Peripheral endocannabinoids regulate skeletal muscle development and maintenance

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Abstract

As a principal tissue responsible for insulin-mediated glucose uptake, skeletal muscle is important for whole-body health. The role of peripheral endocannabinoids as regulators of skeletal muscle metabolism has recently gained a lot of interest, as endocannabinoid system disorders could cause peripheral insulin resistance. We investigated the role of the peripheral endocannabinoid system in skeletal muscle development and maintenance. Cultures of C2C12 cells, primary satellite cells and mouse skeletal muscle single fibers were used as model systems for our studies. We found an increase in cannabinoid receptor type 1 (CB1) mRNA and endocannabinoid synthetic enzyme mRNA skeletal muscle cells during differentiation. We also found that activation of CB1 inhibited myoblast differentiation, expanded the number of satellite cells, and stimulated the fast-muscle oxidative phenotype. Our findings contribute to understanding of the role of the endocannabinoid system in skeletal muscle metabolism and muscle oxygen consumption, and also help to explain the effects of the peripheral endocannabinoid system on whole-body energy balance.

Key Words: Endocannabinoids, skeletal muscle, development, differentiation, metabolism
skeletal muscle by modulating energy homeostasis [8,33]. For example, glucose uptake and oxygen consumption were significantly increased in the isolated soleus of mice treated for 7 days with the CB1 antagonist SR141716 compared with control mice [33]. Further, expression of CB1 mRNA in soleus muscle from obese mice was increased compared with soleus muscle from lean mice [30]. All these findings suggest that CB1 plays an important role in skeletal muscle metabolism, especially in glucose uptake [30,33]. Currently, data on the effects of the ECS on skeletal muscle are much less than for other tissues. The purpose of this study was to investigate the involvement of the peripheral endocannabinoid system on skeletal muscle development and establishment of metabolic function.

Materials and Methods

Pharmacological reagents

R(+) -methanandamide (MAEA) was purchased from Sigma-Aldrich Chemical Co (St. Louis, MO, USA). AM-251 was purchased from Enzo (Plymouth Meeting, PA, USA). Trypsin was purchased from Gibco Invitrogen (Carlsbad, CA, USA). Tissue Tek was purchased from Fisher Scientific (Houston, TX, USA).

Animals

The Male NIH Swiss mice were purchased from Harlan Labs (Indianapolis, IN, USA). Animal care procedures followed approved Purdue Animal Care and Use Committee (PACUC) protocols.

C2C12 cell culture

In Mouse C2C12 myoblasts were maintained in DMEM supplemented with 10% fetal bovine serum, 0.1% gentamycin reagent and 1% antibiotic antitiotics at 37°C and 5% CO2. Differentiation medium contained 2% horse serum instead of 10% fetal bovine serum. When cells reached 70% confluence they were passaged by trypsinization (0.5% trypsin in 0.5 mM EDTA [Invitrogen, Carlsbad, CA, USA]).

GIEMSA staining to assay differentiation

Cells were fixed in 100% methanol for 5 min and subsequently rinsed with PBS. Cells were then stained with a fresh solution of 10% Giemsa (Invitrogen, Carlsbad, CA, USA) in PBS at room temperature for 10 min and then rinsed in PBS for 10 min. For each treatment depicted, 6 random images were captured from each culture using a Leaf Micro-Lumina scanning digital camera. From these images, the following data were obtained: total cell number, number of myofibers (cells with greater than or equal to 3 nuclei); number of nuclei per myofiber (# of nuclei in a cell containing 3 or more nuclei).

Real time PCR (RT-PCR)

Total RNA was extracted from skeletal muscle using Trizol reagent according to the manufacturer’s protocols (Life Technologies, Bethesda, MD, USA). The concentration and purity of the RNA were determined by measurement of the optical densities at 260 and 280 nm and analyzed by gel electrophoresis. Contaminating DNA was removed from total RNA by two 10-min treatments with RQ1 (RNA Qualified) RNase-Free DNase (Promega, Madison, WI, USA). The RNA solutions were diluted to a working concentration of 1 µg/µl in DEPC treated water (0.1% DEPC to water, Invitrogen). CDNA was prepared from RNA samples as following: A 20 µl reaction mix was made of 1x 1st standard buffer (Life Technologies), 10 mM DTT, 1 mM dNTPs, and 5 µM random hexamers. To this, 200 ng of RNA were added and the mixtures were heated to 65°C for 10 minutes. The reactions were then cooled to 25°C for 5 minutes and 1 µl (200 units) superscript II Reverse Transcriptase (Invitrogen) was added. Identical reaction mixtures were made for each RNA sample without adding superscript II Reverse Transcriptase. These reactions served as no-RT controls. The reaction was heated to 37°C for 90 min followed by heat deactivation at 90°C for 10 min. The reaction was then diluted to 100 µl with H2O and stored at -20°C for later use. RT-PCR was performed using a GeneAmp 5700 Sequence Detection System (PE Applied Biosystems, Carlsbad, CA, USA) and the SYBR Green PCR core reagents kit (PE Applied Biosystems). RT-PCR was performed using Integrated DNA Technologies, Inc. primers. Sequences of primers are shown in Table 1. Results are presented as a ratio of target gene mRNA/18S mRNA. Primers were designed using the Primer3 program (Geneious, Auckland, New Zealand) and reactions were performed using primers obtained from Integrated DNA Technologies Inc. (Coralville, IA, USA). Each pair of primers gave a product about 200 bp and spanned an intron.

Primary myoblast cell culture

Forelimbs and hindlimbs were removed from neonatal mice (2-5 d old) and bones were dissected away. The remaining muscle mass was weighed. A few drops of PBS were added and the muscle was minced into a coarse slurry using razor blades. Cells were enzymatically dissociated by the addition of 2 ml per g
**Endocannabinoids regulate skeletal muscle**


**Table 1: Forward and reverse primers used in RT-PCR reactions.**

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer 5 – 3</th>
<th>Reverse primer 5 - 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1</td>
<td>CTTTGCGATACAACTTT</td>
<td>TCACAGTCCTCTTTGATA</td>
</tr>
<tr>
<td>CB2</td>
<td>CATAGCGATACCTTCACCAA</td>
<td>CCAAGTGGTCAGGAAGAA</td>
</tr>
<tr>
<td>MAGL</td>
<td>TGCCAGTGGTATTGATTCCTT</td>
<td>TTAGCAGTGTATGCCAAGCAC</td>
</tr>
<tr>
<td>NAPE-PLD</td>
<td>ATGCAGCAATGTCGGGAGAAC</td>
<td>ACCACCTTTGCTGATACCCAG</td>
</tr>
<tr>
<td>DAGL-Alpha</td>
<td>AATTTCGGACTTTCAACTCTTCG</td>
<td>TCCAGACAGAAGGACCCAGATGT</td>
</tr>
<tr>
<td>FAAH</td>
<td>TAGCTTGCCAGTATGAGCCGTGCT</td>
<td>AGGAAGTAAACGGAGGTGACAA</td>
</tr>
<tr>
<td>18S</td>
<td>CGGCCTACACATGCAAGGAA</td>
<td>GCCTGAATTACCGCGGCT</td>
</tr>
</tbody>
</table>

Isolated muscle fibers

Extensor digitorum longus (EDL) muscles were dissected out by handling tendons so as not to damage the muscle fibers. Muscles were then placed in 0.2% collagenase and incubated in a shaking water bath at 35°C for 45 min. After the fibers had been loosened by this treatment, they were liberated using heat polished glass Pasteur pipettes of various bore size and by viewing under a dissecting microscope. These fibers were transferred to DMEM (containing 10% fetal bovine serum, 10 mM Hepes, 100 units/ml penicillin, and 100 μg/ml streptomycin).

Plasmids

pcDNA3-CB1 was provided by Dr. Beat Lutz (Gutenberg-University Mainz Duesbergweg). pcDNA3 was obtained from Invitrogen. The pcMV-LacZ, Renilla luciferase (pRL-SV40) and myoglobin promoter driven-luciferase reporter were obtained from Promega. Sarco(endo)plasmic reticulum calcium ATPase 1 (SERCA1; −1373 to +172 bp) promoter driven-luciferase reporter was obtained from Dr. Steven Swoap (Williams College, Williamstown, MA, USA). Plasmids used in these studies were purified with an EndoFree plasmid mega kit (Qiagen, Alameda, CA, USA) and diluted in 0.9 % saline to a final concentration of 0.5 μg/μl.

In vitro transfection

For reporter gene experiments, reporter gene constructs were transfected into myoblasts using FuGene 6 (Roche Applied Science, Indianapolis, IN, USA) for 8 h according to the manufacturer’s recommendations. The pRL-SV40 plasmid expressing Renilla luciferase was cotransfected at a ratio of 1:50 to normalize for reporter expression. Mice were subjected to RT-PCR as described above. In another experiment, gastrocnemius, soleus and tibialis anterior muscles were dissected and pooled from 5 week old male mice. These muscles were used for RT-PCR.

In vivo DNA injection and electroporation

Mice were anesthetized with an intraperitoneal injection of 0.01 ml/g BW of a mixture of ketamine (100 mg/ml) and xylazine (20 mg/ml). Fifty μl of a mixture of 15 μg of pcDNA3-CB1, 10 μg of reporter gene (Serca1-luciferase or myoglobin-luciferase) [5, 44] and 1 μg pRL-SV40 Renilla luciferase were injected into the gastrocnemius muscle. In the contralateral gastrocnemius muscle, 50 μl of a mixture of 15 μg of empty pcDNA3, 10 μg reporter genes and 1 μg pRL-SV40 Renilla luciferase was injected. Following the injection, electroporation was performed (200 V/cm, 8 pulses, 1 Hz, 20 ms interval) using a BTX ECM 830 electroporator (BTX Harvard Apparatus, Holliston, MA) as described previously [1]. Four days following electroporation, muscles were dissected and homogenized in passive lysis buffer and analyzed with Dual Luciferase Assay Kit (Promega). All procedures were conducted in accordance with guidelines set by the Purdue Animal Care and Use Committee.

Immunofluorescent staining

Cells or muscle fibers were fixed by incubation with 4% paraformaldehyde in PBS, pH 7.4 at room temperature for 5-10 min. Prior to immunohistochemistry, tissue was blocked by incubation in 5% normal horse serum/PBS for 30 min at room temperature. Primary antibodies were diluted in blocking buffer: MyoD (1:300, Santa Cruz Biotechnology, Santa Cruz, CA); Pax7 (1:70, Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Samples were incubated in primary antibody solutions at room temp for 1h or 4°C overnight. Secondary antibodies were diluted in blocking buffer: MyoD (1:300, Santa Cruz Biotechnology, Santa Cruz, CA); Pax7 (1:70, Developmental Studies Hybridoma Bank, Iowa City, IA, USA). Samples were incubated in secondary antibody at room temp for 30-45 min. Nuclei were counterstained with 0.5 μg/ml DAPI (Sigma-Aldrich) in PBS for 5 min.
Endocannabinoids regulate skeletal muscle

Fig 1. Endocannabinoid receptor CB1 and endocannabinoid synthetic enzyme mRNAs increase during differentiation of C2C12 skeletal muscle cells. Total RNA was isolated from proliferating cells (Prolif) or cells differentiated for 24, 48, 72 or 96 hours. After reverse transcription, cDNAs were assayed by RT-PCR. A) Myogenin mRNA expression was detected in cells differentiated for 72 and 96h, demonstrating that terminal myofiber differentiation was occurring at these time points. (MSE=0.08); B) CB1 mRNA expression was significantly up-regulated 24, 72 and 96h post differentiation. (MSE= 0.04); C) CB2 mRNA expression was significantly down-regulated upon terminal differentiation. (MSE= 0.02); D) Endocannabinoid synthetic enzyme DAGLa expression was significantly increased upon C2C12 myofiber differentiation. (MSE= 0.02); E) Endocannabinoid synthetic enzyme NAPE-PLD expression was significantly increased upon C2C12 myofiber differentiation. (MSE= 0.04); F) The expression of the endocannabinoid degradative enzyme MAGL was significantly decreased upon differentiation. (Mean Standard Error [MSE]= 0.06). n=3 for each mean. Means with different superscripts differ (p<0.01).

Statistics
Data were analyzed by ANOVA using SAS. Means were separated by Least Significance Difference. Statements of significance were based on P-levels as noted. All experiments where repeated twice and a representative experiment is shown in each figure.

Results and Discussion
Endocannabinoid receptor CB1 and endocannabinoid synthetic enzyme mRNAs increase during differentiation of C2C12 skeletal muscle cells
The C2C12 mouse myoblast cell line is a well-established model system for studying myogenesis. C2C12 cells were cultured in a proliferation media and
Endocannabinoids regulate skeletal muscle

induced to differentiate by switching to a proliferation (0h) or differentiated cells at 24h, 48h, 72h, or 96h after switching to a differentiation media.

Fig 2. Cannabinoids inhibit differentiation and myofiber formation in C2C12 cultures. A) MAEA treatment inhibits C2C12 myoblast differentiation. C2C12 skeletal muscle cells were cultured in a proliferation media and induced to differentiate by switching to a differentiation media containing 1 or 10 μM MAEA. Addition of MAEA at 10 μM significantly inhibited differentiation. Administration of the CB1 antagonist AM251 blocked the differentiation-inhibitory effects of MAEA. (MSE = 1.8); B) Effects of MAEA and/or AM251 on total cell number. There was a loss of total nuclei upon addition of MAEA, but this loss alone would not contribute to the majority of reduction of nuclei in differentiated cells (MSE=10.1);
Endocannabinoids regulate skeletal muscle

and assayed by RT-PCR. Myogenin mRNA expression was significantly elevated in cells differentiated for 72 and 96h (Figure 1A). As myogenin is a differentiation specific mRNA [42,48],

C) Effects of AM251 on myoblast differentiation. Addition of AM251 significantly stimulated premature differentiation in C2C12 cells. Five days post-differentiation, significant differences in differentiation were no longer detectable (MSE= 2.1); D) Effects of AM251 on total cell number. Loss of total nuclei number could not solely account for the effects of AM251 on differentiation observed in C (MSE= 8.0); n=6 for each mean. Means with different superscripts differ (p<0.01).
Endocannabinoids regulate skeletal muscle

Fig 3. CB1 ectopic expression inhibits C2C12 differentiation. A CB1 receptor expression construct was co-transfected with a slow (Myoglobin-Luciferase) or fast (Serca1-Luciferase) fiber, differentiation-specific reporter gene into proliferating C2C12 cells. Cells were allowed to proliferate for 24 hrs or switched to differentiation medium for 96 hrs. Myoglobin or SERCA luciferase values were normalized for transfection efficiency (co-transfection of pRL-SV40 Renilla luciferase). Ectopic CB1 over-expression inhibited differentiation of C2C12 cells as indicated by the significantly decreased serca1-luciferase (A, MSE = 0.0018; B, MSE = 0.00014); n=4 for each mean. Means with different superscripts differ significantly (p<0.01).

this demonstrates that terminal myofiber differentiation was occurring in these cells at these time points. CB1 mRNA expression was significantly up-regulated 24, 72 and 96h post differentiation (Figure 1B). In contrast, CB2 mRNA expression was significantly down-regulated upon terminal differentiation of C2C12 cells (Figure 1C). In addition, the mRNA expression of the endocannabinoid synthetic enzyme DAGLa was significantly increased following C2C12 differentiation for 48, 72 and 96 h (Figure 1D). Nape PLD, another endocannabinoid synthetic enzyme, was significantly up-regulated at 24 and 96 h post-induction of differentiation (Figure 1E). However, the endocannabinoid degradative enzyme MAGL was significantly decreased 24, 48, 72 and 96h post differentiation (Figure 1F). CB1 receptors have been detected in skeletal muscle [30]. Our results expand on this observation to demonstrate that expression of the endocannabinoid family is tightly related to myogenic differentiation in vitro, and suggest that the endocannabinoid family might play a role in the functional control of differentiation.

Cannabinoids inhibit differentiation and myofiber formation in C2C12 cultures
MAEA, a synthetic stable activator of CB1, was added to the differentiation media of C2C12 skeletal muscle cells. In the control treated cells, the percent of differentiated cells as measured by GEIMSA staining, was significantly elevated 3 days post-initiation of differentiation and reached a peak of 42% 6 days post-differentiation (Figure 2A). Addition of 1 µM MAEA had no effect on the progression of differentiation. In contrast, addition of MAEA at 10 µM significantly inhibited differentiation, with a maximum differentiation at approximately 10%. To further examine the specificity of the MAEA-mediated inhibition, a CB1 specific antagonist (AM251) was added to the differentiation media in addition to 10 µM MAEA. Administration of the CB1 antagonist AM251 at concentration 1 and 10 µM significantly blocked the effects of cannabinoid ligands (MAEA 10 µM in media) on myoblasts at day 6 after changing to differentiation media. (Figure 2A). To analyze for non-specific cell toxic effects of MAEA, total cell number was also examined. There was some loss of total nuclei upon addition of MAEA, but this loss alone would not contribute to the majority of reduction in nuclei within differentiated cells (Figure 2B). In another set of experiments, the role of endogenous cannabinoids on C2C12 myoblast differentiation was examined by addition of AM251 to differentiation media. Addition of AM251 significantly stimulated premature differentiation in C2C12 cells. However, this stimulation was transient in nature as by 5 days post-differentiation, the stimulation was no longer detectable (Figure 2C). There was some loss of total nuclei upon addition of AM251, but the levels could not account for the differences in differentiation (Figure 2D). Further evidence that endogenous endocannabinoids can regulate C2C12 myoblast differentiation was demonstrated by transient co-transfection of a CB1 receptor expression construct with slow or fast fiber, differentiation-specific reporter gene (serca1-luciferase:fast fiber; or myoglobin-luciferase:slow fiber) into proliferating C2C12 cells.
**Cannabinoids inhibit differentiation and myofiber formation in primary muscle cell cultures**

At 96 hours after the initiation of differentiation, MAEA addition significantly inhibited myoblast differentiation and myofiber formation (A). AM251 significantly blocked the effects of MAEA on primary myoblast differentiation (A; MSE=2.38). Addition of MAEA resulted in increased cell number (B; MSE=2.85). Addition of 5 or 10 µM AM251 did not have an effect on primary muscle cell differentiation. 15 µM AM251 inhibited primary muscle cell differentiation (C; MSE=3.05). Addition of AM251 resulted in decreased total cell number (D; MSE=2.94); n=6 for each mean. Means with different superscripts differ (p<0.01).

**C2C12 cells express both of these expression constructs in a differentiation-specific fashion [41].** Comparing myoglobin or SERCA luciferase values normalized for transfection efficiency (co-transfection of pRL-SV40 Renilla luciferase), it was observed that ectopic CB1 over-expression inhibited differentiation of C2C12 cells (Figures 3A and 3B). These results demonstrate that in the clonal myoblast cell line C2C12, cannabinoids can inhibit myoblast differentiation and myofiber formation. Intriguingly, the expression pattern demonstrated above shows that these differentiation-inhibiting factors are expressed when the cells are in the act of differentiation. These novel results explain why a function of the endocannabinoid family could be to halt or slow differentiation as it is occurring.

**Cannabinoids inhibit differentiation and myofiber formation in primary muscle cell cultures**

To further examine the action of cannabinoids in primary myogenic cells derived from neonatal muscles, MAEA was added to differentiation media of primary myoblast cultures. At 96 hours after the initiation of differentiation, similar to the C2C12 cultures, MAEA addition significantly inhibited myoblast differentiation and myofiber formation (Figure 4A). AM251 significantly blocked the effects of MAEA on primary myoblast differentiation (Figure 4A). In contrast to the C2C12 cultures, addition of MAEA resulted in increased cell number (Figure 4B). Addition of 10 µM AM251 did not have an effect on primary muscle cell differentiation; however high concentration AM251 (15 µM) can inhibit primary muscle cell differentiation (Figure 4C). Addition of AM251 resulted in decreased cell number (Figure 4D). In summary, cannabinoids can inhibit differentiation in primary myoblast cultures similarly in C2C12 cells.

**Cannabinoids regulates satellite cell proliferation in isolated skeletal muscle single fiber culture**

As we have demonstrated, cannabinoids are synthesized by differentiating myoblasts, and these cannabinoids seem to act to inhibit differentiation. This provides evidence for the hypothesis that cannabinoids produced by the differentiated fiber act to reduce the differentiation of surrounding myoblasts. Isolated
myofibers provide a good model to study the activation, proliferation, and differentiation of satellite cells in their native position beneath the basal lamina. This model preserves the potentially important interactions between satellite cells and multi-nucleated myofibers (Figures 5A and 5B). Myofibers were isolated from the extensor digitorum longus (EDL) muscle of adult mice and cultured in suspension growth medium with or without 10 μM MAEA. Satellite cells typically form clusters of myoblast progenies on isolated myofibers after several days of suspended culture. Addition of MAEA resulted in a significant increase in the numbers of nuclei in each cluster (Figure 5C). This suggests an endocannabinoid mediated stimulation of satellite cell proliferation. Addition of AM251 inhibited this MAEA mediated effect on cell number per cluster (Figure 5C). MyoD is expressed in activated satellite cells and an early marker of myogenic differentiation [27]. MyoD is typically highly expressed in myogenic cells entering the differentiation program and in differentiated myocytes and myotubes [27,47]. There was a trend for a reduced number of MyoD positive cells following MAEA treatment (Figure 5D). In adult muscle, Pax7 is expressed by both quiescent and activated satellite cells, but not in differentiated myocytes or myotubes [25,40]. We observed an increased number of Pax7 positive cells in MAEA treated cultures (Figure 5E). Our results suggest that cannabinoids can stimulate proliferation and/or inhibit differentiation of satellite cells, and support the hypothesis, derived from our in vitro expression data that the ECS synthesized by differentiated muscle fibers might act to ensure a population of myogenic stem cells for future regeneration and growth. While some recent studies have shown that cannabinoids regulate proliferation and differentiation of many cell types [13,19,20,34,38,46], these results are the first demonstration of their role in regulating skeletal muscle myoblasts.

The endocannabinoid family is developmentally expressed in skeletal muscle in vivo

The mRNA expression of the endocannabinoid family was analyzed in mice gastrocnemius muscles of different developmental ages. The expressions of CB1, DAGLa, NAPE-PLD and MAGL were significantly decreased with increasing age (Figure 6). In contrast CB2 mRNA expression was not significantly changed with development (Figure 6B). The dynamic expression of these genes involved in the ECS suggests a role for these factors in postnatal muscle growth and aging.

CB1 mRNA expression is greater in fast than slow muscles

Gastrocnemius, soleus and tibialis anterior muscles were dissected and pooled from 5 week old male mice. These muscles were used for RT-PCR analysis of CB1 mRNA expression. The levels of CB1 mRNA were
Endocannabinoids regulate skeletal muscle

Fig 6. Expression of the endocannabinoid family mRNA is developmentally regulated in skeletal muscle. Gastrocnemius muscles were dissected from 2 w, 4 w and 1 year old mice. The endocannabinoid family mRNA was measured in these muscles using RT-PCR. (A) CB1 mRNA expression was significantly down-regulated with increasing age. (MSE= 0.12); (B) CB2 mRNA expression did not change with increasing age. (MSE= 0.17); (C) The endocannabinoid synthetic enzyme DAGla expression significantly decreased upon aging. (MSE=0.076); (D) The endocannabinoid synthetic enzyme NAPE-PLD expression was significantly decreased with increasing age. (MSE= 0.12); (E) The expression of the endocannabinoid degradative enzyme MAGL was significantly decreased as the mice got 1 year old. (MSE = 0.17). n=4 for each mean. Means with different superscripts differ (p<0.01).

Fig 7. CB1 expression is greatest in fast-type skeletal muscles Gastrocnemius, soleus and tibialis anterior muscles were dissected and pooled from 5 week old male mice. These muscles were used for RT-PCR analysis of CB1 mRNA expression. The levels of CB1 mRNA were significantly greater in the tibialis anterior muscle (primarily fast-type) than in the gastrocnemius (mixed), which was significantly greater than that observed in the soleus muscle (primarily slow-type). (MSE=0.00781). n=4 for each mean. Means with different superscripts differ (p<0.05)

CB1 regulates muscle fiber metabolism in vivo
The above studies all examined the role of the cannabinoids during myogenesis by introducing cannabinoids to the proliferating myoblast and determining effects on differentiation. Yet, as determined by the expression studies, cannabinoids and their receptors are made by differentiated fibers. In order to determine what effect the cannabinoid family has on the differentiated fiber a CB1-receptor expression construct was co-electroporated with reporter gene vectors (Sercal1-luciferase and myoglobin-luciferase) into the soleus and gastrocnemius muscle of mice. In contrast to in vitro transfections which target proliferating myoblasts, these electroporations target differentiated fibers [1,17,45]. Ectopic expression of CB1 in these muscles stimulated fast fiber-specific, and inhibited slow fiber specific, reporter activities in vivo (Figures 8A and 8B). As skeletal muscle metabolism is associated with fiber types, these results suggest that the cannabinoids can regulate the metabolism in adult skeletal muscle. It is well-known that slow/oxidative muscle fibers play a very important role in regulating whole-body metabolism [22,49]. Recently, a study showed that an
Endocannabinoids regulate skeletal muscle metabolism

Fig 8. CB1 regulates muscle fiber metabolism in vivo. A CB1-receptor expression construct was co-electroporated with reporter gene vectors (Serca1-luciferase or myoglobin-luciferase) into the gastrocnemius muscles of mice. Ectopic expression of CB1 in these muscles stimulated fast fiber-specific reporter (A; MSE = 0.00399) activity and inhibited slow fiber specific in vivo (B; MSE = 0.0014). n=5 for each mean. Asterisk indicates a significant difference (p<0.01).

Increase of fast/glycolytic muscle mass can regress obesity by altering fatty acid oxidation in remote tissues [23]. Our results suggest that ectopic ECS treatment could help fight obesity by regulating muscle metabolism in a similar fashion. In summary, our findings contribute to the understanding of the role of endocannabinoid system in skeletal muscle metabolism and muscle oxygen consumption, and also help to explain the effects of peripheral endocannabinoid system on whole-body energy balance. In addition, our results suggest caution for ECS use in situations where muscle development is currently ongoing, or regeneration might be needed.

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Endocannabinoids regulate skeletal muscle


Endocannabinoids regulate skeletal muscle


Endocannabinoids regulate skeletal muscle