Helena Rouru

Effects of hormonal contraception on brain CB1 receptor availability in females

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HELENA ROURU: Effects of hormonal contraception on brain CB1 receptor availability in females

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Abstract

The endocannabinoid system is an important modulatory system of the brain. It consists of cannabinoid receptors type 1 and type 2 (CB1R and CB2R), the endogenous cannabinoids that bind to these receptors, and enzymes that synthetize and metabolize endogenous cannabinoids. The endocannabinoid system partakes in brain development, brain plasticity and cognitive function. It has also been associated with several psychiatric disorders. Previous studies conducted with human and animal subjects indicate that there may be gender differences in the endocannabinoid system. This difference has been suggested to be transmitted via gonadal hormones, especially estrogen.

The aim of this study was to examine whether combined oral contraception has impact on brain CB1R availability in healthy women. Somatic diseases were ruled out by laboratory tests, EKG and somatic status. Psychiatric axis I disorders were ruled out by structured psychiatric interview. MRI was done to all subjects to rule out structural anomalities.

PET-imaging were performed using the CB1R specific radioligand [^{18}F]FMPEP-d2. Emission data was gathered first for 60 minutes, and continued for another 30 minutes after a 30 minute break. The activity of the radioligand and its metabolites in blood was measured using arterial blood samples. Continuous blood samples were taken for the first 3.5 minutes, and after that manual samples were drawn from the artery cannula. The radioligand activity concentrations in plasma were corrected to correspond to unchanged radioligand activity in each timepoint. CB1R availability was quantified using modeling of distribution volume with Logan plot. We used repeated measures ANOVA to test the difference between the groups.

In this study we found that women who used combined oral contraception had significantly lower distribution volume compared to women who did not use hormonal contraception.

Our finding supports the previous evidence of gonadal hormones affecting the endocannabinoid system. The phase of ovarian cycle was not known in all subjects and blood estrogen levels were not measured in this study. However, results indicate a need to consider hormonal pharmaceutical use and the hormonal status of human subjects when studying the endocannabinoid system.

Keywords: Endocannabinoid system, CB1-receptor, combined oral contraception

1. Introduction

The endocannabinoid system is a widespread neurotransmitter system of the brain. It consists of two main cannabinoid receptors, endocannabinoid receptors 1 and 2, the endogenous endocannabinoid receptor agonists 2-arachidonoylglycerol (2-AG) and N-arachidonoyl-ethanolamide or anandamide (AEA), and the enzymes that are responsible for the synthesis and degradation of these endocannabinoids (Lu *et al.*, 2016). AEA is mostly degraded by fatty acid amide hydrolase (FAAH), while 2-AG is broken down by both monoacylglycerol lipase (MAGL) and FAAH. Cannabinoid receptors are G-protein coupled receptors serving neuromodulatory functions. Cannabinoid receptor type 1 (CB1R) is most abundant in the central nervous system, while the cannabinoid type 2 receptor (CB2R) is primarily expressed in peripheral tissues (Gorzkiewicz *et al.*, 2018). The effect of the endocannabinoids on neurotransmission is mainly transmitted via the CB1 receptors (Chanda *et al.*, 2019), which is why the role of CB2 receptor is not discussed in this review.

Recent evidence suggests that the endocannabinoid system may play a part in several psychiatric disorders (Ibarra-Lecue *et al.*, 2018). When compared to healthy individuals, differences in the endocannabinoid system have been reported in patients suffering from depression, schizophrenia, PTSD, anxiety diseases and some neurodegenerative diseases such as Alzheimers disease and Parkinsons disease (Sloan *et al.*, 2018). However, some of the evidence is inconclusive (Ibarra-Lecue *et al.*, 2018). Furthermore, in recent years the endocannabinoid system has been investigated as a potential target for pharmacological therapies. (Davies *et al.*, 2019)

Evidence from in vivo human positron emission tomography studies of CB1R is accumulating. However, combined sample sizes in these studies are still relatively small and heterogenous between studies, which hampers drawing conclusions of disease specific associations (Van Laere *et al.*, 2008; Normandin *et al.*, 2015; Laurikainen *et al.*, 2019). For example sex and gonadal hormone status have been suggested to affect the results of CB1R studies (Laurikainen *et al.*, 2019).

1.1. Gender differences and the CB1 receptor

There is evidence of gender differences in the expression of CB1 receptor in certain brain areas. In mice, one study showed significantly higher amounts of CB1 receptor mRNA transcripts in intact males compared to female rats in the anterior pituitary gland (González *et al.*, 2000). Another study reported higher levels of CB1R mRNA in the cerebellum and brain stem of female mice (Xing *et al.*, 2011). Higher levels of mRNA in female rats compared to males have been also reported in the amygdala, hippocampus and prefrontal cortex (Xing *et al.*, 2014). Hippocampal CB1 receptor protein expression has been shown to be higher in male mice compared to female mice (Reich *et al.*, 2009), and CB1 receptor density has been shown to be higher in the mesencephalon and hypothalamus of male versus female rats (de Fonseca *et al.*, 1994; Riebe *et al.*, 2010). There have also been some reports that suggests females to have higher brain CB1 receptor density. One study reported that female rats have higher cannabinoid receptor protein density in the amygdala compared to males (Riebe *et al.*, 2010). Zamberletti and colleagues (2012) reported that female rats have higher overall expression of CB1 protein in the brain. Higher CB1 receptor protein expression has been also reported separately in the frontal cortex, parietotemporal cortex and thalamus of female rats compared to males (Takkinen *et al.*, 2018).

Higher overall CB1R availability in females compared to males has been reported in human in vivo PET studies (Neumeister *et al.*, 2013; Normandin *et al.*, 2015), while contradictory results showing

higher CB1 receptor availability in males compared to females have also been reported (Van Laere *et al.*, 2008; Laurikainen *et al.*, 2019).

To summarize, the results regarding sex differences of the human endocannabinoid system are inconclusive. Methodological differences, such as the use of different radioligands and primary outcome measures in human PET studies, may partly explain the discordant results of the studies outlined above (Neumeister *et al.*, 2013; Laurikainen *et al.*, 2019). Further, the hormonal status of female subjects has been largely overlooked as a potential source of variation when measuring the human endocannabinoid system.

1.2. Gonadal hormones

The gonadal hormones estrogen, progesterone and androgens are lipophilic steroid hormones. They can pass the blood-brain barrier to access the central nervous system by diffusion or via specific transporter molecules (Banks, 2012). Neurosteroid hormones can also be synthetized in the brain from cholesterol and other steroidal precursors (Plassart-Schiess *et al.*, 2001). Neurosteroids have their own specific nuclear and membrane associated receptors in the brain, but they also act as a modulators of other receptors and are thus posed to impact brain function through various mechanisms (Plassart-Schiess *et al.*, 2001).

Estrogen has been shown to associate to memory functions, mood and mental state in humans (Fink *et al.*, 1996). For example, decreased levels of estrogen are associated with depression and premenstrual syndrome (Fink *et al.*, 1996). Oral contraceptive use has also been suggested to affect the mood in some individuals (Skovlund *et al.*, 2016). De Witt et. colleagues (2019) recently reported that female subjects under 16 years old using oral contraceptives scored higher in a depression symptom self-report scale compared to similarly aged females who were not using oral contraceptives.

Gorzalka and Dang (2012) suggested that the endocannabinoid system and gonadal hormones have a bidirectional impact on each other. They stated that gonadal hormones seem to affect functions associated to the endocannabinoid system and that in addition, CB1 receptors of the brain seem to be abundant in brain areas which are also affected by estrogen. Furthermore, various studies imply that endocannabinoid system could be altered by gonadal hormone status, particularly estrogen (de Fonseca *et al.*, 1994; Riebe *et al.*, 2010).

1.3. Gonadal hormones and the endocannabinoid system

In one animal study conducted with rats, ovariectomy (OVX) resulted in decreased cannabinoid receptor protein density in the limbic forebrain (de Fonseca *et al.*, 1994). This effect reported by Fonseca et al. was reversable by the administration of estradiol or progesterone. Further, chronic estradiol treatment seemed to increase the density of cannabinoid receptors in both intact females and ovariectomized females. (de Fonseca *et al.*, 1994). A study conducted by Riebe et al. (2010) showed that OVX female rats had increased CB1R ligand binding in the hippocampus, hypothalamus and decreased binding in the amygdala when compared to intact females and estradiol-treated females. Another study found that estradiol treated OVX rats had significantly lower CB1 receptor-mRNA levels in the anterior pituitary gland (González *et al.*, 2000).

CB1 receptor expression has also been suggested to fluctuate in some brain areas throughout the ovarian cycle (González *et al.*, 2000). For example, CB1 receptor mRNA transcripts measured from

the anterior pituitary gland fluctuated during the different phase of the ovarian cycle (González *et al.*, 2000). Accordingly, another study reported significant variation of cannabinoid receptor binding in the medial basal hypothalamus and the limbic forebrain throughout the phases of the ovarian cycle (de Fonseca *et al.*, 1994). In this study, cannabinoid receptor binding was reported to be lowest during the estrus and highest during the diestrus (de Fonseca *et al.*, 1994). The activity FAAH, the enzyme responsible for metabolizing anandamide, has been reported to be decreased by the effect of estrogen and progesterone in mouse uterus (Maccarrone *et al.*, 2000), while the relocation of estrogen receptor alpha has been reported to downregulate the expression of the FAAH gene (Waleh *et al.*, 2002).

To our knowledge only one study has investigated the effects of oral contraception on CB1R binding (Van Laere *et al.*, 2008). No significant differences between women using oral contraceptives compared to women not using oral contraceptives were found in this study. Unfortunately, the type of oral contraception used by the participants was not explicitly stated so it is possible that the group consisted of subjects using progestin contraception as well as combined contraceptives with both estrogen and progestin effects. These two different oral contraception methods might have a differential effect on the endocannabinoid system.

1.4. Behavioral associations

The behavioral effects of phytocannabinoids have been reported to have gender differences. This could be due to differences in gonadal hormones acting as modulators of phytocannabinoid receptor CB1R, but possibly also due to the developmental effects of gonadal hormones (Craft *et al.*, 2013, 2017). The pharmacological effects of cannabinoids which are more prominent in men include changes in food intake, energy homeostasis and decreased sexual behavior (Fattore *et al.*, 2010). In women exogenous cannabinoids have more prominent effects in analgesia, motor activity, symptoms of depression, catalepsy, anxiety, and increase in sexual behavior (Fattore *et al.*, 2010).

In animal studies the antinociceptive effects of Δ^9-tetrahydrocannabinoid (THC), the primary psychoactive compound of phytocannabinoids, has been reported to be dependent on gonadal hormone status (Wakley *et al.*, 2011; Craft *et al.*, 2017). Estrogen increases the antinociceptive effects of THC in males (Craft *et al.*, 2017) and OVX females (Craft *et al.*, 2008), while it does not enhance antinociceptive effects in hormonally intact females (Craft *et al.*, 2017). There is also support for gonadal hormone involvement in modulation of fear responses based on reports that fear responses of OVX rats were increased compared to intact females (Simone *et al.*, 2015). However, since estrogen replacement therapy did not affect the responses of the OVX rats the response doesn't seem to be directly estrogen dependent. The same study also reported that the fear modifying effect of agonist or antagonist action on the CB1 receptor was not affected by estrogen. (Simone *et al.*, 2015).

The estrogen system seems to be affected by the endocannabinoid system. This has been speculated to be caused by the central downregulation of luteinizing hormone (LH) and gonadotropin releasing hormone (GnRH) expression (Gorzalka *et al.*, 2012) via endocannabinoid effects on the hypothalamus (Tasker *et al.*, 2015). THC administration has been documented to decrease the amount of LH in brain in ovariectomized rats (Tyrey, 1978).

Out of the gonadal hormones estrogen has been most convincingly shown to affect the endocannabinoid system. However, progesterone has been also reported to upregulate the FAAH enzyme in human lymphocytes (Maccarrone *et al.*, 2001, 2003). The implication of gonadal hormone actions in human endocannabinoid system remain unclear. Therefore, in this study, we examine how

combined oral contraceptive pills affect the binding in CB1R. We compared the availability of CB1R in 18-40 year old men, similarly aged women using combined oral contraceptive pills and women with no contraception using positron emission tomography and the specific CB1R radioligand [^{18}F]FMPEP-d2.

2. Methods

We expanded the previously published (Laurikainen *et al.*, 2019) study sample of 11 healthy males and 11 healthy females to include more females using combined contraceptive pill and females without contraception. Recruitment of subjects was done from the national population registry, local educational institutions, and by local newspaper advertisement. Inclusion and exclusion criteria were the as in the study by Laurikainen et al. (2019). Namely, current somatic and lifetime psychiatric illnesses were ruled out by medical examination, blood and urine tests, electrocardiography, and a structured clinical interview for DSM-IV axis I disorders (SCID-I/NP). Lifetime cannabis use was documented, and current use was ruled out with a urine screen prior to the PET scan. Subjects with a lifetime substance-related disorder, DSM-IV Axis I diagnosis, or who had used any illicit substances two months prior to scanning, were excluded. Pregnancy was ruled out by urine and/or blood screening. Structural abnormalities were ruled out in all subjects with a structural MRI using the Philips 3T Ingenuity PET/MR hybrid scanner.

Synthesis of [^{18}F]FMPEP-d2, positron emission tomography, plasma input curve preprocessing, ROI definition and tissue activity curve measurements were done as described previously in Laurikainen et al. (2019). Briefly, the subjects were given an intravenous bolus injection of CB1 receptor specific radioligand [^{18}F]FMPEP-d2. Emission data was gathered with the brain-dedicated high-resolution research tomograph in two parts (60 +30 min) so that the total scan range was 0-120 min. Emission data was reconstructed and head motion was corrected. The MR image was processed with freesurfer (http://surfer.nmr.mgh.harvard.edu/). The resulting individualized anatomical atlas was coregistered to the sum of the realigned PET frames. This atlas was then used to extract masks to define volumes of interest (VOI) on the PET time series. Mean time activity curves were gathered from 17 volumes-of-interest (amygdala, anterior cingulate cortex, brainstem, caudate nucleus, cerebellum, frontal cortex, hippocampus, insula, occipital cortex, orbitofrontal cortex, parahippocampal gyrus, parietal cortex, posterior cingulate cortex, prefrontal cortex, putamen, temporal cortex and thalamus).

Blood data analysis was done as described in Laurikainen et al. (2019). After tracer injection, the activity of whole blood was measured first for 3.5 minutes continuously using Allogg ABSS on-line detector and after that with manual arterial plasma samples at 4.5, 7.5, 11,15, 20, 25, 30, 35, 40, 45, 50 and 60 minutes. Plasma metabolite samples were also manually drawn from artery cannula at 4.5, 11, 15, 20, 30, 45 and 60 minutes. Plasma activity was corrected for the amount of unchanged tracer activity in plasma in each time point. The resulting plasma activity concentration curve was used for modeling. In one test subject the first measurement of radioactive metabolites was unsuccessful, thus the population mean was used to replace it.

Tissue time activity curves were visually inspected for quality. In Laurikainen et al. (2019) there were no differences between hemispheric distribution volume measurements. Thus, the volumes of interest containing both hemispheres were used for statistical testing.

2.1. Statistics

Regional [^{18}F]FMPEP-d2 V_T was calculated using Logan plot. Modeling was done with PMOD 3.4 software (PMOD Technologies, Zurich, Switzerland) using Poisson weighting of residuals. Statistics were performed using IBM SPSS Statistics software. We used Shapiro-Wilks test to test the normality assumption of variables. Normality was not met in the putamen, pallidum, orbitofrontal cortex, anterior cingulate cortex and nucleus caudatus, and therefore these areas were not included in the parametric statistical tests. Repeated measures ANOVA was used to test the difference between the three groups.

3. Results

All subjects were of Finnish ancestry. No significant differences were found in smoking status, handedness, age, years of education, average movement during the PET scan or injected tracer activity. BMI did not differ between male and female subjects or between women who were on oral combined contraceptives and women who were not using any contraception. Lifetime cannabis use was greater in men (p=0.037) but cannabis use during the year preceding PET scanning did not differ between men and women (p=0.063). Lifetime cannabis use did not differ between the female study groups, and none of the female subjects had used cannabis in past year prior the PET imaging.

Table 1. Demographics of male and female subjects

	Male	Female	p-value
Age (years)	27.3±5.9	24.7±5.1	0.198
BMI (kg m-2)	25.3±3.74	23.4±2.49	0.259
Years of education	15.7±3.17	15.5±2.86	0.959
AUDIT score	9.09±5.87	5.14±3.42	0.066
Tobacco smokers (N)	1	1	0.677
Lifetime cannabis use >5 times	3	0	0.037
Past year cannabis use < 6 times	3	0	0.063
Injected [18F]FMPEP-d2 activity (MBq)	199.6±13.00	206.1±11.40	0.134
Average frame-to-frame movement (mm)	0.545±0.225	0.471±0.177	0.354

Values are number or mean±standard deviation. BMI = body mass indeks. AUDIT = alcohol use disorders identification test. P-values shown are counted using non parametric tests expect in average movement where t-test was made.

Table 2. Demographics of female subjects.

	Female without contraception	Female with contraception	p-value
Age (years)	25.0-±6.98	24.4±3.11	0.613
BMI (kg m-2)	23.7±3.1	23.2±2.1	0.955
Years of education	15.9±2.98	15.2±2.90	0.397
AUDIT score	15.16±3.49	5.13±3.60	0.755
Tobacco smokers (N)	1	0	0.467
Lifetime cannabis use >5 times	0	0	-
Past year cannabis use < 6 times	0	0	-
Injected [18F]FMPEP-d2 activity (MBq)	202.0±13.91	209.8±7.87	0.232
Average frame-to-frame movement (mm)	0.558±0.118	0.394±0.191	0.072

Values are number or mean±standard deviation. BMI = body mass indeks. AUDIT = alcohol use disorders identification test. P-values shown are counted using non parametric tests expect in average movement where t-test was made.

3.1. Volume of interest analysis

We found a significant difference in V_T between women who were on oral combined contraceptives and women who were not using any contreception (df=1, F=6.514, p= 0.024). The effect was regionally selective (VOI*oral contraception interaction: df= 2.447, F=5.193, p = 0.008). The V_T of CB1R was significantly higher (p=0.024) in women who did not use combined oral contraception

with regionally specific effects (p=0.008). The effect was largest in the prefrontal cortex (η^2= 0.403, t=2.965, p=0.011), frontal cortex (η^2= 0.380, t=2.820, p=0.014), posterior cingulate cortex (η^2= 0.359, t=2.700, p=0.018) and parietal cortex (η^2= 0.356, t=2.678, p=0.019).

We also tested the difference of V_T between male and female subjects. Similarly to the results reported in Laurikainen et al (2019), a significant difference could be observed between men and women (df =1, F=7.11, p=0.013). The male subjects had significantly higher V_T compared to females with combined contraception in all brain areas (df=1, F=14.873, p=0.001) and the effect was regionally selective (df=2.165, F=8.316, p=0.001). The effect was largest in the occipital cortex (η^2=0.502, t=-4.140, p=0.001), parietal cortex (η^2= 0.502, t=-4.140, p=0.001), posterior cingulate cortex (η^2=0.507, t=-4.180, p=0.001), prefrontal cortex (η^2= 0.496, t=-4.089, p=0.001) and frontal cortex (η^2= 0.474, t=-3.916, p=0.001). However, when female subjects without oral contraception were compared to males, the difference was not significantly different (df=1, F=0.909, p=0.355).

4. Discussion

Cannabinoid receptor type 1 availability was significantly lower in females who were using combined oral contraception compared to women who did not have hormonal contraception. These findings are consistent with previous reports of the effect of gonadal hormones on regulation of the CB1 receptor expression in the brain. Females without oral contraception did not differ in CB1 availability from male subjects. Therefore, the previously reported difference of CB1R availability between females and males could be driven by the female subjects who were using combined oral contraception. This can also be one of the explanations to why the results of human studies concerning sex difference in CB1R availability have been inconsistent since most studies haven't accounted for the hormonal status of female subjects.

The endocannabinoid system has been associated to modulation of cognitive functions. Also oral contraceptive use seems to also affect cognitive test performance (Gogos *et al.*, 2014). For instance, improved memory task performance and visual object recognition after treatment with estradiol has been reported in animal studies using ovariectomized rats (Bimonte *et al.*, 1999; Luine *et al.*, 2003; Lewis *et al.*, 2008). A study using OVX monkeys reported enhanced visuospatial attention after estrogen treatment (Voytko, 2002). However, the effect can be task selective as indicated by a study reporting that face recognition of ovariectomized rhesus monkeys was impaired by admission of ethinylestradiol (Lacreuse *et al.*, 2003).

In humans sex hormones seem to affect verbal memory performance of freely cycling premenopausal women. For instance, better verbal memory was observed in the high estrogen period of the menstruation cycle compared to the other phases of the menstrual cycle (Maki *et al.*, 2002; Rosenberg *et al.*, 2002). Oral contraceptive users have also been found to develop the conditional eyeblink response quicker compared to the non-users in a classically conditioned eyeblink test (Beck *et al.*, 2008; Holloway *et al.*, 2011), and to have enhanced verbal memory in the active pill phase compared to the inactive pill phase when assessed with the California Verbal Learning Test (Mordecai *et al.*, 2008). However, in this study by Mordecai et al. verbal memory performance did not differ throughout the menstrual cycle phases of normally cycling females.

One study compared the recall of emotional memory of a story between naturally cycling women and women using hormonal contraception. The authors found that women using hormonal contraception had better memory of the story gist but could not recall as many details in the emotional compared

with neutral story conditions. Women without hormonal contraception had better memory of details but not the gist in the emotional compared with neutral story conditions (Nielsen *et al.*, 2011) This is particularly interesting since it has been speculated that endocannabinoid system plays a regulatory role in consolidation of emotional memories (Ney *et al.*, 2018).

Studies about mood changes associated with oral contraceptive (OC) use are inconsistent. There are reports of OC use improving the mood in most women (Oinonen *et al.*, 2002), but also some evidence about them increasing depression and anxiety (Robinson *et al.*, 2004; De Wit *et al.*, 2019). However, in most studies women have been allowed to make the choice to continue using OCs, which might lead to overemphasizing of benificial mood changes, since women who have experienced negative effects could have stopped using OCs (Oinonen *et al.*, 2002).

Evidence supporting regulation of mood and anxiety by the endocannabinoid system have also been reported. Cannabinoid receptors are populous in brain areas associated to emotional state processing, such as the amygdala, hippocampus and limbic areas (Ashton *et al.*, 2011). They also seem to be involved in the regulation of emotional states (Witkin *et al.*, 2005). It has been suggested that the endocannabinoid system in the prefrontal cortex may have a critical role in shaping the prelimbic circuits during stress (Worley *et al.*, 2018).

Altogether the currently available evidence suggests that estrogen has domain specific but not global effects on cognitive functions (Gogos *et al.*, 2014) and regulation of emotional states (Ashton *et al.*, 2011). Since the endocannabinoid system is also known to regulate these functions it is well poised to mediate the effects of estrogen. In our study the largest difference between CB1R availability in contraceptive users compared to non-users was on frontoparietal cortices, such as the prefrontal cortex which is involved in inhibiting distracting information, planning, making decisions and working memory (Shanmugan *et al.*, 2014).

Oral combined contraception might also affect the structure of certain brain areas. A study conducted by Plezer et al (2010) reported significantly larger prefrontal cortices, pre- and postcentral gyri, parahippocampal and fusiform gyri and temporal regions in women using hormonal contraceptives compared to women not using contraceptives. Another study found that gray matter volume was larger in the right mid-frontal gyrus of the females using combined oral contraceptives (De Bondt *et al.*, 2013).

It is noteworthy that the estrogen used almost predominantly in combined oral contraceptives is ethinylestradiol, which is a synthetic form of the endogenous E2 hormone (Gogos *et al.*, 2014). Ethinylestradiol has been found to be unable to convert to E1 or other weaker estrogen, which makes it more potent compared to endogenous E2 (Gogos *et al.*, 2014). Estrogen and progesterone both have a negative feedback system through which endogenous hormone levels can be suppressed in women using combined oral contraceptives (Mordecai *et al.*, 2008). It is also important to remark that oral contraceptives have also been found to reduce the blood levels of testosterone (Zimmerman *et al.*, 2014), which also might have additional impacts on brain function and structure. These differences illustrate how hormonal contraception pills could have different impact on the brain compared to endogenous estrogens and progesterone.

The progesterone component in combined oral contraceptive pills is another factor which might affect the results of human studies of oral contraceptives. There are different generations of progestins, some with anti-androgenic properties and some with androgenic properties. There is some evidence that the varying progestins used in OC pills might have different effects on the brain. One study reported that androgenic progestin users performed worse in verbal fluency task when compared to

anti-androgenic progestine users and normally cycling women (Griksiene *et al.*, 2011). Unfortunately, the blood levels of progestines used in combined oral contraception pills were not measured in this study and therefore no further analysis can be made about the role of progestines in the variability of CB1R availability.

Another limitation of this study is the small group sizes, which predisposes to type II error and limits generizability of the results. The estrogen blood levels of the female subjects were not measured, so the associations estrogen levels to CB1R availability could not be studied. Further, the phase of ovarian cycle was not documented in all female subjects so further analysis could not be performed.

5. Conclusions

We found significantly lower CB1R availabilities in females who used oral combined contraception compared to females without contraception. A difference between male and female subject CB1R availability was observed, but there was no significant difference between male subjects and women who were not using oral contraception. Therefore, the difference between men and women seems to be driven by results from the women using combined oral contraception. These results indicate a need to consider hormonal pharmaceutical use and the hormonal status of human subjects when studying the endocannabinoid system.

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