



Endocannabinoids, exercise, pain, and a path to health with aging

Bruce A. Watkins

Department of Nutrition, University of California, Davis, CA, USA

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ABSTRACT

Physical activity is an important lifestyle factor for growth, development, and sustained health throughout life. In recent years, the benefits of physical activity have drawn more attention to its physiological effects on the body, including well-being. The endocannabinoid system (ECS) has emerged as a focal point to ascertain the mechanisms for how exercise benefits the body and how it reduces or controls pain. The ECS, its ligands [the endocannabinoids (eCB)], receptors (CB1 and CB2), enzymes for the synthesis and degradation of eCB, and the polyunsaturated fatty acids (PUFA) that serve as substrates, comprise a powerful biological organization of multiple controls that affects mood, inflammation, pain, and other neurological aspects of the central nervous system and peripheral nervous system. Recently, investigators have reported increases in circulating levels of eCB after exercise, with some eCB exerting analgesic effects from exercise. The focus of this review is to discuss evidence for the role of eCB and the complexities of the ECS in exercise and pain. Some aspects presented herein are production of eCB and activation of the cannabinoid receptors in the brain following exercise; eCB, pain, and physical activity; oxylipins; and joint pain. Future research on the ECS must include mechanistic approaches to endocannabinoid signaling and explain the role of dietary PUFA in altering signaling of the receptors that affects pain. Additionally, how other types of exercise, such as Tai Chi, which is reported to improve well-being, should be investigated to ascertain if changes in eCB mediate the mind and body benefits of Tai Chi. As we age, exercise in the form of play has evolved with the exploration of our body from walking to running, recreational, and competitive sports, to midlife physical activity focusing on maintaining fitness and a healthy body weight. Furthermore, exercise has been a target of investigation to explore various hypotheses to explain the mechanisms for cognitive benefits in the young and in older adults. The science of exercise has matured to a level of importance in the life cycle to reduce pain with aging and include new investigations on the ECS to explain its role in well-being and improved quality of life in later years.

1. Introduction

1.1. The endocannabinoids and the nervous system

The endocannabinoid system (ECS) of the body is comprised of the G-protein-coupled cannabinoid receptors CB1 (type 1) and CB2 (type 2) and the endogenous ligands agonists known as endocannabinoids (eCB) [*N*-arachidonyl ethanolamide (AEA or anandamide) and 2-arachidonylglycerol (2-AG)]. This system includes several enzymes for the synthesis and degradation of the primary ligands derived from arachidonic acid (Kim et al., 2013; Howlett et al., 2011). The endocannabinoid receptors are located in both the central and peripheral nervous systems, and CB1 is involved in excitatory and inhibitory synapses (Mendiguren et al., 2018). Both receptors are also located in many other tissues and cells, with CB2 especially prevalent in immune cells (Tanasescu and Constantinescu, 2010). This system has been studied to understand the role of the eCB in many physiological processes

including appetite (Gatta-Cherifi and Cota, 2016) and pain sensation (Woodhams et al., 2017), as well as control of chronic pain (Donvito et al., 2018), macronutrient metabolism (Kim et al., 2016), mood (Hill et al., 2009) and mood disorders (Micale et al., 2013), and regulation of immune cell functions (Basu and Dittel, 2011). The signaling of eCB can be characterized as autocrine and paracrine stimulators of its receptors in neuropathic pain and neuroinflammation, as well as other biological actions (Miller and Devi, 2011). Because of the lipid nature of eCB and transport mechanisms, the actions of the ligands with its receptor is believed to be localized.

The synthesis and degradation of eCB is achieved by primarily phospholipases and lipases associated with intracellular membranes. AEA is biosynthesized from arachidonic acid by *N*-acylphosphatidyl ethanolamines (NAPE)-specific phospholipase D (NAPE-PLD), while 2-AG is biosynthesized by diacylglycerol lipase alpha (DAGL α) and diacylglycerol lipase beta (DAGL β). The degrading enzymes for AEA and 2-AG are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase

E-mail addresses: bawatkins@ucdavis.edu, baw@purdue.edu.

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Abbreviations

CB1 (type 1)	Cannabinoid receptor 1
CB2 (type 2)	Cannabinoid receptor 2
CNS	Central nervous system
COX-2	Cyclooxygenase-2
AEA	Endocannabinoid N-arachidonylethanolamide or anandamide

2-AG	Endocannabinoid 2-arachidonoylglycerol
eCB	Endocannabinoids
ECS	Endocannabinoid system
FAAH	Fatty acid amide hydrolase
MAGL	Monoacylglycerol lipase
DHEA	N-docosahexaenoyl-ethanolamide
EPEA	N-eicosapentaenoyl-ethanolamide
PUFA	Polyunsaturated fatty acid

(MAGL), respectively. In general, the CB receptors are endogenously activated by AEA and 2-AG, inactivated through a membrane transporter facilitated reuptake, and degraded by an intracellular FAAH for AEA or MAGL for 2-AG (Kano et al., 2009).

The interest in running effects on neurochemical and neurophysiology were hypothesized to be related to monoaminergic (Chaouloff, 1989) and endorphinergic, e.g. the endorphin hypothesis (Hoffmann, 1997), mechanisms for mood, cognition, and behavior outcomes. As the research advanced, other hypotheses were put forward to examine the effects of chronic exercise on the central nervous system in mice, targeting the dentate gyrus (Van Praag et al., 1999a,b) and in cognition in the human (Hillman et al., 2008). As a result, the ECS has become a novel and appealing line of research to understand the neurological benefits of exercise. A recent meta-analysis on exercise and cognitive function that included 30 studies on adults 50 years and older found a significant improvement in cognitive function from participation in aerobic exercise, resistance training, and multicomponent training and Tai Chi (Northey et al., 2018). Although these studies did not examine the ECS, the benefits of exercise on older adults is an important, promising area of investigation.

At present, the ECS is part of the neuromodulatory organization where the two receptors, CB1 and CB2, and the arachidonate-derived endogenous ligands, AEA and 2-AG, participate (Hillard, 2017; Howlett et al., 2011). Both *in vitro* and *in vivo* studies revealed that neural progenitor cells express both CB1 and CB2 receptors and synthesize AEA and 2-AG (Aguado et al., 2006; Pertwee et al., 2010). Rodent studies showed that the ECS supports neuroplasticity (Hill et al., 2010); and in humans, strenuous exercise activates the ECS, resulting in an elevation of AEA in blood and promoting well-being (Dietrich and McDaniel, 2004). The eCB can also reduce pain in osteoarthritis and rheumatoid arthritis (Richardson et al., 2008). Evidence for how exercise affects the ECS with actions on the nervous system and mood will be explored herein to include evidence for eCB role in pain and inflammation.

1.2. The evidence for endocannabinoids in neuroplasticity and their amplification by induced or voluntary exercise

Neurogenesis is the development of new neurons from neural progenitor cells in the dentate gyrus of the hippocampus that undergo mitosis to produce daughter cells of the neuronal phenotype, with some becoming glia (Christie and Cameron, 2006; Zhao et al., 2008). New hippocampal neurons mature and become functional when they extend their structural design within the neuronal network connections of axons and dendrites (Schmidt-Hieber et al., 2004; van Praag et al., 2002). The cytoarchitectural connections, bioelectrical properties, and neuroplasticity are described elsewhere (Bramham et al., 2010; Hill et al., 2010).

The ECS acts in a modulatory fashion on the brain and the spinal cord to influence neurobiology and neurophysiology (Hill et al., 2005, 2009). The CB1 receptor is widely and heavily concentrated in many regions of the brain, such as the basal ganglia, the cerebral cortex, the amygdala, the hippocampus, and the hypothalamus (Herkenham et al., 1990; Katona et al., 1999; Marsicano and Lutz, 1999). The presynaptic terminals of neurons are rich in CB1 receptors, and the binding of the

ligand eCB to the CB1 receptor induces suppression of neurotransmitter release, either transiently or persistently (Kano, 2014). The ECS effects on the nervous system are supported by considerable evidence uniquely targeted within the hippocampus (Aguado et al., 2006; Hashimoto et al., 2007; Zhu, 2006). Recent investigations demonstrate that the ECS is involved in neuroplastic events altering neural connections to improve, strengthen, or delete them while reorganizing neural pathways and synapses (Hill et al., 2010).

Environmental factors such as stress (Mirescu and Gould, 2006) and exercise (Olson et al., 2006) are also reported to influence specific aspects of neural progenitor cell proliferation, neurogenesis, and maturation. Voluntary exercise is a potent stimulator of progenitor cell proliferation and generalized neurogenesis, as well as mood responses in the human (Brellenthin et al., 2017).

The effects of induced exercise on neurogenesis and synaptic plasticity in rats is supported by considerable evidence to include expression of neurotrophic factors in rat brain (Neeper et al., 1996), changes in dendritic structure (Redila and Christie, 2006), and extensive alterations within the hippocampus (Stranahan et al., 2007; Pereira et al., 2007). In support of the physiological effects in rodents subjected to exercise are findings of enhanced nerve activation of synapses (Vaynman et al., 2004) and measurable improvements in learning outcomes (van Praag et al., 1999a,b).

Deletion of the CB1 receptor gene in mice (Jin et al., 2004) results in suppressed proliferation of progenitor cells (Aguado et al., 2006; Kim et al., 2006). Further, genetic deletion of FAAH, the fatty acid amide hydrolase, which is responsible for AEA hydrolysis, results in a significant increase in cell proliferation within the dentate gyrus (Aguado et al., 2006). Thus, alterations in gene expression of the ECS further supports the role of eCB in progenitor cell proliferation.

In addition to the effects of exercise targeting progenitor cell proliferation, the ECS appears to support the release of neurotrophic factors (Khaspekov et al., 2004) and promote axonal growth (Williams et al., 2003). These findings are highly suggestive that environmental factors, such as exercise, could mediate neuroplasticity via the ECS in the hippocampus. In support of the concept that environmental factors can affect the ECS, AEA was reported to increase in the circulation of humans following physical exercise of continued duration (Sparling et al., 2003). In addition, the ECS appears to be involved in the reward outcomes from running and differential responses with selective cannabinoid antagonists (Keeney et al., 2008). Moreover, intense and prolonged exercise in the form of running was reported to increase insulin-like growth factor and binding protein-3 (Schwarz et al., 1996) as well as immune activity and growth factors (Schobersberger et al., 2000). These findings support the premise that the ECS effects are an outcome of specific exercise activity and demonstrate that exercise activates this system, as supported by modifying actions on cannabinoid receptors, involvement of growth factors, and genes.

2. Exercise and ECS actions on the brain and behavior

2.1. Rodent studies

In experiments with mice given free access to a running wheel, Hill et al. (2010) reported increased density of CB1 receptors and AEA in the

hippocampus with accompanied cell proliferation. However, the administration of AM251 (CB1 antagonist) to the animals abolished the cell proliferation in the hippocampus. The authors interpreted their findings in that voluntary exercise in mice supports endocannabinoid signaling, which is likely necessary for the actions of exercise on cell proliferation in the brain. These well-designed studies reveal that the hippocampus is subject to environmental changes of ECS actions via ligand receptor binding signaling of AEA/CB1 and is responsible for exercise-induced brain plasticity, as proposed by the investigators (Hill et al., 2010). In another study to establish the link between CB1 receptors and the control of wheel running in mice, it was revealed that use of CB1 antagonist AM251 increased wheel running distance in mice (Zhou and Shearman, 2004). Thus, blocking the CB1 receptor may result in other downstream events where ECS influences the brain and exercise; however, the duration of antagonist administration, as well as type, may have different outcomes.

Recently, mice selectively bred for high running (HR) activity were used to determine the effect of genetic background on voluntary wheel exercise and circulating levels of eCB (Thompson et al., 2017). Results for the HR group were compared with other lines not selected for high running, which served as controls. Both male and female mice were used in the study. The mean running wheel revolutions were 3 times greater in the HR group compared to the control group. The authors state that the levels of 2-AG in mice followed a 3-way interaction among sex, line type, and wheel access; while AEA showed a line type by wheel access interaction. The *p* values for the differences were less than 0.05 but 0.06 for 2-AG and AEA (Thompson et al., 2017). This is an interesting study, however, it is difficult to interpret the findings for plasma eCB given the relationships and statistical model used. The hypothesis that divergent genetic lines of mice selected for high running versus other traits would affect the ECS is intriguing. Investigations to understand a genetic mechanism for exercise-induced endocannabinoid signaling requires more specific hypothesis testing to better understand the relationships and to confirm differences in the ECS.

In another study with mice, the effects of chronic voluntary wheel running for 6 weeks was examined in the CB1 receptor in wild type (CB1 +/+) and knockout (CB1 -/-) littermates and on behavior and hippocampal neurogenesis (Dubreucq et al., 2010). Mice in the control group had a blocked wheel to prevent running, while those with free wheels were recorded for running variables of speed, distance, and number of running episodes. The CB1 +/+ mice total running distance, time running, and speed were greater than the CB1 -/- mice, as was food intake. Neurogenesis was determined by immunoperoxidase labelling of doublecortin (DCX) microtubule-associated phosphoprotein for newly generated healthy neurons. The investigators suggest that the effect of running on neurogenesis was independent of the presence/absence of CB1 receptors. Moreover, the total amount of running in mice, which was associated with the extent of neurogenesis, was significant. The authors conclude that these experiments with mice subjected to voluntary running and its effects are dependent on CB1 receptors but not its neurogenic consequences in the hippocampus. Further, the findings suggest that CB1 deletion is associated with reduced running activity in mice and lowered hippocampal neurogenesis (Dubreucq et al., 2010). In this research, behavior of memory for aversive events and depression-like behaviors were assessed in control and running CB1 +/+ and CB1 -/- mice. The freezing intensity decreased with time, and effective fear extinction CB1 deletion led to increased freezing scores. While these data were not as clear to interpret, it is most likely that the finding that deficiency of extinction measured in the knockout mice is reasonable given the role of the ECS.

The data on fear memory with respect to exercise in mice can be helpful in understanding the role of the ECS in behavior. For example, Hill and colleagues (Hill et al., 2005, 2006) reported that endocannabinoid signaling is lessened in a rat model of depression, but signaling is increased with an antidepressant. In support of these findings, another study with rats is suggestive of cannabinoid CB1 agonist

showing an antidepressant-like response in dentate gyrus of the hippocampus (McLaughlin et al., 2007).

The mechanisms underlying the decrease in wheel running activity in CB1 deficient mice are not well understood, although exercise does result in reinforced behavior and rewards in rodents (Sherwin, 1998). Furthermore, the reported findings in mice for wheel exercise is not the same activity as running in the human, and the reward aspects might not be facilitated similarly. When running is performed by rodents, it does not duplicate human running on a treadmill or al fresco, and perhaps, follows different aspects of a Circadian rhythm. For example, a large circadian variation of serum 2-AG was reported, thus demonstrating that activity of the ECS is profoundly modulated by circadian rhythmicity and may alter food intake as well during a 24 h period (Hanlon et al., 2015). Therefore, care must be taken when extrapolating mouse wheel running to running in the human, as well as in the interpretation of how CB1 receptors influence exercise performance, motivation, rewards, and well-being.

How and why CB1 receptors affect reward aspects in mice is not well understood, thus, wheel running behavior of mice was investigated in ventral tegmental area (VTA) dopamine (DA) neuronal activity in wildtype (gamma-aminobutyric acid [GABA]-CB1 +/+) and mutant (GABA-CB1 -/-) mice (Dubreucq et al., 2013). The VTA is a group of neurons located close to the midline on the floor of the midbrain, a rich area of dopamine neurons and motivation mesolimbic nucleus accumbens [mesocorticolimbic dopamine (DA) system] or reward areas that would explain the consequences of wheel running. This study focused on mechanisms for the involvement of CB1 receptors in wheel running behavior, and those located on VTA GABAergic terminals appear to exert a permissive control on running performance (Dubreucq et al., 2013). The investigators report that absence of CB1 receptors from GABAergic neurons is associated with inhibitory consequences of running on VTA DA neuronal activity, which may explain a role for the ECS in voluntary exercise. When examined in wildtype mice, the impact of pretreatment with JZL195, an inhibitor of endocannabinoid AEA and 2-AG degradation (both fatty acid amide hydrolase and monoacylglycerol lipase), decreased running performance (Dubreucq et al., 2013). The ECS appears to control wheel running activity in mice mutants missing the CB1 receptors (CB1 -/-) compared to the wild-type littermates that show a significant decrease in wheel running (Dubreucq et al., 2010) but not in locomotor activity (Chaouloff et al., 2011). Since the activation of the CB1 receptor is important for voluntary exercise in mice, the ECS is likely a normal tonus of nerve function stimulus to support the activity of wheel running. The experiments in mice for this study on CB1 receptor targeting of GABAergic neurons stimulation of CB1 receptors might result in increased exercise performance (Dubreucq et al., 2013).

In a study to examine ECS reward effects on the central nervous system in striatal GABA synapses, mice were subjected to treatments of sucrose consumption and wheel running to activate CB1 receptors (De Chiara et al., 2010). Mice were assigned to a control, wheel running, or sucrose groups. The investigators previously reported that opiates activate striatal dopamine signaling (De Chiara et al., 2010), and wheel running activity also is a strong reward to mice (Zhou and Shearman, 2004). The control group never used a running wheel and consumed water without sucrose. In additional experiments, mice were reared in a cage with a blocked wheel to prevent movement. Sucrose preference was measured in mice assigned to the sucrose group. Interestingly, the investigators found similarities in neurophysiology and behavioral effects of voluntary exercise and sucrose intake. Use of AM251 by IP injection abolished the protective action of wheel running and sucrose on stress induced behavior.

To further explore exercise-induced antinociception action of the ECS, rats were subjected to progressive speed on a rodent treadmill (aerobic exercise (AE)) until fatigue (Galdino et al., 2014). The ECS and eCB have been found to reduce pain but the mechanism is not known. Plasma AEA and 2-AG were increased in rats after AE exercise (Galdino

et al., 2014). Activation of CB1 receptors in the rat brain and immunofluorescence analysis demonstrated an increase of activation and expression of CB1. The increase in CB1 receptors were found in neurons of the periaqueductal gray matter (PAG) after exercise. Pretreatment of rats with CB1 and CB2 cannabinoid receptor antagonists (AM251 and AM630) blocked the antinociception induced by AE protocol in both mechanical and thermal nociceptive tests and aerobic exercise. The investigators conclude from their results that the ECS mediates AE-induced antinociception at peripheral and central levels in rats (Galdino et al., 2014).

2.2. Human studies

For decades, the benefits of exercise in the human have been directed at investigating the effects on cognition and health (including mental health and inability to experience pleasure), and in identifying mechanisms for physical activity on well-being. These efforts are particularly important for the aging population. Recent reviews indicate consistent findings of improvements for cognition and memory in adults that participate in moderate exercise (van Uffelen et al., 2008; Northey et al., 2018).

An interesting aspect of the ECS is that beyond the changes in mental state and analgesia, exercise was reported to increase the levels of AEA (Sparling et al., 2003). In a recent study, post-exercise values for slow jog and medium-intensity workouts showed increased blood (EDTA-treated) levels of AEA in self-reported fit, healthy adult men and women when compared to pre-exercise values (Raichlen et al., 2013). The increase in AEA appears to be consistent with earlier reports; however, the sample size was relatively small (10 subjects with both men and women) and the actual presented values are not explained but presumed to be means and error bars (Raichlen et al., 2013). The variation is large for the data presented within each exercise intensity group, and no discussion was provided for possible differences between men and women. The sense of well-being experienced by distance runners is an effect that is thought to maintain elevated aerobic exercise and proposed to be associated with activation of the ECS (Dietrich and McDaniel, 2004). In humans and dogs, running post-exercise resulted in higher AEA but not 2-AG in plasma compared to the levels at pre-exercise (Raichlen et al., 2012). The changes in AEA in men and women appear to be associated with moderate exercise, although not while walking (Raichlen et al., 2013).

Another investigation in humans measured plasma eCB using three study protocols that determined the effects of low and high elevation exercise conditions (Feuerecker et al., 2012). Subjects were 12 healthy men with an average age of 22.6 years (24–38 years) with the study conditions in the South Tyrolean Alps. Hiking conditions included extended times and different conditions but were three protocols referred to low elevation and two of high elevations of active ascent and passive ascent (by helicopter). All subjects completed the three study protocols. The subjects demonstrated an increase in plasma AEA and 2-AG with exercise, and hiking to a high altitude resulted in a three-fold higher level of AEA but not 2-AG. The passive ascent by helicopter did not result in a change of blood AEA and 2-AG. The levels of eCB returned to baseline after the exercise except for AEA in the hiking to a high altitude. The authors conclude that the hiking protocol resulted in the activation of the ECS to support the organ system when engaged in physical activity (Feuerecker et al., 2012).

Physical activity is recognized as a modifier of disease risk and the ECS, including the eCB, are considered factors that mediate some of the physical activity triggers systemically (Tantimonaco et al., 2014). Furthermore, exercise has an effect on positive well-being and could be associated with what was commonly called the “runner’s high” that was observed in some but not all runners (Dietrich and McDaniel, 2004).

Although taken in a different direction, aerobic exercise may have a positive effect on brain size not related to cognitive factors shaping human evolution (Raichlen and Polk, 2013). Evidence for this

hypothesis is associated with increased brain-derived neurotrophic factor (BDNF), insulin growth factor I (IGF-I), and vascular endothelial growth factor (VEGF) in neurogenesis resulting from aerobic exercise (Raichlen and Polk, 2013). The research on exercise and eCB has been extended to BDNF, a possible molecule involved in exercise-induced brain plasticity (Heyman et al., 2012a). The BDNF may be associated with antidepressant effects of exercise resulting from elevated AEA (PEA and OEA but not 2-AG) and cannabinoid signaling in young male cyclists (Heyman et al., 2012a). In this study, the positive correlation with serum cortisol and plasma AEA levels might suggest that cortisol resulted in an increase in AEA after acute exercise in men (Heyman et al., 2012a).

Spaceflight has been reported to result in changes in the ECS resulting from kinetic stress and weightlessness during parabolic flight (Strewe et al., 2012). A small sample size for subjects (4–5) showed increased blood levels of AEA and 2-AG after 4 and 6 months of space flight missions compared to baseline levels; however, the levels returned to baseline after 1 and 7 days, upon return. In this study motion sickness showed that in subjects experiencing low stress undergoing parabolic flight experiments while in-flight had higher 2-AG and low saliva cortisol, while the group experiencing high stress showed high cortisol and low 2-AG. These studies show that opposite associations between 2-AG and cortisol were found between low stress and high stress in subjects for parabolic flight (Strewe et al., 2012). These observations in subjects either during an extended stay in a space station or when subjected to parabolic flight provide additional evidence for the involvement of the ECS in the factors associated with stress and space travel. In a recent study, chronic stress resulted in reduced circulating levels of 2-AG in six male adult subjects (Yi et al., 2016) The volunteer participants were part of the confinement study simulating a flight to Mars conducted at the Institute of Biomedical Problems in Moscow. The isolation showed decreased brain cortical activities and high levels of catecholamine release in the men. Interestingly, only the levels of 2-AG not AEA were reduced in these healthy men. The authors conclude that the lowered 2-AG levels are linked to dysregulation of 2-AG signaling in response to chronic stressors (Yi et al., 2016). However, considering all the factors that contribute to stress and how the body and CNS are affected, much research is needed to better understand the ECS responses during spaceflight and on earth.

An interesting study in healthy adults examined relationships between mood and exercise on the ECS with measurements of eCB blood levels (Brellenthin et al., 2017). The subjects were grouped according to self-reported activity levels of low, moderate, and high. Prescribed exercise was a 10 min warm-up at low to moderate intensity, followed by 45 min at 70–75% VO₂max, and finished with a 5 min walking cool down; while the preferred exercise started with a 10 min warm-up at low to moderate intensity and followed by the subject’s choice of treadmill exercise intensity and duration. Both exercise conditions resulted in positive mood improvements and elevated circulating levels of AEA and 2-AG post-exercise compared to pre-exercise values. No differences were found between the physical activities performed during treadmill exercise (Bruce protocol) levels for the 36 men and women with a mean age of 21 ± 4 yrs. Time effect of exercise was observed for 2-AG and palmitoylethanolamide (PEA), which increased from pre-to post-exercise, while both AEA and oleoylethanolamide (OEA) increased after both exercise conditions. With regards to the subjects for both exercise treatments, they experienced decreased tension, depression, and anger but higher vigor. In this study and others, aerobic exercise activates the endocannabinoid system, resulting in an increase of AEA and 2-AG; however, with some exceptions, as 2-AG is not usually increased post exercise. These findings of AEA with vigor are encouraging to support hypotheses for exploring the relationships for exercise effects on pain and well-being.

In a recent study, Kong et al. (2016) reported findings from a systematic review of Tai Chi (TC) randomized controlled trials (RCT) to ascertain if the exercises reduce chronic pain outcomes. The focus of

this review was to evaluate the evidence for TC for immediate effects (after the treatment and up to 1 day), short-term (1-day to 3 months), intermediate term (3–12 months), and long term (more than 12 months). Tai Chi is an exercise regime of mind-body therapy that has been used to manage chronic pain and believed to improve musculoskeletal strength and joint stability because of the focused, slow movements. In the study on TC, 18 RCT were included in the review, which included 1260 individuals that participated in different forms of TC, with the Yang style being the principle type. The participants included individuals with OA, osteoporosis, rheumatoid arthritis, fibromyalgia, and lower back pain. The authors point out that a flaw with the studies was that the participants and therapists were unblinded to treatments. Based on the analysis, TC of 5 weeks or less did not reduce OA pain in participants, but chronic pain condition was improved between 6 and 10 weeks and beyond 10 weeks. The aggregated results for TC of 3 RCT did find improvements of lower back pain, and 2 RCT reported reduced osteoporosis pain. In many of the studies that were examined, they used an attention control, wellness education or stretching, physical therapy, or another activity (Kong et al., 2016). Although this review of existing studies on TC exercise and pain has many limitations and no clear mechanism of action for the benefits is known, it is tempting to hypothesize that prolonged, focused mind-body exercises might alter endocannabinoid signaling in the nervous system to mediate the benefits of TC. Others have reported that pain was improved in subjects with OA and in those with rheumatoid arthritis using a Yang-style TC (Wang, 2008; Wang et al., 2009). In at least one study, TC improved balance and quality of life (Kim et al., 2015). Although several investigations indicate that pain using WOMAC and VAS pain assessments improved with TC exercises, it is not clear why pain diminished. Some have suggested that TC benefits on pain are likely related to eCB; however, the evidence is not available. It is interesting to speculate that both changes in eCB levels and types as well as reduced inflammatory mediators (cytokines and oxylipins) could be responsible for controlling pain and improving mood.

Evidence suggests that controlling 2-AG concentrations is a target for understanding this ligand in neurons and the CB1 receptor (Howlett et al., 2011). Allosteric and non-competitive antagonists (Busquets et al., 2016) are another approach to block activation of the cannabinoid receptors (Gi/o protein activation). The multiple ligands for the CB1 in brain, the different cell types, and diverse signaling outcomes is complex but also offers unique approaches to control pain (Busquets et al., 2016). Several new compounds that serve as ligands for CB1 are under investigation, and this includes non-cannabinoid receptors and allosteric binding to the receptor as a novel means for therapy (Busquets et al., 2016). An example of this type of approach is the clinical study of Rimonabant, which acts as a competitive antagonist of CB1 to control appetite and reduce abdominal fat, although the side effects of this drug were detrimental. However, Rimonabant and the related compound AM251 are still used in studies to examine the actions on CB1 and other targets (Baur et al., 2012). Although pharmacological research on the control of the cannabinoid receptors can lead to the discovery of specific, targeted actions, studies on dietary PUFA may afford other means to alter eCB levels and receptor signaling for favorable outcomes, as described in the next section of this review.

3. Endocannabinoids, substrates, and enzymes for their production; activation of the cannabinoid receptors; dietary polyunsaturated fatty acids and inflammation

3.1. Endocannabinoids, substrates, and enzymes for their production and receptors

Many of the eCB are synthesized from polyunsaturated fatty acids (PUFA). AEA and 2-AG are derived from arachidonic acid with EPEA and DHEA derived from eicosapentaenoic acid and docosahexaenoic acid, respectively. Thus, the amounts of these eCB can be influenced by

dietary PUFA that affect the concentrations of these substrates. For example, the long chain n-3 PUFA have been effective in lowering substrate for synthesis of AEA and 2-AG (Kim et al., 2014, 2016). Further, a dietary source of docosahexaenoic acid can significantly increase DHEA in mice (Kim et al., 2015) and postmenopausal women, including an increased ratio of DHEA/AEA (Watkins et al., 2016). In a study in which a high fat diet was fed to obese Zucker rats, increasing amounts of krill oil in the diet reduced the levels of arachidonic acid based on the amounts in diacylglycerol (DAG) species in tissues as well as for AEA and 2-AG in tissues, and also resulted in changes in OEA and PEA (Piscitelli et al., 2011). The high fat diet contained a considerable amount of butterfat (21 wt %) and the specific ingredient composition was not disclosed; however, based on the fat content, the addition of increasing amounts of EPA and DHA in the diet had significant effects on the eCB and N-acyl ethanolamines measured. The study would also benefit from a fatty acid composition analysis of tissues used for eCB and NAE analyses. Although the amounts of DAG species are presented and reflect lower proportions of arachidonic acid with krill oil feeding, the differences in eCB and NAE could be due to a low n-6 PUFA (linoleate) content of the diets.

A growing body of evidence suggests that both essential n-6 and n-3 PUFA seem to support a proper functioning or signaling of the ECS and maintain tone for health. One particular action of long chain n-3 PUFA is to act as a homeostatic regulator of eCB levels, especially for 2-AG, and to prevent dysregulation of the system in adipose and liver (McPartland et al., 2014). More recently, a positive association was found for appetite score in female hemodialysis patients and lowering the ratio of linoleoyl ethanolamide/docosahexaenoyl ethanolamide in blood (Friedman et al., 2016).

In recent years, investigators have identified different pathways that lead to the production of eCB. A primary source of AEA is the release from membrane precursors by NAPE and NAPE-PLD (Okamoto et al., 2004). In addition, FAAH facilitates the release of arachidonic acid after cleavage of AEA into AA (Cravatt et al., 1996). In the case of 2-AG, it is formed by the action of the diacylglycerol lipases, DAGL α and DAGL β (Bisogno et al., 2003), and arachidonic acid and glycerol is generated by the action of MAGL (Dinh et al., 2002). Smaller amounts of 2-AG are degraded by MAGL and two hydrolyases ABHD6 and ABHD12 in mouse brain (Blankman et al., 2007). The importance of the ABHD6 and ABHD12 in brain was reviewed more recently (Savinainen et al., 2012). Furthermore, AEA and 2-AG can also be metabolized by cyclooxygenase-2 (COX-2), several lipoxygenase isozymes, and by cytochrome P450, generating oxidized compounds like prostaglandin-ethanolamides and glyceryl esters, hydroxy-anandamides, and hydroxyeicosatetraenoyl-glycerols, respectively. In proliferating C2C12 myoblast cell cultures, COX activity and protein levels are influenced by the endocannabinoid system, specifically by the ligand AEA for CB1 and by inverse agonism of CB2 (Kim and Watkins, 2014).

The review published of late by Micale et al. (2013) describes the role of the ECS in mood disorders, depression, and anxiety, and suggest targeting the system to treat behavioral symptoms, which are dysregulated in mood disorders. The basis for this are changes in CB1 density, the finding of higher serum AEA levels in patients with minor depression (Hill et al., 2008), and variations in FAAH expression with depression (Monteleone et al., 2010), which collectively support merit to this inquiry. The research is complicated since other receptors, such as transient receptor potential vanilloid 1 (TRPV1), besides CB1 and ligand affinity are involved in mood disorders, including new work on CB2 in emotional control (Marco et al., 2011). However, the work on the endocannabinoid system directed to mood supports the connection between pain and exercise, linking systemic physiologic and metabolic effects of the body with well-being that involves the brain and the entire nervous system.

Through the activation of cannabinoid receptors, CB1 and CB2, by the endogenous eCB, initiate multiple responses in the nervous system that include a wide array of analgesic and anti-inflammatory effects

(Chiurchiù et al., 2018). The predominant receptor in the central nervous system (CNS) is CB1 while the CB2 is primarily in the cells of the immune system (Pertwee et al., 2010). The cannabinoid system is involved in cognitive, memory, and motor functions as well as in analgesia and synaptic plasticity. More recently, CB1 appears to be a target for neuroinflammatory diseases such as Multiple Sclerosis, a demyelinating disease of the CNS (Chiurchiù et al., 2018). Furthermore, the eCB are clearly involved in neuropathic pain and nociceptive stimulation of nerve cells, such as inflammation, and in response to physical events (Woodhams et al., 2017).

Although the most widely investigated eCB, AEA and 2-AG, which are derived from arachidonic acid, the long chain n-3 PUFA serve as substrate for the synthesis of ethanolamides namely N-eicosapentaenoylethanolamide (EPEA) and N-docosahexaenoyl-ethanolamide (DHEA) (Artmann et al., 2008; Lucanic et al., 2011). Circulating levels of eCB have a wide array of effects in the body to influence systemic energy metabolism and the immune system (Hillard, 2017); however, those derived from arachidonic acid can be altered by dietary n-3 PUFA (Kim et al., 2013). For example, we recently reported increased levels of EPEA and DHEA in postmenopausal women supplemented with n-3 PUFA (Watkins et al., 2016). Further, we characterized the actions of both EPEA and DHA on genes related to systemic metabolism and specifically glucose uptake in muscle of mice given DHA in a semi-purified diet (Kim et al., 2016) and in myoblast cultures (Kim et al., 2014). At present, the extent that EPEA and DHEA affect the nervous system and cannabinoid signaling is not known.

3.2. Endocannabinoids, immune system and inflammation

The expression of the CB1 is extensive in the CNS (Woodhams et al., 2017) and CB2 in immunocompetent cells (Cabral and Griffin-Thomas, 2009); however, evidence indicates that both CB1 and CB2 are expressed by cells of the immune system and are upregulated in the activation state. The amount of CB2 receptors are greater than those of CB1 in the immune system (Tanasescu and Constantinescu, 2010). Further, decreasing amounts of CB2 are reported in human B cells, natural killer (NK) cells, monocytes, polymorphonuclear neutrophils, and T cells (Tanasescu and Constantinescu, 2010). Interestingly, macrophages, microglia, and osteoclasts express both cannabinoid receptors. As a consequence of CB2 activation in immune cells, the signaling is followed by cytokine release and migration (Tanasescu and Constantinescu, 2010). The biological importance of CB2 signaling in the immune system has been investigated (Jean-Gilles et al., 2015), supporting multiple immune cell actions, and also in *in vivo* studies that highlight great potential for the treatment of inflammation (Basu and Dittel, 2011). Thus, 2-AG and AEA, which both bind to CB2, are potential targets to explore ways to suppress or enhance immune functions, inflammation, and disease. Generally, AEA has high affinity for CB1 compared to CB2. In addition, the concentration of 2-AG is significantly higher than AEA in brain (Stella et al., 1997). The collective endocannabinoid system is one approach to understand and, perhaps control, Multiple Sclerosis and other neurodegenerative diseases in which inflammation is a crucial component (Chiurchiù et al., 2018). Moreover, cannabinoid receptor signaling is yet a promising therapeutic target for inflammatory pain and neuropathy due to multiple synthetic compounds that act as ligands and the eCB that demonstrate a wide range of signaling outcomes (Soethoudt et al., 2017).

The cannabinoid receptors have more than one receptor ligand in the nervous system (Di Marzo and De Petrocellis, 2012). The different ligands that bind to each cannabinoid receptor and the binding of AEA conferring actions on TRPV1 channels is of great scientific inquiry. The interpretation of the dynamic effects of the eCB, AEA, and 2-AG suggests that these ligands are diverse in modifying both physiologic and metabolic events. Moreover, the enzymes for synthesis and degradation of the eCB and nature of broad receptor targets require careful approaches in research to fully understand their actions *in vivo* for

neuroinflammation and chronic pain.

It is clear that activation of the endocannabinoid receptors impact inflammation and influences immune cell regulation (Booz, 2011). In regard to inflammation, it is important to note the relationship that exists between eCB and COX relevant to pain, especially in the nociceptive aspect. Since the eCB can be derived from COX and lipoxygenase pathways, as described herein, it becomes of great interest to study oxylipins in neuropathic pain to better understand the relationships between eCB and lipid mediators of inflammation. As an example, in mice (Kim et al., 2015) and postmenopausal women (Watkins et al., 2016), dietary intakes of n-3 PUFA are associated with not only changes in AEA and DHEA in mice and DHEA in women, but also in the levels of pro-inflammatory oxylipins. It is not yet clear how this relationship works, that is if the change in specific eCB result in downstream alterations of oxylipin levels or vice versa. The endocannabinoid system and its responses upon activation appear to be an opportunity to manipulate eCB signaling and inflammatory factors to improve pain by physical activity, cannabinoid receptor modifiers, and dietary lipids.

Endocannabinoid tone is the conceptual model integrating the ECS for biochemical and physiological control of obesity and diabetes (Heyman et al., 2012b; Watkins and Kim, 2015; Kim et al., 2016). AEA is an orexigenic factor in homeostatic control of appetite, and high levels of AEA are reported in obese subjects (Gatta-Cherifi et al., 2012). Over-expression of CB1 is associated with adult obesity, accompanied with insulin resistance (Bordicchia et al., 2010). In a recent review, the ECS was examined in a study focusing on the effects of food intake and energy metabolism on obesity (Gatta-Cherifi and Cota, 2016). The review highlights newer aspects of the ECS in taste, gastrointestinal physiology, and systemic metabolism, with emphasis on AEA and CB1. Further, the review by Heyman et al. (2012b) discussed the need to explore CB1 receptor signaling, the eCB and PPARs in a comprehensive examination of pathways to better understand obesity and exercise (Heyman et al., 2012b).

Tone of the ECS is considered important for optimal responses of the body to physical activity to include oxidative stress and inflammatory processes (Tantimonaco et al., 2014). Endocannabinoid tone and constitutive activity of cannabinoid receptors is a complex interaction of all the constituents of the endocannabinoid system, but the study of eCB signaling with recognized receptors CB1 and CB2 is revealing many opportunities to improve human health and prevent or control disease (Howlett et al., 2011). The involvement of environmental factors such as physical activity (degree of exercise intensity and the psychological changes) (Dietrich and McDaniel, 2004) and dietary PUFA (Kim et al., 2016) are vital approaches for understanding eCB and endocannabinoid tone and dysregulation by over stimulation of the CB1 by increased AEA and its physiological consequences. Although some investigators have reported that AEA is increased following exercise (Raichlen et al., 2013, Sparling et al., 2003), no comprehensive analysis of all eCB are reported.

4. Endocannabinoids, pain and inflammation

The eCB act in mediating pain but also behave as analgesics in models of both acute nociception and clinical neuropathy. The basis for eCB analgesic effects is associated with the cannabinoid receptors found in cells and tissues of the nervous system responsible for pain processing and in immune cells that regulate the neuro-immune interactions that convey the inflammatory hyperalgesia (Donvito et al., 2018).

A recent study in women focused on analysis of anti-inflammatory lipid mediators [N-acylethanolamines (NAE)] associated with different aspects of pain of the musculoskeletal system (Stensson et al., 2016). The women experiencing chronic widespread musculoskeletal pain (CWP) (n = 17) were compared to healthy controls (n = 19). A published microdialysis procedure was performed by placing the catheter in the trapezius muscle for collection of samples and the perfusion rate was 5 mL/min and dialysate was collected in 20 min intervals.

Oleylethanolamide (OEA) and stearoylethanolamide (SEA) levels in the dialysate were significantly higher in women with CWP compared to the controls at five time points. The investigators also reported a positive correlation between SEA and pain intensity in CWP. This well-designed and conducted study revealed interesting relationships between pain and NAE. The findings have implications for tissue specific effects of pain and inflammatory mediators, and their levels are influenced by dietary lipids. Furthermore, the NAE are related to the eCB in that they share some common metabolic pathways to AEA and are agonists for peroxisome proliferator-activated-receptor alpha, in addition to having recently been investigated in major depression (Hill et al., 2009). A study of women diagnosed with depression, serum and basal levels of AEA and 2-AG were lower but not in palmitoylethanolamide (PEA) and OEA when compared to healthy matched controls (Hill et al., 2009). However, after a Trier Social Stress Test (TSST), levels of AEA and 2-AG showed unchanged and increased levels, respectively, compared to baseline. Furthermore, serum levels of PEA and OEA were lower than baseline 30 min after ending the TSST. The investigators conclude that the relationship of the ECS and NAE signaling pathways may be involved in the stress exposure network (Hill et al., 2009).

4.1. Nociceptive and neuropathic pain

The two cannabinoid receptors CB1 (in the CNS, localized in the plasma membrane) and CB2 (located in the peripheral tissues), and the enzymes for the synthesis and degradation of 2-AG are part of the complex mechanisms involved in pain processing to include both nociceptive and neuropathic pain (Woodhams et al., 2017). Activation of the CB1 receptor is associated with neuronal inhibition. Early studies focused on AEA in pain, but the research has progressed to studies on 2-AG. Moreover, the attention on inhibition of FAAH and MAGL has revealed new targets to control pain (Woodhams et al., 2017; Donvito et al., 2018). Integration of the pain experience includes sensory, emotional, and cognitive components in pain of the inflammatory aspects and chronic pain (Donvito et al., 2018). Approaches to perturb the ECS is an attractive means to control and reduce pain. In the current review, the actions and use of opioids are not discussed, only the eCB and exercise and forms of physical activity are presented in regard to pain.

Neuropathic pain is mediated in microglia of the spinal cord and brain and associated with the release of pro-inflammatory cytokines, such as interleukins and TNF α (Baron et al., 2010), and a target for treating inflammatory neuropathic pain (Donvito et al., 2018). Neuropathic pain must be better understood because it is common in cancer, diabetes, Multiple Sclerosis, and peripheral nerve injury. The endocannabinoid degradative enzymes, FAAH and MAGL, are logical targets for drug development because AEA suppresses pro-inflammatory cytokines, and inhibiting MAGL affords some anti-nociceptive and anti-inflammatory actions by increasing levels of 2-AG (graphically illustrated by Donvito et al., 2018).

A recent comprehensive review on chronic pain (allodynia) characterized as neurological dysfunctional responses, might be controlled by modifying the ECS (Luongo et al., 2017). Targeting the molecular and biochemical pathways of the ECS might afford some benefits to patients that experience this type of pain, which affects quality of life. Taking a pharmacological approach to manipulate the ECS via the cannabinoid receptors and block enzymes of eCB, FAAH and MAGL, are promising lines of investigation. The high expression of CB1 in the CNS and CB2 in immune cells can and have afforded greater insights in both animal and human studies to control pain via the nervous system and inflammatory pathways of neuropathic pain (Luongo et al., 2017). Furthermore, the broad approach to characterize how to modify the biosynthesis and degradation of eCB and related compounds is a means to better understand the ECS in the signaling and propagation of pain.

Besides cytokines and other mediators of inflammation, some new

evidence indicates that reactive oxygen species are increased by microglial cells and might be an initiating factor in pain (Kim et al., 2010). In addition to the recognized relationship between AEA and 2-AG as well as COX enzymes and the collective pathways of oxylipin synthesis as described herein, another approach is to direct elaboration of anti-inflammatory or less inflammatory oxylipins (Ulu et al., 2013). Although the oxylipins is a family of numerous compounds and are secondary from the primary targets of the eCB on their receptors downstream, the oxylipins can modulate immune cells activities and pain responses (Wagner et al., 2011).

4.2. Inflammatory joint pain and the endocannabinoid system

Osteoarthritis (OA) is a widespread disease for many adults (Ruiz-Romero and Blanco, 2010). The etiology of OA is a consequence of trauma and strain of the joint followed by immune responses of inflammation involving cytokines, specifically interleukins in human cartilage degradation (Honorati et al., 2002). The severity of OA is associated with interleukin 17 levels that likely contribute to pain, and regulation of this cytokine may help control and reduce pain (Lui et al., 2015). The endocannabinoid system's involvement in OA pain (LaPorta et al., 2014) and emotional aspects (LaPorta et al., 2015) have been reported. However, a rat model of knee joint OA was used to study the relationships between gene expression of ECS components and matrix metalloproteinase (MMP) with OA-pain (Pajak et al., 2017). In the rat model, the progression of OA-pain correlated with increased expression of ECS proteins and matrix metalloproteinases (MMP).

In OA, the loss of articular cartilage, the chondrocytes and extracellular matrix, occurs by proteolytic enzymes such as MMP. However, altering the signaling of CB1 and CB2 may reduce MMP activity, as recently shown in the OA rat model (Pajak et al., 2017). The endocannabinoid system and actions on MMP seem to be involved in OA pain. Interestingly, AEA was found to have analgesic properties in animal models to study pain (Guindon and Hohmann, 2009). In the progression of OA in the rat model, both eCB and MMP were involved in the inflammatory condition leading to pain (Pajak et al., 2017). The pain associated with OA may occur by up-regulating cannabinoid receptors and expression of enzymes degrading AEA (FAAH) and oxidative enzymes of eCB, COX, and LOX. Such changes were observed in synovial tissue biopsies of OA patients (Richardson et al., 2008). Evidence was presented for targeting the CB2 receptor to better understand pain, and perhaps, improve pain associated with OA via the peripheral immune system and the neuro-immune physiologic connection (Burston et al., 2013). A therapeutic approach to treat pain might be linked to the activation of CB2, which attenuates spinal neuronal processing of pain in the rat model investigated (Burston et al., 2013). Although this work is of great merit for pain research, much needs to be elucidated from cannabinoid signaling, eCB, and the enzymes that control the levels of AEA and 2-AG as well as the n-3 PUFA derived eCB.

In a recent investigation on 58 healthy adults, an equal number of women and men aged 18–40 years, exercise-induced hypoalgesia and eCB were evaluated (Crombie et al., 2018). Experimental pain testing and isometric exercise were interventions, and both AEA and OEA increased in blood post-pain while 2-AG increased post-exercise. This work suggests that eCB may contribute to nonopioid exercise-induced hypoalgesia.

Besides the eCB, the oxylipins are well recognized lipid-derived mediators of inflammation and immune cell functions (Lundström et al., 2013). The eCB and oxylipin levels in postmenopausal women (PMW) are modifiable by dietary n-3 PUFA (Watkins et al., 2016). PMW supplemented with n-3 PUFA showed increased serum DHEA but lower docosatetraenoyl ethanolamide and 2-oleoyl ethanolamide, while some oxylipins derived from 20:5n3 were higher and those from arachidonate lower (Watkins et al., 2016). Furthermore, profiling of oxylipins during exercise and in diseases, such as osteoarthritis, could confirm differences compared to normal subjects (German et al., 2013) and in

those with joint inflammation (Brouwers et al., 2015). Thus, understanding endocannabinoid tone and its constituents is, in part, an important, functional aspect of controlling pain and inflammation. Moreover, exploring the complexity of the binding of ligands to the cannabinoid receptors and downstream signaling is key for developing novel approaches to control pain as well as the synthesis and degradation and transport of 2-AG and AEA in cells of the nervous system (Howlett et al., 2011).

Considering the important role eCB and the cannabinoid receptors play in the multiple pathways of processing pain, the question is can n-3 PUFA modulate pain via eCB and downstream to influence inflammatory mediators? It is clear that n-3 PUFA have an established role in prostanoid production via the COX enzyme and inflammatory mediators. Moreover, the endocannabinoids affect COX. However, it is not clear how n-3 PUFA and the eCB derived from eicosapentaenoic acid and docosahexaenoic acid affect pain and the complete family of lipid-derived oxylipins. How the n-3 PUFA collectively or synergistically impact eCB types and levels, endocannabinoid signaling, and inflammation via the oxylipins is worthy of investigation for controlling pain. These investigations should also consider the effects of n-3 PUFA supplements in subjects using medications such as glucocorticoids, acetaminophen, NSAIDs, opiates, which have some actions on the endocannabinoid system and enzymes like COX and LOX (McPartland et al., 2014).

5. Future research

To summarize, the involvement of environmental factors such as physical activity and diet are vital approaches to understanding the ECS and its neurological and physiological consequences. Others have evaluated the efficacy of n-3 PUFA on pain and found similar outcomes of improvement compared with NSAIDs on arthritic pain in patients (Maroon and Bost, 2006). Although some investigators have reported that AEA is increased after exercise (Raichlen et al., 2013; Raichlen and Polk, 2013; Sparling et al., 2003), no comprehensive analysis of most eCB have been reported and little, if any, data is available on Tai Chi exercises and eCB. Besides the eCB, the oxylipins are well recognized lipid-derived mediators of inflammation and immune cell functions (Lundström et al., 2013), and the eCB and oxylipins levels in PMW are modifiable by diet (Watkins et al., 2016). The primary eCB and some oxylipins share common substrates and pathways (Kim and Watkins, 2014). The relationships between eCB and oxylipins were recently reviewed to explain common pathways for the metabolism of AEA (Maccarrone, 2017) and the comprehensive analysis of both eCB and oxylipins in human studies to understand exposure to environmental pollutants (Gouveia-Figueira et al., 2017) and a rat model of osteoarthritis (Wong et al., 2014). Furthermore, profiling of oxylipins in osteoarthritis confirms differences compared to normal subjects (Gierman et al., 2013) and in those with joint inflammation (Brouwers et al., 2015). In addition, moderate intensity exercise, such as Tai Chi, may be particularly beneficial for improving functional and clinical outcomes, as eCB signaling seems to be intensity-dependent, with significant changes in circulating eCB observed following moderate intensities only since very high and very low intensity exercises may not significantly alter circulating eCB levels (Raichlen et al., 2013; Raichlen and Polk, 2013). Low levels of moderate exercise are positively associated with plasma AEA levels and reduced anxiety in women (Fernández-Aranda et al., 2014). Chronic or repeated stress results in a prolonged elevation of endogenous corticosterone via the hypothalamic-pituitary-adrenocortical (HPA) axis. Future research is needed to confirm how chronic stress affects the ECS system, the eCB AEA and 2-AG levels and related compounds in signaling pathways. Changes in CB1 expression are perhaps more labile. Interventions, such as Tai Chi, as a form of exercise that includes aspects of stress management may reverse the effects of chronic stress via eCB signaling in adults and older adults (McPartland et al., 2014).

A thorough analysis of eCB levels after exercise is certainly warranted given the promising consistent finding that AEA is increased after exercise. However, one aspect that must be explored is how modifying plasma membrane lipids with dietary n-3 PUFA can result in increased levels of EPEA and DHEA ethanolamides and decreased AEA and 2-AG. Such changes could influence pain or mood after exercise since the actions of EPEA and DHEA are not well known. In this case, the amounts, and perhaps sources, of dietary n-3 PUFA and diet composition are worthy pursuits for research. Furthermore, it is not entirely clear if n-3 PUFA affects FAAH and MAGL expression and enzyme levels, or their activities are changed with exercise and n-3 PUFA supplementation that might affect pain. Research focused on eCB, the enzymes for the degradation, FAAH and MAGL, are logical next steps in understanding pain and inflammatory mediators. Such work should consider the family of oxylipins, known to be pro-inflammatory and some less inflammatory, but likely linked to cannabinoid receptor signaling for CB1 in the nervous system and CB2 in the immune system.

From a molecular and biochemical perspective, understanding how cannabinoid receptor signaling works under conditions of exercise to elaborate analgesic effects is important, in addition to how this may occur to improve conditions of nociceptive and neuropathic pain. Such investigations should include cytokine and oxylipin changes during cannabinoid receptor signaling will likely expand our understanding of the nociceptive and neuropathic pain processes.

With regard to specific exercises and intensity, it is reasonable to determine how Tai Chi versus more aerobic exercises, such as moderate treadmill running, alter eCB and oxylipins. It is necessary to elucidate how Tai Chi reduces pain and if the types of controlled movement and mind activities are directly related to eCB and cannabinoid signaling or changes in inflammatory markers. While Tai Chi exercises reduce pain, enhance physical strength/flexibility, and improve quality of life in adults suffering with knee osteoarthritis, the mechanism is not known.

6. Conclusions

Herein, a brief overview of the ECS was presented to describe some current aspects of exercise, behavior, pain, and inflammation in rodents and the human. Exercise is encouraged to improve physical mobility and maintain muscle mass. The eCB AEA is reported to increase in blood after moderate and intense aerobic exercise, and Tai Chi is one group of exercises involving mind and body that reduces pain and improves well-being but without a known mechanism. The ECS and its collective components are widely found in the CNS and peripheral system, as well as the immune system. These systems are clearly involved in pain and the neuro-inflammatory pathways associated with pain. Specific inflammatory diseases, such as OA of the knee, are debilitating and movement of the joint is painful. Considerable research is necessary to fully understand the ECS and its role in exercise and pain in the human. In many respects, the responses of wheel exercise in mice demonstrating increased levels of eCB in blood, neuroplasticity, and improved behavior appear to occur to some extent in the human. The translation of rodent work to the human is encouraging but considerable research is needed to better understand the complex role of the ECS in voluntary exercise, control of pain, and mood in humans.

Exercise for many was pursued in youth for the rewards of running or competitive sports. As we move to adult status, exercise is a means to stay fit, maintain a healthy body weight and muscle mass, and to delay or prevent disease. The common goal is to be healthy and enjoy an active lifestyle. Perhaps with aging and the possibility of chronic diseases such as inflammatory joint disease, the goal for exercise is to reduce pain and maintain muscle strength and mass. Now, studies on eCB and the collective ECS will be a path of research to elucidate mechanisms, both molecular and genetic, to explain the benefits of exercise on the body and mind.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mam.2018.10.001>.

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