Investigating Steroid Hormone Changes After Short-term High-Dose Exposure to Cannabidiol in Healthy Subjects

Qingchen Zhang MS[1] ; Josephine M. Raeuscher[1]; Christopher A. Singleton[2,3]; Philip W. Melchert PharmD[1]; David J. Greenblatt MD[3]; John S. Markowitz PharmD[1,4]

[1]Department of Pharmacotherapy and Translational Research, College of Pharmacy, University of Florida, Gainesville, FL, USA.
[2]Callen Analytical Service, Boston, MA, USA
[3] Program in Pharmacology and Drug Development, Tufts University School of Medicine, Boston, MA, USA
[4]Center for Pharmacogenomics and Precision Medicine, College of Pharmacy, University of Florida, Gainesville,, FL, USA

BACKGROUND

Epidiolex® is the first FDA-approved drug containing purified cannabidiol (CBD) derived from the cannabis plant, approved for the treatment of **seizures associated with Lennox–Gastaut syndrome and Dravet syndrome** [1]. Moreover, CBD is a non-psychoactive cannabinoid that is widely used as an **over-the-counter supplement** and can be a component of medical cannabis [2].

The metabolism of CBD in humans has been published previously [3]. After oral administration, CBD is extensively metabolized to its major active metabolite, **7-hydroxy-cannabidiol (7-OH-CBD)**, primarily by the cytochrome P450 enzymes CYP2C19 and CYP2C9 [4,5]. Seven-OH-CBD showed comparable pharmacological activity to the parent compound [5] and is subsequently metabolized to **7-carboxy-cannabidiol (7-COOH-CBD)** by CYP3A4 [6]. Notably, 7-COOH-CBD, an inactive metabolite, reaches plasma concentrations higher than CBD itself (Figure 1) [7].

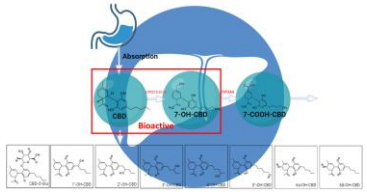


Figure 1. Primary metabolic pathway for CBD in humans.

Figure 2. Epidiolex®.

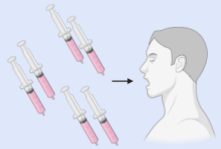
The understanding of the pharmacology of CBD continues to evolve. Clinically, CBD's primary therapeutic effects include sedation and anticonvulsant properties [8]. Its central nervous system (CNS) activity is largely attributed to its role as a negative allosteric modulator of the type 1 cannabinoid receptor (CB1) [9]. CB1 is predominantly located on GABAergic and glutamatergic neurons in the hippocampus, and functions as a presynaptic receptor [10]. Antagonism of CB1 enhances the release of GABA and glutamate, which increases inhibitory neural activity and leads to psychological sedation [11]. Besides CNS, the CB1 receptor is also distributed in the adrenal cortex [12] and peripheral neurons [13].

Numerous studies have explored the biological role of CB1 in steroid regulation. Research indicates that **CB1 plays a key role in modulating the hypothalamic-pituitary-adrenocortical (HPA) axis**, which primarily governs the regulation of circulating glucocorticoids [14] [12] [15]. Additionally, in vitro studies suggest that CBD may affect both male and female reproductive systems by influencing sex-related steroid hormones [16,17]. **However, a comprehensive clinical investigation into the effects of CBD on circulating steroid hormones has yet to be conducted.**

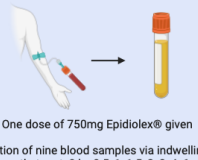
METHODS

Study Design and Drug Administration

- The study protocol was approved by the University of Florida Investigational Review Board
- 12 healthy subjects (6 male, 6 female) completed the entire protocol
- CBD was well-tolerated. No significant side effects reported

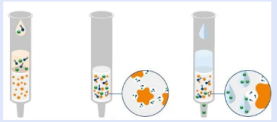


Day 1 - Day 3: Self-administration of 750mg Epidiolex® twice a day, taken orally



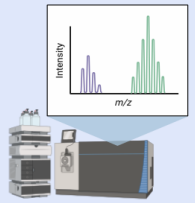
Day 4: One dose of 750mg Epidiolex® given orally
Collection of nine blood samples via indwelling venous catheter at 0 hr, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h after administration.
Plasma samples were collected to be analyzed

Bioanalytical Method



1. Triple solid supported liquid-liquid extraction using a solution of 1:1 Methyl t-butyl ether:Ethyl acetate
2. Dried under nitrogen
3. Resuspended in 50:50 Water:Methanol

- Gradient method LC-MS/MS
  Mobile phase A: 0.1% formic acid in LC-MS grade water
  Mobile phase B: 0.1% formic acid in LC-MS grade acetonitrile
- Internal standard: tolbutamide



Statistical analysis

- Statistical Analysis was performed by SAS9
- A t-test was conducted for each hormone with the CBD and placebo groups for male and female subjects
- Each data point represented the average plasma concentration of 9-time points

SELECT RESULTS

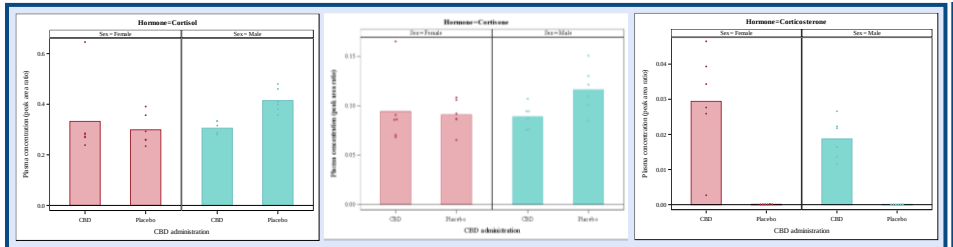


Figure 3. Glucocorticoid plasma concentration changes with CBD administration.

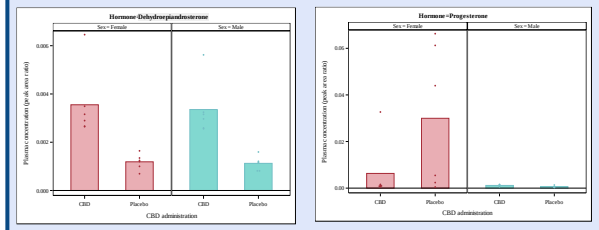


Figure 4. Sex-related steroid plasma concentration changes with CBD administration.

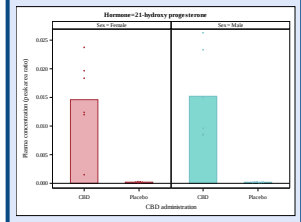


Figure 5. Mineralocorticoid plasma concentration changes with CBD administration.

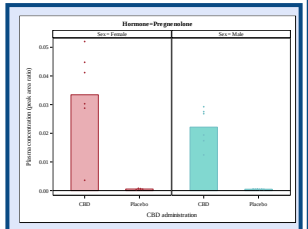


Figure 6. Precursor steroid plasma concentration changes with CBD administration.

Type	Hormone	Sex	CBD administration	P-value (CBD vs Placebo)
Glucocorticoids	Corticosterone	Female	CBD	0.0008
			Placebo	
		Male	CBD	<.0001
			Placebo	
	Cortisol	Female	CBD	0.6359
			Placebo	
		Male	CBD	0.0005
			Placebo	
	Cortisone	Female	CBD	0.8247
			Placebo	
		Male	CBD	0.0288
			Placebo	
Mineralocorticoid	21-hydroxy progesterone	Female	CBD	0.0011
			Placebo	
		Male	CBD	0.0009
			Placebo	
Precursor steroid	Pregnenolone	Female	CBD	0.0008
			Placebo	
		Male	CBD	<.0001
			Placebo	
Sex-related steroids	Dehydroepian drosterone	Female	CBD	0.0031
			Placebo	
		Male	CBD	0.0009
			Placebo	
	Progesterone	Female	CBD	0.1129
			Placebo	
		Male	CBD	0.0239
			Placebo	

Table 1. T-test results for hormone concentrations following CBD administration.

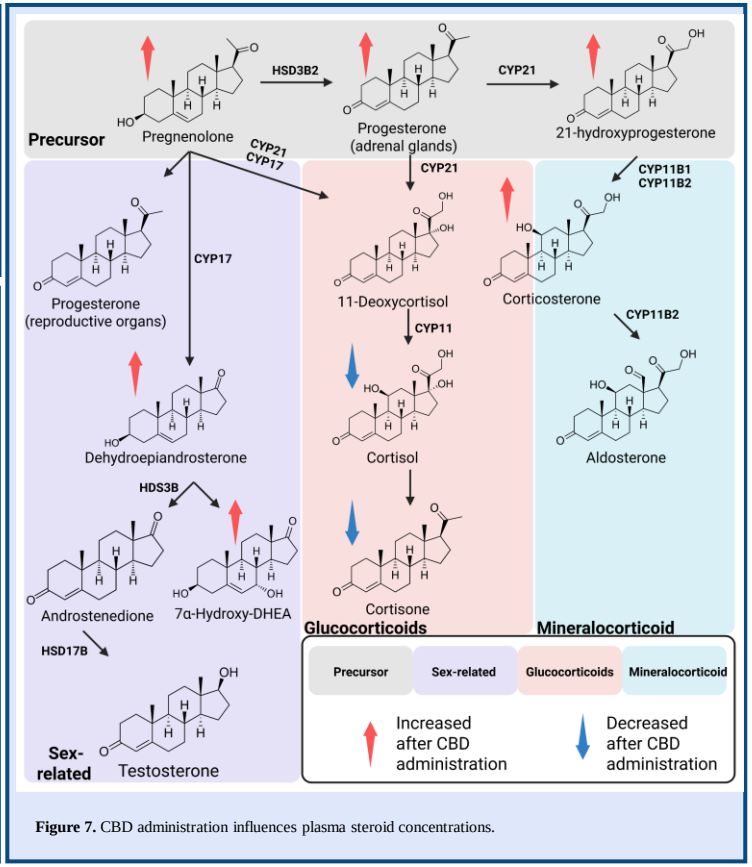


Figure 7. CBD administration influences plasma steroid concentrations.

CONCLUSIONS

- Exposure to short-term high-dose oral CBD was associated with the following steroid hormone changes:
- In males: **Significant decrease in cortisol, cortisone, progesterone**
- In both sexes: **Significant increase in pregnenolone, DHEA, 7α-hydroxy-DHEA, corticosterone, and deoxycorticosterone**
- No significant changes observed in: 11-deoxycortisol, testosterone, androstenedione, or aldosterone
- These changes may be related to the inhibition of CB_1 receptors within the HPA axis

LIMITATIONS

- The sample size was relatively small (n = 12)
- Inter-individual variability in hormone levels, particularly across time and female menstrual cycles, requires further investigation
- Certain hormones, such as estrogens, were not measured due to analytical challenges
- Hormone concentrations were expressed in relative units, preventing direct comparison with established clinical reference ranges

REFERENCES

FUTURE DIRECTIONS

- Comprehensive analysis of circulating estrogen levels to explore sex-specific responses
- Validation of findings through replication in a larger populations
- In vitro enzyme kinetics studies to assess CBD's impact on key CYP enzymes involved in steroidogenesis
- Mechanistic pharmacology investigations of CBD's influence on steroidogenesis via CB_1 receptor activation

ACKNOWLEDGEMENTS

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