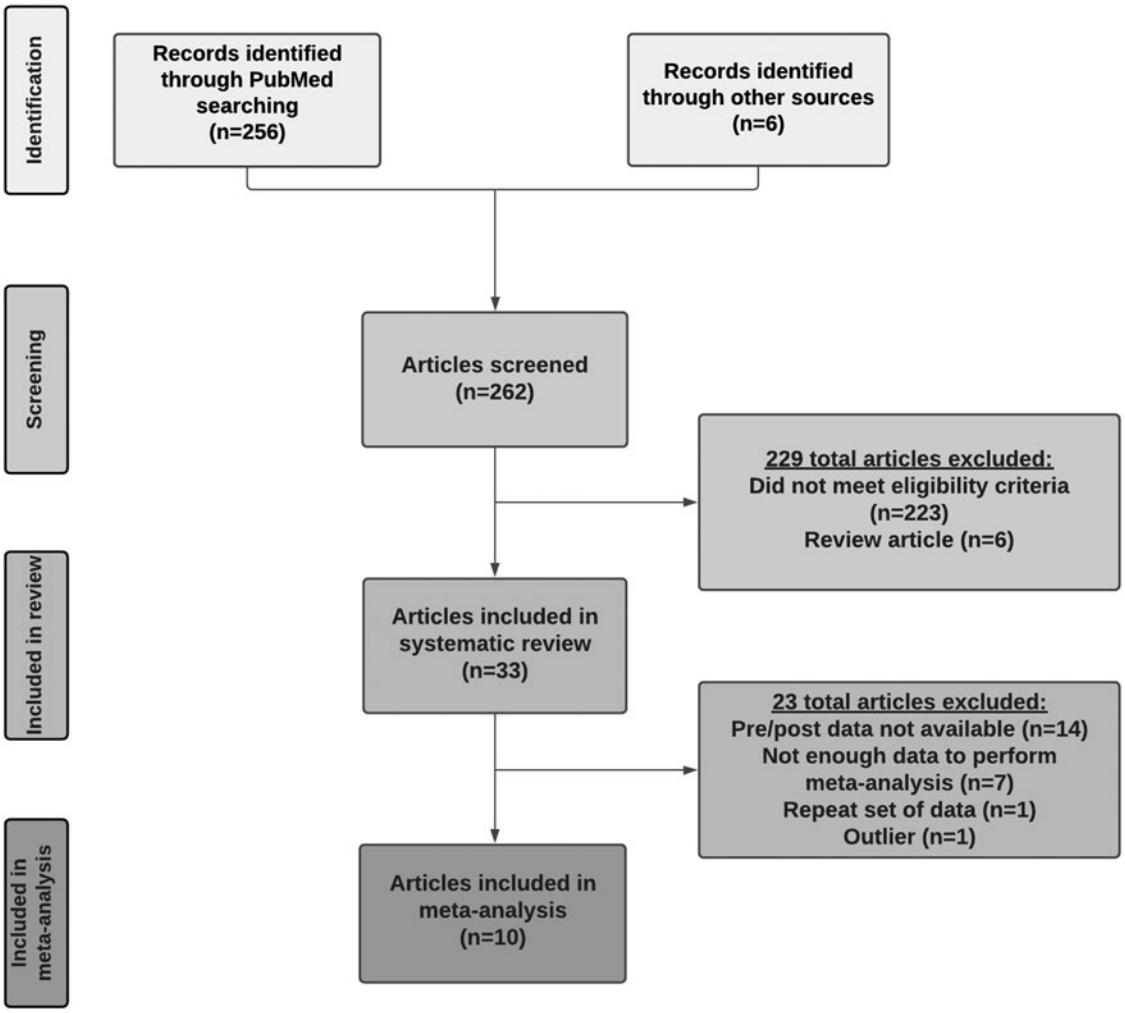

FIG. 1. Flowchart of literature search strategy, and identification of articles for the systematic review and meta-analysis.

Table 1. Included samples on acute exercise ($N = 40$ samples reported in $N = 22$ articles)

Type	First author (year)	Condition	Strain of animal	Sample size (n)	Age (mean ± SD)	Gender (% female)	Race/ ethnicity (%)	Type of exercise	Exercise modality
Human studies									
Patients, including those with pre-existing conditions									
	Crombie (2020)[88]	Patients with PTSD	N/A	HC: $n = 12$ TE: $n = 14$ PTSD: $n = 14$	HC: 21.4 ± 3.9 TE: 23.0 ± 5.4 PTSD: 27.6 ± 6.6	HC: 100% F TE: 100% F PTSD: 100% F	HC: 92% W, 8% B, 0% A, 0% M, 0% O TE: 86% W, 0% B, 7% A, 0% M, 7% O PSTD: 64% W, 21% B, 7% A, 0% M, 7% O	Endurance	Treadmill running
	Meyer (2019)[58]	Patients with MDD	N/A	$n = 17$	40.8 ± 14.8	100% F	N/R	Endurance	Electronically braked cycle ergometer
	Crombie (2019)[80]	Patients with PTSD	N/A	HC: $n = 10$ PTSD: $n = 10$	HC: 22.0 ± 6.1 PTSD: 25.2 ± 8.1	HC: 70% F PTSD: 80% F	HC: 70% W, 0% B, 20% A, 10% M, 0% O PTSD: 90% W, 0% B, 10% A, 0% M, 0% O	Endurance	Treadmill running or incline walking
	Stensson (2019)[49]	Patients with CNSP	N/A	HC: $n = 11$ CNSP: $n = 21$	HC: 37.7 ± 15.9 CNSP: 50.8 ± 12.9	HC: 55% F CNSP: 76% F	N/R	Endurance	Arm cycling
	Brellenthin (2019)[44,b]	Patients with Substance Abuse Disorder	N/A	Treatment as usual with exercise: $n = 21$	35.1 ± 10.2	45% F	64% W, 2% B, 0% A, 1% M, 1% O	Endurance	Treadmill incline walking
	Crombie (2018)[30]	Patients with PTSD	N/A	HC: $n = 12$ PTSD: $n = 12$	HC: 22.0 ± 4.7 PTSD: 26.1 ± 6.7	HC: 75% F PTSD: 75% F	N/R	Endurance	Treadmill running or incline walking
Healthy volunteers									
	Marin Bosch (2020)[46]	Healthy volunteers	N/A	$n = 15$	23.2 ± 4.2	0% F	N/R	Endurance	Cycling
	Hughes (2020)[89]	Healthy, recreationally active volunteers	N/A	$n = 12$	29.0 ± 6.0	17% F	N/R	Strength	Light load RE, BFR-RE, BFR-RE with high pressure, and heavy load RE
	Stone (2018)[90]	Healthy volunteers	N/A	$n = 9$	61.0	100% F	N/R	Endurance	Cycling and dancing

Exercise duration	Exercise intensity	eCB collection method	eCB collection timepoints	Significant reported change in AEA	Significant reported change in 2-AG	Significant reported change in other eCBs	Additional outcomes[a]
30 min of running on a treadmill at a moderate-intensity exercise with 5 min warm-up and cool-down	70–75% AAMHR	Standard blood draw	Collected immediately before and after exercise	HC: AEA ↑ TE: AEA ↑ PTSD: AEA ↑	HC: 2-AG ↑ TE: 2-AG ↑ PTSD: 2-AG ↑	HC: OEA ↑ PEA ↑ 2-OG ↑ TE: OEA ↑ PEA ↑ 2-OG ↑ PTSD: OEA ↑ PEA ↑ 2-OG ↑	HC: Anxiety ↓ Fear ↓ Improvements in mood TE: Anxiety ↓ Fear ↓ Improvements in mood PTSD: Anxiety ↓ Fear ↓ Improvements in mood
30 min of cycling, either (1) at a moderate intensity or (2) at a preferred intensity, each with 5 min warm-up and cool-down	Ratings of perceived exertion of "13"	Standard blood draw	Collected immediately before and as quickly as possible after the session ended, always within 10 min post-exercise	Prescribed: AEA ↑ Preferred: No AEA changes	Prescribed: No 2-AG changes Preferred: No 2-AG changes	Prescribed: OEA ↑ No PEA, 2-OG changes Preferred: No OEA, PEA, 2-OG changes	Prescribed: Improvements in mood related to eCB changes Preferred: Improvements in mood not related to eCB changes
30 min of running or walking on a treadmill at a moderate-intensity exercise with 5 min warm-up and cool-down	70–75% AAMHR	Standard blood draw	Collected immediately before and after exercise	HC: AEA ↑ PTSD: AEA ↑	HC: 2-AG ↑ PTSD: No 2-AG changes	HC: OEA ↑ No PEA, 2-OG changes PTSD: OEA ↑ No PEA, 2-OG changes	N/A
A 30-min session of arm cycling	N/R	Standard blood draw	Collected immediately before and 60 min after exercise	HC: AEA ↓ CNSP: No AEA changes	HC: No 2-AG changes CNSP: No 2-AG changes	HC: No OEA, PEA, SEA changes CNSP: No OEA, PEA, SEA changes	HC: Glutamate/AEA ratio ↑ CNSP: No glutamate/AEA ratio changes
30 min of moderate-intensity treadmill incline walking	70–75% AAMHR	Standard blood draw	Collected within 5 min before and after exercise	AEA ↑	No 2-AG changes	N/A	Acute improvements in mood and reductions in perceived stress and drug craving
30 min of walking or running on a treadmill at a moderate intensity with 10 min warm-up	70–75% AAMHR	Standard blood draw	Collected immediately before and after exercise	HC: AEA ↑ PTSD: AEA ↑	HC: 2-AG ↑ PTSD: 2-AG ↑	HC: OEA ↑ No PEA, 2-OG changes PTSD: OEA ↑ No PEA, 2-OG changes	HC: Improvements in mood Pain ↓ PTSD: Improvements in mood Pain ↓
A 30-min session of cycling at a moderate intensity and 15 min of cycling at a high intensity the next day, each with a 3 min warm-up and cool-down	Moderate intensity: 70% MHR High intensity: 80% MHR	Standard blood draw	Collected immediately before and after exercise	Moderate intensity: AEA ↑ High intensity: AEA ↑	N/A	N/A	Hippocampus activation ↑ Motor sequence learning ↑
Light load RE, BFR-RE, BFR-RE with high pressure: 4 sets (30, 15, 15, and 15 repetitions, respectively) of unilateral leg press exercise with 30 sec interset rest periods. Heavy load RE: 4 sets of 10 repetitions of unilateral leg press exercise with 53 sec interset rest periods.	Light load RE, BFR-RE, BFR-RE with high pressure: 30% one rep max Heavy load RE: 70% one rep max	Standard blood draw	Collected immediately before, 5 min after, 10 min after, and 24 h after exercise	N/A	Light load RE, BFR-RE, BFR-RE with high pressure, and heavy load RE: No 2-AG changes (decrease for heavy load RE after 24 h)	N/A	Beta-endorphin concentration ↑ Pain ↓
One 30-min session of cycling and one 30 min session of dancing	N/R	Standard blood draw	Collected immediately before and after exercise	Cycling: No AEA changes Dancing: No AEA changes	N/A	Cycling: OEA ↑, no PEA changes Dancing: no OEA changes, no PEA changes	Cycling: ↓ hunger/appetite Dancing: Improved mood

(*continued*)

Table 1. (Continued)

Type	First author (year)	Condition	Strain of animal	Sample size (*n*)	Age (mean ± SD)	Gender (% female)	Race/ethnicity (%)	Type of exercise	Exercise modality
	Crombie (2018)[91,c]	Healthy volunteers (placebo condition)	N/A	*n* = 58	21.0 ± 3.0	50% F	57% W, 17% B, 14% A, 12% M, 0% O	Strength	Isometric handgrip
	Brellenthin (2017)[40]	Healthy volunteers	N/A	Low PA exercisers (preferred and prescribed): *n* = 11 Moderate PA exercisers (preferred and prescribed): *n* = 12 High PA exercisers (preferred and prescribed): *n* = 13	Low PA exercisers (preferred and prescribed): 20.6 ± 2.4 Moderate PA exercisers (preferred and prescribed): 19.8 ± 1.1 High PA exercisers (preferred and prescribed): 22.6 ± 5.5	Low PA exercisers (preferred and prescribed): 50% F Moderate PA exercisers (preferred and prescribed): 50% F High PA exercisers (preferred and prescribed): 50% F	N/R	Endurance	Prescribed treadmill exercise and preferred intensity treadmill exercise. Order was randomized and counterbalanced across participants
	Cedernaes (2016)[47]	Healthy volunteers	N/A	*n* = 16	22.9 ± 0.7	0% F	N/R	Endurance	Ergometer cycling
	Koltyn (2014)[29]	Healthy volunteers (placebo condition only)	N/A	*n* = 58	21 ± 3.0	50% F	57% W, 17% B, 14% A, 12% M, 0% O	Strength	Isometric handgrip
	Raichlen (2013)[31]	Healthy volunteers	N/A	*n* = 10	31.9 ± 12.1	40% F	N/R	Endurance	Treadmill running or walking
	Raichlen (2012)[59]	Healthy volunteers	N/A	*n* = 10	N/R	N/R	N/R	Endurance	Treadmill running or walking
	Feuerecker (2012)[48]	Healthy, trained hikers	N/A	*n* = 12	27.6	0% F	N/R	Endurance	Hiking
	Heyman (2012)[56]	Healthy, trained cyclists	N/A	*n* = 11	23.3 ± 5.1	0% F	N/R	Endurance	Cycling
	Sparling (2003)[41]	Healthy, trained volunteers	N/A	Running: *n* = 8 Cycling: *n* = 8	23.8 ± 9.4	Running: 0% F Cycling: 0% F	N/R	Endurance	Running or cycling
Animal studies									
	King-Himmelreich (2017)[64]	Mice treated with CB1R antagonist and AMP-activated protein kinase	C57BL/6 mice	N/R	6–8 weeks	N/R	N/R	Endurance	Treadmill running

Exercise duration	Exercise intensity	eCB collection method	eCB collection timepoints	Significant reported change in AEA	Significant reported change in 2-AG	Significant reported change in other eCBs	Additional outcomes[a]
Isometric handgrip exercise for 3 min	25% MVC	Standard blood draw	Collected immediately before and within 5 min after exercise	AEA ↑	2-AG ↑	OEA ↑ PEA ↑ 2-OG ↑	See Koltyn (2014)[29]
Prescribed exercise: 45 min session of treadmill walking or running Preferred exercise: participants' choice of treadmill exercise intensity and duration	Prescribed: 70–75% VO2max Low PA preferred: 74.7 ± 7.8 Moderate PA preferred: 80.4 ± 7.6 High PA preferred: 74.6 ± 15.5	Standard blood draw	Collected immediately before and within 5 min after exercise	Low PA exercisers (preferred and prescribed): AEA ↑ Moderate PA exercisers (preferred and prescribed): AEA ↑ High PA exercisers (preferred and prescribed): AEA ↑	Low PA exercisers (preferred and prescribed): 2-AG ↑ Moderate PA exercisers (preferred and prescribed): 2-AG ↑ High PA exercisers (preferred and prescribed): 2-AG ↑	Low PA exercisers (preferred and prescribed): OEA ↑ PEA ↑ Moderate PA exercisers (preferred and prescribed): OEA ↑ PEA ↑ High PA exercisers (preferred and prescribed): OEA ↑ PEA ↑	Low PA exercisers (preferred and prescribed): Improvements in mood Moderate PA exercisers (preferred and prescribed): Improvements in mood High PA exercisers (preferred and prescribed): Improvements in mood
Ergometer cycling for 35 min	Watts equivalent to 75% of VO2 reserve	Standard blood draw	Collected immediately before, 15 min after, and 4 h after exercise	No AEA changes	2-AG ↑	OEA ↑ No PEA changes	Sleep deprivation ↑2-AG levels but no modulation in exercise response
Isometric handgrip exercise for 3 min	25% MVC	Standard blood draw	Collected immediately before and within 5 min after exercise	AEA ↑	2-AG ↑	OEA ↑ PEA ↑ 2-OG ↑ DHEA ↑	PPT ↑ PPR ↓
30 min of treadmill walking or running at one of four intensities. Intensities were randomized across four separate laboratory visits.	Intensity 1: < 50% AAMHR Intensity 2: 70% AAMHR Intensity 3: 80% AAMHR Intensity 4: 90% AAMHR	Standard blood draw	Collected immediately before and after exercise	Intensity 1: No AEA changes Intensity 2: AEA ↑ Intensity 3: AEA ↑ Intensity 4: No AEA changes	Intensity 1: No 2-AG changes Intensity 2: No 2-AG changes Intensity 3: No 2-AG changes Intensity 4: No 2-AG changes	N/A	Improved positive affect
30 min of treadmill walking on day 1 and 30 min of treadmill running on day 2	Running: 72.5% ± 2.54% MHR (Froude of 0.71 ± 0.03) Walking: 44.6% ± 1.25% MHR (Froude of 0.26 ± 0.01)	Standard blood draw	Collected immediately before and after exercise	Running: AEA ↑ Walking: No AEA changes	Running: No 2-AG changes Walking: No 2-AG changes	N/A	N/A
Protocol A: Hiking at lower altitude for 4–4.5 h Protocol B: Hiking at higher altitude for 3.5–5.5 h	Protocol A: Hiking at lower altitude of ~1650 m Protocol B: Hiking at higher altitude of ~3380 m (1780 m difference compared with lower altitude)	Standard blood draw	Collected before, immediately after, 18 h after, and 24 h after exercise	Protocol A: AEA ↑ Protocol B: AEA ↑	Protocol A: No 2-AG changes Protocol B: No 2-AG changes	N/A	Hypoxia (higher altitudes) ↑ eCB system activation
60 min of pedaling on an ergometric bicycle, followed by predetermined amount of work equal to 30 min at higher intensity. The total exercise period was 90 min	55% of maximal power output for 60 min, followed by 75% of maximal power output for 30 min	Standard blood draw	Collected at rest, after 60 min of exercise at 55% maximal power output, immediately after the 90 min time trial, and 15 min after exercise.	AEA ↑ after a 90-min exercise period	No 2-AG changes	N/A	BDNF levels ↑ significantly during exercise and ↓ during the 15 min of recovery BDNF and AEA concentrations correlated after exercise
45 min of prescribed exercise	70–80% MHR	Standard blood draw	Collected immediately before and after exercise	Running: AEA ↑ Cycling: AEA ↑	Running: No 2-AG changes Cycling: No 2-AG changes	N/A	N/A
60 min of treadmill running	N/R	Standard blood draw	Collected immediately after, 30 min after, 2 h after, 4 h after, and 24 h after exercise	AEA ↑	N/A	N/A	CB1 ↑, FAAH ↓ Found a significant ↓ in the nociceptive response after a single bout of 1 h treadmill running

(*continued*)

Table 1. (Continued)

Type	First author (year)	Condition	Strain of animal	Sample size (*n*)	Age (mean ± SD)	Gender (% female)	Race/ ethnicity (%)	Type of exercise	Exercise modality
	Fuss (2015)[92]	Mice	C57BL/6J mice	$n=16$	10 weeks	N/R	N/R	Endurance	Wheel running
	Galdino (2014)[93]	Rats injected with CB receptor inverse agonists, eCB metabolizing enzyme inhibitors and an AEA reuptake inhibitor	Male Wistar rats	N/R	N/R	0% F	N/R	Strength	RE (i.e., electrical stimulation to the animal's tail causing them to flex their leg muscl
	Galdino (2014)[94]	Rats injected with CB receptor inverse agonists, eCB metabolizing enzyme inhibitors and an AEA reuptake inhibitor	Male Wistar rats	N/R	N/R	0% F	N/R	Endurance	Treadmill running
	Raichlen (2012)[59,d]	Dogs and ferrets	Mixed-breed dogs and ferrets	Dogs: $n=8$ Ferrets: $n=8$	N/R	N/R	N/R	Endurance	Treadmill running and walking

Reported sample size is only for the sample that performed the exercise condition.
[a]Outcomes, including mood, anxiety, stress, pain, fear, hunger, cravings, etc. were all self-reported unless otherwise stated.
[b]Also appears in chronic exercise section.
[c]Same sample as in Koltyn (2014).[29]
[d]Same article as human study by Raichlen (2012).[31]
2-AG, 2-arachidonoylglycerol; 2-OG, 2-oleoylglycerol; A, Asian; AA, arachidonic acid; AAMHR, age-adjusted maximum heart rate; AEA, anandamide; AMP, adenosine monophosphate; B, Black; BDNF, brain-derived neurotrophic factor; BFR-RE, blood flow restriction resistance exercise; CB1R, cannabinoid type 1 receptor; CNSP, chronic neck and shoulder pain; DHEA, docosahexaenoyl ethanolamide; eCB, endocannabinoid; F, female; FAAH, fatty acid amide hydrolase; HC, healthy control; M, mixed; MDD, major depressive disorder; MHR, maximum heart rate; MVC, maximum voluntary contraction; N/A, not applicable; N/R, not reported; O, other; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPR, pressure pain ratings; PPT, Pressure pain thresholds; PTSD, post-traumatic stress disorder; RE, resistance exercise; SD, standard deviation; TE, trauma-exposed individuals without PTSD; W, White.

FAAH inhibition have been shown to attenuate autonomic stress reactivity and improve fear extinction memory recall in healthy adults.[21] Similarly, administration of low doses of exogenous cannabinoids that act on CB1Rs, such as Δ^9-tetrahydrocannabinol, reduces fear and anxiety-like behaviors in animal models,[22] potentiates fear extinction memory recall in healthy adults,[23] and reduces corticolimbic responses to threat in adults with PTSD.[24] Several ongoing or recently completed clinical trials have examined drugs that target the eCB system for stress-related psychiatric disorders, including anxiety (NCT02432703), depression (NCT02498392), substance use disorders (SUDs; NCT03787628), and PTSD (NCT04080427).

Growing research suggests that *nonpharmacological* approaches—particularly exercise—can boost eCB signaling. Historically, the euphoric, analgesic, anxiolytic, and mood-elevating effects of exercise were thought to be mediated, at least in part, by the endogenous opioid system (i.e., exercise-induced increases in beta-endorphins).[25,26] However, recent studies have challenged the role of endorphins in mediating the beneficial effects of exercise. For example, there is evidence that endorphins cannot cross the blood–brain barrier[27] and blockage of endorphin signaling using an opioid receptor antagonist (e.g., naltrexone) does not block the post-exercise euphoric, analgesic, and anxiolytic effects.[28,29]

Rather, emerging data indicate that the eCB system plays a pivotal role in mediating some of the well-documented effects of exercise, including reductions in pain and anxiety, and improvements in cognitive functioning and mood.[30,31] Indeed, studies suggest a role for eCBs in regulating hippocampal neurogenesis[32,33]; therefore, elevations in circulating eCBs may explain some of the reported beneficial effects of exercise on memory and cognitive functioning. There is also evidence that eCBs may contribute to the

Exercise duration	Exercise intensity	eCB collection method	eCB collection timepoints	Significant reported change in AEA	Significant reported change in 2-AG	Significant reported change in other eCBs	Additional outcomes[a]
5 h of wheel running	N/R	Mice tissues (e.g., heart, lung, skeletal muscle, brain), cerebrospinal fluid, and plasma	Collected 5 h after exercise	AEA ↑	2-AG ↑	OEA ↑ PEA ↑ AA ↑	Anxiety-like behavior ↓ Pain (thermal sensitivity) ↓ Absence of CB1R on GABAergic neurons and blockade of central and peripheral CBRs inhibits anxiolytic and analgesic effects, respectively.
15 sets of 15 repetitions, with a 120 sec rest between each set. The total exercise period was 7.5 min	Load was 70% of one rep max	Cardiac puncture	Collected immediately after exercise	AEA ↑	2-AG ↑	OEA ↑ PEA ↑	Injection of AM251 and AM630 prevents the antinociceptive effect induced by acute RE
Animals ran with a progressive speed at 20 m/min (average time of 49.06 min) and 0% inclination until fatigue	N/R	Cardiac puncture	Collected immediately after exercise	AEA ↑	2-AG ↑	OEA ↑ PEA ↑	Injection of AM251 and AM630 prevents the antinociceptive effect induced by acute aerobic exercise
30 min of walking on day 1 and 30 min of running on day 2	Running (Dogs and Ferrets): Froude numbers of ~0.70 Walking (Dogs and Ferrets): Froude numbers of ~0.25	Standard blood draw	Collected immediately before and after exercise	Dogs: AEA ↑ Ferrets: No AEA changes	Dogs: No 2-AG changes Ferrets: No 2-AG changes	N/A	N/A

motivational or rewarding aspects of exercise through modulation of synaptic activity in reward-related brain regions.[34,35] Furthermore, peripheral eCB levels are readily measured in lipid extracts of plasma or serum obtained from venous blood,[36] making circulating eCBs an excellent potential biomarker for tracking the efficacy of exercise interventions.

While the number of studies examining the impact of exercise on eCB signaling has increased by 200% over the past 5 years (Supplementary Fig. S1), no meta-analyses to date have synthesized the current literature on the impact of exercise on eCB signaling. Furthermore, while previous reviews have been published on the eCB system in general,[36] the role of eCBs in exercise focusing on regulation of skeletal muscle reponse to exercise,[37] implications for the prevention and treatment of neurological disorders,[37] and molecular and cellular pathways related to aerobic exercise,[38] there have been no comprehensive reviews on the effects of exercise on eCB signaling and potential moderators of these effects.

To address this, we performed a systematic review and meta-analysis of all studies on acute (i.e., single bout) and chronic exercise measuring eCBs. Effects of acute and chronic exercise on eCB concentrations were examined separately, given evidence that they may have different effects.[39] Finally, this is the first review to explore various factors that may influence eCB response to exercise, including acute versus chronic exercise, duration, intensity, modality, patient groups (i.e., with and without pre-existing health conditions), and species (i.e., humans, animal models).

Methodology

Search strategy

We searched the MEDLINE (PubMed) database for original articles published through December 31, 2020. Articles were included in the systematic review if they

Table 2. Included studies on chronic exercise ($N = 18$ samples reported in $N = 12$ articles)

Type	First author (year)	Condition	Strain of animal	Sample size (n)	Age (mean ± SD)	Gender (% female)	Race/ethnicity (%)	Type of exercise	Exercise modality
Human studies									
Patients, including those with pre-existing conditions									
	Stensson (2020)[45]	Patients with FM	N/A	HC: $n = 33$ FM: $n = 37$	HC: 50.3 ± 12.8 FM: 50.1 ± 10.5	HC: 100% F FM: 100% F	N/R	Strength	RE
	Belitardo de Oliveira (2019)[95]	Patients with Episodic Migraines	N/A	HC: $n = 12$ Migraines: $n = 13$	HC: 34.7 ± 12.0 Migraines: 37.4 ± 13.8	HC: 83% F Migraine: 85% F	HC: 92% W, 0% B, 8% A, 0% M, 0% O Migraines: 92% W, 0% B, 8% A, 0% M, 0% O	Endurance	Treadmill jogging or walking
	Brellenthin (2019)[44]	Patients with Substance Abuse Disorder	N/A	Treatment as usual with exercise: $n = 21$	35.1 ± 10.2	45% F	64% W, 2% B, 0% A, 1% M, 1% O	Endurance	Treadmill incline walking
	Fernández-Aranda (2014)[96]	Patients who are obese	N/A	HC: $n = 16$ Obese: $n = 30$ Morbid obese: $n = 43$	HC: 27.6 ± 7.9 Obese: 44.9 ± 12.9 Morbid obese: 43.5 ± 10.2	HC: 100% F Obese: 100% F Morbid obese: 100% F	N/R	Endurance	PA
Healthy volunteers									
	Koay (2021)[97]	Healthy, newly enlisted soldiers	N/A	$n = 52$	22.0 ± 4.0	0% F	N/R	Endurance and strength	Combined strength and endurance program and occupational specific activities such as marching.
	Sadhasivam (2020)[98]	Healthy volunteers	N/A	$n = 148$	≥ 18.0	Blood draws only: N/R Full sample: 49% F	16% W, 2% B, 74% A, 2% M, 6% O	Balance	Yoga (BSP)
	Belitardo de Oliveira (2019)[99]	Healthy volunteers	N/A	$n = 17$	39.0 ± 13.4	60% F	41% W, 1% B, 1% A, 0% M, 35% O	Endurance	Treadmill running or walking
Animal studies									
	Dos Santos (2019)[100]	Mice that received carrageenan injection	C57BL/6 female mice	Exercise: $n = 6$ Cg+ exercise: $n = 6$	8–10 weeks	Exercise: 100% F Cg+ exercise: 100% F	N/R	Endurance	Swimming
	Thompson (2017)[101]	Mice from HR lines	Trained mice from HR lines	HC: $n = 50$ HR: $n = 50$	N/R	HC: 50% F HR: 50% F	N/R	Endurance	Wheel running
	Biedermann (2016)[39]	Mice	C57BL/6 mice	Runners: $n = 10$ Restricted runners: $n = 10$	10 weeks	Runners: 0% F Restricted runners: 0% F	N/R	Endurance	Wheel running

Exercise duration	Exercise intensity	eCB collection method	eCB collection timepoints	Significant reported change in AEA	Significant reported change in 2-AG	Significant reported change in other eCBs	Additional outcomes[a]
60-min sessions of RE, 2 times/week for 15 weeks	Weeks 1–2: 40% of one rep max, 15–20 reps, 1–2 sets Weeks 3–5: 60% one rep max, 10–12 reps, 1–2 sets Weeks 6–15: 80% one rep max, 5–8 reps, 1–2 sets	Standard blood draw	Collected before (within 1–7 days) the start of the 15-week exercise interventions and after the intervention (within 1–7 days)	HC: No AEA changes FM: AEA ↑	HC: No 2-AG changes FM: No 2-AG changes	HC: No OEA, PEA changes SEA ↑ FM: No OEA, PEA changes SEA ↓	HC: No pain, depression scorings, or muscle strength changes FM: Pain ↓ Depression scorings ↓ Muscle strength ↑
40-min sessions of walking or jogging on a treadmill, 3 times/week for 12 weeks	Heart-rate equivalent to each individual's ventilatory threshold—additional information N/R	Standard blood draw	Collected before exercise and between 2 and 5 days after the last exercise session or 48 h after the last exercise session within the late follicular phase of the menstrual cycle	HC: AEA ↓ Migraines: AEA ↓	N/A	N/A	HC: No migraine attacks or mood changes Migraines: Migraine attacks ↓ Improvements in mood
30 min of moderate-intensity treadmill incline walking, 3 times/week for 6 weeks	70–75% AAMHR	Standard blood draw	N/A	N/R	N/R	N/A	Acute improvements in mood and reductions in perceived stress and drug craving
PA was monitored using accelerometry for 6 days	Time accumulated in MVPA during waking hours	Standard blood draw	Collected between 8 and 9 am after at least 12 h of fasting	AEA ↑ (all groups combined[b])	No 2-AG changes	OEA ↑ (all groups combined[b])	High PA associated with low harm avoidance and high novelty seeking (temperament trait)
80-day physical training program with about 1.3 h of PA each day	68% of the PA in the program was of moderate intensity (4.3–6 METs), while 32% was high intensity (METS >6)	Standard blood draw	Collected after an 80-day program	N/A	2-AG ↓	N/A	Indole-3-propionate ↑ Fatty acids and ketone body substrates ↓ Increases in dimethylguanidino valeric acid associated with increases in several cardiovascular risk factors Shifts in metabolism across many different substrates
4-day, 3-night yoga retreat	N/R	Standard blood draw	Collected before (up to 2 days before program began) and after exercise (within 2 days after program ended)	AEA ↑	2-AG ↑	DEA ↑ 1-AG ↑ OEA ↑	BDNF levels ↑ Depression ↓ Anxiety ↓ Happiness ↑
40 min of treadmill running or walking, 3 times/week for 12 weeks	Heart rate equivalent to each individual's ventilatory threshold—additional information N/R)	Standard blood draw	Collected before program and within 2–5 days after the last exercise session in program	AEA ↓	N/A	N/A	Reduction in AEA associated with weight loss Improvements in mood
5 days/week of swimming for 3 weeks: Week 1: 15 min Week 2: 30 min Week 3: 45 min	N/R	Mice spinal cord tissue	Collected immediately after exercise program	Exercise: No AEA changes Cg + exercise: AEA ↑	N/A	N/A	Spinal CB2R participate in exercise-induced antinociception in mice
Wheel running access for exercise group for 6 days	N/R	Cardiac puncture	Collected after exercise program	HC: AEA ↑ HR: AEA ↓	HC: 2-AG ↓ HR: 2-AG ↓	N/A	HC: Female mice had ↓2-AG levels and ↑ AEA levels compared with male mice HR: Female mice had ↓2-AG levels and ↑ AEA levels compared with male mice
Free access wheel running (all day) or restricted access wheel running (6 h/day) for 9 weeks	N/R	Cardiac puncture	Collected after exercise program	Runners: AEA ↓ Restricted runners: AEA ↓	Runners: No 2-AG changes Restricted runners: No 2-AG changes	Runners: No OEA, PEA, 1-AG, and AA changes Restricted Runners: No OEA, PEA, 1-AG, and AA changes	Runners: Hippocampal gray matter volume ↑ Immature hippocampal neurons ↑ Restricted runners: Immature hippocampal neurons ↑

(*continued*)

Table 2. (Continued)

Type	First author (year)	Condition	Strain of animal	Sample size (*n*)	Age (mean ± SD)	Gender (%)	Race/ethnicity (%)	Type of exercise	Exercise modality
	Gamelin (2016)[102]	Rats	Male Winstar rats	HC: $n=7$ HFD: $n=7$	15 weeks	HC: 0% F HFD: 0% F	N/R	Endurance	Treadmill running
	Hill (2010)[32]	Rats (treated with CB1R antagonist in experiment 2)	Sprague/Dawley rats	$n=35$	12 weeks	0% F	N/R	Endurance	Wheel running

[a]Outcomes, including mood, anxiety, stress, pain, fear, hunger, cravings, etc., were all self-reported unless otherwise stated.
[b]Did not report separate results for groups, treated as one sample.
1-AG, 1-arachidonoylglycerol; BSP, Bhava Spandana Program; CBR2, cannabinoid type 2 receptor; DEA, docosatetraenylethanolamide; FM, fibromyalgia; HFD, high-fat diet; HR, high runner; MVPA, moderate-vigorous physical activity; OEA, oleoylethanolamide; PA, physical activity; SEA, stearoylethanolamide.

met the following inclusion criteria: (1) measured eCB concentrations in circulation or in tissue, (2) examined eCBs before and after exercise (i.e., within-subject design) or compared eCBs in an exercise versus a non-exercise control group (i.e., between-subject design), (3) examined effects of acute (i.e., single bout) or chronic exercise (e.g., 10-week running program). We included studies on aerobic (e.g., running, cycling, dancing) and resistance (e.g., isometric handgrip, resistance training, yoga) exercise. We included studies in humans, including those with and without pre-existing health conditions (e.g., depression, PTSD, SUDs, fibromyalgia), and animal models, of all ages. Although our search focused on AEA and 2-AG as the most well-characterized eCBs, we also examined eCB-like N-acylethanolamides (NAEs) and related compounds (e.g., oleoylethanolamide [OEA], palmitoylethanolamide [PEA], and 2-oleoylglycerol [2-OG]). See Supplementary Material for more information on search strategy and article screening, and Figure 1 for a flowchart of identified articles and exclusions.

A total of 33 articles were included in the systematic review. Of note, 12 articles included results of separate groups (PTSD vs. trauma-exposed controls, dogs vs. ferrets) and 8 included effects of separate exercise conditions (e.g., preferred vs. prescribed exercise),[40] for a total of 57 samples/conditions. The earliest published study was in 2003.[41] First, we summarize results from all articles on acute and chronic exercise. Next, we report results on subgroups, including (1) articles on healthy humans and (2) articles reporting on moderate-intensity aerobic exercise on humans.

Meta-analysis

The 33 articles included in the systematic review were subsequently examined for inclusion in the meta-analysis. Full data were available for 10 articles (reporting on 24 samples) that examined (1) eCB concentrations before and after acute exercise (within-subject design). There were not enough data (<5 articles) to perform meta-analyses on (2) within-subject effects of chronic exercise, (3) between-subject effects of chronic exercise, or (4) between-subject effects of acute exercise. Thus, we performed a meta-analysis on the articles that examine within-subject effects of acute exercise on eCBs. If an article reported effects in different samples separately (e.g., PTSD, healthy control), samples were considered as unique data points. Given the limited number of articles with data from multiple post-exercise time points (four articles), the meta-analysis focused on the first available measurement of eCBs following acute exercise.

First, we extracted data from each of the 10 articles (24 samples) on eCB concentrations before (pre) and after (post) acute exercise. Extracted data included means, standard deviations (SDs), and sample size (*N*). Change scores were calculated as post-exercise mean minus pre-exercise mean; therefore positive scores represent increases while negative scores represent decreases in eCBs. SDs for the change scores were estimated from pre- and post-exercise SDs (SDpre and SDpost).[42,43] See Supplementary Material for more information. Meta-analyses were conducted for AEA and 2-AG separately, using RevMan software v.5.4 (Cochrane Community, London, United Kingdom) and fixed effect models. Forest plots were generated for sample-specific effect sizes along with 95% confidence intervals (CIs) and pooled effects. Results were considered significant at $p<0.05$ and heterogeneity between studies was tested using a chi-square test for heterogeneity and the I^2 statistic. Follow-up analyses were performed in the following subgroups: (1) articles

Exercise duration	Exercise intensity	eCB collection method	eCB collection timepoints	Significant reported change in AEA	Significant reported change in 2-AG	Significant reported change in other eCBs	Additional outcomes[a]
Treadmill running 1 h/day, 5 days/week for 12 weeks	70–80% of the maximal aerobic velocity	Cardiac puncture	Collected after exercise program	HC: No AEA changes HFD: No AEA changes	HC: No 2-AG changes HFD: No 2-AG changes	HC: No OEA and PEA changes HFD: No OEA and PEA changes	HC: No CB1R, CB2R, or FAAH changes HFD: No CB1R, CB2R, or FAAH changes
Access to wheel for 8 days	N/R	Brain tissue in rats	Collected after exercise program	AEA ↑	No 2-AG changes	N/A	Voluntary exercise ↑ proliferation of progenitor cells in the dentate gyrus of the hippocampus Effect of proliferation was prevented following AM251 treatment

on healthy humans (i.e., adults without a clinical diagnosis) and (2) articles reporting on moderate-intensity aerobic exercise on humans.

Results of Systematic Review

Overview of studies included in systematic review

Thirty-three articles reporting on 57 samples were included in the systematic review ($n = 1021$ participants, see Table 1. Nine of the 33 articles included patients with pre-existing physical or mental health conditions (e.g., PTSD, fibromyalgia), 15 included strictly healthy volunteers (e.g., no current physical or mental health diagnosis), and 10 included animal models (Tables 1 and 2 and Supplementary Material).

The majority of the 33 articles ($n = 22$ articles, 66.7%) focused on the effects of acute exercise on eCB concentrations (Table 1), and 12 articles examined chronic exercise (Table 2). One article examined both acute and chronic exercise.[44] The most common modalities were walking and running, followed by cycling (Supplementary Fig. S2 and Tables 1 and 2). For human studies of acute exercise, the most frequently tested exercise duration (excluding warm-up and cool-down) was 30 min (55.6%).

Of the 33 articles, 93.9% reported on AEA concentrations, 81.8% reported on 2-AG, 51.5% on OEA, 51.5% on PEA, 18.2% on 2-OG, and few (< 7%) examined other related molecules (Tables 1 and 2). The majority of articles ($n = 25$) measured eCBs in circulation (plasma and/or serum) using a blood draw with standard venipuncture procedures (75.8%). For articles on acute exercise, the majority ($n = 21$, 63.6%) measured eCBs immediately after or within 5 min following acute exercise. However, eCBs were also measured 15 min–24 h later. For articles on chronic exercise, the time that eCBs were measured varied from within 5 min[44] to within 1–7 days post-exercise.[45] All studies included adults (i.e., ages 18 +) or mature/adult animals. Around 33.3% of studies reported male-only participants and subjects, 39.4% reported both, and 15.2% reported female only (12.1% did not report). For further description of included studies, see the Supplementary Material.

Systematic review results for AEA concentrations

Acute exercise. Twenty-one of the 22 of the articles (39 samples) that examined the effects of acute exercise measured AEA concentrations. Twenty-nine of the 39 (74.4%) samples demonstrated a significant increase in AEA, 1 (2.6%) showed a significant decrease, and 9 (23.1%) revealed no change in AEA.

Acute exercise: healthy individuals only. Sixteen of the 22 articles on acute exercise examined solely healthy humans or included a healthy control group and 15 of these (25 samples) measured AEA concentrations. Eighteen samples (72%) reported an increase in AEA compared with baseline levels, six samples (24.0%) reported no AEA changes, and one sample (4.0%) reported a decrease in AEA concentrations.

Acute exercise: moderate-intensity aerobic exercise: humans only. Twelve of the 22 acute exercise articles (18 samples) measuring AEA concentrations included a moderate-intensity aerobic acute exercise condition (i.e., running or bicycling between 70% and 80% maximum heart rate [MHR]). Seventeen samples (94.4%) reported an increase in AEA, one reported no AEA changes (5.6%), and none reported a decrease in AEA.

Acute exercise: effects of intensity. Seven of the 22 articles (16 samples) on acute exercise examined the impact of various exercise intensities on AEA concentrations. All seven studies (100%) indicated that

moderate-intensity exercise was associated with greater increases in AEA as compared with less-intense exercise (i.e., < 50% MHR). Of note, Marin Bosch et al.[46] found that exercise performed at 80% MHR produces greater increases in AEA concentrations as compared with exercise performed at 70% MHR. However, one study by Raichlen et al.[31] also examined vigorous-intensity exercise (i.e., > 90% age adjusted MHR). In that study, vigorous-intensity exercise elicited lower AEA as compared with moderate exercise intensities (70–80% MHR). Two studies compared preferred and prescribed moderate exercise conditions (see Supplementary Material for discussion).

Chronic exercise. Eleven of 12 articles (17 samples) examined the effects of chronic exercise on AEA concentrations. However, one[44] did not report AEA before and following the intervention (i.e., pre and post-exercise eCB concentrations averaged across acute exercise sessions throughout [baseline and week 6] the intervention were reported). Therefore, only 10 articles (16 samples) were considered in this section. In particular, we report on AEA concentrations before and after chronic exercise. Six samples (37.5%) revealed a significant increase in AEA, six (37.5%) demonstrated a significant decrease, and four (25%) showed no change in AEA.

Chronic exercise: healthy individuals only. Five of the 12 articles on chronic exercise solely examined healthy humans or included healthy control groups, and four of these (4 samples) measured AEA concentrations. One sample reported an increase in AEA, two reported a decrease in AEA, and one reported no changes in AEA following the exercise program.

Chronic exercise: moderate-intensity aerobic exercise. There were not enough data to evaluate.

Systematic review results for 2-AG

Acute exercise. Nineteen articles (35 samples) reported 2-AG concentrations following acute exercise. Twenty samples (57.1%) showed no 2-AG changes, 15 samples (42.9%) exhibited a significant increase in 2-AG following acute exercise, and no studies showed a decrease.

Acute exercise: healthy individuals only. Sixteen of the 22 articles on acute exercise solely examined healthy humans or included a healthy control group and 14 of these (22 samples) measured 2-AG conentrations. Thirteen samples (59.1%) reported no 2-AG changes compared with baseline levels, nine samples (40.9%) reported an increase in 2-AG, and no samples reported decreases in 2-AG concentrations.

Acute exercise: moderate-intensity aerobic exercise: humans only. Eleven of the 22 acute exercise articles (17 samples) used a moderate-intensity aerobic exercise (e.g., running or bicycling between 70% and 80% MHR) and measured 2-AG concentrations. Nine samples reported no 2-AG changes (59.1%), eight samples reported an increase in 2-AG (40.9%), and no samples reported a decrease in 2-AG.

Acute exercise: effects of intensity. Seven of the 22 articles (14 samples) examined the impact of exercise intensity on 2-AG concentrations. None of the 14 samples demonstrated significant effects of exercise intensity on 2-AG concentrations.

Chronic exercise. Eight articles (12 samples) reported 2-AG concentrations following chronic exercise. One sample (8.3%) demonstrated a significant increase in 2-AG following chronic exercise, three (25.0%) showed a significant decrease, and eight (66.7%) showed no changes.

Chronic exercise: healthy individuals only. Four of the 12 articles on chronic exercise solely examined healthy humans or included a healthy control group and 3 of these (3 samples) measured 2-AG concentrations. One sample reported an increase in 2-AG, one reported a decrease in 2-AG, and one reported no 2-AG changes following an exericse program.

Chronic exercise: moderate-intensity aerobic exercise. There were not enough data to evaluate.

Systematic review for eCB-like NAEs and related compounds

Seventeen articles measured OEA and PEA (13 articles acute exercise, 4 chronic exercise). Other eCB-like compounds (e.g., 2-OG, SEA, 1-AG) were measured infrequently. Results for these compounds were inconsistent and showed no clear trend for increases, decreases, or no changes in concentrations. However, one study reported elevations in OEA that lasted 4 h after an acute (30 min) cycling session.[47] See Supplementary Material and Table 1 for full summary.

Results of Meta-Analysis: Within-Subject Effects of Acute Exercise

Acute exercise AEA

Eleven articles (24 samples) were included in the meta-analysis ($n = 335$ participants). The meta-analysis

revealed a significant increase (mean difference = 0.15 a.u., 95% CIs = 0.15 to 0.15) in AEA concentrations following acute exercise ($z = 1227.58$, $p < 0.00001$; Fig. 2a). Using the studies that reported AEA concentrations in units of pmol/mL (Fig. 2a), this mean difference was 0.03 pmol/mL (95% CIs = 0.02 to 0.03). However, there was also substantial heterogeneity in the effect ($I^2 = 100\%$), suggesting that the magnitude of the increase varied across articles. Inspection of the effect sizes (Fig. 2a) suggests that moderate-intensity exercise is associated with greater increases in AEA as compared with low-intensity exercise, as revealed by the systematic review.[48] Consistent with observations from the systematic review, in general, prescribed moderate-intensity exercise was associated with greater increases in AEA as compared with preferred (Fig. 2a). In addition to comparing preferred versus prescribed, Brellenthin et al.[40] examined the impact of regular physical activity on exercise-related changes in eCBs across three groups: low (≤ 60 min MVPA per week), moderate (150–299 min MVPA per week), and high (≥ 300 MVPA per week) physical activity groups. Although the authors reported no significant main effects or interactions with group[40] inspection of effect sizes (Fig. 2a) for moderate prescribed sessions suggest that the high moderate vigorous physical activity (MVPA) group demonstrated the greatest exercise-related increases in AEA, followed by the moderate, then low MVPA group. Importantly, participants in that study were asked to abstain from exercise within 24 h of testing to minimize potential carryover effects.[40] Therefore, although acute exercise increased AEA among inactive to highly active individuals, prior exercise history may further augment eCB system activation in response to exercise. Post-exercise collection time may also contribute to the observed heterogeneity. While most studies measured post-exercise eCB concentrations immediately after or within 15 min following exercise, Stensson and Grimby-Ekman[49] collected the post-exercise blood sample 60 min after finishing the exercise and demonstrated smaller effect sizes (Fig. 2a). We observed no other patterns related to modality, collection method, subject group, or other factors.

Acute exercise: healthy humans only

We repeated the meta-analysis using 15 samples that included healthy humans and found a significant increase (mean difference = 0.18 a.u., 95% CIs = 0.18 to 0.18) in AEA concentrations following acute exercise ($z = 1245.84$, $p < 0.00001$). Using the studies that reported AEA concentrations in units of pmol/mL, this mean difference was 0.2 pmol/mL (95% CIs = 0.2 to 0.2). Heterogeneity remained high ($I^2 = 100\%$).

Acute exercise: moderate-intensity aerobic exercise: humans only

We repeated the meta-analysis using 15 samples that reported effects of a moderate-intensity aerobic exercise condition. There was a significant increase (mean difference = 0.14 a.u., 95% CIs = 0.13 to 0.14) in AEA concentrations following acute moderate exercise ($z = 205.11$, $p < 0.00001$). Using the studies that reported AEA concentrations in units of pmol/mL, this mean difference was 0.14 pmol/mL (95% CIs = 0.14 to 0.14). Heterogeneity remained high ($I^2 = 100\%$).

Acute exercise 2-AG

Twenty-four samples were included in the 2-AG meta-analysis ($n = 335$). Similar to results for AEA, the meta-analysis showed a significant increase in 2-AG (mean difference = 0.02 a.u., 95% CI = 0.02 to 0.02) following acute exercise ($z = 458.18$, $p < 0.00001$; Fig. 2b). Using the studies that reported 2-AG concentrations in units of pmol/mL (Fig. 2b), this mean difference was 0.9 pmol/mL (95% CIs = 0.9 to 0.9). There was also substantial heterogeneity across samples ($I^2 = 100\%$). Brellenthin et al.[40] examined both preferred and moderate prescribed exercise conditions and reported larger effect sizes for prescribed relative to preferred conditions (Fig. 2b). However, the authors reported no group-by-time interactions in 2-AG concentrations.[40] Breakdown of studies by modality, duration, sample, and timing of sample collection did not reveal any clear patterns in terms of effects on 2-AG (Fig. 2b).

Acute exercise: healthy humans only

We repeated the meta-analysis using 15 samples that included healthy humans and found a significant increase (mean difference = 0.02 a.u., 95% CIs = 0.02 to 0.02) in 2-AG concentrations following acute exercise ($z = 459.95$, $p < 0.00001$). Using the studies that reported 2-AG concentrations in units of pmol/mL, this mean difference was 0.02 pmol/mL (95% CIs = 0.02 to 0.02). Heterogeneity remained high ($I^2 = 100\%$).

Acute exercise: moderate-intensity aerobic exercise: humans only

We repeated the meta-analysis using 15 samples that reported effects of a moderate-intensity aerobic exercise condition. There was a significant increase (mean difference = 0.02 a.u., 95% CIs = 0.02 to 0.02) in 2-AG

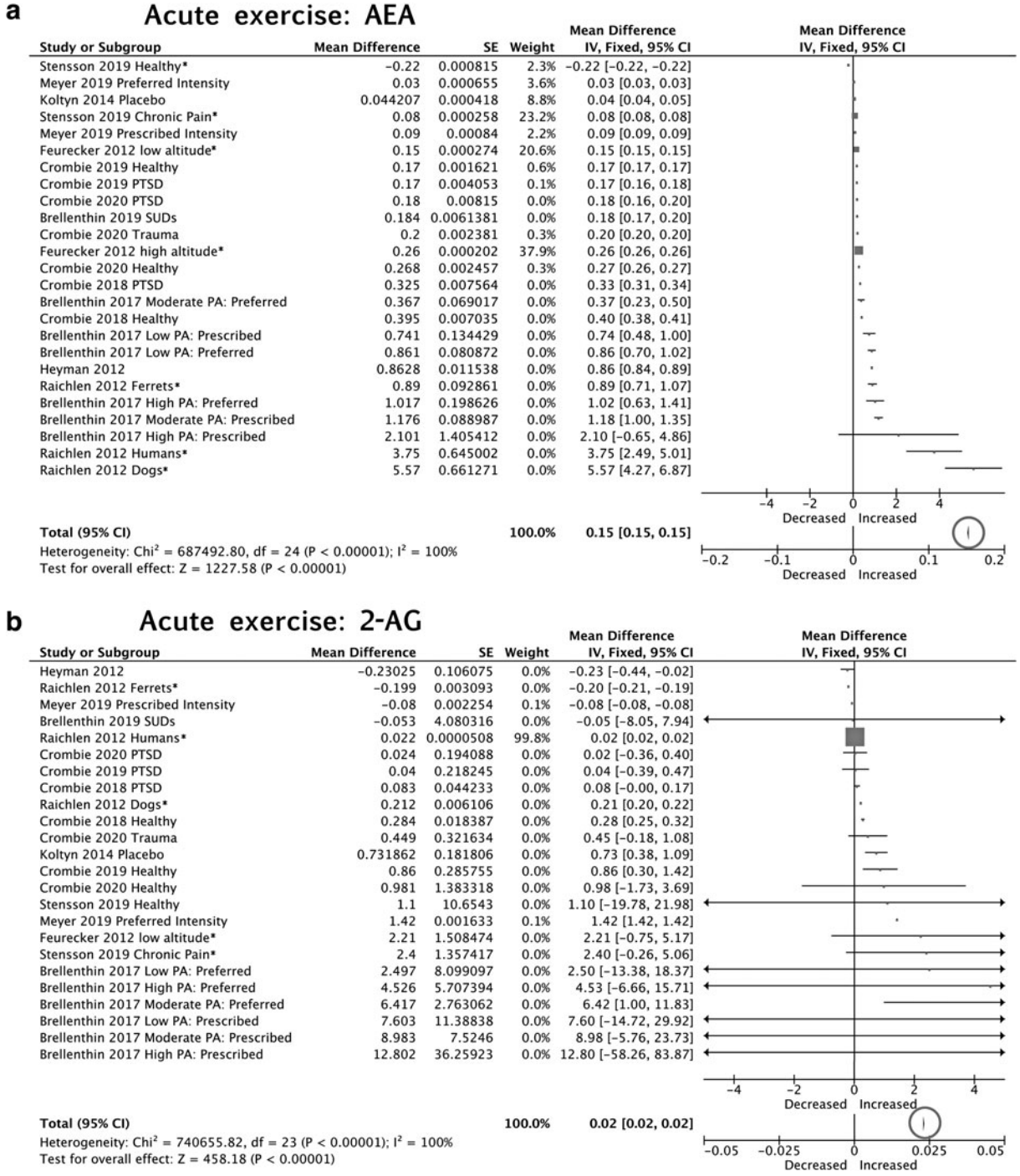

FIG. 2. Forest plots for meta-analyses on effects of acute exercise on **(a)** AEA and **(b)** 2-AG concentrations (pmol/mL). Note: some articles reported effects in different samples separately (e.g., PTSD and healthy control groups); therefore, these articles are entered more than once in the meta-analysis and treated as separate subgroups. The total effects (bottom) are reported on a different scale than the study effects. Individual studies show mean difference scores and the pooled results show weighted mean differences and 95% confidence intervals. The size of the block indicates the weight assigned to that study in the meta-analysis and the horizontal line depicts the confidence interval. Thus, larger block sizes indicate studies with larger weight (typically with narrower confidence intervals), which contribute more to the calculation of the summary result. The summary result is presented as a diamond at the bottom. The total around that diamond is circled to draw attention to the total effect. 2-AG, 2-arachidonoylglycerol; AEA, anandamide; PTSD, post-traumatic stress disorder.

concentrations following acute moderate exercise ($z = 205.18$, $p < 0.00001$). Using the studies that reported 2-AG concentrations in units of pmol/mL, this mean difference was −0.07 pmol/mL (95% CIs = −0.08 to −0.07). Heterogeneity remained high ($I^2 = 97\%$).

ECB-like NAEs and related compounds

Eight articles (19 samples) reported PEA concentrations, 7 articles (13 samples) reported OEA, and 6 articles (10 samples) reported 2-OG. Acute exercise was associated with a significant increase in both PEA ($z = 681.21$, $p < 0.00001$) and OEA concentrations ($z = 75.79$, $p < 0.00001$); but these results showed significant heterogeneity ($I^2 = 100\%$). In contrast, acute exercise was associated with a significant *decrease* in 2-OG concentrations ($z = 26.78$, $p < 0.00001$) with lower heterogeneity ($I^2 = 28\%$). See Supplementary Figures S1–S3 for full results and forest plots.

Discussion

Our systematic review revealed a consistent increase in AEA concentrations following acute exercise, which was confirmed by our meta-analysis. This increase in AEA was observed across modalities (e.g., running, cycling, resistance exercise) as well as, across pre-clinical and clinical studies, including patients with and without pre-existing health conditions (e.g., depression, PTSD). However, there was substantial variation in magnitude of the increase across samples, which may be related to exercise intensity, timing of sample collection, or other factors. These possibilities are discussed below, and we provide some recommendations for future studies.

With regard to 2-AG, effects of acute exercise were less consistent. In the systematic review, half of the samples demonstrated a significant increase in 2-AG concentrations following acute exercise, while half reported no significant changes. However, the meta-analysis supported a small but significant increase in 2-AG concentrations following acute exercise, with substantial heterogeneity across samples. Both the increase in AEA and 2-AG remained significant when considering the subgroup of healthy adults and moderate-intensity aerobic exercise subgroup, only. Overall, the effects of *chronic* exercise on circulating eCB concentrations were inconsistent, which may be due to the smaller number of studies examining chronic exercise and substantial heterogeneity across studies.

In this study, we provide the first meta-analytic evidence to support the notion that acute exercise mobilizes eCBs.[36] Post-exercise eCB mobilization in the periphery is thought in part to be related to the need to replenish energy stores,[36] given that CB1Rs are widely distributed in adipose tissues, liver, and skeletal muscle. Centrally, eCB mobilization may contribute to the wide range of cognitive, emotional, and physiological processes associated with exercise, through modulation of the HPA axis, neural progenitor cell proliferation, and glutamate and GABA signaling (for a comprehensive review, see Forteza et al.[38]). Indeed, studies included in this review frequently report that elevations in eCBs are associated with, or accompanied by, mood elevations, reductions in stress, anxiety, and pain, and enhanced cognitive functioning (Tables 1 and 2).

Given evidence that skeletal muscle expresses enzymes that synthesize eCBs,[50,51] the observed increase in circulating eCBs following acute exercise may be produced by skeletal muscle. Our meta-analysis revealed that acute exercise increases AEA, PEA, and OEA concentrations (but not 2-OG, which showed a significant decrease). This is consistent with prior studies showing that PEA and OEA are cosynthesized with AEA (NAPE), but not with 2-OG.[52,53] Although PEA and OEA do not bind to CB1Rs, they may be involved in other well-documented effects of exercise, such as appetite suppression, reduced inflammation, and reduced nociception.[54,55] In addition, exercise has been shown to increase circulating glucocorticoids, which may increase AEA concentrations in the brain.[32] Circulating concentrations of other biomarkers (e.g., brain-derived neurotrophic factor [BDNF]) have also been found to be elevated following acute exercise,[56] which may contribute (independently or in conjunction with eCBs) to neuroplastic and antidepressant effects of exercise.

Although the overall effect size was much smaller than for AEA, our meta-analysis revealed an increase in 2-AG concentrations following acute exercise (Fig. 3). The small effect size may explain some of the inconsistencies in reported results in the literature, particularly for studies with smaller sample sizes. 2-AG biosynthesis occurs separately from AEA (e.g., DAGL) and plays a role in reducing HPA axis activation in response to stressors.[57] A few studies reported that elevations in 2-AG (but not AEA) were associated with reductions in tension, mood disturbance, and negative affect following acute exercise (Tables 1 and 2). Therefore, elevations in 2-AG may have additional or complementary benefits from AEA.

Variation in exercise intensity may explain some of the observed heterogeneity in AEA (but not 2-AG).

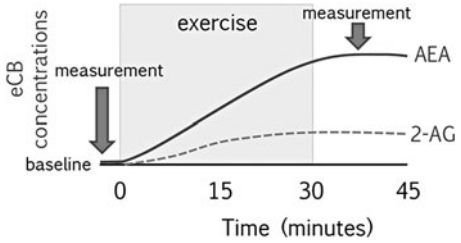

FIG. 3. Proposed increase in AEA and 2-AG concentrations following moderate acute exercise.

Indeed, several studies report that moderate-intensity exercise is associated with greater increases in AEA as compared with other intensities (Fig. 3).[31,40,46,48,56,58,59] There is also initial evidence for an inverted U-shaped effect of exercise intensity on AEA concentrations, such that moderate intensity is associated with the greatest increases; however, this notion is based on results of one study that examined high-intensity exercise (i.e., ~90% age-adjusted MHR).[31] However, the idea that the optimal eCB response may be at moderate-intensity exercise fits with prior data on other outcomes. Indeed, prior studies that compare effects of exercise intensity commonly report greater improvements in mood and cognitive performance, reductions in stress, and enhanced neurobiological reward response following moderate-intensity compared with low- or high-intensity exercise.[60–62] This also fits with the dual-mode model, proposed by Ekkekakis et al., which suggests that moderate-intensity exercise seems to be optimal for improvements in affect.[63]

There is also some evidence that AEA concentrations continue to increase to 15 mins post-exercise.[31] One study in mice suggest that AEA concentrations remain elevated (with respect to a no-exercise control group) for up to 2 h.[64] However, the duration, shape, and recovery of eCB concentrations following exercise remain open questions (Fig. 3). In addition, we did not find consistent effects of *chronic* exercise on eCB concentrations. Thus, the potential for chronic exercise to modulate basal eCB concentrations or eCB responses to acute stress—including exercise stress—is unclear.

In addition to exercise intensity, several other factors may contribute to the observed variation in eCB mobilization following acute exercise. We found initial evidence that the timing of the post-exercise eCB measurement is important, with at least one study measuring eCBs after a 60-min rest period demonstrating smaller effect sizes than studies that measured eCBs immediately after exercise. Exercise history may also modify eCB responses, with initial evidence that more physically active individuals may show a more robust AEA response to exercise as compared with those with low physical activity. eCB tone or response to stress may also be affected by fasted state, last meal timing and content, recent exercise, circadian timing of exercise, BMI, sex, gender, pre-existing health conditions, genetic variance in the eCB system (e.g., *FAAH, CNR1*), sleep restriction, psychotropic medications, oral contraceptives, menstrual stage, recent cannabis or alcohol use, injury or inflammation, or racial/ethnic background.[30,65–73]

Future studies should characterize eCB system functioning at baseline and responses to exercise in various patient or risk groups (e.g., trauma-exposed, first-degree relative with depression), which may inform the pathophysiology of these disorders or inform exercise interventions. Future studies should also standardize the duration, timing of measurements and exercise, time-of-day, fed state (e.g., fasted or standard breakfast), and ask participants to refrain from exercising or using substances (e.g., cannabis, alcohol) before the session. Furthermore, future studies are needed to identify the most effective modalties and durations of exercise for enahcing eCB concentrations, as most of the modalties examined in this review were primarily aerobic in nature. We also found no studies that examine the impact of exercise on children, adolescents, or juvenile animals. Thus, the observed effects of acute exercise on eCBs in adults may not generalize to younger populations.

The involvement of stress in the neurobiology of anxiety, depression, PTSD, and SUDs is well described,[74–76] and emerging data implicate eCB system hypoactivity in the pathophysiology and/or maintenance of these disorders.[16,77,78] Indeed, pre-clinical studies suggest that acute, repeated psychological stress exposure is associated with increased 2-AG and *reduced* AEA concentrations in the brain. Clinical studies have reported increases in circulating concentrations of 2-AG in response to acute social stress.[65,79,80] However, lower basal 2-AG concentrations have been reported in individuals with PTSD as compared with trauma-exposed healthy controls, and lower basal AEA and 2-AG concentrations in unmedicated depressed women as compared with controls.[79,81,82] Given the critical role of eCB signaling in regulating anxiety,

stress, and fear-related behavior, the eCB system has garnered attention as a potential therapeutic target for the prevention and treatment of stress-related psychopathology.

Exercise may be a potent nonpharmacological intervention for a variety of stress-related disorders that could benefit from elevations in eCBs and associated effects on mood, stress, anxiety, pain, cognitive functioning, inflammation, sleep, and appetite. Exercise may also be beneficial for conditions associated with eCB system *hyperactivity*, for example, diabetes, obesity, and inflammation. Given that the eCB system responds to stimuli (i.e., stressors), and due to the fact that exercise is a form of physical stress, administering an acute bout of exercise may be useful for probing eCB system stress reactivity in those at risk for psychopathology.[30] Furthermore, eCB signaling is considered to be critical for fear extinction learning and recall, and prior studies indicate that individuals with stress-related disorders (e.g., PTSD) have impaired fear extinction learning and/or recall.[83] Therefore, the ability of exercise to augment eCB signaling may have significant benefits for the management of stress-related disorders, for example, as an adjunct to extinction-based exposure therapy.[84–87]

Limitations should be mentioned. First, there were not enough data to perform meta-analysis on the effects of chronic exercise on eCB concentrations, or to examine studies on acute exercise separately by species, modality, duration, measurement timing, sex, or other factors. We did, however, examine the potential impact of various factors on results of the systematic review and meta-analysis, to identify potential patterns for future study. Furthermore, we focused on the impact of exercise on eCB concentrations; future studies should also examine the role of endorphins, BDNF, or other signaling systems in mediating or moderating acute exercise effects either in isolation from or in conjuction with the eCB system.

Conclusions

In this study, we provide evidence from a systematic review and meta-analysis that acute exercise activates the eCB system, that is, increases circulating concentrations of eCBs. Given that disruptions in eCB signaling are linked to several stress-related diseases (e.g., anxiety, depression, PTSD, obesity, and diabetes) exercise is a promising nonpharmacological approach for the prevention and treatment of stress-related psychopathology through its potential impacts on eCB signaling. However, the wide heterogeneity observed across studies to-date suggests that there is a critical need for standardized, well-powered studies to examine (1) the usefulness of exercise as a probe for eCB system functioning in disease or at-risk states, (2) the shape of the eCB response and recovery following acute exercise, (3) factors that modify eCB response to exercise, (4) ability for chronic exercise to modulate basal eCB concentrations and eCB response to stress, and (5) exercise as a preventive or therapeutic intervention.

Authors' Contributions

S.D., B.B., and H.M. performed the systematic review. C.C. and H.M. performed the analyses. S.D., B.B., C.C., K.C., C.R., M.H., and H.M. contributed to writing and reviewing. All authors approved the article.

Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Data
Supplementary Figure S1
Supplementary Figure S2
Supplementary Figure S3

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Abbreviations Used

1-AG = 1-arachidonoylglycerol
2-AG = 2-arachidonoylglycerol
2-OG = 2-oleoylglycerol
A = Asian
AA = arachidonic acid
AAMHR = age-adjusted maximum heart rate
AEA = anandamide
AMP = adenosine monophosphate
B = Black
BDNF = brain-derived neurotrophic factor
BFR-RE = blood flow restriction resistance exercise
BSP = Bhava Spandana Program
CB1R = cannabinoid type 2 receptor
CB2R = cannabinoid type 2 receptor
CIs = confidence intervals
CNSP = chronic neck and shoulder pain
DAGL = diacylglycerol lipase
DEA = docosatetraenylethanolamide
DHEA = docosahexaenoyl ethanolamide
eCB = endocannabinoid
F = female
FAAH = fatty acid amide hydrolase
FM = fibromyalgia
HC = healthy control
HFD = high-fat diet
HR = high runner
M = mixed
MDD = major depressive disorder
MHR = maximum heart rate
MVC = maximum voluntary contraction
MVPA = moderate-vigorous physical activity
N/A = not applicable
N/R = not reported
NAE = N-acylethanolamide
NAPE = N-acylphosphatidylethanolamine phospholipase D
O = other
OEA = oleoylethanolamide
PA = physical activity
PEA = palmitoylethanolamide
PPR = pressure pain ratings
PPT = pressure pain thresholds
PTSD = post-traumatic stress disorder
RE = resistance exercise
SD = standard deviation
SEA = stearoylethanolamide
SUDs = substance use disorders
TE = trauma-exposed individuals without PTSD
W = White