

# Hemp seed oil inhibits adipocyte differentiation of human mesenchymal stem cells by suppressing cannabinoid type 1

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## Abstract

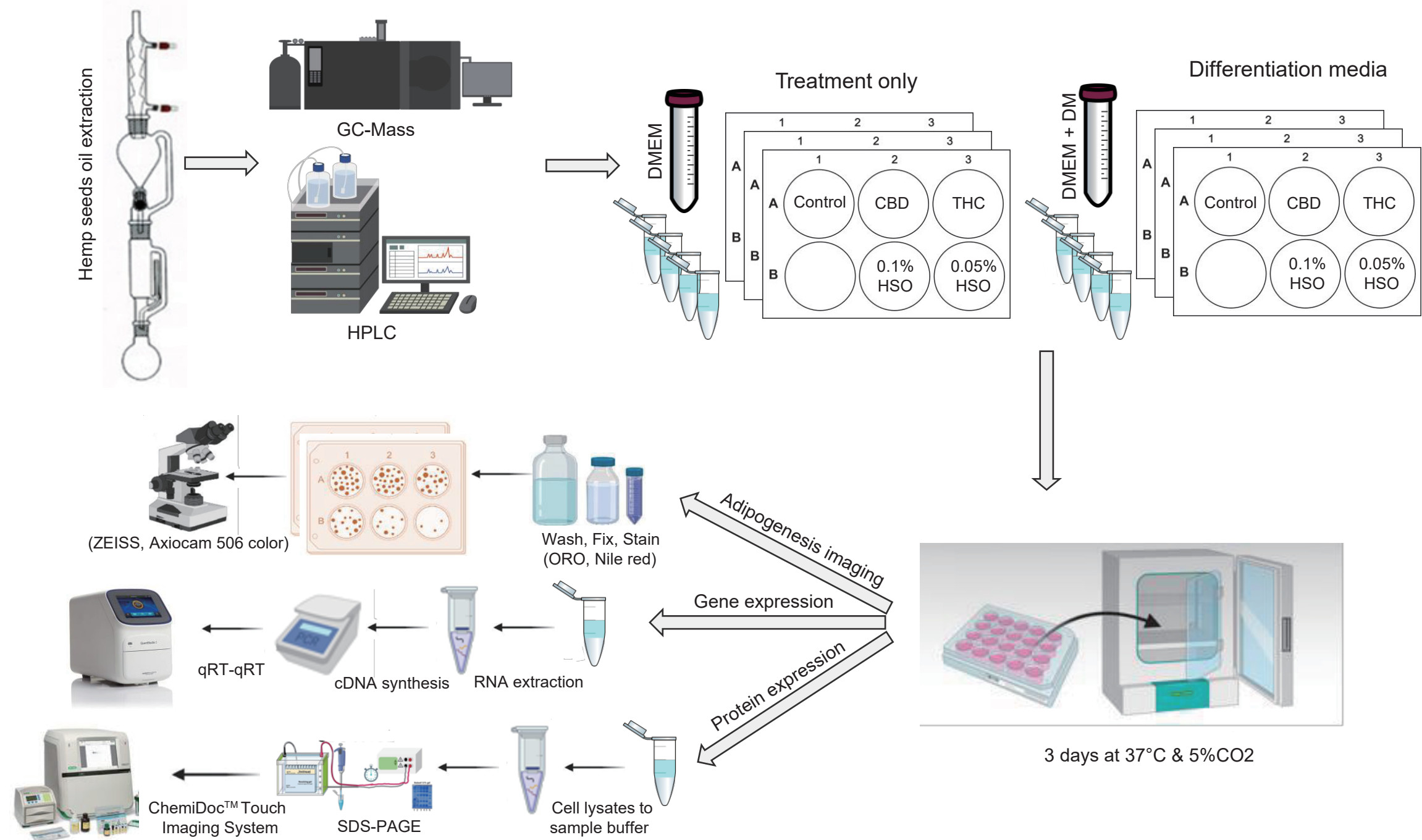
Central and peripheral mechanisms of the endocannabinoid system (ECS) favor energy intake and storage. ECS, especially cannabinoid type 1 (CB1), controls adipocyte differentiation (hyperplasia) and lipid accumulation (hypertrophy) in adipose tissue. In white adipose tissue, CB1 stimulation increases lipogenesis and inhibits lipolysis; in brown adipose tissue, it decreases mitochondrial thermogenesis and biogenesis. This study compared the availability of phytocannabinoids (CBD and THC) and polyunsaturated fat acids (omega 3 and omega 6) in different hemp seed oils (HSOs). The study also examined the effect of HSO on adipocyte lipid accumulation by suppressing cannabinoid receptors in adipogenesis-stimulated human mesenchymal stem cells (hMSCs). Most importantly, Oil-Red-O and Nile red tests showed that HSO induced adipogenic hMSC differentiation without differentiation agents. Additionally, HSO-treated cells showed increased PPAR $\gamma$  mRNA expression compared to controls (hMSC). HSO reduced PPAR $\gamma$  mRNA expression after differentiation media (DM) treatment. After treatment with HSO, DM-hMSCs had significantly lower CB1 mRNA and protein expression than normal hMSCs. HSO treatment also decreased TRPV1, FAAH, and MGL mRNAs in hMSCs and DM-hMSCs. HSO treatment significantly decreased CB1, CB2, TRPV1, and GPCR55 protein levels in DM-hMSCs compared to hMSCs in western blot analysis. In this study, HSO initiated adipogenic differentiation in hMSCs without DM, but it suppressed CB1 gene and protein expression, potentially decreasing adipocyte lipid accumulation and lipogenic enzymes.

## Introduction

The complex cell-signaling endocannabinoid system (ECS) regulates appetite, mood, pain, and immune function. ECS involvement in adipocyte physiology was recently discovered. Matias et al. (2016) found that ECS regulates preadipocyte proliferation and phenotype. Endocannabinoids, receptors for cannabinoids and non-cannabinoids, and enzymes make up the ECS (Bermudez-Silva et al., 2010).

The ECS is altered by compounds found in hemp seeds, such as polyunsaturated fatty acids ( $\omega$ 3 and  $\omega$ 6), tetrahydrocannabinol (THC), and cannabidiol (CBD). Understanding how hemp seeds affect the ECS balance is important because of its growing recreational use. Hemp seed oil (HSO) may regulate the ECS, causing adipogenesis, fat accumulation, and preadipocyte hMSC differentiation. This study examined its effect on adipogenic differentiation in hMSCs and the role of ECS system downstream gene and protein signaling cascade molecules during differentiation. Our findings suggest that hemp seeds and their derivatives may alter the ECS system and lipid metabolism during early adipocyte differentiation.

## Methods



## Results

Cold-pressed HSO contained around a 0.5:1 ratio of  $\omega$ 3: $\omega$ 6 and 0.327 ppm CBD.

Table 1. GC-MS analysis of hemp seed oil extracted using different methods.

|                    | DyM    |        | Sox     |         | CEM    |        | HSO<br>Cold pressed |
|--------------------|--------|--------|---------|---------|--------|--------|---------------------|
|                    | S      | NS     | S       | NS      | S      | NS     |                     |
| $\Omega$ 3         | 30.65% | 14.46% | 28.78%  | 34.08%* | 1.24%  | 0.69%  | 13.36%              |
| $\Omega$ 6         | 41.98% | 20.4%  | 64.74%* | 55.02%  | 54.06% | 60.71% | 25.09%              |
| Vitamin E          | 0      | 0      | 0       | 0       | 0      | 2.65%* | 0                   |
| Palmitic acid      | 0      | 0      | 4.13%   | 6.78%   | 7.68%* | 5.71%  | 5.02%               |
| Stearic acid       | 1.79%  | 0      | 0.89%   | 2.20%*  | 0      | 0      | 1.87%               |
| Pentadecanoic acid | 0      | 0      | 0       | 0       | 0      | 0      | 3.80%*              |

Table 2. CBD concentration in hemp seed oil as determined using HPLC

| DyM   |       | Sox   |       | CEM   |       | HSO<br>Cold pressed |
|-------|-------|-------|-------|-------|-------|---------------------|
| S     | NS    | S     | NS    | S     | NS    |                     |
| 0.131 | 0.141 | 0.162 | 0.144 | 0.101 | 0.109 | 0.327               |

DyM: dynamic maceration; Sox: Soxhlet; CEM: cannabinoid extraction method; HSO: hemp seed oil (cold pressed); S: shelled hemp seeds; NS: not-shelled hemp seeds. \* The highest among all extraction methods.

HSO initiated adipocyte differentiation in hMSCs without the differentiation media in a dose-dependent manner.

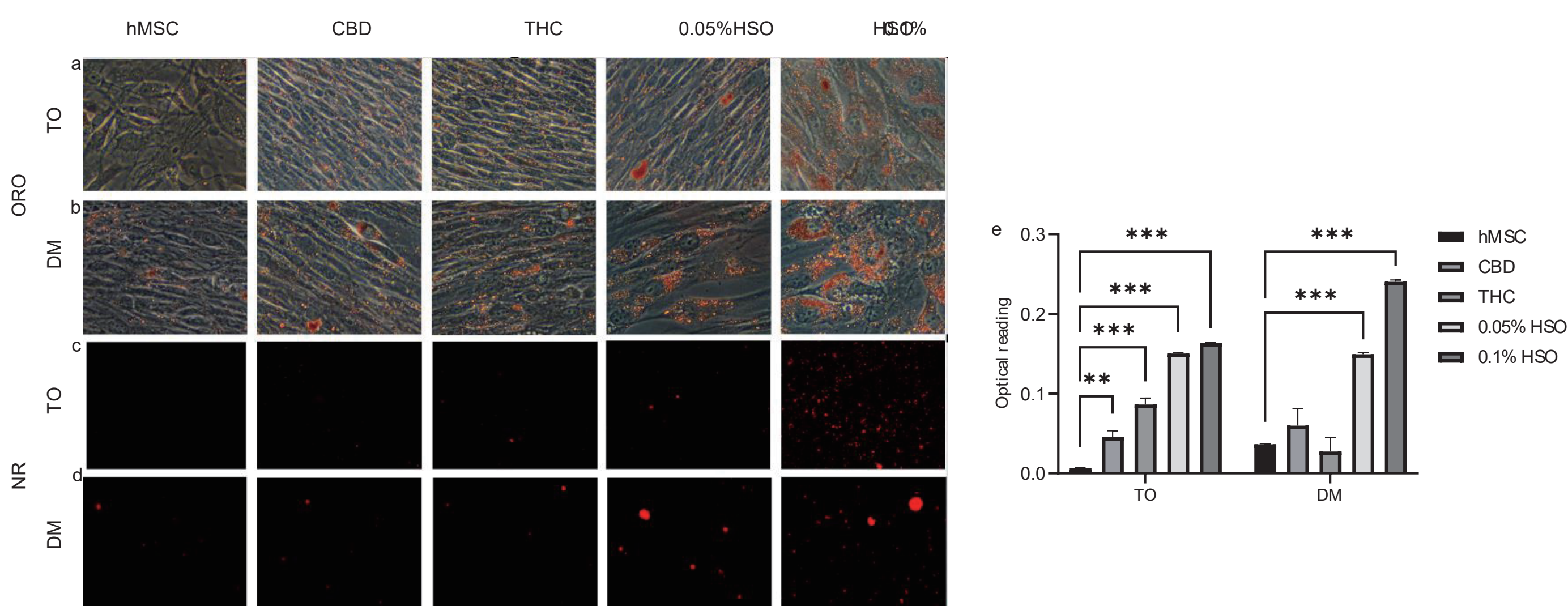


Figure 1. Adipocyte differentiation of hMSCs after 72h incubation with CBD, THC, 0.05% HSO, or 0.1% HSO in either differentiation media (DM) or treatment only (TO). (a) (b) Oil Red O (ORO) stain. (c) (d) Nile Red stain. (e) Intracellular lipid content represented by optical density of ORO in 100% absolute isopropanol; the mean  $\pm$  SD are shown; \*\*P  $\leq$  0.01, \*\*\*P  $\leq$  0.001.

HSO decreased PPAR $\gamma$  expression and increased CEBP- $\alpha$  expression in the presence of DM.

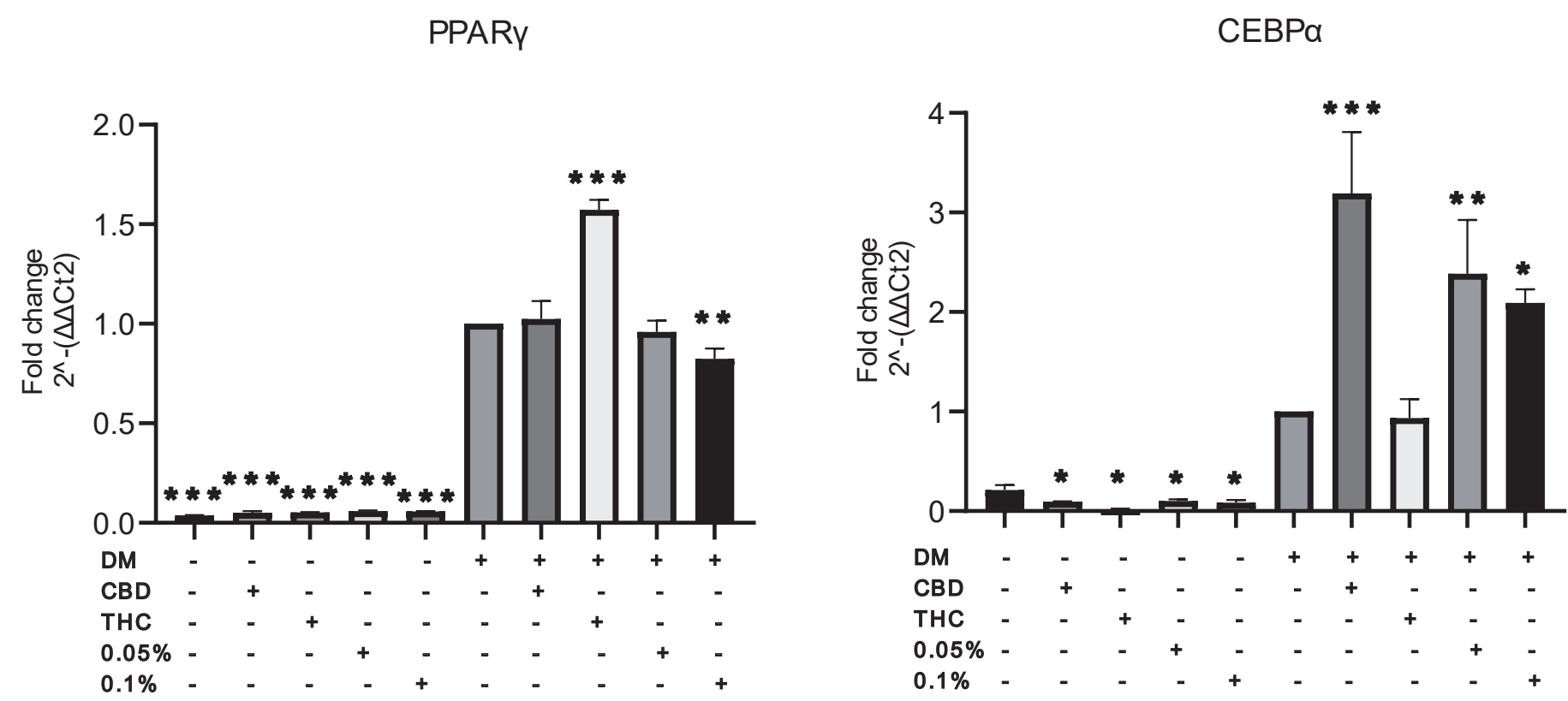


Figure 2. Relative mRNA levels of the adipogenic genes PPAR $\gamma$  and CEBP $\alpha$  determined by quantitative RT-PCR after 72 h of culturing with CBD, THC, 0.05% HSO, or 0.1% HSO treatments with or without DM; the mean  $\pm$  SD are shown relative to the DM control; \*p  $\leq$  0.05, \*\*p  $\leq$  0.01, \*\*\*p  $\leq$  0.001, compared with the corresponding control. 0.1% HSO decreased PPAR $\gamma$  expression by 17.7% in the presence of DM. HSO treatments increased CEBP $\alpha$  expression in the presence of DM but decreased CEBP $\alpha$  expression in the absence of DM.

HSO regulated the expression of ECS genes.

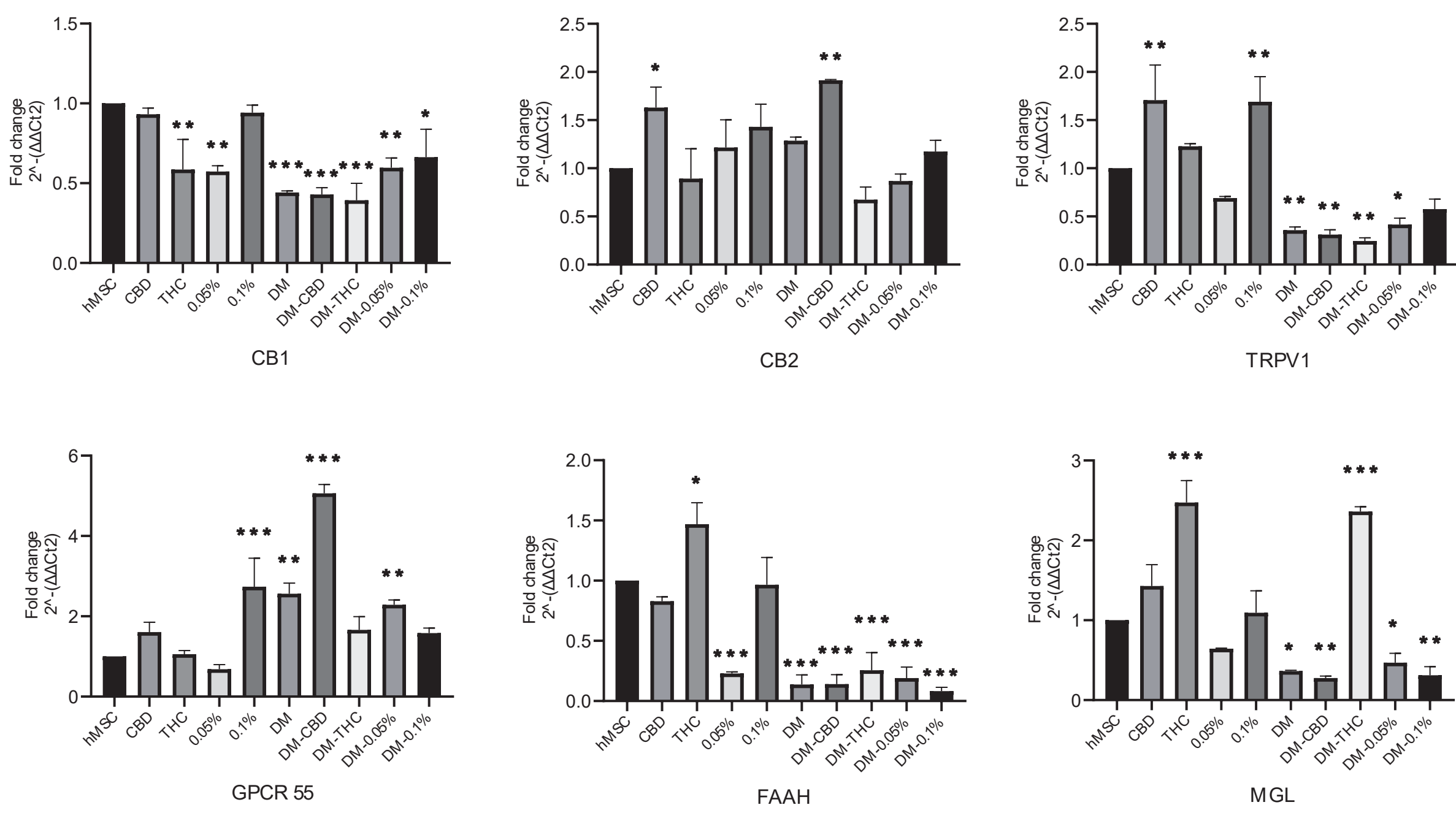


Figure 3. Relative mRNA levels of the ECS genes CB1, CB2, TRPV1, GPCR55, FAAH, and MGL determined by quantitative RT-PCR after 72 h of culturing with CBD, THC, 0.05% HSO, or 0.1% HSO treatment with or without DM; the mean  $\pm$  SD are shown; \*p  $\leq$  0.05, \*\*p  $\leq$  0.01, \*\*\*p  $\leq$  0.001 compared with hMSC. High dose of HSO increased the expression of all of these genes compared with low dose of HSO in the absence of DM. In the presence of DM, HSO treatments increased CB1 and TRPV1 expression but decreased CB2 and GPCR55 expression.

HSO treatment reduced protein levels of CB1, CB2, TRPV1, and GPCR55.

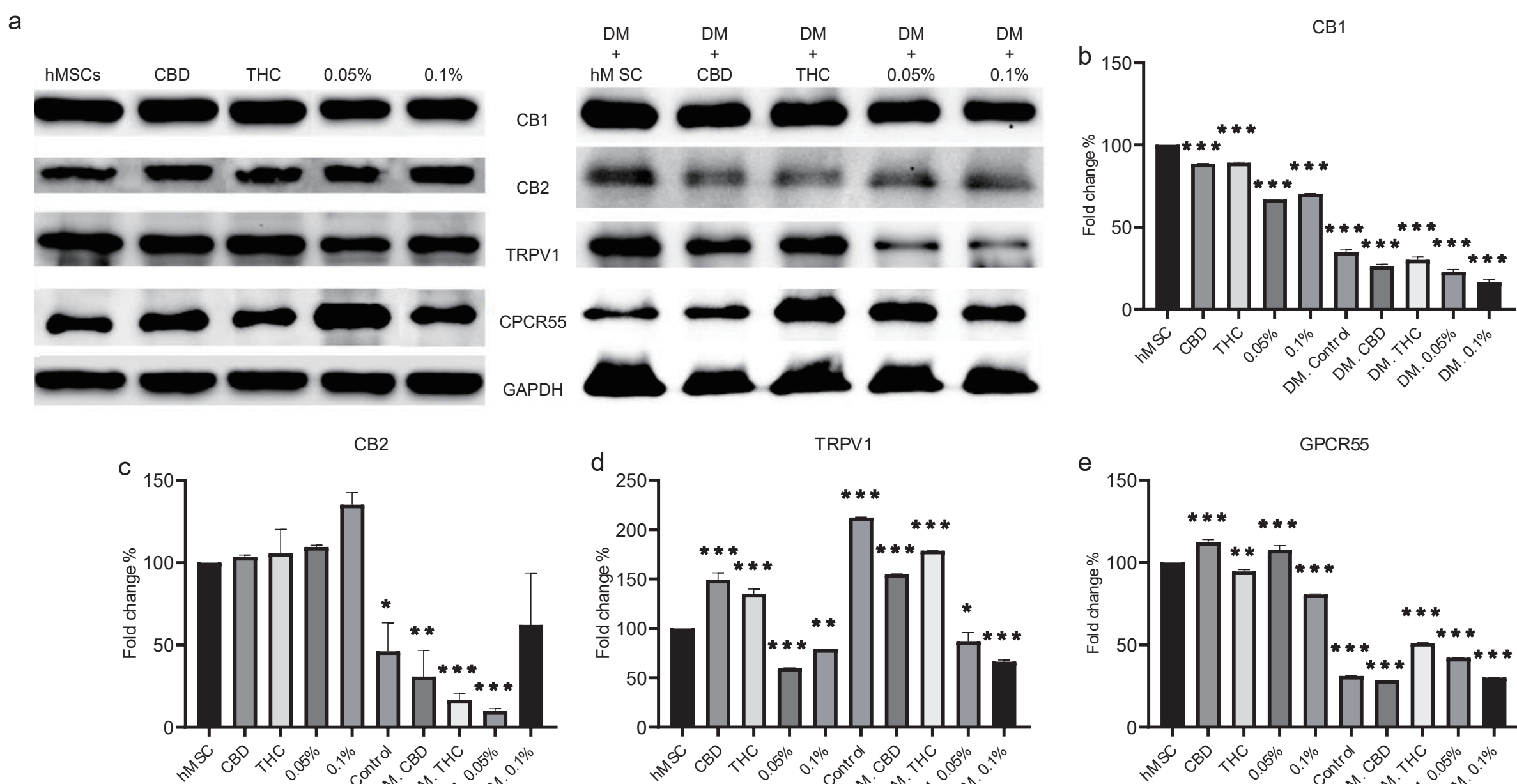


Figure 4. ECS receptor protein levels in hMSCs following CBD, THC, 0.05% HSO, or 0.1% HSO treatments with or without DM. Representative western blot images are shown in (a). Densitometry analysis of CB1, CB2, TRPV1, and GPCR55 are shown in (b), (c), (d), and (e), respectively. The means  $\pm$  SD are shown; \*p  $\leq$  0.05, \*\*p  $\leq$  0.01, \*\*\*p  $\leq$  0.001 compared with hMSC. CB1 level was significantly decreased in all treatments and the highest decrease was among the DM-treated cells.

## Conclusions

- HSO alone initiated adipogenic differentiation and upregulated the adipogenic gene PPAR- $\gamma$ , but it inhibits CB1, TRPV1, and PPAR- $\gamma$  when differentiation is induced by DM.
- HSO effectively downregulated CB1 mRNA and protein expression and reduced the endogenous ligand TRPV1.
- Endogenous CB1 inhibition reduced FAAH and MGL expression, which play a role in endocannabinoid degradation.
- HSO did not show a significant effect on ECS hemostasis at the early stage of differentiating hMSCs to preadipocytes.

## Future Directions

- Hemp seed oil (HSO) may be considered as a natural agent for hMSC adipogenic differentiation.
- A longer period of study is recommended to determine whether HSO will continue to inhibit CB1 and define the adipocyte type.

## Acknowledgments

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## References

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