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Role of the endocannabinoid system in the regulation of the skeletal muscle response to exercise

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Exercise is a valuable tool in the prevention and treatment of cardiometabolic diseases like obesity and type 2 diabetes. Interestingly, endocannabinoids (eCBs), involved in a large range of physiological processes, are elevated with both obesity and acute exercise. In this review we outline this paradox overlap in the context of metabolic health and delineate the transcriptomic response of skeletal muscle to acute and chronic aerobic and resistance exercise in relation to the endocannabinoid system by utilizing a meta-analyses tool. We show that exercise modulates the expression of receptors and enzymes involved in the synthesis and breakdown of eCBs and discuss that eCBs possibly interfere with the anti-inflammatory effect of exercise.

The endocannabinoid system (ECS), consisting of certain endogenous lipids (i.e. endocannabinoids), their receptors and associated metabolic enzymes, is involved in the modulation of a plethora of cognitive and physiological processes. Besides its role in the control of, for example, mood formation and immune responses, the ECS takes part in the regulation of appetite and energy metabolism [1,2]. In this current opinion review we will focus on the increased activity of the ECS that is associated with cardiometabolic diseases like obesity and type 2 diabetes (T2D), which paradoxically overlaps with the acute physiological response to exercise. After 1) outlining the role of the ECS in metabolic health, we will 2) discuss the link between endocannabinoid (eCB) action in skeletal muscle and cardiometabolic disease, 3) investigate how exercise modulates the gene expression of ECS components in skeletal muscle and 4) delineate the impact of the ECS on the immune response by skeletal muscle.

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The endocannabinoid system in metabolic health

The circulating endogenous endocannabinoids (eCBs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) bind to G protein-coupled receptors expressed in different tissues. Currently, two cannabinoid receptors are well described, type 1 (CB1R) and 2 (CB2R). CB1R is widely expressed in the central nervous system and peripheral tissues, including white and brown adipose tissues, liver, myocardium and skeletal muscle. CB2R, on the other hand, shows a more limited expression pattern and can be found mainly in immune cells and to a lesser extent in the cerebral cortex, cerebellum and the gastrointestinal tract [3]. The existence of alternative cannabinoid receptors is still a topic of debate; however, the receptors GPR18, GPR55 and GPR119 respond to a variety of both endogenous and exogenous cannabinoid ligands such as phytocannabinoids and are therefore considered novel cannabinoid receptors or at least cannabinoid receptor-related receptors [4–7]. The confirmation and discovery of such new cannabinoid receptors, potentially specific to peripheral tissues or cell types, could lead to the development of new pharmacological inhibitors to combat obesity and cardiometabolic diseases.

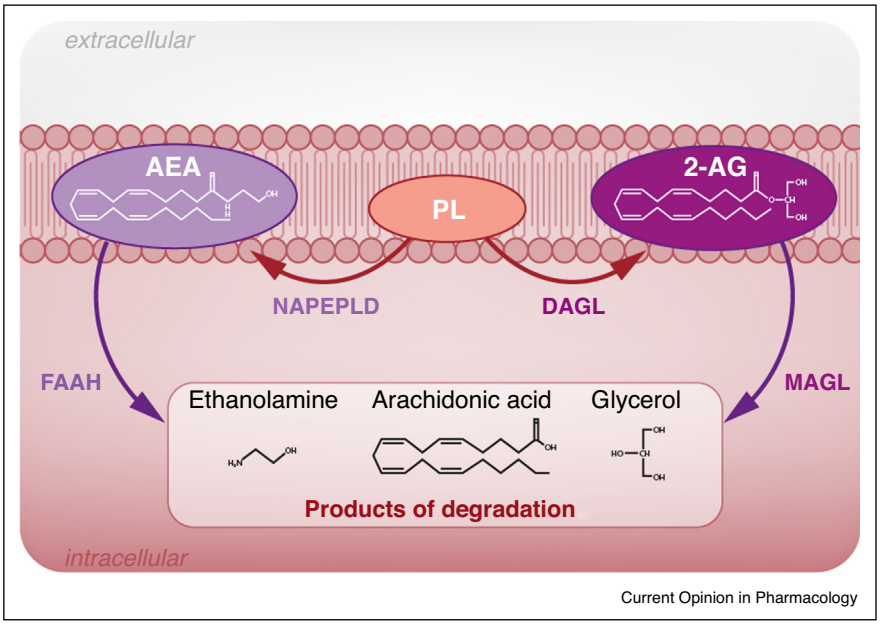
An increased eCB tone (i.e. circulating eCB levels) in obese postmenopausal women was first discovered by Engeli *et al.* in 2005 who reported elevated levels of AEA (+35%) and 2-AG (+52%), in comparison to lean postmenopausal women [8]. In the following years, several studies found that specifically 2-AG levels in blood and tissues positively correlate with body mass index (BMI) and visceral adiposity in both men and women [9–12]. Even independently of other cardiometabolic risk factors, insulin resistance and T2D have directly been associated with elevated eCBs [12,13]. A chronically increased eCB tone is considered to aggravate metabolic disorders as eCBs stimulate food intake, increase adipogenesis in liver and adipose tissue, and further reduce peripheral insulin sensitivity [14]. In accordance, a year-long lifestyle intervention in obese men involving caloric restriction and exercise that led to weight loss also lowered the eCB tone, reducing plasma concentrations of AEA (−7%) and 2-AG (−62%) [15]. Accordingly, we reported that caloric restriction during 16 weeks lowered AEA (−15%) in obese T2D patients with coronary artery disease [16•].

Besides caloric restriction, exercise is a valuable tool in the treatment of obesity and cardiometabolic diseases and is increasingly prescribed by physicians in the form of supervised training. Interestingly, and counterintuitively, acute aerobic exercise elevates blood levels of eCBs. Particularly AEA was found to double or even triple following 30–45 min of moderate intensity exercise while 2-AG levels remain mostly stable [17,18,19•,20]. Two AEA-related molecules, *N*-oleylethanolamine (OEA) and *N*-palmitoylethanolamine (PEA), were described to respond similarly to acute exercise [21]. This is not surprising as AEA, OEA and PEA share similar synthesis and metabolic pathways while 2-AG biosynthesis depends on other precursors and enzymes [21]. Curiously, preclinical studies showed that increased eCB signaling in skeletal muscle directly reduces glucose uptake and AMPK activity, both of which arguably hamper the metabolic capacity and recovery of the muscle [11,22], potentially reducing exercise performance and capacity. Nonetheless, the exercise-induced increase of AEA, which is particularly observed with moderate intensity exercise, was only found in cursorial species 'made for running' such as humans and dogs but not in non-cursorial ferrets [18,23]. Rather than linking this AEA increase to peripheral metabolic effects, it has instead been associated with the positive central effects of exercise such as analgesia and the so-called 'runner's high', which supposedly also mainly occurs with moderate intensity exercise [18,21]. While peripheral tissues, especially adipose tissue depots, are a known source of eCBs that are synthesized from membrane phospholipids [24] (Figure 1) it remains unclear whether skeletal muscle and other tissues increase the biosynthesis and secretion of eCBs upon exercise stimulation and whether skeletal muscle, for instance, is a main target of those eCBs. Of note, in high fat-fed rats, 12 weeks of exercise training reduced the diet-induced AEA increase particularly in the glycolytic skeletal muscle EDL but not in the oxidative soleus, potentially aligning with a specific improvement of skeletal muscle glucose metabolism with exercise in the context of obesity [25]. Interestingly, in the same rat cohort exercise training was found to induce *Cnr1* expression in the hippocampus, previously associated with hyperphagia, to a greater extent than high-fat feeding alone, again outlining a paradox exercise response in relation to the ECS [26]. The authors linked this finding to an increase in hippocampal neuroplasticity with chronic exercise that is supposedly mediated by increased eCB turnover and signaling.

Endocannabinoids in skeletal muscle: an unexplored link to cardiometabolic disease

Skeletal muscle has autocrine, paracrine and endocrine functions, although these are not very well described yet. It is not understood what role the proteins involved in synthesis, binding or degradation of eCBs in skeletal

Figure 1



Synthesis and breakdown of endocannabinoids.
N-acyl phosphatidylethanolamine-specific phospholipase D (NAPEPLD) and diacylglycerol lipase (DAGL) synthesize the main endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) from phospholipids (PL), respectively. AEA and 2-AG are degraded by fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Adapted from Ref. [14].

muscle have in the development of cardiometabolic diseases. In a recent study we observed that the eCB tone in South Asians, an ethnicity that is prone to obesity, dyslipidaemia, and T2D, was higher compared to Europids [27]. We investigated whether the ECS-related gene expression in adipose tissue and skeletal muscle was different between both ethnicities and discovered that South Asian men had a lower expression of the genes encoding the eCB degrading enzymes MGLL, FAAH1 and FAAH2 in skeletal muscle compared to BMI-matched Europid men [28••]. Interestingly, we also found that the expression of the genes (*CNR1, CNR2*) encoding both classical receptors (*CB1R, CB2R*) was much lower in South Asians, possibly as a compensation for reduced eCB degradation within skeletal muscle. Unexpectedly, we did not find any of these differences in adipose tissue, suggesting that particularly the eCB metabolism in skeletal muscle plays an important role in the unfavourable metabolic phenotype of South Asians.

While the ECS in skeletal muscle has not been sufficiently explored as a possible therapeutic target for cardiometabolic diseases, some mechanistic insight on the function of the ECS in muscle can be gained from the studies of other diseases. It was shown that the ECS participates in the development of degenerative muscle disease, such as Duchenne muscular dystrophy, through effects on muscle differentiation, regeneration and repair processes [29]. This appears to be primarily connected to the cannabinoid receptor expression, as the highest degree of CB1R expression in skeletal muscle was detected at disease onset in murine and human models, and then observed to decline over time. Iannoti *et al.* showed that the stimulation of CB1R by 2-AG inhibits myoblast proliferation and differentiation *in vitro* and during early muscle development [30]. Treatment with the CB1R inverse agonist Rimonabant delayed the onset of Duchenne muscular dystrophy in mice, demonstrating one aspect of the pharmacological value of CB1R inhibition. However, Rimonabant, upon market introduction primarily prescribed for its food intake and weight reducing effects, is no longer approved for clinical use due to severe psychiatric side effects. The search for novel periphery-specific cannabinoid receptor antagonists is ongoing [31,32]. In the context of muscle disease it is evident that muscle-specific cannabinoid receptor antagonists would be of value, however, whether targeting the classical or novel cannabinoid receptors in skeletal muscle are promising strategies in combatting the development of cardiometabolic diseases is not clear. Isolated human myotubes from obese individuals were as sensitive to CB1R inhibitor treatment as myotubes from lean donors and responded with a downregulation of PDK4 that acts as a metabolic brake, indicating that obesity does not render the regulatory network the ECS taps into insensitive [33]. While the activation of CB1R in adipose tissue directly downregulates the production of adiponectin, an insulin sensitizing adipokine, it is unknown whether the production and secretion of, for instance, immunomodulatory myokines by skeletal muscle is affected in a similar fashion [33].

Exercise modulates the ECS-related gene expression in skeletal muscle in humans

Recently, Pillon *et al.* published an online tool (MetaMEx) that meta-analyzes 66 skeletal muscle transcriptome data sets from human exercise interventions and gives easy access to the expression response of genes of interest to acute or chronic aerobic or resistance exercise in healthy, metabolic syndrome and type 2 diabetic individuals (www.metamex.eu) [34••]. While exercise is associated with altered circulating eCB levels it has not been addressed yet whether exercise interventions modify ECS-related gene expression in human skeletal muscle. We took advantage of MetaMEx to study the effects of acute and chronic resistance, aerobic or combined exercise interventions on the expression of the main enzymes involved in the synthesis and hydrolysis of AEA and 2-AG (i.e. *FAAH1*, *FAAH2*, *MGLL*, *DAGLA*, *DAGLB*, *NAPEPLD*), as well as the gene expression of the classical (*CNR1* and *CNR2*) and novel cannabinoid receptors (*GPR18*, *GPR55* and *GPR119*) in skeletal muscle.

We found that the gene expression of the classical and novel cannabinoid receptors was not affected by chronic exercise training (Table 1), independently of the type and duration of the exercise intervention. In contrast, the *CNR2* gene expression was upregulated in healthy individuals acutely after resistance training (Table 1, Fig. S1B), possibly representing increased resident and immigrating immune cells involved in the innate immune response of the muscle to repair muscle damage, whereas the expression of *GPR55* and *GPR119* was upregulated specifically after acute aerobic training (Table 1, Fig. S1C and D) [33]. Altogether, this data suggests that not all cannabinoid receptors are modified acutely by exercise, and various types of exercise (resistance versus aerobic) induce different effects on the expression and abundance of the cannabinoid receptors. Albeit poorly understood, the fact that circulating eCB concentrations [18,20–23] and gene expression of some cannabinoid receptors increase after acute exercise training suggest that these circulating eCBs, possibly derived from adipose tissue and other unknown sources, play a role in skeletal muscle function during exercise or recovery, although further studies are needed to confirm this hypothesis and reveal which role.

Analyzing the expression of genes encoding the main enzymes in the anabolic pathways of the synthesis of eCBs showed that the expression of *DAGLA*, encoding

Table 1

Effect of acute and chronic resistance, aerobic or combined exercise interventions on expression of endocannabinoid system-related genes in skeletal muscle of healthy, type 2 diabetes and metabolic syndrome individuals using the online tool MetaMEx (www.metamex.eu) [32]

Gene	Protein	Role	Function	Acute resistance	Acute aerobic	Chronic resistance	Chronic aerobic	Chronic combined
CNR1	CB1R	Receptor	Classical cannabinoid receptor	Not affected	Not affected	Not affected	Not affected	Not affected
CNR2	CB2R	Receptor	Classical cannabinoid receptor	Upregulated in healthy individuals ($P = 0.042$, Fig. S1B)	Not affected	Not affected	Not affected	Not affected
GPR18	GPR18	Receptor	Novel cannabinoid receptor	Not affected	Not affected	Not affected	Not affected	Not affected
GPR55	GPR55	Receptor	Novel cannabinoid receptor	Not affected	Upregulated in healthy individuals ($P = 0.0085$, Fig. S1C)	Not affected	Not affected	Not affected
GPR119	GPR119	Receptor	Novel cannabinoid receptor	Not affected	Upregulated only in athletes ($P < 0.001$, Fig. S1D)	Not affected	Not affected	Not affected
DAGLA	DAGL- α	Anabolism	Responsible for the hydrolysis of DAGs to 2-AG	Not affected	Not affected	Not affected	Not affected	Not affected
DAGLB	DAGL- β	Anabolism	Responsible for the hydrolysis of DAGs to 2-AG	Not affected	Downregulated in healthy individuals, mainly athletes ($P = 0.025$, Fig. S1E)	Downregulated in healthy and metabolic syndrome individuals ($P = 0.003$, Fig. S1F)	Downregulated in healthy and metabolic syndrome individuals ($P = 0.0006$, Fig. S1G)	Downregulated in healthy and metabolic syndrome individuals ($P = 0.00015$, Fig. S1H)
NAPEPLD	NAPE-PLD	Anabolism	Synthesis of AEA	Downregulated in healthy individuals, mainly athletes ($P = 0.0022$, Fig. S1I)	Not affected	Upregulated in healthy and metabolic syndrome individuals ($P = 0.019$, Fig. S1J)	Not affected	Not affected
FAAH1	FAAH1	Catabolism	Responsible for the breakdown of AEA and secondarily 2-AG	Downregulated in healthy individuals ($P = 0.0048$, Fig. S1K)	Downregulated in healthy individuals ($P = 0.00022$, Fig. S1L)	Downregulated in healthy and metabolic syndrome individuals ($P = 0.0071$, Fig. S1M)	Downregulated in healthy and metabolic syndrome individuals ($P = 0.0087$, Fig. S1N)	Downregulated in healthy and metabolic syndrome individuals ($P = 0.015$, Fig. S1O)
FAAH2	FAAH2	Catabolism	Responsible for the breakdown of AEA and secondarily 2-AG	Downregulated in healthy individuals ($P = 0.00049$, Fig. S1P)	Not affected	Not affected	Not affected	Not affected
MGLL	MAGL	Catabolism	Responsible for ~85% of the breakdown of 2-AG	Not affected	Not affected	Not affected	Not affected	Downregulated in healthy and metabolic syndrome individuals ($P = 0.012$, Fig. S1Q)

AEA: anandamide; 2-AG: 2-arachidonoylglycerol; CB1R: cannabinoid receptor 1; CB2R: cannabinoid receptor 2; DAGL: diacylglycerol lipase; FAAH: fatty acid amide hydrolase; GPR: G-protein coupled receptor; MAGL: monoacylglycerol lipase; NAPEPLD: *N*-acyl phosphatidylethanolamine-specific phospholipase D.

diacylglycerol lipase A that synthesizes 2-AG (Figure 1), was not modified by any exercise interventions (Table 1). In contrast, chronic resistance, combined aerobic and resistance and chronic and acute aerobic exercise interventions downregulated the gene expression of the similarly functioning *DAGLB* in skeletal muscle (Table 1; Fig. S1E, F, G and H). Additionally, acute resistance training downregulated *NAPEPLD* gene expression, implying reduced biosynthesis of AEA by its gene product *N*-acyl phosphatidylethanolamine phospholipase D (NAPEPLD) (Figure 1), whereas chronic resistance training upregulated the expression of this gene (Table 1; Fig. S1I and J). Taken together, this data suggests that skeletal muscle is probably not contributing to the secretion of eCBs following acute exercise, although further studies are needed to confirm this hypothesis.

Similarly, the gene expression of the main enzymes involved in the breakdown of AEA and 2-AG, *FAAH1*, *FAAH2* and *MGLL*, is affected by exercise. Acute and chronic endurance and resistance exercise downregulate the expression of *FAAH1* (Fig. S1K, L, M, N and O), whereas only acute resistance exercise downregulates the expression of *FAAH2* (Fig. S1P), both indicating decreased breakdown of AEA in skeletal muscle and thereby possibly supporting the notion of acutely elevated circulating eCBs. Surprisingly, we did not observe any effect of acute exercise interventions on the expression of *MGLL*, encoding the 2-AG degrading MGLL (Table 1) but noted a downregulation with chronic combined exercise training (Table 1; Fig. S1Q). The expression levels of *FAAH, MGLL and NAPEPLD* returned to baseline a few hours following acute exercise, suggesting that the downregulation is linked to the acute exercise adaptation rather than the recovery of skeletal muscle (Fig. S1R, S and T). The chronic effect of exercise on eCB levels independently of weight loss has not been studied, it is hence not evident whether the downregulation of the expression of eCB degrading enzymes in skeletal muscle is primarily weight loss-related [19•]. It is, however, plausible that this decreased gene expression in skeletal muscle is a secondary response to an overall reduced eCB production by other tissues such as adipose tissue with chronic exercise. Indeed, we recently observed that 24 weeks of moderate intensity combined training in a cohort of young healthy adults [35] was able to decrease AEA, 2-AG, PEA and OEA baseline blood concentrations compared to control and vigorous intensity exercise groups independently of weight loss (unpublished).

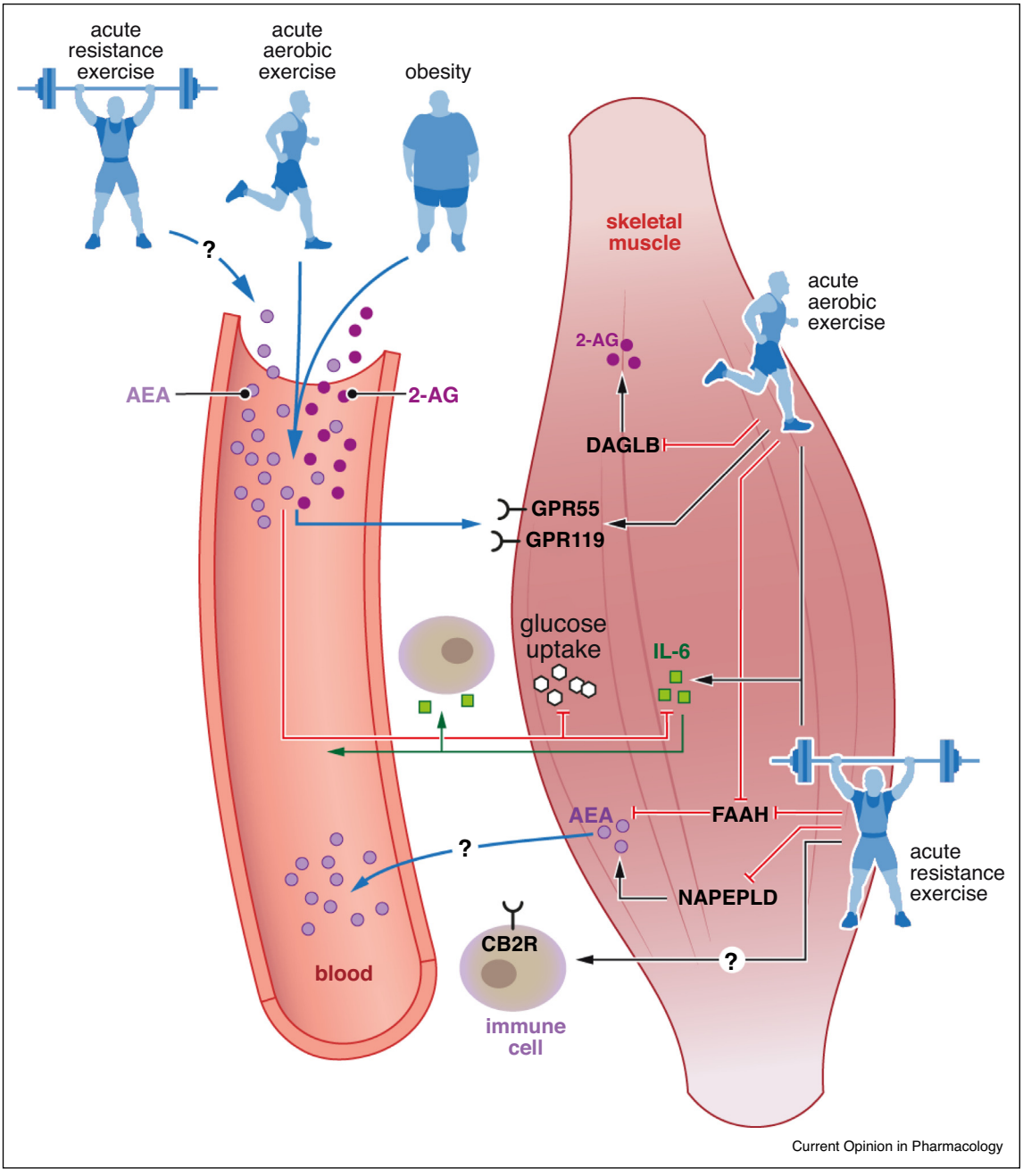
Taken together, it is conceivable that eCBs in skeletal muscle play a role in the adaptation to exercise. However, many aspects of this involvement remain elusive and it is unknown i) whether skeletal muscle contributes to the circulating pool of eCBs during exercise; ii) whether these circulating eCBs play a signalling role in skeletal muscle during either acute or chronic exercise; and iii) how different types (resistance versus aerobic), duration (acute versus chronic) or even intensities of exercise interventions could improve cardiometabolic health through the ECS. This line of research should be further explored in future studies.

Role of endocannabinoids in the regulation of immune responses in exercise training

Regular exercise reduces cardiometabolic risk, at least in part because exercise exerts anti-inflammatory effects [36]. These effects may be mediated via both a reduction in visceral adiposity and the induction of anti-inflammatory cytokines with each bout of exercise [37]. Exercising skeletal muscle contributes to this anti-inflammatory environment by producing and releasing myokines such as interleukin-6 (IL-6) as paracrine and endocrine factors to enhance muscle growth and regulate physiological processes in other tissues (e.g. liver or adipose) [37,38]. Of note, the ablation of CB1R in skeletal muscle of mice was shown to markedly increase the secretion of IL-6 and decrease the expression of myostatin, an inhibitor of muscle growth [39]. This indicates again that the acutely increased blood eCB levels with exercise may not align with the positive effects of exercise in skeletal muscle. However, in cardiac muscle 2-AG was found to not only increase glucose uptake via AMPK activation but also to reduce TNFα-induced pro-inflammatory markers, pointing towards essential discrepancies in the eCB response of different muscle types in the body [40]. The identification of more exercise-responsive myokines may help to better understand the role of eCBs in skeletal muscle and the communication with other tissues, as data on this topic is currently scarce.

Exercise training can moreover cause damage to skeletal muscle tissue as a physiological adaptation to the stimulus, depending on, among other factors, intensity and duration as extensively reviewed elsewhere [41,42]. In the processes of muscle regeneration, the innate and adaptive immune systems play a crucial role in the debris clearance and microenvironment modification by secreting various growth factors, cytokines and enzymes [43]. It has been documented that eCBs play a critical role during inflammation and immune response, as they modulate the activation and migration of immune cells as well as the expression of inflammatory cytokines. In this context, eCBs regulate the expression of pro-inflammatory cytokines and AEA was shown to reduce lymphocyte proliferation by markedly inhibiting the release of IL-2 by activated T-lymphocytes [44]. Interestingly, in vascular smooth muscle cells AEA was found to epigenetically regulate inflammatory gene expression and to reduce the expression of the macrophage-attracting C–C motif

Figure 2



Overview of the proposed role of endocannabinoids in the exercise response of skeletal muscle.
Obesity, acute aerobic exercise, and possibly acute resistance exercise increase the levels of the endocannabinoids (eCBs) anandamide (AEA) and 2-arachidonoylglycerol (2-AG) in the blood [16•,17,18,19•]. Elevated eCBs arguably impair skeletal muscle function as they inhibit the uptake of glucose [10,21] and the synthesis of the anti-inflammatory exercise-responsive myokine interleukin-6 (IL-6) [39]. In response to acute aerobic or resistance exercise, the gene expression of the components of the endocannabinoid system is modulated in skeletal muscle, as evident from transcriptomic meta-analyses (www.metamex.eu, [34••]). The expression of the G-protein coupled receptors (GPR) 55, 119 and the cannabinoid receptor 2 (CB2R) is increased while the expression of eCB synthesis enzymes diacylglycerol lipase B (DAGLB) and *N*-acyl phosphatidylethanolamine-specific phospholipase D (NAPEPLD) and the degrading enzymes fatty acid amide hydrolase (FAAH) is downregulated. CB2R is primarily expressed in cells of the immune system [3], the increased expression may therefore reflect an increased influx of inflammatory cells as a response to resistance training. By these mechanisms, skeletal muscle may thus contribute to the pool of AEA in the blood.

chemokine ligand 2 (CCL2), suggesting anti-inflammatory properties of eCBs in the vasculature [45]. In a unique study, Gasperi *et al.* demonstrated that IL-6 leads to the activation of the *FAAH* promoter in lymphocytes of physically active subjects, thus enhancing FAAH activity and thereby reducing the eCB tone compared to sedentary individuals [46]. Taken together, this data suggests a link between the ECS and exercise- and regeneration-related cytokines but the function of this interaction needs further investigation, which potentially aids the development of new pharmacological treatments for muscular disorders and injuries.

Conclusion

The skeletal muscle expression of components of the ECS involved in synthesis, degradation and signalling responds to various forms of exercise, as summarized in Figure 2. It is, however, unclear to what extent the ECS activity in skeletal muscle is involved in the development of cardiometabolic diseases. The elevated eCB tone observed with both obesity and acute exercise indicates a peripheral misalignment between the negative metabolic effects of eCB signalling and the positive effects of exercise. We show for the first time that exercise modifies the gene expression of cannabinoid receptors and enzymes involved in the biosynthesis and breakdown of eCBs. Additionally, eCBs are conceivably involved in the regulation of the immune response to exercise and thereby modulating the communication between skeletal muscle and other tissues. The therapeutic value of these interactions needs to be further explored.

Conflicts of interest statement

Nothing declared.

CRediT authorship contribution statement

Milena Schönke: Conceptualization, Data curation, Writing - original draft, Writing - review & editing, Formal analysis. **Borja Martinez-Tellez:** Conceptualization, Data curation, Writing - original draft, Writing - review & editing, Formal analysis. **Patrick CN Rensen:** Supervision, Writing - review & editing.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.coph.2020.05.003.

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- • of special interest
- •• of outstanding interest

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