Delta-9-Tetrahydrocannabinol, Cannabidiol and Psychosis

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ABSTRACT

Use of cannabis has been linked to an increased risk of psychotic disorders and to impairments in cognitive function. Both of these effects have been attributed to its main psychoactive constituent, delta-9-tetrahydrocannabinol (THC). However, much of the evidence for these effects comes from studies that involved retrospective estimates of cannabis use, which may introduce a recall bias, particularly in people with psychosis. One way to address this issue is to study the effects of cannabis use prospectively in people at clinical high risk (CHR) for psychosis, a proportion of whom will subsequently develop psychosis.

The second major constituent of cannabis is cannabidiol (CBD). CBD is non-intoxicating, and there is some evidence that it may attenuate the adverse effects of THC. This possibility can be investigated by assessing the effects of cannabis containing different doses of THC and CBD.

This thesis aims to address two key questions: i) What are the consequences of cannabis use on the clinical outcomes and cognitive performance of individuals at CHR? ii) What effect does CBD have on the effects of THC on endocannabinoid signalling?

Chapter 1 provides an overview of the current literature on cannabis, THC, CBD, and the endocannabinoid system, focussing on the impact of cannabis use on the risk of psychosis and on cognitive functioning. It also describes the CHR state and its potential utility in studies of cannabis in psychosis.

In **Chapters 2** and **3**, I use data from a large, multisite, naturalistic study of individuals at CHR to examine the relationship between cannabis use and i) clinical outcomes and ii) cognitive performance. I found no evidence that cannabis use was related to the later onset of psychosis, the persistence of symptoms, or level of functioning. Although cannabis use has been associated with cognitive deficits in the general population, I found that impairments in cognitive performance in CHR individuals who had used cannabis were less severe than in those who had never used cannabis.

In **Chapter 4**, I examine the effects of cannabis containing varying ratios of CBD:THC on the peripheral endocannabinoid system in healthy volunteers. I found that THC altered the plasma concentration of endocannabinoid signalling molecules and biologically related lipids, and that these effects were not influenced by the co-administration of CBD.

Finally, in **Chapter 5**, I bring together the findings from **Chapters 2**, **3** and **4**. I discuss their collective implications, review the limitations of each study. I then consider key challenges to future research on cannabis in relation to psychosis.

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STATEMENT OF PERSONAL CONTRIBUTION

There are three experimental chapters included within this thesis. **Chapters 2** and **3** present data collected from a multi-national research project, the European Network of National Schizophrenia Networks Studying Gene-Environment Interactions (EU-GEI) High Risk study. For **Chapter 4**, I conducted recruitment and data collection alongside study clinicians, and independently conducted data management and cleaning. In addition, I led the management of the biological samples and organised their transport on dry ice to laboratories in London and Finland.

I developed the aims and hypotheses for this thesis, under the supervision of Prof Philip McGuire and Dr Matthew Kempton. The planning and implementation of the statistical analyses in this thesis were conducted by myself with advice from my supervisors and relevant experts. Finally, I wrote this thesis in its entirety, with the following exceptions: after being drafted by me, i) the published article[1] and one article 'under revision' had input from co-authors and underwent peer-review in the publication process, and ii) the article 'in preparation' had input from co-authors which led to editing of the manuscript. The study in **Chapter 4**[1] was conceived of and designed by Amir Englund, who is credited as joint first author in the published article.

1. Chester LA, Englund A, Chesney E, et al. Effects of Cannabidiol and Delta-9-Tetrahydrocannabinol on Plasma Endocannabinoid Levels in Healthy Volunteers: A Randomized Double-Blind Four-Arm Crossover Study. *Cannabis Cannabinoid Res*. 2022;ahead of p. doi:10.1089/can.2022.0174

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LIST OF ABBREVIATIONS

2-AG	2-Arachidonoyl glycerol
AA	Arachidonic acid
ABHD	α/β hydrolase domain-containing protein
AEA	Anandamide
aLEA	Alpha-linolenoylethanolamide
APS	Attenuated psychotic symptoms
ARA-S	N-Arachidonoyl-L-serine
AUC	Area under the curve
AVLT	Rey Auditory verbal learning task
BAC	Blood alcohol concentration
BLIP	Brief limited intermittent psychotic symptoms
BMI	Body mass index
BP	Blood pressure
CAARMS	Comprehensive Assessment of At-Risk Mental State
CBD	Cannabidiol
CBR1	Cannabinoid receptor type-1
CBR2	Cannabinoid receptor type-2
CEQ	Cannabis Experience Questionnaire
CHR	Clinical High Risk for psychosis
CNS	Central Nervous System
CO	Carbon monoxide
COX-2	Cyclo-oxygenase-2
CRF	Clinical Research Facility
CSF	Cerebrospinal fluid
CTQ	Childhood Trauma Questionnaire
CU	Cannabis user
CUD	Cannabis use disorder
DAGL	sn-1-Diacylglycerol lipase
DAST-20	Drug Abuse Screening Test
DEA	Docosatetraenylethanolamide

DSM	Diagnostic and Statistical Manual of Mental Disorders
ECS	Endocannabinoid system
EMCDDA	European Monitoring Centre for Drugs and Drug Addiction
EMM	Estimated marginal mean
EU-GEI	European Network of National Schizophrenia Networks Studying Gene-Environment Interactions study
FAAH	Fatty acid amide hydrolase
FEP	First-episode psychosis
GAF	Global Assessment of Functioning
GDE-1	Glycerophosphodiester phosphodiesterase I
GEST	Rosner's generalised extreme Studentised deviate test
GMP	Good Manufacturing Practice
GPR	G-protein coupled receptor
GRD	Genetic risk and deterioration syndrome
HPA	Hypothalamic–pituitary–adrenal axis
HR	Hazard Ratio
IQ	Intelligence Quotient
IV	Intravenous
lyso-PI	lysophosphatidylinositol
lyso-PLC/D	lysophosphatidylinositol-selective phospholipase C/D
MAGL	Monoacylglycerol lipase
MCAR	Missing completely at random
MICE	Multiple imputation chain equations
MS	Mass spectrometry
NAAA	N-Acylethanolamine-hydrolysing acid amidase
NAE	N-Acylethanolamine
NAPE	N-Acylphosphatidylethanolamine
NAPE-PLD	N-Acylphosphatidylethanolamine-specific phospholipase D
NAT	N-Acyltransferase
NIHR	National Institute of Health Research
NHS	National Health Service
NU	Non-cannabis-user

OEA	Oleoylethanolamide
OR	Odds Ratio
PANSS	Positive and Negative Syndrome Scale
PBMC	Peripheral blood mononuclear cell
PET	Positron emission tomography
PI	Phosphatidylinositol
PIP2	PI-4,5-biphosphate
PPARα	Peroxisome proliferator activated receptor-α
PSI	Psychotomimetic states inventory
SCID	Structured Clinical Interview for DSM-IV
SEA	Stearoylethanolamide
SES	Socioeconomic status
THC	Delta-9-tetrahydrocannabinol
TMT	Trail Making Test
TRPV1	Vanilloid receptor 1
UHPLC	Ultra-High Pressure Liquid Chromatography
VERFL	Verbal fluency test
WAIS-III	Wechsler Adult Intelligence Scale-III

PREFACE

This thesis is a "thesis incorporating publications", referring to the fact that three chapters comprise of journal articles of which I am the first author. Publications relating to the work presented in this thesis include one published article, one article 'under revision' in the peer-review process and one article 'in preparation', all of which are reproduced in full:

Chapter 2, **Paper 1**: **Chester LA**, Valmaggia LR, Kempton MJ, Chesney E, Oliver D, Hedges EP, Klatsa E, Stahl D, van der Gaag M, de Haan L, Nelson B, McGorry P, Amminger GP, Riecher-Rössler A, Studerus E, Bressan R Barrantes-Vidal N, Krebs MO, Glenthøj B, Nordentoft M, Ruhrmann S, Sachs G, McGuire PK, for the EU-GEI High Risk Study Group. Influence of cannabis use on incidence of psychosis in people at clinical high risk. *Psychiatry Clin Neurosci.* 2022 [In Revision]

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Chapter 4, **Paper 3**: **Chester LA**, Englund A, Chesney E, Oliver D, Wilson J, Sovi S, Dickens AM, Oresic M, Linderman T, Hodsoll J, Minichino A, Strang J, Murray RM, Freeman TP, McGuire PK. Effects of Cannabidiol and Delta-9-Tetrahydrocannabinol on Plasma Endocannabinoid Levels in Healthy Volunteers: A Randomized Double-Blind Four-Arm Crossover Study. *Cannabis Cannabinoid Res*. 2022;ahead of print. doi:10.1089/can.2022.0174

CHAPTER 1 - GENERAL INTRODUCTION

1.1 CANNABIS SATIVA

The *Cannabis sativa* plant has been used medicinally and recreationally for thousands of years.[1] Today, cannabis has more users worldwide than all other illicit drugs combined.[2,3] An estimated 30% of the public in the UK have tried cannabis at some point in their lives.[4]

1.1.1 DELTA-9-TETRAHYDROCANNABINOL

Delta-9-tetrahydrocannabinol (THC) is one of almost 150 cannabinoids produced by the *Cannabis sativa* plant, and one of the few produced in substantial quantities.[5] Gaoni and Mechoulam were the first to extract THC from hemp in 1964 (see **Supplementary Figure 4-2** of **Chapter 4** for molecular structure).[6] THC mainly exerts its effects via the endocannabinoid system (ECS), acting as a partial agonist at the type-1 and type-2 cannabinoid receptors, CB1R and CB2R.[7,8] THC is known for producing a euphoric 'high' or 'stoned' effect, as well as adverse reactions, such as psychotomimetic effects.[9]

The phytocannabinoids, including THC, are produced by the glandular trichomes (specialised hairs) of the female cannabis flowers. Cannabis is most commonly consumed by smoking the dried flowers, or 'buds', in a joint or bong. Dried buds can also be vaped or cooked into food and eaten, or trichomes can be extracted as a resin (commonly known as hash) or, less commonly, into high-potency concentrates such as 'shatter' or 'butane hash oil'.[10] Recent decades have seen a dramatic increase in the potency, i.e. percentage THC content, of cannabis on the market.[11] Higher potency strains, sometimes known as 'sinsemilla', or more colloquially as 'skunk' in the UK or 'nederwiet' in the Netherlands, have much greater THC concentrations and extremely low or negligible CBD concentrations compared with the low-potency 'herbal' varieties that were once much more common.[10,12] Sinsemilla, meaning 'without seeds', is produced by segregating female plants indoors to prevent pollination, forcing more of the plants' energy resources into the production of trichomes.[10] Investigations into cannabis seized by police suggest that high-potency skunk now dominates the illicit market in the Europe, with the mean THC content of cannabis buds doubling from an estimated 5% to 10% from 2006 to 2016.[10] In the USA, where a number of states have

legalised the use of cannabis for either medicinal or recreational purposes, the mean THC concentration of plant material confiscated by the Drug Enforcement Administration rose from 6.0% in 2008 to 13.6% in 2017.[13] THC concentrations in resins typically vary much more than in herbal cannabis material but were until recently considered to be a less potent alternative. In most Europe now, however, cannabis resin is around twice as potent as herbal cannabis, having increased from an estimated average of 8% THC in 2006, to 17% in 2016, to between 20 and 28% in 2019.[10,11] In the USA cannabis resins are typically even more potent, and mean THC concentrations of confiscated materials have range from 22.8% in 2008, to 15.5% in 2016 with a subsequent sudden rise to their highest recorded level of 45.9% in 2017.[13] As resins are formed from the concentration of trichomes, the potency of the finished product is highly dependent on the plant material it is produced from. Recent reports suggest that the Moroccan resin production has moved away from traditional 'kif' crops to more potent strains, leading to the market increases seen in Europe.[14,15]

The psychoactive, intoxicating, and physiological effects of cannabis are mainly due to its THC content.[9] Cannabis users report an array of uplifting mood effects. THC intoxication can last for several hours (the length of time depending on administration route)[16] and can cause feelings of euphoria, calmness, creative thinking, heightened sensory perception and social disinhibition.[17–19] In addition to its pleasurable effects, cannabis use can lead to harms both acutely and with repeated use. Common adverse reactions to THC intoxication include dry mouth, dizziness, nausea and vomiting, memory problems, lack of motor coordination, and confusion, as well as acute anxiety, paranoia, dissociation, and psychotic-like symptoms.[20–24] Tolerance to the acute effects of THC intoxication builds with repeated use.[20,23] However, with more use comes a higher risk of long-term adverse effects including cannabis dependence, cognitive decline, depression and, possibly, psychotic disorder.[9,25–27] Around 35% of all people entering specialised drug treatment in Europe now do so for problems related to cannabis use.[11]

1.1.2 CANNABIDIOL

CBD, the second most abundant cannabinoid produced by cannabis, has quite different effects to THC despite their similar molecular structure (see **Supplementary Figure 4-2** of **Chapter 4** for molecular structure).[5,28] CBD is non-intoxicating and has very low affinity for

the orthosteric sites of the cannabinoid receptors.[7,8,29] However, there is some evidence that it may act as a non-competitive inverse agonist at CB1R[30–32] and as an inverse agonist of CB2R.[31] A number of non-cannabinoid receptor targets of CBD have also been suggested.[33–39] Its broad spectrum of pharmacological activity may account for its widespread effects including anti-inflammatory,[40] antiepileptic,[41] cytotoxic (in cancer cell lines),[42] anxiolytic[43] and possible antipsychotic properties.[44]

Antipsychotic effects

CBD has been found to be non-inferior to antipsychotic medication in a 4-week clinical trial in acute schizophrenia,[45] and improved psychotic symptoms when used as an add-on therapy for schizophrenia during a 2017 clinical trial.[46] However, in a 6-week phase II, placebo controlled trial in patients with chronic schizophrenia, CBD was well tolerated but did not lead to any improvement in psychotic symptoms in stable antipsychotic-treated patients with chronic schizophrenia.[47]

Whilst several mechanisms of action of CBD's antipsychotic effect have been proposed, so far none have been confirmed.[48,49] One plausible mechanism is via the enhancement of ECS signalling via upregulation the endogenous cannabinoid ligand N-arachidonoyl ethanolamine, or anandamide (AEA). In vitro experimentation has shown that CBD can reduce AEA degradation by inhibiting both its cellular reuptake via the 'anandamide membrane transporter' and its hydrolysis catalysed the intracellular enzyme fatty acid amide hydrolase (FAAH).[36] In a clinical trial conducted by Leweke and colleagues, CBD was found to increase plasma AEA concentrations in patients with schizophrenia, an effect which was correlated with less severe psychotic symptoms.[45] In patients with schizophrenia, increased AEA levels have been linked with less severe psychotic symptoms and remission from acute psychosis. In addition, increased AEA signalling in individuals identified as being at high risk of psychosis has been associated with a lower rate of transition to a psychotic disorder.[50]

Neuroprotective effects

It has been suggested that CBD has neuroprotective effects in cannabis.[44,51] Results from cross-sectional studies have found that individuals with detectable levels of THC and CBD in their hair had fewer psychosis-like experiences and memory-impairing effects associated

with cannabis use than those with THC alone.[52–54] Experimental trials, however have yielded mixed results.[55] While two double-blind, randomised studies have shown that pre-treatment with CBD (either 5mg intravenously or 600mg orally) ameliorates the psychotomimetic effects of THC,[28,56] two others found no such effect.[57,58] Results are equally inconsistent when assessing THC-induced cognitive deficits. For example, while Englund and colleagues[56] found that pre-treatment with 600mg CBD (orally) ameliorated impairment of delayed recall in a verbal learning task from 1.5mg of intravenous THC, Morgan and colleagues[57] found the same level of episodic memory impairment when vaporised THC (8mg) was given in combination with vaporised CBD (16mg) or alone.

If CBD does reduce the adverse effects of cannabis, it does not appear to do so by altering the pharmacokinetics of THC.[55] It is possible that CBD acts as a neuroprotectant against the psychotomimetic effects of THC by upregulating AEA signalling, as chronic cannabis use is associated with reduced AEA levels.[59–61] An alternative hypothesis could be that, as CBD and THC are produced in different ratios from their shared precursor molecule cannabigerol, THC levels are diminished in high-CBD cannabis strains and *visa-versa*,[62] resulting in reduced THC consumption and therefore reduced THC-induced psychotomimetic effects for consumers when compared to high-potency, low-CBD strains.

1.2 THE ENDOCANNABINOID SYSTEM

The ECS is a signalling network expressed throughout the central nervous system (CNS) and periphery.[63,64] It consists of endogenous cannabinoids, enzymes which catalyse their synthesis and degradation, and cannabinoid receptors.[8,65]

1.1.2 CB1 AND CB2 RECEPTORS

THC and endogenous cannabinoids bind principally to the type-1 and type-2 cannabinoid receptors, CB1R and CB2R.[7,8] These 7-transmemberante G-protein coupled receptors vary greatly in distribution- CB1R is expressed primarily in the central nervous system and CB2R is found in peripheral and immune cells.[66,67]

CB1R is the most highly expressed G-protein coupled receptor in the CNS, expressed most abundantly in the cerebral cortex, the amygdala, hippocampus, cerebellum, and basal ganglia.[66,68] These receptors alter neuronal excitability and neurotransmitter release in order to modulate a number of different functions, including neurodevelopment, synaptic plasticity, pain, immune response, stress response, mood, reward learning and motivation.[8,69–74] CB1Rs are mainly located presynaptically in both excitatory and inhibitory neurons where they inhibit the release of GABA and glutamate either via inhibition of N- and P/Q-type Ca^{2+} channels or direct inhibition of vesicular transmitter release.[71,75–78] In addition, CB1R expressed in liver and adipose tissue have been shown to activate lipogenesis.[79] Though CB1R antagonists were proven effective in clinical trials of obesity and metabolic syndrome, they were withdrawn from the market due to increases in patient anxiety, depression, and suicidal ideation.[80]

As CB2R are predominantly located in the immune tissues and cells, including monocytes, macrophages, B- and T-lymphocytes and microglial cells,[81,82] the receptor is thought to be a potential target for the treatment of inflammatory and autoimmune diseases (see Di Marzo 2018[83] for a list of CB2R targeted drugs currently in development).[84–86] While CB2R expression in neurons is limited in comparison with CB1R, they have been detected the brain stem,[87] cerebellum,[88] and substantia nigra[89] of human brains, making CBR2 a potential therapeutic target for neurodegenerative disorders such as Parkinson's disease.

ECS perturbations have been observed in patients with schizophrenia and other psychotic illnesses. Two studies have found an association between CB1R receptor polymorphism and hebephrenic schizophrenia,[90,91] though others refute this link.[92–94] An association has also been found between a CB2R polymorphism and increased susceptibility to schizophrenia.[95] Studies have produced conflicting findings of both increased[96–99] and reduced[100–104] levels of CB1R in the CNS of people with psychosis from in vivo positron emission tomography (PET) and post-mortem autoradiography. In the periphery, a systematic review and meta-analysis by Minichino and colleagues[105] found increased expression of CB1R in peripheral blood mononuclear cells (PBMCs) people with schizophrenia compared to healthy controls (HCs), as well as evidence that both severity of symptoms and poorer cognitive function was correlated with increased PBMC expression of both CB1R and CB2R of antipsychotic naïve schizophrenia patients. In particular, a study by Bioque and colleagues found that, while schizophrenia patients who had a history of prolonged cannabis use did not differ from non-using patients in PBMC CB2R levels, schizophrenia patients with a history of cannabis use had significantly lower PBMC CB2R from HCs.[106] CB1R expression in PBMC could not be reliably detected in this study.

In the general population, a systematic review by Jacobson and colleagues found that PBMC CB1R expression was elevated in current cannabis users, both with and without cannabis use disorder (CUD), but not in past users, compared to non-using HCs.[107] This contrasts with findings of reduced expression of CB1R in the cortical regions,[108–111] hippocampus,[111–113] amygdala,[111,113] and basal ganglia[108–112] of cannabis users found in PET and post-mortem autoradiography studies. These changes in brain CB1R expression reverse after an abstinence period thought to be between 5 days and 2 weeks.[107] In summary, chronic cannabis use leads to compensatory downregulation of CB1R expression in the brain, a change which appears to reverse soon after stopping cannabis use. It is likely that this is the mechanism by which tolerance to THC develops in persistent users.[20,23]

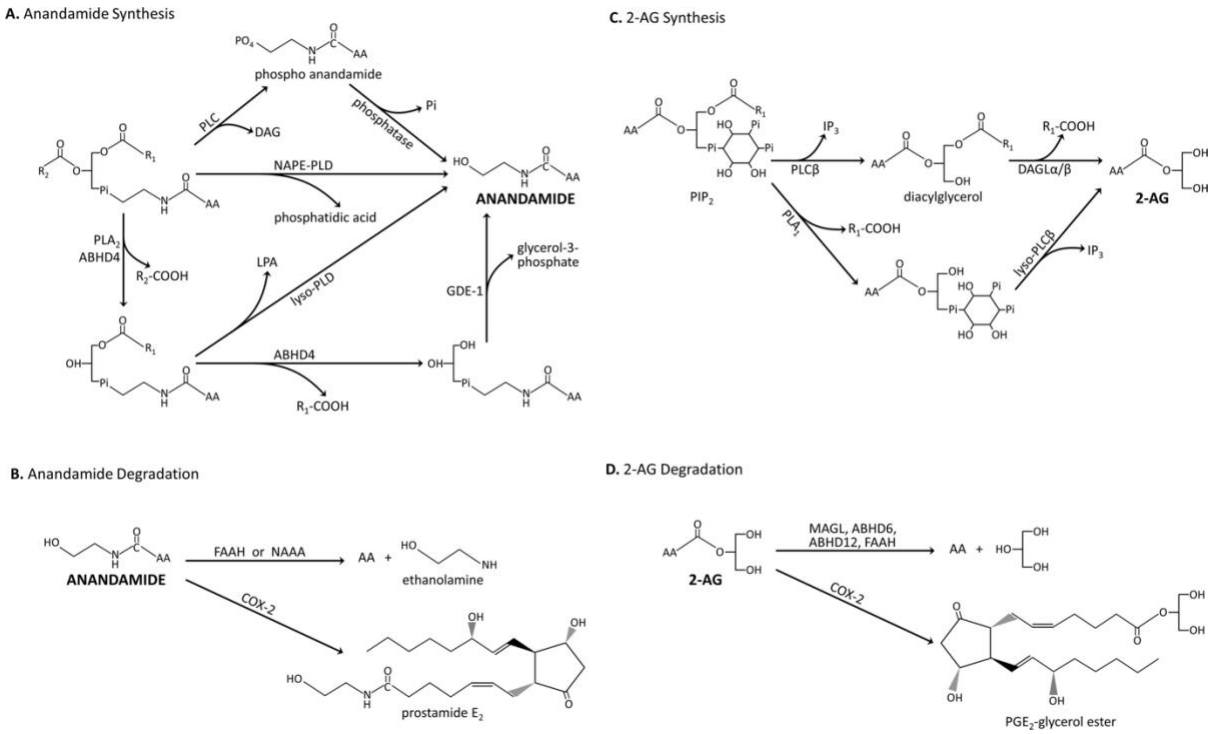
1.2.1 ENDOGENOUS CANNABINOIDS: ANANDAMIDE AND 2-ARACHIDONOYL GLYCEROL

AEA and 2-arachidonoyl glycerol (2-AG) are lipophilic signalling molecules first isolated 1992 and 1995, respectively (see **Supplementary Figure 4-2** of **Chapter 4** for molecular structures).[114,115] Both act as retrograde neuromodulators by binding to CB1R, while only 2-AG also binds with high affinity to CB2R.[116] AEA and 2-AG in the CNS have numerous roles; some examples of neuromodulatory systems that they are involved with include regulation of synaptic plasticity,[117] pain,[118–120] and anxiety and stress responses.[118,121–125]

Both AEA and 2-AG are synthesised on demand through several metabolic routes, outlined in **Figure 1-1**.[126] The production of endocannabinoids from neuronal cell membranes is activity-dependent, permitting a fine feedback control on synaptic release.[78]

Numerous changes in endocannabinoid levels in psychosis have been found. De Marchi and colleagues[127] found significantly higher concentrations of AEA in whole blood extracts of schizophrenic patients compared to HCs, with clinical remission being accompanied by a significant fall in the levels of AEA and the mRNA transcript for FAAH. Whether peripheral levels of AEA accurately reflect levels in the brain, where the majority of CB1Rs are expressed, is under debate. A study by Leweke and colleagues found no correlation between plasma and cerebrospinal fluid (CSF) AEA levels.[128] Animal models of psychosis have produced mixed results when measuring AEA and 2-AG levels in brain regions relevant for schizophrenia,[129–132] and experiments in rats have shown that the pharmacological blockade of AEA degradation improves psychotic-like behaviours.[130,133] Levels of AEA in the CSF of humans have been found to be elevated in antipsychotic-naïve schizophrenic patients,[128,134,135] while 2-AG could not be detected in significant levels in either patients or controls.[134] Interestingly, Giuffrida and colleagues[135] found that symptom severity in patients with psychosis was negatively correlated with AEA concentration and normalised with treatment with first generation but not second-generation antipsychotics, suggesting a possible compensatory action of AEA in the CNS. CSF levels of AEA have also been found to be elevated in patients in the prodromal stage of psychosis compared to HCs, while patients with lower AEA levels were at greater risk of transitioning to psychosis.[50]

Figure 1-1. *Primary synthetic and degradative pathways for AEA and 2-AG.*



A. The principal pathway of synthesis of anandamide (AEA) involves first the generation of N-acylphosphatidylethanolamine (NAPE) catalysed by Ca^{2+} dependent N-acyltransferase (NAT), and then it's hydrolysation via the enzyme N-acylphosphatidylethanolamine-specific phospholipase D (NAPE-PLD).[136] Other synthetic pathways include via lysophosphatidylinositol-selective phospholipase D (lyso-PLD) and glycerophosphodiester phosphodiesterase I (GDE-1) enzymes.[137] **B.** AEA is primarily degraded intracellularly by fatty acid amide hydrolase (FAAH) enzymes to ethanolamine and arachidonic acid (AA).[138] Other routes of metabolism include via N-acylethanolamine-hydrolysing acid amidase (NAAA)[139] and cyclo-oxygenase-2 (COX-2).[140] **C**. 2-AG is synthesised from membrane phosphatidylinositols (PIs), such as PI-4,5-biphosphate (PIP2), by first converting the PI into either lysophosphatidylinositol (lyso-PI) or diacylglycerol before hydrolysing via the enzyme lysophosphatidylinositol-selective phospholipase C (lyso-PLC) or sn-1-diacylglycerol lipase (DAGL), respectively.[296] **D**. 2-AG is primarily degraded by monoacylglycerol lipase (MAGL) in the presynaptic neuron into glycerol and AA.[141] 2-AG can also be hydrolysed by α/β hydrolase domain-containing protein 6 and 12 (ABHD-6 AND ABHD-12),[142] or deoxygenated by COX-2 enzymes.[140]

(Reprinted from H. C. Lu and K. MacKie. An introduction to the endogenous cannabinoid system. *Biol. Psychiatry*. 79(7):516–525. © 2016, with permission from Elsevier)

Cannabis use has been shown to alter endocannabinoid levels both acutely and with repeat dosing. Acute cannabis administration has been shown to increase plasma AEA and 2-AG,[143,144] though these effects aren't observed in daily cannabis users.[61] This may be due to chronic cannabis use leading to compensatory adaptations in the ECS, including reductions in circulating endocannabinoids. Di Marzo and colleagues showed that chronic dosing of cannabinoids led to decreased levels of AEA and 2-AG in the striatum of rats.[59] In humans, Morgan and colleagues showed that frequent cannabis users had lower CSF AEA and lower levels of serum 2-AG than infrequent users (no change in CSF 2-AG or serum AEA), and that CSF AEA levels inversely correlated with state psychotic symptoms.[60] Frequency of cannabis use was again shown to be negatively correlated with plasma 2-AG levels, but not plasma AEA, in a study of regular cannabis users.[61] Finally, frequent cannabis use was found to down-regulate AEA levels in the CNS of patients with schizophrenic patients but not in HCs in the Leweke and colleagues' 2007 study.[128]

1.2.2 ENDOCANNABINOID-LIKE LIPIDS

A number of endogenous lipids with similar chemical structures to the endogenous cannabinoids have been characterised. These molecules show little or no activity at cannabinoid receptors, but their levels can still be altered by cannabis use and in patients with psychotic disorders. See **Supplementary Figure 4-2** of **Chapter 4** for molecular structures.

Arachidonic acid

Arachidonic acid (AA) is an omega-6 poly-unsaturated fatty acid that is obtained from foods such meat, fish, and eggs.[145–148] It typically found in the membrane phospholipids of mammalian cells, and is one of the most abundant poly-unsaturated fatty acids present in human tissue.[145,149] AA plays a crucial role in neuron function, synaptic plasticity, and long-term potentiation in the hippocampus.[150–154] AEA and 2-AG produce AA and either ethanolamine or glycerol, respectively, when metabolised.[126]

N-acylethanolamines

The N-acylethanolamine (NAE) family of lipids includes AEA as well as docosatetraenylethanolamide (DEA), oleoylethanolamide (OEA), stearoylethanolamide SEA, and alpha- and gamma-linolenoylethanolamide (aLEA and gLEA). All are

biogenetically related and share synthetic and catabolic enzymes (see **Figure 1-1**).[7,155] The non-endocannabinoid NAE's bind to other G-protein coupled receptors, ion channels and nuclear receptors, including peroxisome proliferator activated receptor-α (PPARα),[156,157] vanilloid receptor 1 (TRPV1),[158] and the orphan G-protein coupled receptors GPR55[7,159] and GPR119.[160] OEA has been proven to have vasorelaxant effects[161] and to regulate food intake,[162] and SEA to be pro-apoptotic,[163] and anorexic.[164] Like AEA, levels of other NAEs alter in response to stress,[125] cannabis use,[60,144] and in patients with psychosis.[134,135,165]

N-arachidonoyl-L-serine

N-arachidonoyl-L-serine (ARA-S) is an N-acyl-amino acid first isolated from the bovine brain in 2006.[166] ARA-S shows weak affinity at CB1R or CB2R, but is an activator of GPR55,[167] N-type calcium channels,[168] and large conductance calcium-activated potassium channels.[169] ARA-S has vasorelaxant effects[161,166] and may be neuroprotective after traumatic brain injury.[170]

1.3 CANNABIS AND THE RISK OF PSYCHOSIS

The notion that cannabis use is linked with mental health disorders has been discussed for over a century, with the first English-language accounts of cannabis induced 'insanity' being written in a report by British doctors in India in 1873.[171,172] Cannabis use is more common among people with psychosis that it is in the general public, with an estimated 26.2% of patients with schizophrenia having cannabis use disorder at some point in their lives.[173] In addition, cannabis use has been linked to poorer functioning, greater risk of relapse and more intensive psychiatric treatment in psychosis patients who continue to use after diagnosis.[174–178]

There is a wealth of data indicating that cannabis use is associated with psychotic-like experiences in HCs.[22,179] Between 20 and 50% of users are thought to experience cannabis-induced transient, attenuated psychotic symptoms at some point,[180] with individuals who score highly on a measure of schizotypy being at increased risk of experiencing psychosis-like phenomena at the time of use.[181,182] Some adolescent users of the drug report persistent psychotic symptoms several years after stopping use.[183] Cannabis, like cocaine, amphetamines and hallucinogens, is also associated with substance-induced psychotic episodes that can last between a few days and months,[184,185] and are associated with significantly increased risk of developing a persistent psychotic condition in the future.[186] Whether or not cannabis can trigger a primary psychotic disorder such as schizophrenia remains controversial.

A study in Swedish conscripts was the first to provide evidence of increased rates of schizophrenia in previously healthy individuals who reported using cannabis at recruitment.[187] Since then, a meta-analysis of prospective studies by Moore and colleagues has reported a reliable increase in incidence of psychosis in people who had used cannabis compared to never-use.[188] This is supported by results from cross-sectional studies comparing retrospective reports of use in individuals with and without a diagnosis of a psychotic disorder.[189–192] Despite the plethora of epidemiological evidence of a link between cannabis use and developing psychosis, causality remains hotly debated.

1.3.1 DOSE

As THC is the constituent of cannabis thought to be responsible for the psychosis-inducing effects,[9] it would be expected that larger cumulative doses, from high-potency cannabis strains and more frequent use, would have a greater impact on the risk of developing psychotic disorder.

Two separate studies that compared patients experiencing a first-episode of psychosis (FEP) and HCs and found that users of high-potency 'skunk' varieties of cannabis were significantly more likely to be in the patient group, and that daily users of high-potency cannabis were at a considerably higher risk again.[189,191]

A meta-analysis published in 2016 found a 2-fold increase in risk of psychosis in 'average' cannabis users compared to non-users, and a 4-increased risk for the heaviest users.[193] Since then, a multicentre case-control study based on a cohort of 901 FEP patients and 1237 controls from a number of countries found that daily cannabis use, particularly of high-potency cannabis, was associated with increased odds of psychotic disorder compared with never-use.[192] Interestingly, the availability of high-potency cannabis across the different sites showed a strong correlation to the patient attributable factor (an estimate of the proportion of disorder that would be prevented if the exposure were removed, assuming causality) of daily use in that area; from 44% in Amsterdam just 6% in Palermo.

1.3.2 AGE OF FIRST USE

Puberty, a time of increased activity of the central ECS, may represent a 'critical period' when individuals may be more vulnerable to the adverse effects of cannabis.[194,195] According the UN's World Drug Report an estimated 13.8 million young people aged 15–16 years used cannabis in 2020, equivalent to a rate of 5.8%.[196] Cannabis exposure during adolescence may disrupt the formation of neural connections, though the full consequences of this have yet to be clarified.[195] This may put young people at greater risk of health problems associated with cannabis use.[197]

A number of epidemiological studies have shown a relationship between adolescent cannabis use and psychotic symptoms later in life.[183,198–200] According to Di Forti and colleagues,[192]

use of high-potency cannabis by age 15 years doubles risk of later developing psychosis compared to never use. Similarly, a birth cohort study based in Dunedin, New Zealand, showed that individuals using cannabis at the age of 15 years had a significantly greater risk of schizophreniform disorder at age 26 compared with non-users.[201] However, this could be due to a greater cumulative exposure to cannabis rather than use at a younger age. There may be an underlying factor, such as poverty or hardship in childhood, which predisposes to both cannabis use in adolescence and psychotic illness.[199,200] It may also be that a genetic predisposition to psychosis is required for cannabis to have a 'triggering' effect.[9,202,203]

1.4 CANNABIS AND COGNITION

The ECS plays a role in memory consolidation, reward processing and motivation, and is crucial for certain forms of neuronal plasticity.[9] Hence when considering the potential harms of cannabis use, it is important to consider both the transient and longer-term effects of the drug on cognitive functioning.

1.4.1 ACUTE EFFECTS

Short-lived cognitive impairments during cannabis intoxication have been well documented. The most significant effects are on memory, possibly due to THC interfering with signalling in CB1R-rich memory-associated brain regions such as the hippocampus, amygdala, and prefrontal cortex.[204–208] Cannabis use leads to dose-dependent impairment of both working and episodic memory, specifically the encoding of new memories during intoxication.[209,210] A 2021 meta-analysis by Zhornitsky and colleagues[211] confirmed that cannabis also has acute impairing effects on the domains of processing speed, executive function, impulsivity and attention, though with comparatively small effect sizes.

The acute effects of THC on cognition appear to be contingent on the extent of previous use, possibly due to downregulation of central CB1R expression.[108–113] For example, individuals who use cannabis more than once weekly show tolerance to the psychomotor and memory impairments associated with intoxication.[212,213] There is also some evidence that a higher proportion of CBD in cannabis could reduce the acute memory impairments during intoxication, as discussed in section 1.1.2 on page 17.[56,214] Finally, there is mixed evidence as to whether adolescents may be more or less sensitive to certain cognitive effects of THC intoxication.[215,216]

1.4.2 LONG-TERM EFFECTS

Whether or not regular cannabis use can produce long-term impairments in cognitive functioning remains controversial.[9] The 5 most recent meta-analyses assessing the effects of long-term, regular cannabis use in adults on specific cognitive domains have produced largely similar results,[217] finding impairments with small effect sizes in executive function,[218–220] verbal learning and recall,[218–222] and episodic memory.[219–222] However, results

for measures of attention, working memory and processing speed/visuomotor speed were mixed.[218–222]. In addition, a meta-analysis of longitudinal studies found a significant association between frequent and dependent cannabis use in adolescence and a reduction in verbal measures of IQ,[223] and a systematic review by Badalla and colleagues[224] determined that persistent cannabis use is associated with changes in the structure and function of adult and adolescent brains. As with all epidemiological research of this nature, causality cannot be inferred.

It is unclear how much the above results reflect a lingering effect of acute intoxication, and how long individuals would have to abstain from cannabis before cognitive deficits were no longer detectable. PET studies have demonstrated that by the 4th week of abstinence, the downregulation of brain CB1Rs associated with chronic cannabis use reverses to normal levels.[108] A 2018 meta-analysis by Scott and colleagues[219] found that studies that required an abstinence period of 72 hours or more did not find any residual effects of cannabis use on cognition. However, Lovell and colleagues[218] found that studies requiring 25 or more days of abstinence produced similar results to those that required only 12 hours of abstinence, with the exception of executive functions no longer showing impairment after 25 days of abstinence. It may be that the length of abstinence required for recovery of function is dependent on which cognitive domain is assessed.[225]

In common with cannabis's psychotomimetic effects, the residual cognitive effects appear to be greater with both more frequent use[221,226,227] and use in early adolescence.[228,229] In a cohort study of over 1000 individuals followed from birth, individuals with more persistent cannabis use or cannabis dependence showed greater cognitive impairment, particularly in the domains of executive functioning and processing speed, and adolescents onset users presented with a greater decline in IQ than adult-onset users by age 38 years.[230] Interestingly, the meta-analysis of Lovell and colleagues[218] found no evidence that cannabis use duration or age of onset influenced cognitive outcomes in persistent cannabis users. Adolescence is a time when the functioning of the ECS is critical for neurodevelopment and maturation, including synaptic pruning and cerebral white-matter development, processes which may be perturbed by THC.[231,232] A number of case-control and cohort studies have demonstrated an association between cannabis use in adolescence and poorer educational attainment.[233–238] A Mendelian

randomisation study by Chen and colleagues[239] also found evidence of a genetic link between liability to cannabis use disorder and lower education attainment, independent of IQ; smoking or diagnosis of attention deficit hyperactivity disorder. However, that this may be due to unmeasured confounding factors. For example, several studies have shown that adjusting for use of other substances, including tobacco and alcohol, offset the association between adolescent cannabis use and educational achievement.[240–242]

1.4.3 IN PSYCHOSIS

Cognitive deficits are a key feature of psychotic disorders such as schizophrenia and cognitive impairments are a major determinant of functional and clinical outcomes.[243–247] Individuals in the early stages of psychosis present with global cognitive impairments, with the most affected domains being verbal memory, executive function and IQ (though effect sizes vary between studies; for one set of estimates see **Table 1-1**).[248,249]

There is some evidence that the ECS is involved with cognitive function in early psychosis, particularly memory and executive function.[129,250,251] For example, the expression of the 2-AG synthesizing enzyme sn-1-diacylglycerol lipase (DAGL) and the AEA metabolising enzyme FAAH in PBMCs are correlated with performance in tasks measuring short-term verbal memory.[250] THC may interfere with CB1R signalling and the release of AEA and 2-AG to worsen these impairments, according to an animal model of schizophrenia.[129] Indeed, a paper by Solowij and Michie[252] highlights the similarity between the cognitive deficits found in regular cannabis users and those seen in schizophrenia, possibly suggesting a shared underlying neuropathology. The cognitive impairing effects of acute cannabis intoxication also appear to be greater in people with schizophrenia than in healthy individuals.[212] It would therefore be expected for cannabis use to be associated with greater residual cognitive impairments in patients with psychosis than the general population.

Multiple studies in the 1990s and early 2000s produced evidence that cognitive functioning in schizophrenia patients with comorbid substance use disorders is less impaired than in patients without substance use disorders.[253–257] In 2008 Potvin and colleagues published a meta-analysis[258] showing that preferential use of cannabis over alcohol, cocaine or poly-drug use was associated with the least impairment in global cognition compared to non-drug users.

Since then, four more meta-analyses have found that cannabis use is associated with less impaired general cognitive ability, attention, executive abilities, and memory in individuals with psychosis.[259–262]

The effects of moderating factors such as abstinence from cannabis, use in adolescence and frequency of use differ extensively between studies. Yücel and colleagues[261] found that studies reporting lifetime use of cannabis rather than current use showed the strongest cognitive sparing effects of the drug. Similarly, Schoeler and colleagues[221] found that at least 10 days of sobriety were required for cannabis-using patients with psychosis to perform better on memory tasks than non-using patients. It could be concluded then that cannabis use acts as a marker for superior premorbid cognitive functioning in this population.[260] This would be consistent with the results of a study by Ferraro and colleagues,[263] who found that only psychosis patients who used cannabis up to more than once a week had a higher IQ than non-users. It however contrasts with other studies which found that frequency of use was positively associated with executive function performance in people at high risk of psychosis[264] and either a negligible or positive association with memory and attention performance in psychosis patients.[221,265] Although it might be expected that beginning cannabis use at an early age would predict worse cognitive performance in people with psychosis, a study by Yücel and colleagues[261] found that FEP patients who began cannabis use before the age of 16 performed significantly better than non-using patients in cognitive tests. Thus, the effects of different patterns of cannabis use on cognition in psychosis has still yet to be fully established.

1.5 CLINICAL HIGH-RISK FOR PSYCHOSIS

The onset of psychosis is often preceded by a prodromal stage, characterised by "attenuated' psychotic symptoms and a marked deterioration in overall functioning.[266–268] Specialised clinical services have been established to identify individuals who may be in this early stage of psychosis development, with the aims of ameliorating psychotic symptoms and the associated distress and dysfunction, reducing the number of people who transition to a psychotic disorder, and minimising the period of untreated illness in those who do develop a disorder.[269] As not all individuals identified by these early intervention services will go on to develop a psychotic disorder, the term clinical high-risk for psychosis (CHR) has been termed to describe the state of high, but not inevitable risk of developing psychosis.[270,271] Around 20% of people with this clinical syndrome develop a psychotic disorder within 2 years.[270,271]

1.5.1 COGNITION IN THE CHR STATE

Cognitive functioning is not as impaired in the CHR state as it is in patients with psychotic disorders[272] but deficits are significant as compared to HCs.[244,273] The pattern of cognitive deficits in CHR individuals is similar to that seen in psychosis, with impairments in verbal learning, reasoning and problem solving, visual learning and memory, verbal learning and memory, working memory, executive functioning, general intelligence, processing speed and attention.[272,273]

The severity of cognitive dysfunction in the CHR state has been associated with later transition to psychosis, though meta-analytical evidence is mixed.[272,274–277] Only two studies have examined the association between cannabis use and cognitive performance in CHR subjects.[264,278] Neither reported significant associations, but sample sizes were small, limiting their statistical power.

Table 1-1. *Patient versus healthy control differences in cognitive domains*

Cognitive Domain	Drug-naïve schizophrenia patients, effect size (95% CI)[a]	Clinical high-risk patients, effect size (95% CI)[b]
Verbal Memory	−1.03 (−1.44, −0.63)	−0.45 (−0.67, −0.22)
Speed of Processing	−1.03 (−1.23, −0.82)	−0.39 (−0.56, −0.21)
Working Memory	−0.97 (−1.25, −0.69)	−0.44 (−0.57, −0.31)
Attention	−0.80 (−0.95, −0.65)	−0.39 (−0.49, −0.29)
Visual Memory	−0.78 (−1.21, −0.34)	−0.45 (−0.77, −0.13)
Executive Functioning	−0.74 (−0.85, −0.62)	−0.42 (−0.60, −0.24)
Reasoning and Problem Solving	--	−0.46 (−0.74, −0.19)
General Intelligence	--	−0.39 (−0.57, −0.22)

All effects sizes indicate better performance in healthy controls. A value of -0.20 to -0.50 corresponds to small effect sizes, -0.50 to -0.80 to medium, and a value less than -0.80 to large effect sizes.
a Data adapted from Fatouros-Bergman et al. 2014.[279] Effect size as Cohen's d.
b Data adapted from Catalan et al. 2021.[272] Effect sizes as Hedges' g.
Hedges' g is equivalent to Cohen's d with correction for small sample sizes. This correction is small (<5%), so comparing the values is informative.

1.5.2 CANNABIS USE AND OUTCOMES

Unlike other environmental risk factors for psychosis, such as childhood trauma or migrant status, cannabis use is amenable to change through intervention.[12] Both CUD and lifetime cannabis use are higher in individuals at CHR than in the general population.[280] CHR patients who use cannabis have also been found to suffer from more positive psychotic symptoms (such as hallucinations, delusions and paranoia) than non-using patients.[280] While a 2016 meta-analysis found that lifetime cannabis use was associated only at the trend level with transition to psychosis, the association between current cannabis abuse or dependence and transition was significant.[281] Valmaggia and colleagues[282] were the first to assess the effects of different patterns of cannabis use on transition in individuals at CHR, and found that both use before the age of 15 years and weekly or more frequent use were associated with increased risk of developing a psychotic disorder. These findings, however, were not replicated by Buchy and colleagues,[283] nor by McHugh and colleagues,[284] though the latter study did find a significant association between cannabis-induced psychotic symptoms and transition from CHR to psychosis.

Among CHR subjects who do not transition to psychosis, around half (95%CI: 39.3–58.2%) will have achieved remission from the CHR state after 36 months.[285] While the majority of non-transitioning CHR individuals will have improvements in psychotic symptoms and

general functioning over time, around 45% are functionally impaired after 6 years.[286] Relatively little research has been conducted on the impact of cannabis use on these outcomes, and the results have been mixed.[287–290]

1.5.3 THE EU-GEI HIGH RISK STUDY

The European Network of National Schizophrenia Networks Studying Gene-Environment Interactions study (EU-GEI) recruited 334 individuals at CHR and 67 controls from 11 centres in Europe, South America, and Australia.[291] The study had a naturalistic, prospective design. Participants were assessed in detail at baseline and followed for up to 5 years to determine clinical outcomes. They received the usual clinical care provided by the teams that recruited them, which usually involved case management and psychological input, and only rarely the use of antipsychotic medications. Further methodological details of this study are available in **Appendix A**.

In this thesis, I examined the relationship between detailed measures of cannabis use in CHR subjects from this study and a) clinical outcomes and b) cognitive performance.

1.6 SUMMARY AND THESIS AIMS

The main question that underpins this thesis is: "How does cannabis contribute to the development and presentation of psychotic conditions?" Multiple studies have shown an association between cannabis use and psychosis, indicating that this effect may be modified certain factors such as the he frequency and quantity of THC and CBD consumption, as well as specific sociodemographic traits that could increase or decrease susceptibility to effects of the drug.[191–193,292] To address this fundamental question, the thesis is organized around three interrelated objectives, examined across three complementary studies:

i) Determine the effects of cannabis use on clinical outcomes in individuals at CHR;
ii) Assess the effects of cannabis use on cognitive functioning in individuals at CHR;
iii) Examine the effects of THC and CBD on the plasma levels of endocannabinoids.

First, the use of prospective studies on cannabis consumption in individuals at heightened risk of psychosis, such as the EU-GEI CHR cohort studied in Chapter 2, provide a way of examining how particular patterns of cannabis use might causally contribute to the emergence of a psychotic disorder. Secondly, there is evidence linking cannabis use in the general population to deficits in cognitive function.[217] However, cannabis use in patients with psychosis has been linked to less severe cognitive impairments than in patients who do not use cannabis.[259–262] The nature of the association between cannabis use and cognition in the CHR population has yet to be established, and is examined in Chapter 3. Finally, cannabis produces its main effects via interaction with the ECS, and yet the acute effects of THC and CBD on endocannabinoids levels in humans are not well understood.[61,143,144,293] It has been theorised that THC my induce psychotic symptoms by disrupting the equilibrium of the endocannabinoid system, and CBD shows promise both as a potential antipsychotic therapy[294,295] and as a neuroprotectant against the psychotomimetic effects of THC.[52–54] Examining the effects of THC and CBD on endocannabinoid signalling in humans may clarify their mechanisms of action.

The hypotheses tested in this thesis are:

i) Cannabis use is associated with an increased incidence of psychosis in people at CHR;

ii) Cannabis use is associated with non-remittance from the CHR state (persistence of symptoms);

iii) Cannabis use is associated with poor functional outcome for people at CHR;

iv) People at CHR show cognitive impairments when compared to HC participants;

v) Having ever used cannabis is associated with better performance in cognitive assessments in people at CHR;

vi) Administration of THC leads to a transient increase in plasma AEA and 2-AG;

vii) Co-administration of CBD modulates the effect of THC on plasma AEA in a dose-dependent manner.

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CHAPTER 2 - INFLUENCE OF CANNABIS USE ON INCIDENCE OF PSYCHOSIS IN PEOPLE AT CLINICAL HIGH RISK

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Influence of Cannabis Use on Incidence of Psychosis in People at Clinical High Risk

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2.1 ABSTRACT

2.1.1 AIMS

Evidence for case-control studies suggests that cannabis use is a risk factor for the development of psychosis. However, there have been limited prospective studies and the direction of this association remains controversial. The primary aim of the present study was to examine the association between cannabis use and the incidence of psychotic disorders in people at clinical high risk of psychosis. Secondary aims were to assess associations between cannabis use and the persistence of psychotic symptoms, and with functional outcome.

2.1.2 METHODS

Current and previous cannabis use were assessed in individuals at clinical high risk of psychosis (n=334), using a modified version of the Cannabis Experience Questionnaire. Participants were assessed at baseline and followed up for 2 years. Transition to psychosis and persistence of psychotic symptoms were assessed using the Comprehensive Assessment of At-Risk Mental States criteria. Level of functioning at follow up was assessed using the Global Assessment of Functioning disability scale.

2.1.3 RESULTS

During follow up, 16.2% of the clinical high-risk sample developed psychosis. Of those who did not become psychotic, 51.4% had persistent symptoms and 48.6% were in remission. There was no significant association between any measure of cannabis use at baseline and either transition to psychosis, the persistence of symptoms, or functional outcome.

2.1.4 CONCLUSIONS

These findings contrast with epidemiological data that suggest that cannabis use increases the risk of psychotic disorder.

Key Words: clinical high-risk, longitudinal, psychotic disorders, substance use, THC

2.2 INTRODUCTION

There is a considerable body of evidence linking cannabis use with an increased risk of developing a psychotic disorder. Cannabis use is more common in patients with psychosis than in the general population,[1–3] and the risk may be higher if use begins in adolescence,[4–6] is frequent,[7–10] and involves cannabis with a high delta-9-tetrahydrocannabinol (THC) content.[2,6,11] However, the direction of this association remains controversial:[12] the presence of a psychotic disorder may increase the likelihood of cannabis use,[13] patients with psychotic disorders use cannabis to relieve psychotic symptoms,[14–16] and genetic factors that increase the likelihood of cannabis use may be more common in patients with psychosis than the general population.[17,18] Much of the data relating cannabis use to psychosis have been derived from interviewing patients after they have developed a psychotic disorder.[2,7,11] These data thus reflect patients' retrospective assessments of their premorbid cannabis use, and recall accuracy may be influenced by the effects of time and of the disorder.[19] Only a few prospective studies have examined cannabis use and the incidence of psychosis in general population samples, although these have found some associations between cannabis use and the later onset of psychosis, the large scale of these studies (which involved thousands of participants) precluded a detailed assessment of cannabis use.[5,20,21]

The Clinical High-Risk (CHR) state is a clinical syndrome that typically occurs in adolescents and young adults. It is associated with a very high risk of developing a psychotic disorder, with around 19% of CHR individuals becoming psychotic within 2 years of presentation.[22] To date, only a limited number of studies have investigated the relationship between cannabis use in CHR individuals and the subsequent incidence of psychosis, and the findings have been inconsistent. A recent meta-analysis[23] did not find a significant difference in risk of transition to psychosis between CHR cannabis users and non-users, but highlighted the need to assess cannabis use in more detail. Further meta-analytical results suggest that whilst lifetime use of cannabis is not significantly associated with transition rates, the relative risk is greater in those with cannabis abuse or dependence, likely a marker for heaver cannabis use.[24] Results from the few studies which have specifically measured frequency of cannabis use and age of first use have been mixed,[25–27] with only Valmaggia *et al.* 2014[25] finding a significant association with risk of psychosis.

The primary aim of the present study was to examine the association between cannabis use and the incidence of psychosis in people at clinical high risk. Secondary aims were to assess associations between cannabis use and the persistence of psychotic symptoms, and with functional outcome. In a prospective design, cannabis use was comprehensively assessed in a large sample of CHR subjects that was then followed for 2 years to determine clinical outcomes. Based on the previous literature in CHR subjects, we hypothesised that neither current nor previous cannabis use would be associated with an increased incidence of later psychosis, but that a high frequency of cannabis use, use before the age of 16, the use of high potency (>10% THC) cannabis strains, and current cannabis dependence would be. Secondary hypotheses were that cannabis use would be linked with non-remittance from the CHR state (persistence of symptoms) and a poor functional outcome.

2.3 METHODS

2.3.1 RECRUITMENT OF PARTICIPANTS

Participants were recruited to a multi-centre prospective study of people at CHR for psychosis.[28] 344 CHR participants meeting Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria[29] for an ultra-high risk state were enrolled from eleven centres in Europe, Australia and South America. In addition, sixty-seven healthy controls (HCs) were recruited from London, Amsterdam, Den Haag, and Melbourne. The HC sample was not included in the following analysis and will be described elsewhere (see Chapter 3).

Inclusion and exclusion criteria

The study guidelines recommended that participants should be 16-35 years old. While most of the sample (95.0%) was in this age range, a few sites included individuals who were slightly older (n=3) or younger (n=14) than this range as the local clinical services for CHR subjects employed a slightly broader age range. Exclusion criteria were: previous diagnosis of a psychotic disorder, as defined by the Structural Clinical Interview for DSM Disorders;[30] exceeding the 'Psychosis Threshold' or 'Antipsychotic Treatment Threshold', defined by the CAARMS;[29] an estimated IQ < 60 as measured by the shortened Wechsler Adult Intelligence Scale-III (WAIS-III);[31] being unwilling to give a blood or saliva sample for genetic analysis. In addition, CHR subjects were excluded if their psychotic symptoms could be explained by an organic disorder or substance misuse (other than alcohol or cannabis). Written, informed consent was provided by all participants.

2.3.2 ETHICS STATEMENT

The study protocol was approved by the relevant research ethics committees at each study site. All procedures conductive to the present work are in compliance with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

2.3.3 BASELINE ASSESSMENTS

Cannabis use was assessed using a modified form of the Cannabis Experience Questionnaire (EU-GEI$_{CEQ}$).[7] Participants were first asked if they had ever used cannabis. If the answer was yes, they were asked if they were a current or an ex-user, and to describe their typical pattern

of use. Age at first cannabis use was estimated by the participant, with collateral information from informants if available. The presence of cannabis dependence in the year prior to baseline was assessed using DSM-IV criteria for substance dependence.[32] Participants were also asked to describe the type of cannabis that they used the most. This description was used by the investigators to classify the cannabis used as having either a high (>10%) or low (<10%) THC content, using data published by the European Monitoring Centre for Drugs and Drug Addiction 2016 report[33] and national data reports (see **2.10.2. Supplementary Methods, Supplementary Table** 2-1).[34–53]

Global functioning was assessed using the Global Assessment of Functioning (GAF) disability subscale.[54] Use of tobacco and alcohol were recorded using the Composite International Diagnostic Interview.[55] Use of other recreational drugs were collected using the EU-GEI$_{CEQ}$. Sociodemographic data were collected using the Medical Research Council Sociodemographic Schedule.[56]

2.3.4 ASSESSMENT OF CLINICAL OUTCOMES

Participants had face-to-face assessments at baseline, 12 and 24 months. When a CHR individual developed psychosis, a follow-up assessment was conducted as close to psychosis onset as possible. The primary outcome was transition to psychosis within 2 years, defined according to CAARMS criteria.[29] Secondary outcomes included persistence of symptoms, defined as still meeting CAARMS criteria for the CHR state or having transitioned to a psychotic disorder, and level of functioning at the latest available follow up timepoint.

2.3.5 STATISTICAL ANALYSIS

CHR participants for whom there were no cannabis use data (n=10) were excluded from analysis. Baseline sociodemographic and clinical differences between outcome groups were assessed using either independent t-tests or ANOVA models for continuous data, and either Pearson's chi squared test or Fisher's exact test for categorical data.

Cannabis use variables were coded as follows: Cannabis use status – 0= never used, 1= past user, 2= current user; Age of first cannabis use – 0= aged 16 years or older, 1= aged 15 years or younger; Frequency of cannabis use – 0= less than once weekly, 1= more than once

weekly/less than daily, 2= daily; THC content of most used cannabis type – 0= less than 10% THC, 1= more than 10% THC; Cannabis dependence – 0= no cannabis dependence in past 12 months, 1= cannabis dependent in past 12 months. Participants who had never used cannabis were excluded from the age of first use, frequency of use, THC content and cannabis dependence variables, such that cannabis users were compared with each other.

For the primary outcome, we completed survival analyses with the outcome of time to psychosis onset, with outcomes censored at 2 years post baseline. Kaplan-Meier survival curves for each cannabis predictor variable, without covariates, were inspected to assess for proportional hazards. Variables which met our threshold ($p < 0.2$) for univariate analyses were included in multilevel Cox regression analyses, using the coxme package for R. Site was included as a random effect to account for clustering. Effect sizes were quantified as hazard ratios (HR) and 95% confidence intervals.

For persistence of symptoms, cannabis variables which met our threshold ($p < 0.2$) in chi-square or Fisher's exact test analyses were input into multilevel logistic regression models using the lme4 package for R. Site was included as a random effect. Effect sizes for the remission outcome were quantified as odds ratios (ORs) with 95% confidence intervals.

For functional outcome, we used Spearman Rank Correlation and t-tests with the outcome of GAF score at the latest follow-up assessment. Cannabis variables which met our threshold ($p < 0.2$) in univariate analyses were input into multilevel linear regression models using the lme4 package for R. Time (in days) from baseline to the last GAF assessment was added as a covariate to account for possible deviation around the planned assessment date. Site was included as a random effect. To analyse the difference between the mean change scores of GAF from baseline to follow-up, baseline GAF score was added as a covariate to the multilevel models. Fixed effect parameter estimates were quantified with 95% confidence intervals (*see* ***2.10.2. Supplementary Methods***).

Potential confounders were identified from recent meta-analyses,[57–59] and included age, gender, ethnicity, tobacco, alcohol and other substance use. Potential confounders were not included as a priori defined covariates in all analyses to prevent overfitting. Instead, confounding variables which met our threshold ($p < 0.2$) in univariate analyses were included

in sensitivity analyses. Potential confounders were added to each multilevel model in a forward stepwise fashion and the maximum log likelihood of the new and old models was compared. Confounders which significantly improved the model were retained, and the process was repeated with the next confounder.

All analyses were performed using R version 4.0.3 and SPSS version 25. Statistical significance was defined at the 0.05 level. For post hoc power and sensitivity calculations see **2.10.2 Supplementary Methods**.

2.4 RESULTS

2.4.1 DESCRIPTION OF CHR POPULATION

The baseline sociodemographic and cannabis use patterns of participants is summarised in **Table 2-1**. 9.3% of the CHR group were taking an antipsychotic medication. 248 (74.3%) of CHR participants had ever used cannabis, of whom 90 (26.9%) were current users at baseline (**Table 2-1**).

***Table 2-1**. Demographic, clinical and cannabis use features of CHR participants.*

	CHR (n=334)
Age, years (SD)	22.4 (5.0)
Male gender	177 (53.0%)
Ethnicity	--
White	239 (71.6%)
Black	33 (9.9%)
Other	62 (18.6%)
Taking antipsychotic medication	32 (10.3%)
Current tobacco use	180 (55.4%)
Other substance use (ever)	125 (37.5%)
Cannabis use status	--
Current user	90 (26.9%)
Ex-user	158 (47.3%)
Never	86 (25.7%)
First cannabis use ≤15 years	117 (49.2%)
Frequency of cannabis use	--
Daily	78 (33.1%)
More than once weekly	33 (14.0%)
Less than once weekly	125 (53.0%)
High (>10%) THC content of most used cannabis type	125 (76.2%)
Cannabis dependence	36 (17.9%)

Abbreviations: CHR, clinical high risk; HC, healthy control.
P values for χ^2 tests. Data as mean (SD) or n (%). Significant (<0.05) p values in bold.

2.4.2 CANNABIS USE AND CLINICAL OUTCOMES

There were no socio-demographic differences between CHR participants who completed follow-up and those with missing follow-up data (**Supplementary Table 2-2**). Cannabis users were on average older than non-users (past users +2.4 years, current users +2.8 years) and used more tobacco products, alcohol, and other substances. Current cannabis users were more likely to be male than non-users, and used more tobacco and other substances than past users (**Supplementary Table 2-3**).

Onset of psychosis

62 (18.6%) of 334 CHR participants developed psychosis during follow up. The mean time to transition was 380 days (SD= 411.6), with an interquartile range of 121-496 days (**Supplementary Figure 2-1**). There were no significant differences in demographic or clinical features between subjects who did or did not subsequently develop psychosis (**Supplementary Table 2-4**), save that more of the former were taking antipsychotic medications at baseline (HR 2.375 [95% CI: 1.185 to 4.758], p= 0.015).

In univariate survival analyses, only use of cannabis by age 15 years (HR 0.62 [95% CI: 0.32 to 1.18], p= 0.142) met our threshold (p< 0.2) for inclusion in subsequent multivariate analyses (**Table 2-2**, **Figure 2-1**). In an unadjusted mixed-model Cox regression analysis, which used site as a random effect, the association was not significant (HR = 0.61 [95% CI: 0.32 to 1.17] p= 0.135). No potential confounding variables met our threshold (p< 0.2) for inclusion in multivariate analyses (**Supplementary Table 2-4**).

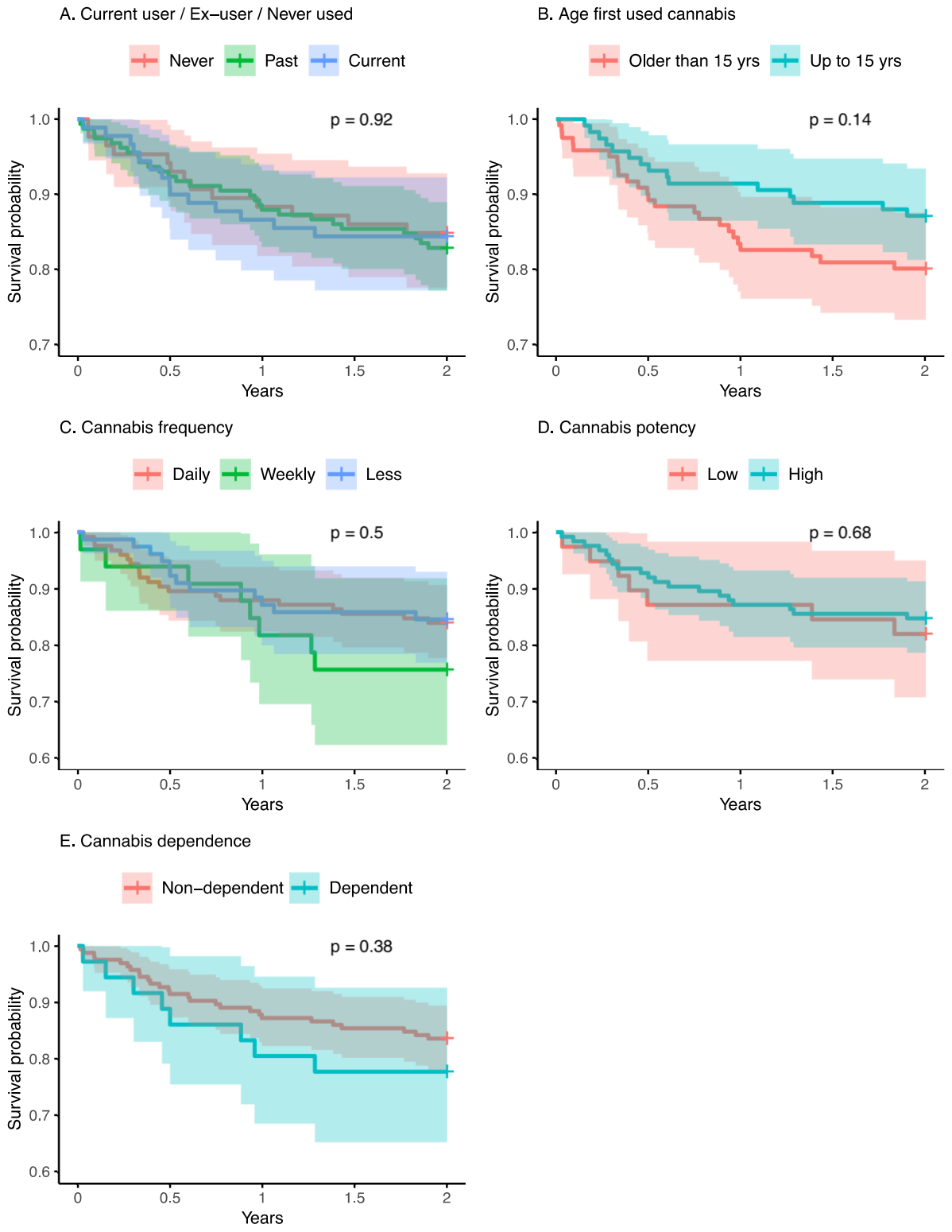
Table 2-2*. Relationship between cannabis use and time to transition to psychosis*

	Crude HR (95% CI)	P value	Fully adjusted HR (95% CI)	P value
Cannabis use status				
Current user	1.04 (0.49 – 2.22)	0.914	--	--
Ex-user	1.14 (0.59 – 2.20)	0.707	--	--
Never	1 (ref.)	--	--	--
Age first used cannabis				
≤15 years	0.62 (0.32 – 1.18)	0.142	0.61 (0.32 – 1.17)	0.135
>15 years	1 (ref.)	--	1 (ref.)	--
Frequency of cannabis use				
Daily	0.95 (0.46 – 1.93)	0.876	--	--
More than once weekly	1.55 (0.68 – 3.51)	0.297	--	--
Less than once weekly	1 (ref.)	--	--	--
THC content of most used cannabis type				
High (>10% THC)	0.83 (0.35 – 1.98)	0.679	--	--
Low (<10% THC)	1 (ref.)	--	--	--
Cannabis dependence				
Dependent	1.42 (0.41 – 1.42)	0.383	--	--
Not dependent	1 (ref.)	--	--	--

Abbreviations: HR, Hazard Ratio; ref., reference category.
Crude HRs are unadjusted for confounders, fully adjusted HRs are adjusted for site as a random effect. Only variables with crude HR p<0.2 added to adjusted, multilevel model, to reduce error from multiple testing.

Figure 2-1. *Kaplan-Meier survival curves showing relationship between cannabis use at baseline in the CHR group and transition to psychosis.*

There was no significant association with any measure of cannabis use, including user status (current/ex-/never), age at first use, frequency of use, THC content of most used cannabis type, or cannabis dependence.



Persistence of symptoms

Among subjects for whom CAARMS follow up data were available (n=209), 137 (65.6%) either still met CAARMS criteria for the CHR state or had transitioned to a full-blown psychotic disorder, and 72 (34.4%) were in symptomatic remission. In univariate analyses, two cannabis use variables met our threshold ($p< 0.2$) for inclusion in subsequent multilevel analyses: use of high potency cannabis ($\chi^2= 3.566$, $p=0.059$) and cannabis dependence ($\chi^2= 3.262$, $p= 0.071$) (**Table** 2-3). In unadjusted multilevel logistic regression models, which included site as a random effect, neither of these two measures was significantly associated with persistence of psychotic symptoms (OR 0.60 [95%CI 0.14 – 2.26], p=0.459; OR 3.15 [95%CI 1.04 – 11.38], p=0.054). Three potentially confounding variables were identified in univariate analyses: alcohol use ($t= 1.551$, $p= 0.123$), current drug use ($\chi^2= 3.827$, $p= 0.050$) and current drug dependence ($p= 0.170$, Fisher's exact test) (**Supplementary Table 2-5**). None of these improved the accuracy of the final multilevel models when added as covariates.

Level of functioning at follow-up

In CHR subjects for whom GAF disability data were available (n=215), the mean score at final follow-up was 61.5 (SD= 14.6), with an interquartile range of 50.0 to 73.0. GAF disability score at follow-up was significantly associated with GAF disability score at baseline ($R= 0.329$ $p<0.001$).

In univariate analyses, two cannabis use variables met our threshold ($p< 0.2$) for inclusion in subsequent multivariate analyses: cannabis dependence ($t= 1.630$ $df= 136$, $p= 0.105$) and frequency of cannabis use ($F(2,159)= 1.861$, $p= 0.159$). In multilevel linear regression models, which included time of follow-up assessment as a covariate and site as a random effect, the association with cannabis dependence was not significant (estimate= -5.1 [95%CI -11.2 to 1.1], p=0.105). Daily use of cannabis was significantly associated with level of functioning at follow-up compared to less than weekly use (estimate= -5.8 [95%CI -11.0 to -0.6], p=0.029), and compared to less than daily use (estimate= -5.7 [95%CI -10.7 to -0.6], p=0.027). However, these associations were no longer significant after adjusting for baseline GAF disability score (**Table** 2-4).

Three potentially confounding variables were identified in univariate analyses: age (R=

-0.098, p= 0.153), lifetime use of other drugs (t= -1.692 df= 210, p= 0.092) and drug dependence within year to baseline (t= 1.728 df= 213, p= 0.085) (**Supplementary Table 2-6**). Although adjusting for lifetime drug use improved the accuracy of the multilevel linear regression model for frequency of use (χ^2= 6.5771 p= 0.010), the association with functional outcome remained non-significant. Similarly, adjusting for lifetime drug use improved the accuracy of the multilevel linear regression model for cannabis dependence (χ^2= 6.3143 p= 0.012), but the association with functional outcome remained non-significant **(Table** 2-4).

Table 2-3. Relationship between cannabis use and persistence of symptoms vs. symptomatic remission

	CHR-R (n=72)	CHR-NR (n=137)	P value	Fully adjusted OR (95% CI)	P value
Cannabis use status			0.304		
Current user	16 (22.2%)	44 (32.1%)		--	--
Ex-user	37 (51.4%)	64 (46.7%)		--	--
Never	19 (26.4%)	29 (21.2%)		--	--
Age first used cannabis			0.866		
≤15 years	26 (49.1%)	53 (50.5%)		--	--
>15 years	27 (50.9%)	52 (49.5%)		--	--
Frequency of cannabis use			0.245		
Daily	12 (23.5%)	39 (36.8%)		--	--
More than once weekly	8 (15.7%)	15 (14.2%)		--	--
Less than once weekly	31 (60.8%)	52 (49.1%)		--	--
THC content of most used cannabis type			0.059		
High (>10% THC)	36 (90.0%)	48 (75.0%)		0.60 (0.14 – 2.26)	0.459
Low (<10% THC)	4 (10.0%)	16 (25.0%)		1 (ref.)	--
Cannabis dependence			0.071		
Dependent	5 (10.9%)	21 (23.9%)		3.150 (1.04 – 11.38)	0.054
Not dependent	41 (89.1%)	67 (76.1%)		1 (ref.)	--

Abbreviations: CHR-R, clinical high risk remission subgroup; CHR-NR, clinical high risk persistent symptoms subgroup; OR, odds ratio; ref, reference category. Fully adjusted ORs are adjusted for site as a random effect. Only variables with $p<0.2$ in χ^2 tests added to adjusted, multilevel model, to reduce error from multiple testing.

Table 2-4. Relationship between cannabis use and functional outcome

	GAF score at follow up (95% CI)	P value	Crude estimate (95% CI)	P value	Fully adjusted estimate (95% CI)	P value
Cannabis use status		0.346				
Current user	59.3 (55.5 to 63.1)		--	--	--	--
Ex-user	62.6 (59.7 to 65.5)		--	--	--	--
Never	62.0 (58.2 to 65.8)		--	--	--	--
Age first used cannabis		0.496				
≤15 years	61.0 (57.4 to 64.5)		--	--	--	--
>15 years	62.6 (59.6 to 65.5)		--	--	--	--
Frequency of cannabis use		0.159				
Daily	57.9 (53.4 to 62.5)		-3.3 (-8.4 – 1.9)	0.213	-4.4 (-9.5 – 0.8)	0.094
More than once weekly	62.3 (56.0 to 68.7)		-1.2 (-7.1 – 4.8)	0.698	-1.2 (-7.0 – 4.6)	0.674
Less than once weekly	63.1 (60.1 to 66.2)		0 (ref.)	--	0 (ref.)	--
THC content of most used cannabis type		0.548				
High (>10% THC)	63.2 (60.2 to 66.2)		--	--	--	--
Low (<10% THC)	60.8 (51.0 to 70.5)		--	--	--	--
Cannabis dependence		0.105				
Dependent	57.0 (50.2 to 63.8)		-3.5 (-9.3 – 2.3)	0.240	-5.3 (-11.2 – 0.62)	0.079
Not dependent	62.2 (59.6 to 64.8)		0 (ref.)	--	0 (ref.)	--

Abbreviations: GAF, global assessment of functioning score; ref, reference category.

GAF score at follow up given as mean (95% confidence interval), where higher scores represent higher levels of functioning.

Estimates represent difference in mean GAF scores from reference group. Crude estimates are adjusted for baseline GAF score, days from baseline to final GAF assessment and for site as a random effect. Fully adjusted estimates are additionally adjusted for lifetime drug use. Only variables with $p<0.2$ in t test or ANOVA added to adjusted, multilevel models, to reduce error from multiple testing.

2.5 DISCUSSION

Our primary hypothesis was that cannabis use in CHR subjects would be associated with an increased rate of later transition to psychosis. However, there was no significant association with any measure of cannabis use. These results are in keeping with the study by Buchy *et al.*,[26] who followed 362 CHR subjects for 2 years and found no association between either the frequency of use, or the age at first use of cannabis and transition to psychosis. Conversely, Valmaggia *et al.*[25] in a study of 182 CHR subjects reported that both frequent use and use before age 15 years were linked to later onset of psychosis. 52.2% of CHR participants in that study reported using cannabis at least once per week, compared to 32.6% of CHR participants who were current more-than-weekly users in the study of Buchy *et al.*[26] (who did not find an association between frequency of use and transition), and 47.0% of CHR participants using more than once weekly in the present study. Another study in 341 CHR individuals found an association between cannabis use and transition, but this was no longer significant after controlling for alcohol use.[42] In the present study, alcohol use did not significantly influence the findings. Although the total number of studies that have examined the link between cannabis use in CHR individuals and transition to psychosis is still modest, meta-analyses of data from these studies have not found a significant association.[23,24,60]

The lack of an association between cannabis use and psychosis onset contrasts with data from cross-sectional studies that have examined cannabis use in patients with a psychotic disorder and controls. These suggest that initiation of use at an early age,[5–7] frequent use,[7,10] and the use of high-THC preparations[2,7] are associated with an increased risk of psychosis. For example, di Forti *et al.*[7] found that a greater proportion of patients with first episode psychosis than healthy controls had used cannabis by age 15 (FEP = 28.6% vs. HC = 13.7%), used more than once per week (41.4% vs 14.2%) and used cannabis with estimated ≥10% THC (37.1% vs 19.4%). In the present study, 49.2% of CHR participants had used cannabis by age 15, 47.0% used more than weekly and 76.2% used high potency cannabis. As well as having the risk of recall bias, associations found by these cross-sectional studies might be confounded by the effects of other risk factors for psychosis, such as social adversity, genetic risk, and use of other substances,[12,61] Mendelian randomisation studies, which can control for such effects, indicate a causal relationship between initiation of cannabis use and schizophrenia,[13,62], although the effect of schizophrenia risk on cannabis initiation may be even stronger. This is consistent with a study by Power *et al.*[63] which

reported an association between genetic risk for schizophrenia and both age of initiation of cannabis use and the amount of cannabis consumed.

Most CHR subjects do not develop psychosis, but these individuals may still have adverse clinical outcomes in the form of persistent symptoms and an impaired level of functioning.[64,65] Our secondary hypotheses were that cannabis use would also influence the likelihood of these two outcomes. However, we found no evidence of significant associations between any cannabis measures and either outcome. Only one previous study has examined the association between cannabis use and persistence of the CHR state, and this also found no association.[66] The small number of studies examining the association between cannabis use and functional outcomes in CHR subjects have produced mixed results. A cross-sectional study by MacHielsen *et al.*[67] found no difference in GAF scores between CHR participants with and without a cannabis use disorder. In a cross-sectional study of 731 CHR and non-CHR help-seeking individuals, Carney *et al.*[68] found that participants who showed signs of cannabis dependence and 'high risk' cannabis use presented with lower social and occupational functioning. However, Auther *et al.*[69] reported that lifetime cannabis use in 101 CHR subjects was associated with a higher level of social functioning at follow-up.

The present study also found that while most (74%) CHR subjects had used cannabis before, only around a third of the sample were using cannabis at the time of presentation. These observations are consistent with data from previous studies which reported that between 43-55% of CHR subjects had ever used cannabis, and between 22-30% were current users.[23,25,26,68–74] This suggests that a large proportion of CHR individuals have stopped using cannabis before they seek clinical help. Insight is less impaired in CHR subjects than in patients with psychosis,[75] and some CHR subjects may stop using cannabis because they think that it exacerbates their symptoms.[25,74] It is possible that differences in level of insight and the pattern of use could explain differences between findings in studies of cannabis use and psychosis risk in CHR populations and in patients with psychotic disorders. For example, If CHR subjects tend to discontinue cannabis use, this could reduce the influence of cannabis use on the risk of psychosis in this population.[76]

Strengths of the present study were the large size of the CHR sample and the availability of detailed information on previous and current cannabis use. Although we cannot exclude the

possibility that an association between cannabis use and transition to psychosis might have been evident if the follow up period had been longer than 2 years, the great majority of transitions occur within this timeframe,[22] The present study examined the relationship between cannabis use and transition to psychosis in a sample of people at CHR for psychosis. However, the CHR population appears to be heterogenous,[77] and the nature of the relationship between cannabis use and psychosis risk may vary between different CHR subgroups. People are categorised as being at CHR for psychosis because of subthreshold psychotic symptoms, but the causes of these symptoms may differ between each person.[78] For example, some people at CHR might experience attenuated psychotic symptoms due to genetic and environmental factors other than cannabis use. In others, their symptoms may be related to cannabis use, even if this is not necessary or sufficient for the development of a psychotic disorder.[78] As both these subgroups have an increased risk of psychosis, it may be difficult to find a difference in the incidence of psychosis when cannabis users and non-users within a CHR sample are compared. In addition, many of those who may be experiencing cannabis induced attenuated psychotic symptoms could have already stopped using cannabis before baseline assessment. One way to examine this theory would be to investigate the temporal relationship between within-subject changes in cannabis use and clinical outcomes.[79] However, this is not possible in the present study, as follow up data on cannabis use were not available in 36% of the cohort. Moreover, in almost all of the participants who transitioned to psychosis, the follow up assessments of cannabis use were made *after* the point of transition. As a result, it is not possible to know whether longitudinal changes in cannabis occurred before or after the onset of psychosis. It was thus not possible for us to address this issue in the present dataset. Because it was also not possible to collect information on clinical outcome for the entire sample, there is a risk that subjects with adverse clinical outcomes might have been more likely to be lost to follow up. However, there were no significant socio-demographic or clinical differences between those who completed follow-up and those who did not.

The present study did not include biological measures of cannabis and other substances, and future investigations could be enhanced by collecting serial urine or blood samples to corroborate interview data. Finally, although we examined cannabis use prior to the onset of psychosis, the mean age of the participants was 22 years. Our measures of cannabis use in

childhood and adolescence were therefore retrospective and might not have been accurate enough to detect associations between very early use and clinical outcomes in adulthood.

2.5.1 CONCLUSIONS

There was no evidence that cannabis use in people at high risk for psychosis had a significant effect on the incidence of psychosis or other adverse clinical outcomes. These findings are not consistent with epidemiological data linking cannabis use to an increased risk of developing psychosis.

2.6 ACKNOWLEDGMENTS

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2.7 DISCLOSURE STATEMENT

The authors declare no conflict of interest.

2.8 AUTHOR CONTRIBUTIONS

LC, PM, MK, DS, planned the analyses, LC conducted the analyses, LC, PM, MK, EC, DO drafted the manuscript along with the other authors. All authors contributed to the interpretation of the results. The authors given final approval of the current version of the manuscript.

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2.10 SUPPLMENTARY MATERIAL

2.10.1. EU-GEI High Risk Study Group

2.10.2. Supplementary Methods

Supplementary Table 2-1. Classification of cannabis products as high (>10%) or low (<10%) THC content

Supplementary Table 2-2. Subgroup analyses assessing CHR sample characteristics and missingness of follow-up data

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Supplementary Table 2-6. Relationship between demographic and clinical features of CHR participants and GAF score at follow-up

2.10.3. Supplementary References

2.10.1 EU-GEI HIGH RISK STUDY GROUP

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2.10.2 SUPPLEMENTARY METHODS

Sensitivity and power calculations

The original *a priori* sample size calculation for the EU-GEI High Risk project was an estimate based on a previous study,[1] suggesting that a sample of n=400 subjects with an CHR would provide sufficient statistical power for the project's primary outcome, genetic influences on clinical outcome.

Post hoc sensitivity and power calculations were conducted for the current study using the R package *epiR* version 2.0.61.[2]

Power

We considered the primary outcome to be current cannabis use vs. non-use, transition to psychosis. With HR = 1.04, a significance criterion of $\alpha = .05$, and a total sample size of 176 (90 current cannabis users, 86 non-users), the calculated power = 0.038.

Sensitivity

With power = 0.80, a significance criterion of $\alpha = .05$ and a total sample size of 176 (90 current cannabis users, 86 non-users), the minimum detectable HR for this study was <0.655 or >1.526, equivalent to either a 34.5% decrease in risk or a 52.6% increase in risk of transitioning to psychosis within a 2-year period.

Multilevel linear regression parameter estimates

Fixed effect parameter estimates are interpreted the same way as one would interpret estimates from a traditional ordinary least squares linear regression. For instance, for a categorical predictor with a coefficient of 1.0, the mean GAF score of the predictor group of is 1.0 points higher than the mean GAF score of the reference group.

THC content of cannabis variable

Participants were asked to name in their own language the type of cannabis they mostly used during their period of use. Types named included hash (cannabis resin/solid), imported herbal cannabis, home-grown skunk/ sensimilla / super skunk, and the Dutch geïmporteerde wiet (imported herbal cannabis), Nederwiet (Dutch herbal cannabis), geïmporteerde hasj (imported cannabis resin) and Nederhasj (cannabis resin made of Nederwiet). Other named types or answers that could not be clearly grouped, such as "all of them" or "don't know", were excluded from this analysis.

The THC content variable was created using a cut off of 10% THC, consistent with previous research.[3,4] Data published by the European Monitoring Centre for Drugs and Drug Addiction (EMCDDA) reports[5-11] as well as other national reports on cannabis potency[12-21] was stratified according to country and cannabis type and used to classify the types of cannabis named by participants as either high (>10%) or low (<10%) THC content (see **Supplementary Table** 2-1 for more details).

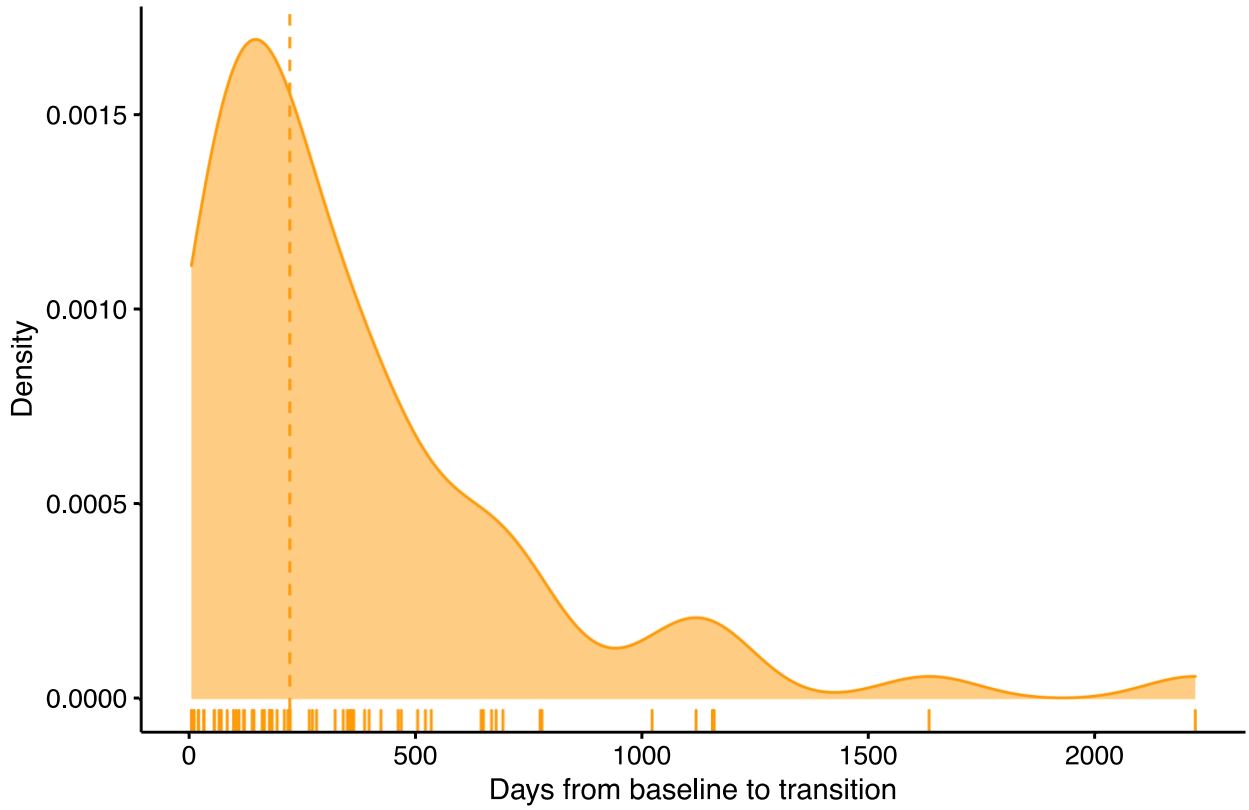
Supplementary Table 2-1. Classification of cannabis products as high (>10%) or low (<10%) THC content

	Named types of cannabis products						
Site	Hash (cannabis resin/solid)	Imported herbal cannabis	Home-grown skunk/ Sensimilla/ Super skunk	Geïmporteerde hasj	Nederhasj	Geïmporteerde wiet	Nederwiet
London	Low	Low	High				
Vienna	Low	Low					
Basel	High	Low	High				
Cologne	Low	High					
Melbourne	High		High				
Copenhagen	High	High					
Paris	High	High					
Barcelona	High	High	High				
Sao Paulo		Low					
Amsterdam				High	High	Low	High
Den Haag				High	High	Low	High

High = high estimated THC content (>10%); Low = low estimated THC content (<10%).
Geïmporteerde hasj = Dutch imported cannabis resin; Nederhasj = Dutch cannabis resin made of Nederwiet; Geïmporteerde wiet = Dutch imported herbal cannabis; Nederwiet = Dutch herbal cannabis.
Blank cells indicate that no participants from the site named that type of cannabis product.

Supplementary Figure 2-1. Density plot of time to transition to psychosis.

Dashed lines mark median time to transition (223 days) and 2-years post-baseline respectively.



Supplementary Table 2-2. Subgroup analyses assessing CHR sample characteristics and missingness of follow-up data

	Followed-up (n=259)	No follow-up (n=75)	P value
Age, years (SD)	22.6 (4.9)	21.8 (5.2)	0.262
Male gender	142 (54.8%)	35 (46.7%)	0.212
Ethnicity	--	--	0.167
White	186 (71.8%)	53 (70.7%	--
Black	29 (11.2%)	4 (5.3%)	--
Other	44 (17.0%)	18 (24.0%)	--
Taking antipsychotic medication	23 (9.4%)	9 (13.8%)	0.294
Tobacco use, daily	144 (57.6%)	34 (46.6%)	0.096
Alcohol, drinks per week (SD)	5.4 (9.5)	6.3 (12.9)	0.509
Other substance use, ever	101 (39.5%)	23 (30.7%)	0.167
Other substance use, past year	76 (32.2%)	21 (28.4%)	0.536
Other substance dependence, ever	21 (8.1%)	7 (9.3%)	0.736
Other substance dependence, past year	11 (4.2%)	4 (5.3%)	0.689

P values for χ^2 tests. Data as mean (SD) or n (%).

Supplementary Table 2-3. Demographic and clinical features of CHR participants by cannabis use status

	Never used (n=86)	Past user (n=158)	Current user (n=90)	P value
Age, years	20.5 (4.7)	22.9 (4.5)[a]	23.3 (5.5)[a]	**<0.001**
Male gender	38 (44.2%)	81 (51.3%)	58 (64.4%)[a]	**0.022**
Ethnicity	--	--		0.175
White	60 (69.8%)	113 (71.5%)	66 (73.3%)	
Black	5 (5.8%)	21 (13.3%)	7 (7.8%)	
Other	21 (24.4%)	24 (15.2%)	17 (18.9%)	
Antipsychotic use	8 (10.5%)	15 (9.9%)	9 (10.8%)	0.974
Tobacco use, daily	9 (10.8%)	99 (64.3%)[a]	70 (81.4%)[a, b]	**<0.001**
Alcohol, drinks per week	1.7 (5.1)	6.4 (12.3)[a]	8.1 (9.4)[a]	**<0.001**
Other substance use, ever	3 (3.5%)	66 (42.0%)[a]	55 (61.8%)[a, b]	**<0.001**
Other substance use, past year	2 (2.4%)	51 (34.9%)[a]	44 (55.0%)[a, b]	**<0.001**
Other substance dependence, ever	0 (0%)	1 (8.9%)[a]	14 (15.6%)[a, b]	**<0.001**
Other substance dependence, past year	0 (0%)	6 (3.8%)	9 (10.0%)[a]	**0.004**
GAF disability score	56.2 (14.2)	55.9 (12.1)	53.7 (10.7)	0.393
CAARMS positive symptom score	35.0 (19.8)	37.2 (20.8)	38.7 (17.8)	0.332
CAARMS negative symptom score	27.9 (20.4)	30.3 (17.8)	29.4 (17.8)	0.359

Abbreviations: CAARMS, Comprehensive Assessment of At-Risk Mental State; GAF, Global Assessment of Functioning.

P values for χ^2, Fisher-Freeman-Halton Exact, one-way ANOVA or Kruskal-Wallis tests. Data as mean (SD) or n (%). Significant (<0.05) p values are presented in bold.

[a] Significantly different from never used group, after Bonferroni correction for multiple comparisons.

[b] Significantly different from past user group, after Bonferroni correction for multiple comparisons.

Supplementary Table 2-4. Demographic and clinical features of transitioned and non-transitioned CHR participants

	Hazard Ratio (95% CI)	P value
Age, years	1.01 (0.95 – 1.06)	0.836
Gender	--	
Male	1.10 (0.64 – 1.88)	0.729
Female	1 (ref.)	--
Ethnicity	--	
White	1 (ref.)	--
Black	1.36 (0.61 – 3.04)	0.456
Other	0.90 (0.43 – 1.86)	0.771
Taking antipsychotic medication	--	
Yes	2.38 (1.19 – 4.76)	**0.015**
No	1 (ref.)	--
Tobacco use, daily	--	
Yes	0.83 (0.48 – 1.44)	0.509
No	1 (ref.)	--
Alcohol, drinks per week	1.00 (0.98 – 1.03)	0.846
Other substance use, ever	--	
Yes	1.12 (0.64 – 1.94)	0.695
No	1 (ref.)	--
Other substance use, past year	--	
Yes	1.14 (0.64 – 2.05)	0.653
No	1 (ref.)	--
Other substance dependence, ever	--	
Yes	1.13 (0.45 – 2.84)	0.791
No	1 (ref.)	--
Other substance dependence, past year	--	
Yes	1.30 (0.41 – 4.16)	0.661
No	1 (ref.)	--

Abbreviations: ref., reference category.
P values for Cox regression analyses. Significant (<0.05) p values in bold.

Supplementary Table 2-5. Demographic and clinical features of CHR participants with and without persistent symptoms at last follow-up

	CHR-R (n=72)	CHR-NR (n=137)	P value
Age, years	22.6 (4.7)	23.1 (5.3)	0.447
Male gender	35 (48.6%)	78 (56.9%)	0.251
Ethnicity	--	--	0.838
White	52 (72.2%)	97 (70.8%)	--
Black	7 (9.7%)	17 (12.2%)	--
Other	13 (18.1%)	23 (16.8%)	--
Taking antipsychotic medication	5 (7.5%)	14 (10.7%)	0.466
Tobacco use, daily	36 (50%)	77 (56.2%)	0.253
Alcohol, drinks per week	4.0 (10.5)	6.3 (9.4)	0.123
Other substance use, ever	26 (36.1%)	59 (43.1%)	0.310
Other substance use, past year	15 (23.8%)	49 (38.0%)	0.050
Other substance dependence, ever	6 (8.3%)	14 (10.2%)	0.660
Other substance dependence, past year	1 (1.4%)	9 (6.6%)	0.170

Abbreviations: CHR-R, clinical high risk remission subgroup; CHR-NR, clinical high risk persistent symptoms subgroup.
P values for χ^2 or Fisher's Exact test. Data as mean (SD) or n (%).

Supplementary Table 2-6. Relationship between demographic and clinical features of CHR participants and GAF score at follow-up

	GAF at follow up	P value
Age, years	R= -0.098	0.153
Gender	--	0.339
Male	60.7 (57.9 – 63.4)	--
Female	62.6 (59.8 – 65.4)	--
Ethnicity	--	0.209
White	62.6 (60.2 – 64.9)	--
Black	57.4 (50.1 – 64.8)	--
Other	59.5 (55.4 – 63.7)	--
Taking antipsychotic medication	--	0.179
Yes	56.8 (49.1 – 64.5)	--
No	61.8 (59.6 – 64.0)	--
Tobacco use, daily	--	0.922
Yes	61.6 (58.9 – 64.3)	--
No	61.4 (58.4 – 64.4)	--
Alcohol, drinks per week	R= -0.058	0.415
Other substance use, ever	--	0.092
Yes	63.6 (60.5 – 66.6)	--
No	60.2 (57.6 – 62.8)	--
Other substance use, past year	--	0.243
Yes	63.3 (60.0 – 66.8)	--
No	60.8 (58.2 – 63.3)	--
Other substance dependence, ever	--	0.428
Yes	59.0 (51.7 – 66.3)	--
No	61.8 (59.7 – 63.8)	--
Other substance dependence, past year	--	0.085
Yes	53.8 (45.9 – 61.7)	--
No	61.9 (59.9 – 63.9)	--

Abbreviations: Global Assessment of Functioning score.
Data are Spearman's rho (continuous variables) or mean (95% confidence interval) (categorical variables). P values for Spearman's rank correlation, ANOVA or student's t test analyses.

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CHAPTER 3 - EFFECTS OF CANNABIS USE ON COGNITION IN PEOPLE AT CLINICAL HIGH RISK FOR PSYCHOSIS

Paper 2: Chester LA, Kempton MJ, Tognin S et al. Effects of cannabis use on cognition in people at clinical high risk for psychosis. *Neuropsychopharmacology* 2022 [In Preparation]

Effects of Cannabis Use on Cognition in People at Clinical High Risk for Psychosis

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3.1 ABSTRACT

3.1.1 BACKGROUND

Despite evidence that cannabis use leads to cognitive deficits, studies show that patients with psychosis who use cannabis have less impaired cognition than non-using patients. We sought to assess the relationship between cannabis use and cognitive function in individuals at clinical high risk for psychosis.

3.1.2 METHODS

A multi-site sample of individuals at clinical high risk of psychosis (CHR; n=326) and healthy controls (HC; n=65) completed a comprehensive battery of cognitive tests. Multilevel linear regression models were used to assess the effects of subject status (CHR vs. HC) and lifetime cannabis use in the CHR group (ever used vs. no use) on cognitive performance. In exploratory analyses, cognition was compared in subgroups of CHR participants based on a) current or past use, b) frequency of use, c) age of first use and d) cannabis dependence.

3.1.3 RESULTS

CHR participants showed significant cognitive deficits compared the HCs in the domains of general intelligence, executive function, working memory, and verbal fluency. In CHR participants, lifetime cannabis use was associated with better performance in a measure of executive functioning. Results from the exploratory analysis suggest that participants who used cannabis less often than once per week were the least cognitively impaired subgroup in the CHR sample, though were not superior to the HC sample.

3.1.4 DISCUSSION

These results are consistent with findings in individuals with psychosis, indicating that cannabis using patients show less impaired cognition than non-using patients. Cannabis users may represent a subgroup of CHR individuals with comparatively good premorbid cognitive function.

3.2 INTRODUCTION

In healthy volunteers, cannabis and its constituent tetrahydrocannabinol (THC) can produce acute impairments in cognitive functioning, with effects on verbal learning, working memory, executive function and processing speed.[1,2] There is also evidence that cognitive deficits persist with regular cannabis use,[3–6] particularly in frequent users,[7,8] and in people who start to use cannabis in adolescence.[9,10]

Cannabis use is particularly common among people with psychosis, with as many as 80% of patients reporting use of the drug at some point in their life,[11] Cognitive impairments are a feature of psychotic disorders[12–14] and are linked with poor functional and clinical outcomes.[15–17] Although chronic cannabis use in the general population has been associated with impairments in cognitive performance,[4,5] five of seven recent meta-analyses found that patients with schizophrenia who reported having ever used cannabis performed better than patients who had not used cannabis on tests of general cognitive ability, attention, memory, and executive function.[18–22] A number of different explanations for this counter-intuitive finding have been proposed. One is that the subgroup of patients in whom psychosis is associated with cannabis use have a relatively high level of premorbid cognitive functioning, education, and/or socioeconomic status.[23,24] Another is that sourcing illicit cannabis requires motivation, planning, organisation and social skills, and that cannabis use may be a proxy indicator of these.[5,25]

The onset of psychotic disorders is often characterised by the emergence of sub-threshold psychotic symptoms and a decline in social and occupational functioning.[26,27] Individuals with these symptoms can classified as being at clinical high-risk for psychosis (CHR).[28] When compared with healthy controls, like patients with psychotic disorders, the CHR population show impaired performance across all domains,[14,29] although the magnitude of the impairments is smaller.[30] A key advantage of studying cognitive functioning in CHR subjects is that the majority have never been treated with antipsychotic medications. This minimises the potentially confounding effects of treatment on cognitive performance that is typically a concern in studies involving patients with psychosis.[31]

As in individuals with psychotic disorders, cannabis use is more common in people with a CHR state than in the general population.[32] There have only been two previous studies of the

effects of cannabis use on cognitive performance in this population. Both Bugra *et al.*[33] and Korver *et al.*[34] found no differences between individuals at CHR who regularly used cannabis performed and non-users of cannabis on tests of general intelligence, executive function, attention, verbal learning or memory. However, both studies had relatively small samples sizes (n=74 and n=63, respectively), limiting their power to detect differences.

The present study examined the relationship between cannabis use and cognitive function in a large cohort of CHR subjects. We first tested the hypothesis that CHR subjects, like patients with psychosis, would show impairments in cognition compared to healthy controls across a range of domains. Our second prediction was that, as in patients with psychosis, performance in CHR subjects who had used cannabis would be less impaired than in CHR subjects who had never used cannabis. Finally, within CHR subjects that used cannabis, we performed exploratory analyses of the relationship between frequency of use, early onset of use and cannabis dependence and cognitive performance.

3.3 METHODS

3.3.1 PARTICIPANTS

Participants were recruited to a multi-centre prospective study of people at clinical-high risk for psychosis.[35] 344 people meeting Comprehensive Assessment of At-Risk Mental States (CAARMS) criteria[28] for a CHR state were enrolled from centres in London, Amsterdam, Den Haag, Barcelona, Basel, Cologne, Copenhagen, Paris, Vienna, Melbourne and Sao Paulo. 67 HCs were recruited from four of the sites; London, Amsterdam, Den Haag, and Melbourne. The HC sample matched (at group level) the CHR cohort in terms of age and gender.

Most participants (95.0%) were aged 16-35 years, though 17 participants outside of this range were included as some of the local clinical services employed a slightly wider age range. Inclusion criteria for all participants were an adequate understanding of the language local to each study site and provision of written, informed consent. Exclusion criteria included: previous diagnosis of a psychotic disorder as defined by the Structural Clinical Interview for DSM Disorders,[36] exceeding the 'Psychosis Threshold' or 'Antipsychotic Treatment Threshold' defined by the CAARMS,[28] estimated IQ < 60 as measured by the shortened Wechsler Adult Intelligence Scale-III (WAIS-III),[37] unable or unwilling to give a blood or saliva sample for genetic analysis. Additionally, CHR participants were excluded if their psychotic symptoms could be explained by substance misuse or an organic disorder, and HC were excluded if they met CAARMS criteria for a CHR state.

3.3.2 ETHICS STATEMENT

All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/patients were approved research ethics committees at each site.

3.3.3 MEASURES

Data on demographic characteristics were collected using the Medical Research Council Sociodemographic Schedule.[38] Global functioning was assessed using the Global Assessment of Functioning (GAF) disability subscale.[39] Socio-economic status (SES) was defined as the

father's social class at participant's birth. Fathers who were long-term unemployed were classified according to their last main paid job and those who never worked or were full-time students were excluded (n=2). SES was categorised into a three-class model: salariat, intermediate and working class.[40]

Cannabis use was assessed using a modified form of the Cannabis Experience Questionnaire (EU-GEI$_{CEQ}$).[41] Participants who had used cannabis at any point in their life were categorised as a Cannabis User (CU). Participants who reported they had never used cannabis were classed as a Non-Cannabis User (NU). Participants who reported any cannabis use were subdivided into current or past users depending on whether they were using cannabis at baseline. The pattern of cannabis use (either current or past) was characterised as either i) occasional (less than once per week) ii) at least once per week, or iii) daily. Age at first cannabis use was estimated by the participant and categorised as either early (up to 15 years old) or not early (16 years or later). The presence of cannabis dependence in the year prior to baseline assessment was assessed on the basis of the participant's report of their use, using DSM-IV criteria for substance dependence.[42]

Use of alcohol and tobacco were recorded using the Composite International Diagnostic Interview.[43] Use of other recreational drugs, including amphetamines, cocaine, crack, hallucinogens, inhalants, ketamine, opioids, sedatives, or other illicit substances, were collected using the EU-GEI$_{CEQ}$.

3.3.4 COGNITIVE TASKS

The present study selected cognitive tasks that assess domains for which there is evidence of higher performance in patients with psychosis who are cannabis users compared to those who are not.[20]

Trail Making Test (TMT)

The TMT is a widely used test of executive ability. Trail A (TMT-A) involved asking participants to draw a line connecting consecutive numbers from 1 to 25. Trail B (TMT-B) involved asking participants to draw a similar line, connecting alternating numbers and letters in sequence (i.e., 1-A-2-B etc). The time to complete each trail was recorded. Trail A is designed to primarily measure visual scanning and motor speed, while trail B also assesses

the sustained attentional control processes involved in the rapid alteration of tasks. The TMT-B/A ratio score was calculated to provide an indicator of executive control function independent of visual scanning and motor speed.[44]

Rey Auditory verbal learning task (AVLT)

The AVLT was used to test verbal memory.[45] Participants were asked to listen to a list of 15 words and repeat them back. The number of words recalled over five consecutive trials were recorded as immediate recall, a measure of working memory. Participants were then asked to repeat the list again 20-25 minutes later, and the number of words recalled was recorded as delayed recall, a measure of episodic memory.

Verbal fluency test (VERFL)

The VERFL was used to evaluate verbal fluency.[46] In 60 second trials, participants were asked to name as many animals as possible (semantic fluency) or generate as many words as possible for a given letter (phonetic fluency). The score was recorded as the number of correct words produced in the different categories. The letter subtests differed between sites to reflect letter frequencies within each language (e.g., FAS was used in London and SNA in Amsterdam and Den Haag).

Wechsler Adult Intelligence Scale-III

A shortened version of the WAIS-III was used to estimate general cognitive ability and intelligence (IQ).[47] It comprised four subtests of Arithmetic, Block Design, Digit Symbol, and Information.[48]

3.3.5 STATISTICAL ANALYSIS

Analyses were performed using R version 4.0.3[49] and SPSS version 28.[50] Sociodemographic and clinical differences between groups were assessed using Pearson's chi squared, Student's t- or Mann-Whitney U tests. Post-hoc pairwise comparisons were conducted using the Student's t-test, adjusted using the Bonferroni correction for multiple comparisons.

Performance in each cognitive assessment in CHR participants was first compared to the HC group using multilevel logistic regression models (lme4 package version 1.1-26).[51] Subject status (CHR or HC) was included as a fixed effect, with site as a random effect to account for

clustering. The effect of having ever used cannabis in the CHR group were investigated by removing HC data and replacing subject status with the lifetime cannabis use variable (NU or CU) in the models.

All regression models were adjusted for age, sex, and SES (0=salariat, 1=intermediate, 2=working class). Confounders were chosen *apriori* based on literature on the subject.[18,22,25,31,33,52–55] As the use of alcohol, tobacco and other substances were highly skewed between groups and are known influences of cognitive functioning, we adjusted for these variables in a sensitivity analysis. Alcohol and tobacco product use were coded as continuous variables (average number of alcoholic drinks per week; average number of tobacco products [cigarettes, pipe tobacco, chewing tobacco and snuff; not including e-cigarette or vape products] per day), and use of other illicit substances in the past 12 months apart from cannabis was binary (0=no use, 1=any use). Alcohol, tobacco, and other illicit substance use variables were not included in the initial models as 19.2% of participants (CHR n=70, HC n=9) were missing data from one or more of these variables, reducing statistical power. Years of education was not chosen as a covariate as 33 CHR participants (11.3%) and only 1 HC (1.5%) were under the age of 18, making age a strong predictor of education level.

For the exploratory analysis, we also examined the effects of patterns of cannabis use within the CHR group by replacing the lifetime cannabis use variable with variables for a) cannabis use status (0=NU, 1=past user, 2=current user); b) frequency of use (0=NU, 1=occasional user, 2=weekly user, 3=daily user), c) age at first cannabis use (0=NU, 1=age 16 years or older, 2=age 15 years or younger), and d) cannabis dependence (0=NU, 1=non-dependent, 2=cannabis dependent). In a post-hoc analysis, we repeated the frequency of use analysis limiting the CU group to only current users, to account for possible effects of abstinence. Pairwise comparisons were not used in order to minimize family-wise error rate. One group (NU) was selected as the reference category and estimated marginal mean (EMM) differences were calculated between the NU and CU groups only. P values were corrected for multiple comparisons using the multivariate t distribution adjustment method.[56,57]

In a final post-hoc analysis, performed after other analyses had been completed and results were known, we compared the best performing CHR subgroup with the HC group in order to

reassess the relative cognitive functioning in this CHR subgroup. P values have not been presented as groups were not defined *a priori*.

For figures, cognitive assessment scores were converted into standardised z-scores to better allow comparisons between cognitive tests. Z-scores were based on the HC sample as a reference group using the formula below, where χ is the raw score, μ is the mean of the HC sample and σ is the standard deviation of the HC sample.

$$\frac{\chi - \mu}{\sigma}$$

EMM differences are presented along with p-values and 95% confidence intervals. Statistical significance was defined at the 0.05 level. For post hoc power and sensitivity calculations see **Supplementary Results**.

3.4 RESULTS

CHR participants for whom there were either no cannabis use data and/or no cognitive assessment data available were excluded (n=18). Excluded individuals did not differ from the included participants (n=326) in terms of age, proportion of male gender, ethnicity, childhood trauma questionnaire (CTQ) score, SES, number of years in education, use of antipsychotics, use of tobacco, alcohol or other drugs, GAF disability score or CAARMS total symptom score (**Supplementary Table** 3-1). The majority (91.1%) of included CHR were not taking antipsychotic medications at the time of the cognitive assessments.

3.4.1 SOCIODEMOGRAPHIC AND CLINICAL FEATURES: HC AND CHR GROUPS

Compared to the HC group, CHR participants were more likely to be working class and less likely to be in the salariat class. CHR had on average 1.7 less years of education, used more tobacco products (+4.1 per day), and had poorer global functioning scores than HC. There were no differences in the age, gender, ethnicity, alcohol use or other substance use of the HC and CHR groups. More CHR participants than HC had ever used cannabis, but CHR were not more likely than HC to be current users of cannabis (**Supplementary Table** 3-2).

3.4.2 COGNITIVE IMPAIRMENT IN CHR

Compared to the HC group, CHR participants had a lower WAIS-III estimated IQ ($t(318)=5.403$, $p<0.001$), poorer performance on the TMT-B ($t(289)=3.943$, $p<0.001$), and had a higher TMT-B/A ratio ($t(243)=2.420$, $p=0.016$). They also had lower scores for AVLT immediate recall ($t(303)=3.067$, $p=0.002$), phonetic verbal fluency ($t(309)=4.382$, $p<0.001$), and semantic verbal fluency ($t(310)=2.390$, $p=0.017$). There were no significant group differences on the TMT-A or AVLT delayed recall tests (**Table** 3-1). Results did not differ significantly when adjusting for the use of alcohol, tobacco, and other substances in a sensitivity analysis (**Supplementary Table** 3-3).

Table 3-1. Cognitive assessment scores between healthy controls and CHR participants

Cognitive assessment		Raw cognitive assessment score, mean (SD) HC (n=65)	CHR (n=326)	EMM difference, HC-CHR (95% CI)[a]	P value[a]
WAIS-III	IQ	112.9 (18.1)	98.3 (17.0)	14.5 (9.2, 19.8)	**<0.001**
TMT	Trail A	26.64 (12.76)[b]	30.13 (12.16)[b]	2.67 (-1.39, 6.73)	0.197
	Trail B	56.29 (18.8)[b]	73.27 (30.56)[b]	16.60 (7.33, 25.88)	**<0.001**
	Trail B/A Ratio	2.255 (0.591)[b]	2.575 (0.960)[b]	0.354 (0.066, 0.642)	**0.016**
AVLT	Immediate Recall	56.27 (8.50)	51.30 (10.00)	4.42 (1.59, 7.26)	**0.002**
	Delayed Recall	11.45 (3.48)	10.59 (3.03)	0.95 (-0.04, 1.94)	0.060
VERFL	Phonetic Fluency	43.97 (14.43)	35.45 (12.54)	8.55 (4.71, 12.38)	**<0.001**
	Semantic Fluency	22.83 (8.00)	21.47 (5.96)	2.38 (0.42, 4.35)	**0.017**

Abbreviations: AVLT, Rey Auditory Verbal Learning Test; CHR, clinical high-risk group; EMM, Estimated Marginal Mean; HC, healthy control group; TMT, Trail Making Task; VERFL, Verbal Fluency Test; WAIS-III, shortened Wechsler Adult Intelligence Scale-III.
Statistically significant rows ($p<0.05$) are presented in bold.
[a] Adjusted for age, gender, and Father's SES, and site as random effect.
[b] Raw Trail Making Test times not reversed, higher score = poorer performance.

3.4.3 SOCIODEMOGRAPHIC AND CLINICAL FEATURES: CHR CANNABIS USE

In the CHR group, compared to never users (NU), the cannabis users (CU) group were older (+2.3 years), more likely to be male and less likely to be working class. Cannabis users also used more alcohol (+5.2 drinks per week) and tobacco products (+7.9 per day), and other illicit substances. There were no significant differences in ethnicity, years in education, antipsychotic use, GAF disability or CAARMS symptom scores (**Table** 3-2).

3.4.4 EFFECTS OF CANNABIS USE ON COGNITION

Of 287 CHR participants, 247 (75.8%) had used cannabis at least once in their lifetime. **Figure 3-1** shows the estimated marginal mean (EMM) difference between the standardised scores of the CU and NU groups for each cognitive assessment measure. CU participants performed better than the NU group on the TMT-B ($t(247)=2.273$, $p=0.024$), but there were no differences on the TMT-A, TMT-B/A ratio, WAIS-III IQ, AVLT or VERFL test scores (**Table** 3-3). Results did not differ significantly when adjusting for the use of alcohol, tobacco, and other substances in a sensitivity analysis (**Supplementary Table** 3-4).

Table 3-2. Sociodemographic and clinical features of cannabis using and non-using CHR participants

	NU (n=79)	CU (n=247)	P value
Age, years	20.8 (4.8)	23.1 (4.9)	< **0.001**
Male gender	34 (43.0%)	138 (55.9%)	**0.047**
Ethnicity	--	--	0.166
White	55 (69.6%)	179 (72.5%)	
Black	5 (6.3%)	28 (11.3%)	
Other	19 (24.1%)	40 (16.2%)	
SES			**0.017**
Salariat	20 (28.6%)	78 (35.9%)	
Intermediate	19 (27.1%)	82 (37.8%)	
Working class	31 (44.3%)	57 (26.3%)[a]	
Years in education	13.8 (3.1)	14.6 (3.1)	0.051
Antipsychotic use	7 (10.0%)	24 (10.3%)	0.942
Tobacco used, daily	1.0 (3.7)	8.9 (9.6)	**<0.001**
Alcohol use, weekly	1.7 (5.3)	6.9 (11.3)	**<0.001**
Other substance use	2 (2.6%)	95 (42.2%)	**<0.001**
GAF disability	56.3 (14.8)	55.1 (11.6)	0.508
CAARMS positive symptoms	34.1 (19.8)	37.7 (19.7)	0.161
CAARMS negative symptoms	27.7 (20.2)	30.0 (17.7)	0.378

Abbreviations: CAARMS, Comprehensive Assessment of At-Risk Mental State; CU, cannabis-using CHR group; GAF, Global Assessment of Functioning; NU, non-cannabis-using CHR group; SES, socioeconomic status.

P values for χ^2, independent t- or Mann-Whitney U tests (denoted by *) where assumption of homogeneity of variances was broken. Data as mean (SD) or n (%). Significant (<0.05) p values are presented in bold.

[a] Significantly different from NU group at the .05 level after Bonferroni correction for multiple comparisons (t-test of z-score).

3.4.5 PATTERNS OF CANNABIS USE

From the sample of 247 CHR participants in the CU group, 158 (64.0%) were classified as past users, and 89 (36.0%) as current users. Former cannabis users had last consumed the drug a median of 12 months before the assessment data, with a range of 1-120 months. 125 (50.6%) of the CU group reported using cannabis less than once per week (occasional users), 33 (13.4%) used at least once per week but not daily, and 77 (31.2%) used daily (data missing for 12 participants). 36 (14.6%) of the CU group were cannabis dependent at some point in the year prior to baseline assessment (data missing for 46 participants). The average age at which CHR first used cannabis was 15.9 years old (SD=2.89, data missing for 12 participants). Sociodemographic and clinical differences between CHR cannabis use subgroups are reported in **Supplementary Table** 3-5 and **Supplementary Table** 3-6.

Table 3-3. Cognitive assessment scores between CHR lifetime cannabis users and non-users

Cognitive assessment		Raw cognitive assessment score, mean (SD) NU (n=79)	CU (n=247)	EMM difference NU-CU (95% CI)[a]	P value[a]
WAIS-III	IQ	97.5 (19.1)	99.0 (16.6)	-3.3 (-8.3, 1.7)	0.191
TMT	Trail A	32.62 (14.43)[b]	29.59 (11.74)[b]	-3.72 (-0.01, 7.45)	0.050
	Trail B	79.60 (35.21)[b]	70.90 (28.70)[b]	-10.49 (-19.58, -1.40)	**0.024**
	Trail B/A Ratio	2.543 (0.910)[b]	2.550 (0.927)[b]	-0.016 (-0.299, 0.267)	0.912
AVLT	Immediate Recall	50.43 (10.52)	51.21 (10.02)	-0.60 (-3.24, 2.03)	0.653
	Delayed Recall	10.78 (2.74)	10.35 (3.14)	0.31 (-0.55, 1.16)	0.481
VERFL	Phonetic Fluency	33.85 (13.84)	35.81 (12.39)	-2.33 (-5.68, 1.03)	0.173
	Semantic Fluency	20.63 (6.47)	21.64 (5.91)	-1.06 (-2.69, 0.58)	0.203

Abbreviations: AVLT, Rey Auditory Verbal Learning Test; CU, cannabis-using CHR group; EMM, Estimated Marginal Mean; NU; non-cannabis-using CHR group; TMT, Trail Making Task; VERFL, Verbal Fluency Test; WAIS-III, shortened Wechsler Adult Intelligence Scale-III.
Statistically significant rows ($p<0.05$) are presented in bold.
[a] Adjusted for age, gender, and SES, and site as random effect.
[b] Raw Trail Making Test scores not reversed, higher score = poorer performance.

Figure 3-2 illustrates the cognitive performance measures of the CHR cannabis subgroups and the NU group. See **Supplementary Tables 3-7**, **3-8**, **3-9**, **3-10** and **3-11** for EMM differences, confidence intervals, and p values for both the initial models and the fully adjusted sensitivity analyses.

Overall, the best performing subgroup of CHR subjects were those who were current occasional cannabis users (n=39). **Supplementary Table** 3-12 in the supplementary materials provides EMM differences and confidence intervals of the differences in cognitive performance between CHR current occasional cannabis users and the total HC group.

Figure 3-1. Effect of lifetime cannabis use on standardised cognitive assessment scores.

Abbreviations: CU; cannabis using CHR group; EMM, estimated marginal mean; NU, non-cannabis using CHR group.

Circles represent EMM z-scores of the CHR NU and CU groups, adjusted for age, gender, and socioeconomic status, and site as random effect. Error bars show 95% confidence intervals. Grey dotted line represents the mean z-score for the HC group, for reference. – sign signifies reverse coded assessments.

** = p<0.05 difference between NU and CU group.*

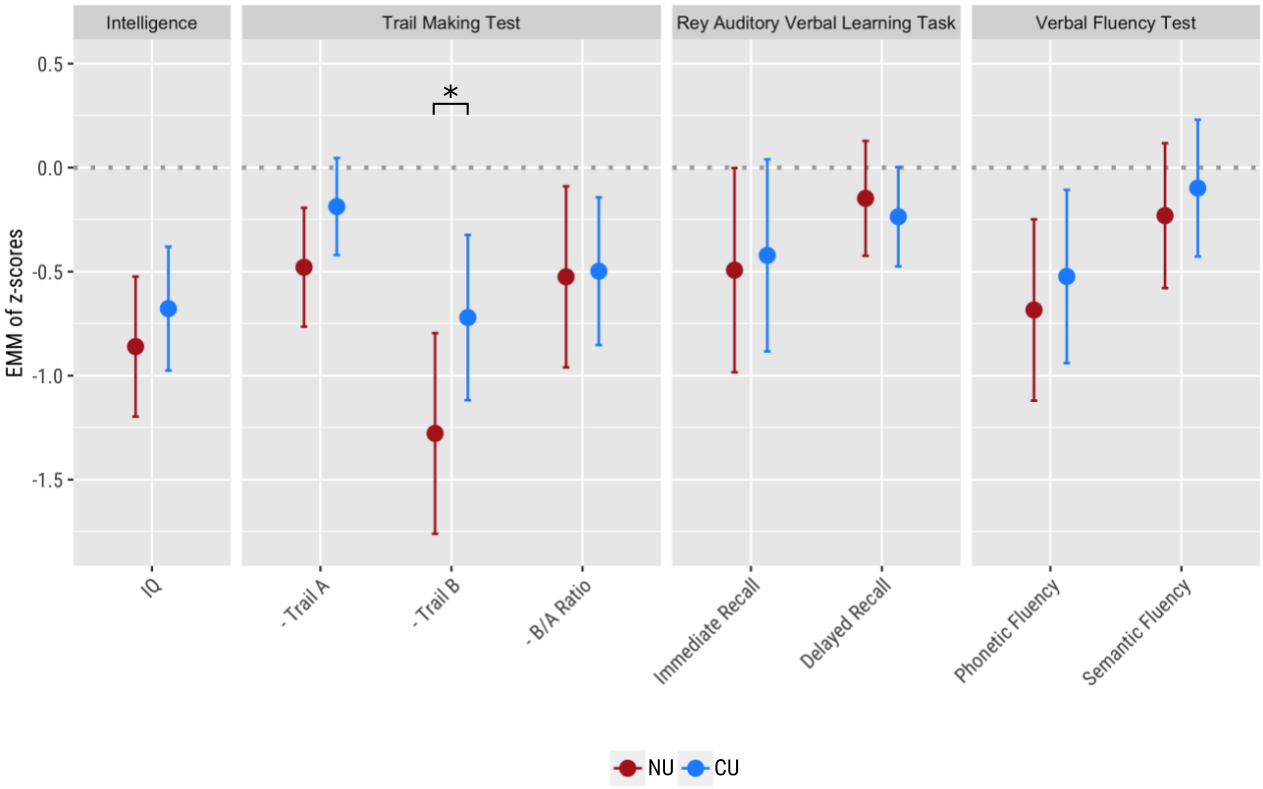


Figure 3-2. Effect of cannabis use patterns on cognitive assessment scores.

a) Estimated IQ; b) TMA-A, reversed; c) TMT-B, reversed.
Abbreviations: CU, cannabis-using CHR group; EMM, estimated marginal mean; NU, non-cannabis-using CHR group.
Circles represent EMM z-scores of the CHR NU and CU groups, adjusted for age, gender, and socioeconomic status, and site as random effect. Error bars show 95% confidence intervals. Grey dotted line represents the mean z-score for the HC group, for reference. – sign signifies reverse coded assessments.
** = p<0.05 difference between NU and CU group, after adjustment for multiple comparisons.*

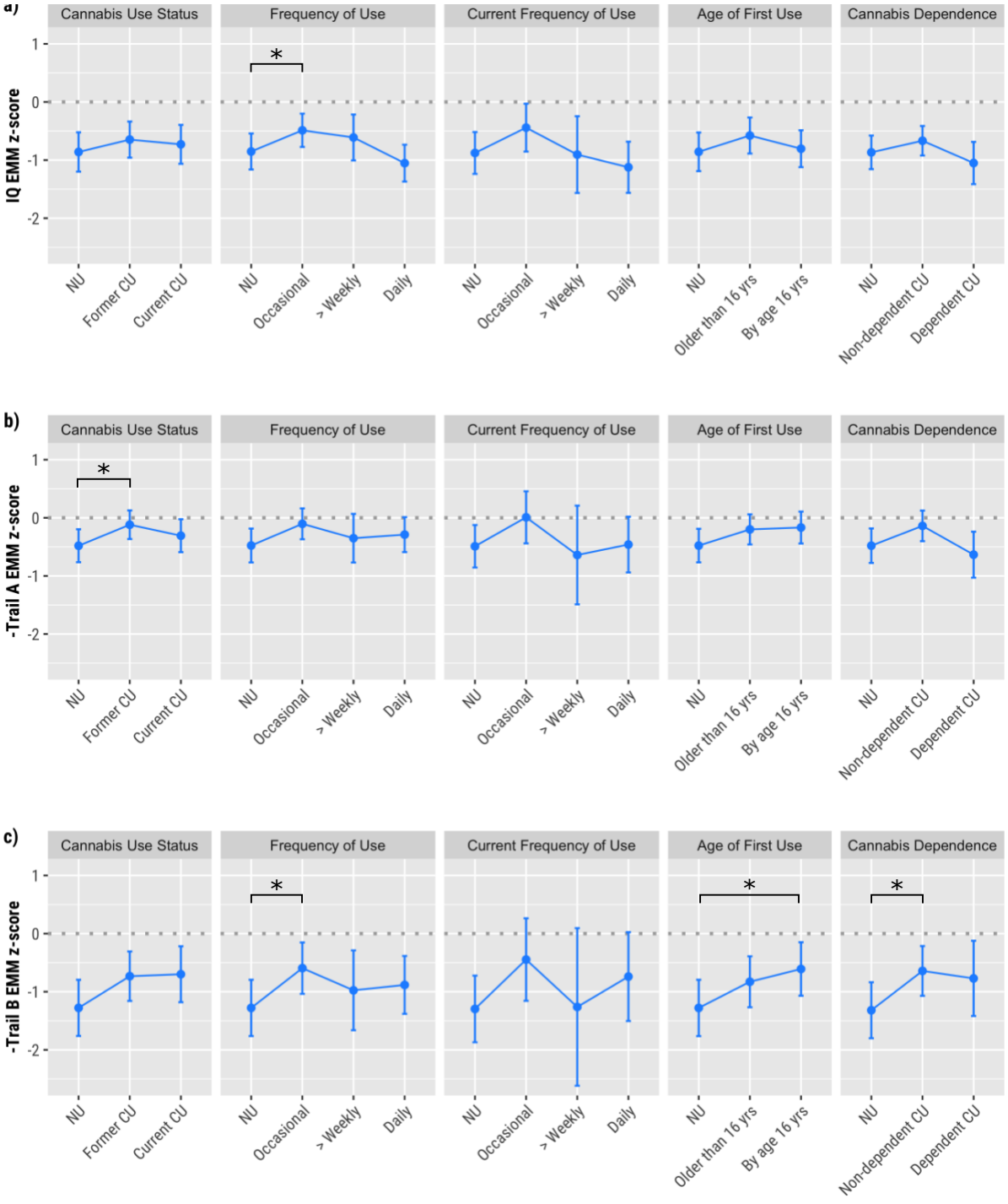


***Figure 3-2 continued**. Effect of cannabis use patterns on cognitive assessment scores.*

d) TMT-B/A Ratio; e) AVLT Immediate Recall, f) AVLT Delayed Recall.

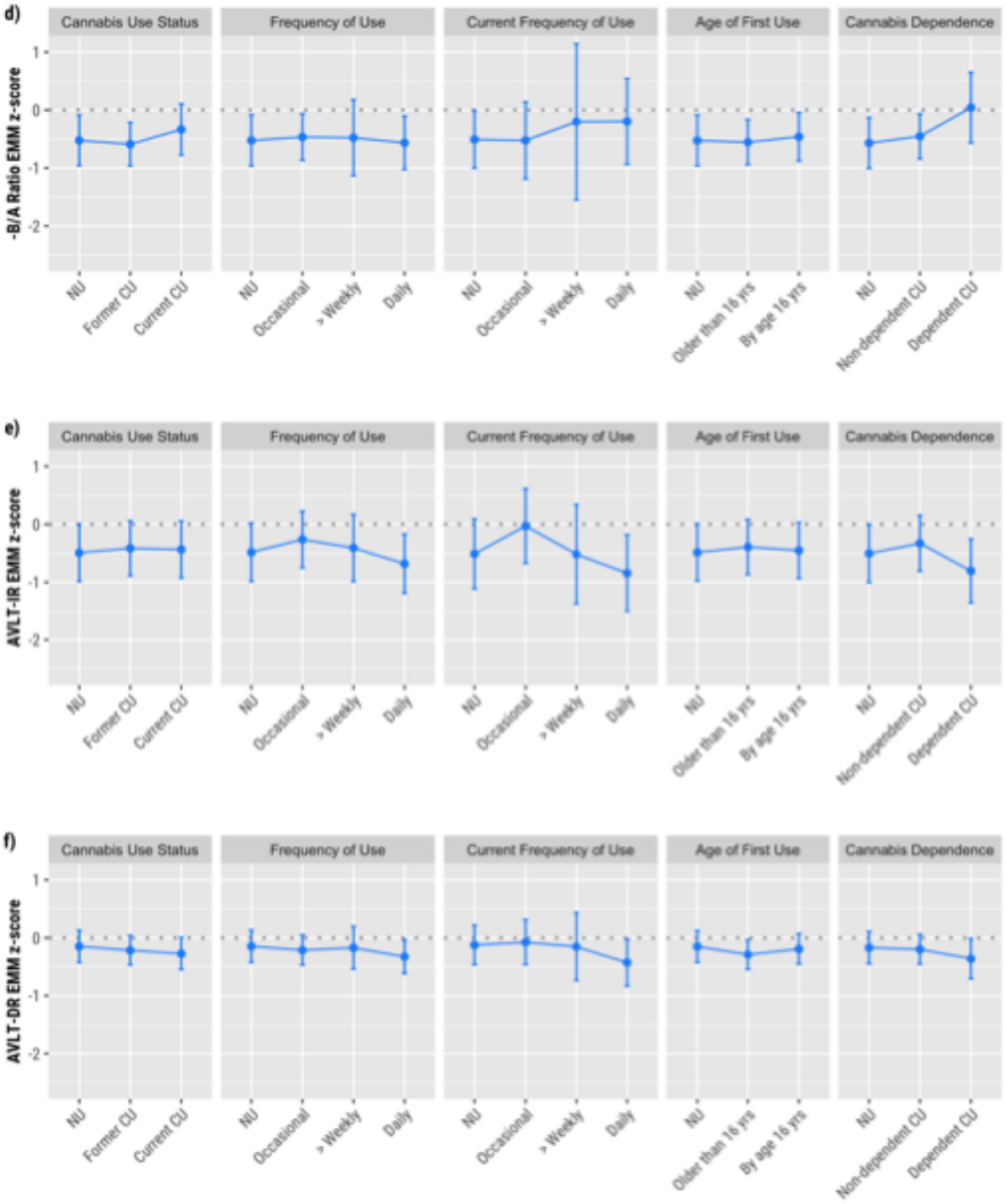
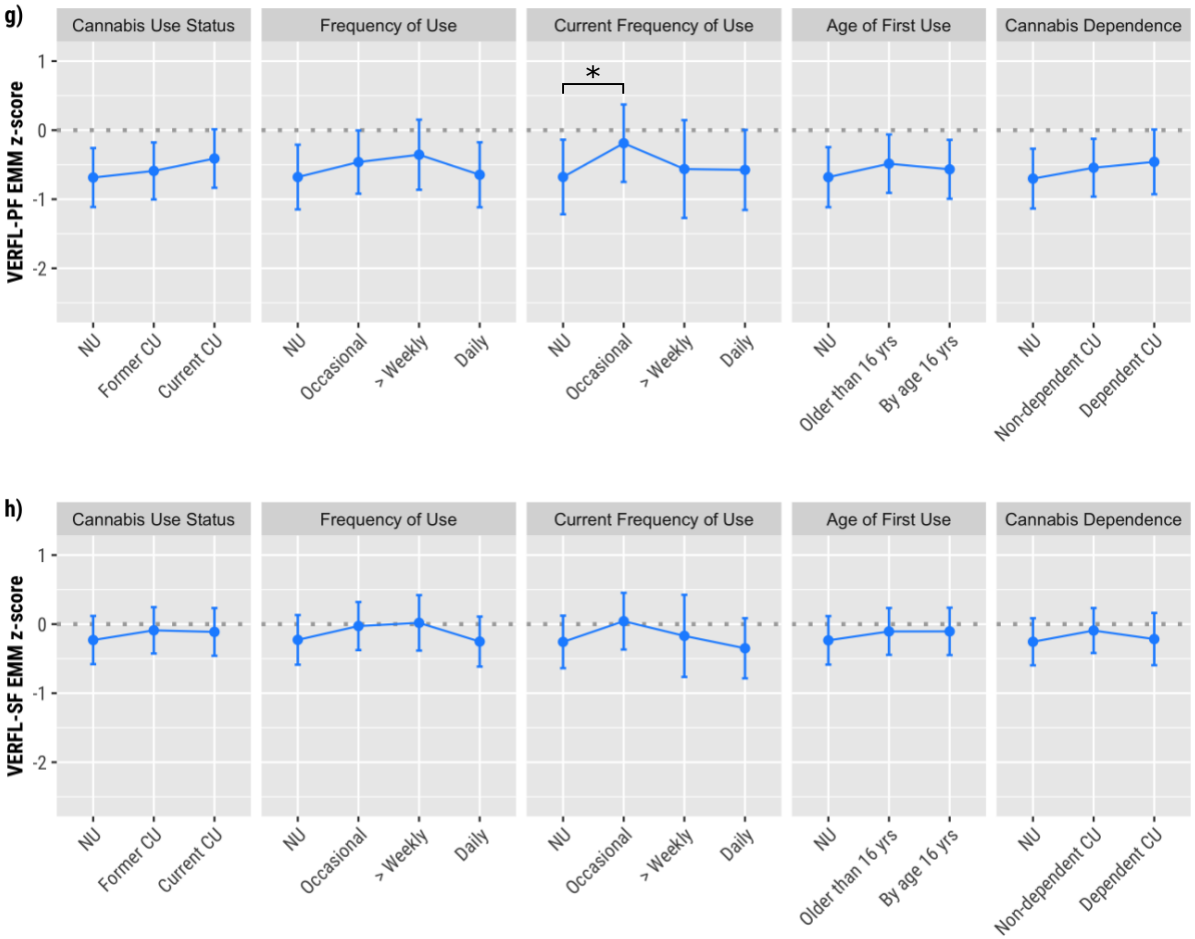


***Figure 3.2 continued**. Effect of cannabis use patterns on cognitive assessment scores.*

g) VERFL Phonetic Fluency; h) VERFL Semantic Fluency.



3.5 DISCUSSION

The present study investigated the relationship between cannabis use and cognitive functioning in people at CHR for psychosis. As expected, CHR participants showed impairments across several cognitive domains compared to controls. Our main finding was that CHR individuals who had ever used cannabis performed better than CHR individuals who had not used cannabis on most cognitive tests, significantly so for the TMT-B. However, even the highest performing CHR subgroup (current, occasional cannabis users) did not outperform the HC sample.

Two previous studies have examined cannabis use and cognition in CHR subjects.[33,34] Although neither found significant differences in cognitive performance between cannabis users and non-users, both involved relatively small CHR samples (n=63 and n=74, respectively), so may have lacked the power to detect true associations. There is a larger literature on the relationship between cannabis use and cognition in people with a psychotic disorder, including 7 meta-analyses,[8,18,20–22,52,58] and our findings are more in line with evidence from these studies. Rabin *et al.*, Potvin *et al.* and Donoghue and Doody all found small to medium effect sizes denoting superior cognitive performance in cannabis-using patients compared to non-using patients,[18,20,22] while Sánchez-Gutiérrez *et al*. found no effect.[52] In their 2012 meta-analysis, Yucel *et al*.[21] found that a lifetime history of cannabis use was associated with relatively better cognitive functioning in psychosis, whereas there was a trend for current use to be associated with poorer performance. Schoeler *et al*.[8] found that cannabis-using patients with psychosis performed better than non-using patients on memory tasks, but only if they had been abstinent from cannabis use for at least 10 days prior to testing. In the present study there was no clear effect of abstinence (current vs. former cannabis use) on cognitive performance in the CHR sample, with the exception of past cannabis users performing better on the TMT-A than non-users, while current users scored similarly to non-users. However, these analyses were exploratory in nature and require replication to be confirmed.

The most pronounced effect of cannabis use in the CHR cohort in the present study was on part B of the TMT task. Part A of the TMT is designed to measure visual scanning and motor speed, whereas part B also requires executive control of alternating-task demands and sustained attentional control.[44] This suggests that cannabis use was associated with better

performance on tasks that require executive and attentional control. It has been suggested that the TMT-B/A ratio provides an index of executive control independent of visual scanning and motor speed, but we did not find any differences on this measure in relation to cannabis use. Meta-analyses of patients with psychosis have found either no effect or a small to medium positive effect size of lifetime cannabis use on speed of processing,[18,21,52,58] attention,[20,22,52,58], psychomotor speed,[22] and cognitive flexibility/executive function[20,22,52,58] compared to non-users. Conversely, attention, processing speed and executive function have all been shown to be negatively affected in both acute and long-term cannabis use in healthy participants.[1,6,59,60] While this could suggest that cannabis effects those with or at risk of a psychotic disorder differently than those who are not, it should be noted that at least one study has shown that acute cannabis intoxication causes greater cognitive impairment in patients with psychosis than in healthy control subjects, not less.[61]

In the general population, occasional or discontinued cannabis use has been associated with a higher premorbid IQ, whereas frequent cannabis use has been linked to lower premorbid IQ.[62] The is in line with the results of present study, in that 'occasional' cannabis users (i.e., those who used less than once per week) showed better performance on TMT-B and had a higher IQ than non-users, but these differences were not evident in more frequent users. While these analyses were exploratory in nature, they suggest that heavier cannabis use may be negatively correlated with visuomotor speed, executive function, and attention in the CHR population. These findings are also consistent with those from patients with psychotic disorders, such as Ferraro *et al.*,[25] who found that only patients who used cannabis less than daily had a higher IQ than non-users, and a meta-analysis from Bogaty *et al.*,[58] who reported that patients who used at least weekly performed worse than non-users on verbal working memory tasks, and had a lower premorbid and current IQ. Taken all together, evidence suggests that more frequent exposure to cannabis is associated with residual cognitive deficits in individuals with psychotic symptoms in a similar to fashion to in healthy populations,[20]

In view of the literature linking cannabis use to impaired cognitive functioning,[3,7] an association with improved cognitive performance in people at CHR for psychosis is counter intuitive. One explanation that has been proposed is that the process of sourcing illicit cannabis requires motivation, planning, organisational and social skills, and that cannabis is use may be a proxy indicator of these skills.[5,25] Another suggestion is that there is a subgroup

of psychosis patients in whom the disorder is particularly associated with cannabis use, and have relatively less biological predisposition for the disorder and a relatively high level of premorbid cognitive functioning.[23,24] Fewer neurological soft signs have been reported in schizophrenia patients with comorbid cannabis misuse than non-using patients.[63] Both Bugra *et al.* and Korver *et al.* found that estimated premorbid intelligence was the same in their cannabis using and non-using CHR sample,[33,34] while studies of patients with psychosis have produced mixed result.[58,64,65] While premorbid IQ or premorbid level of functioning (i.e., before onset of CHR) were not assessed in the present study, we found that cannabis users had spent more time in education than non-users ($p>0.05$). Thus, it is possible that our findings were driven by a subgroup of cannabis users with comparatively high pre-morbid cognitive functioning.

Strengths of the present study include the use of a mainly antipsychotic-free sample, minimising the confounding effects of antipsychotic use on cognition.[31] The large, globally representative sample of CHR individuals allowed for detailed analysis of cannabis use, clinical, and functional data with superior statistical power than previous studies of this kind. Limitations include the lack of accurate measurements of premorbid functioning and IQ, which limits the interpretation of our results. More precise assessments of cannabis use patterns could have improved our ability to detect and interpret associations. For example, the assessment of cannabis use in early adolescence were retrospective in nature, therefore possibly being subject to recall bias.[66] Measurements of time since last use of cannabis, for example using a follow-back questionnaire,[66] and precise calculations of the quantity of THC consumed by participants could have allowed more accurate assessments of the dose and abstinence-related effects of cannabis on cognition.[5]

3.5.1 CONCLUSIONS

Among subjects at CHR for psychosis, cannabis use was associated with less severe impairments in TMT-B task performance. It is unclear if cannabis users represent a subgroup with relatively good premorbid cognitive function, or if the ability to source illicit cannabis is a proxy for less severe cognitive impairment.

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3.7 SUPPLEMENTARY MATERIALS

3.7.1 Supplementary Results

Supplementary Table 3-1. Sociodemographic and clinical features of excluded and included CHR participants

Supplementary Table 3-2. Sociodemographic and clinical features of HC and CHR participants

Supplementary Table 3-3. Sensitivity analysis: cognitive assessment scores in healthy controls and CHR participants

Supplementary Table 3-4. Sensitivity analysis: cognitive assessment scores in CHR lifetime cannabis users and non-users

Supplementary Table 3-5. Sociodemographic and clinical features stratified by cannabis use status

Supplementary Table 3-6. Sociodemographic and clinical features stratified by frequency of cannabis use

Supplementary Table 3-7. Cognitive assessment scores in CHR current and past cannabis users versus non-users

Supplementary Table 3-8. Cognitive assessment scores in CHR occasional, weekly, and daily cannabis users versus non-users

Supplementary Table 3-9. Cognitive assessment scores in CHR current cannabis users, subdivided as occasional, weekly, and daily users, versus non-users

Supplementary Table 3-10. Cognitive assessment scores in CHR early and late cannabis user initiators versus non-users

Supplementary Table 3-11. Cognitive assessment scores in CHR non-dependent and dependent cannabis users versus non-users

Supplementary Table 3-12. Cognitive assessment scores between healthy controls and CHR current, occasional cannabis users

3.7.2. Supplementary References

3.7.1 SUPPLEMENTARY METHODS

Sensitivity and power calculations

The original *a priori* sample size calculation for the EU-GEI High Risk project was an estimate based on a previous study,[1] suggesting that a sample of n=400 subjects with an CHR would provide sufficient statistical power for the project's primary outcome, genetic influences on clinical outcome.

Post hoc sensitivity and power calculations were conducted for the current study using G*Power version 3.1.9.4.[2]

Power

We calculated the power of the key finding from this study, the effect of having ever used cannabis on Task B of the Trail Making Test in CHR participants. With an effect size (standardised EMM difference) of 0.306, a significance criterion of $\alpha = .05$, n of group 1 (NU) = 79 and n of group 2 (CU) = 247, the calculated power = 0.67.

Sensitivity

With a significance criterion of $\alpha = .05$, power = 0.80, n of group 1 (NU) = 79 and n of group 2 (CU) = 247, the minimum detectable effect size (d) in for this study was 0.363, considered to be a small effect size using Cohen's (1988) criteria.

3.7.2 SUPPLEMENTARY RESULTS

Sociodemographic and clinical features: cannabis use status

Cannabis users were on average older than the NU group; past users by 2.1 years and current users by 2.5 years. Current cannabis users were more likely to be male than NUs. Ex-cannabis users, but not current users, spent an average 1.1 years longer in education and were less likely to be working class than NUs. Both past and current cannabis users consumed more alcohol and tobacco products than NUs and were more likely to have used other illicit substances in the past year. Current cannabis users were also more likely to have used other illicit substances in the past year than past cannabis users. Also compared to past cannabis users, current users used cannabis more frequently, began using cannabis on average 0.9 years younger, and were more likely to have reached the DSM-IV threshold for cannabis dependence in the past 12 months. Ethnicity, use of antipsychotic medications, GAF disability scores and CAARMS symptoms scores did not differ between cannabis user groups and NUs (***Supplementary Table*** 3-5).

Sociodemographic and clinical features: cannabis use frequency

Daily cannabis users consumed more tobacco and had poorer global functioning than both weekly and occasional cannabis users. Daily users also began using cannabis at a younger age than occasional users and were more likely to be cannabis dependent than weekly users, who were in turn more likely to be cannabis dependent than occasional users. Age, ethnicity, socio-economic status, years in education, use of antipsychotic medications, alcohol use, other substance use in the past 12 months, and CAARMS symptoms scores did not differ between occasional, weekly, and daily cannabis users (***Supplementary Table*** 3-6).

Supplementary Table 3-1. Sociodemographic and clinical features of excluded and included CHR participants

	Excluded (n=18)	Included (n=326)	P value
Age, years	20.4 (3.7)	22.5 (4.9)	0.075
Male gender	13 (72.2%)	172 (52.8%)	0.107
Ethnicity	--	--	0.771
White	13 (72.2%)$_a$	234 (71.8%)$_a$	
Black	1 (5.6%)$_a$	33 (10.1%)$_a$	
Other	4 (22.2%)$_a$	59 (18.1%)$_a$	
SES			0.145
Salariat	0 (0%)$_a$	98 (34.1%)$_a$	
Intermediate	4 (66.7%)$_a$	101 (35.2%)$_a$	
Working class	2 (33.3%)$_a$	88 (30.7%)$_a$	
Years in education	13.3 (1.5)	14.4 (3.1)	0.295
Antipsychotic use	1 (7.7%)	31 (10.2%)	1.000
Tobacco used, daily	2.8 (5.7)	6.9 (9.2)	0.179
Alcohol use, weekly	4.7 (9.3)	5.6 (10.4)	0.793
Other substance use	0 (0%)	97 (32.1%)	0.061
GAF disability	56.7 (7.0)	55.4 (14.4)	0.420
CAARMS positive symptoms	40.6 (17.1)	36.8 (19.8)	0.453
CAARMS negative symptoms	28.9 (20.9)	29.4 (18.4)	0.913

Abbreviations: CAARMS, Comprehensive Assessment of At-Risk Mental State; GAF, Global Assessment of Functioning; SES, socioeconomic status.

P values for χ^2, Fisher's Exact, independent t- or Mann-Whitney U tests. Data as mean (SD) or n (%). Significant (<0.05) p values in bold.

Subscript letters denote subsets whose column proportions do not differ significantly from each other at the .05 level after Bonferroni correction for multiple comparisons.

Supplementary Table 3-2. Sociodemographic and clinical features of HC and CHR participants

	HC (n=65)	CHR (n=326)	P value
Age, years	22.9 (4.2)	22.5 (4.9)	0.556
Male gender	33 (50.8%)	172 (52.8%)	0.769
Ethnicity			0.318
White	41 (63.1%)	234 (71.8%)	
Black	10 (15.4%)	33 (10.1%)	
Other	14 (21.5%)	59 (18.1%)	
SES			**0.002**
Salariat	29 (52.7%)	98 (34.1%)[a]	
Intermediate	21 (38.2%)	101 (35.2%)	
Working class	5 (9.1%)	88 (30.7%)[a]	
Years in education	16.1 (2.8)	14.4 (3.1)	**<0.001**
Antipsychotic use	0 (0%)	31 (10.2%)	**0.010**
Tobacco used, daily *	2.8 (6.3)	6.9 (9.2)	**<0.001**
Alcohol use, weekly	4.8 (6.4)	5.6 (10.4)	0.569
Other substance use	20 (31.7%)	97 (32.1%)	0.954
GAF *	84.9 (9.1)	55.4 (12.4)	**<0.001**
Cannabis use status			**0.023**
Never used	26 (40.0%)	79 (24.2%)[a]	
Past user	22 (33.8%)	158 (48.5%)[a]	
Current user	17 (26.2%)	89 (27.3%)	

Abbreviations: CAARMS, Comprehensive Assessment of At-Risk Mental State; CHR, clinical high-risk for psychosis group; GAF, Global Assessment of Functioning disability subscale; HC, healthy control group; SES, socioeconomic status.

P values for χ^2, independent t- or Mann-Whitney U tests (denoted by *) where assumption of homogeneity of variances was broken.

Data as mean (SD) or n (%). Significant (<0.05) p values are presented in bold.

[a] Significantly different from HC group at the .05 level after Bonferroni correction for multiple comparisons (t-test of z-score).

Supplementary Table 3-3. Sensitivity analysis: cognitive assessment scores in healthy controls and CHR participants

Cognitive assessment		EMM difference, HC-CHR (95% CI)	P value
WAIS-III	IQ	15.0 (9.3, 20.7)	**<0.001**
TMT	Trail A	2.53 (-1.95, 7.00)	0.267
	Trail B	18.50 (8.51, 28.50)	**<0.001**
	Trail B/A Ratio	0.414 (0.110, 0.718)	**0.008**
AVLT	Immediate Recall	4.52 (1.44, 7.61)	**0.004**
	Delayed Recall	0.98 (-0.10, 2.06)	0.077
VERFL	Phonetic Fluency	10.05 (6.02, 14.08)	**<0.001**
	Semantic Fluency	2.78 (0.67, 4.89)	**0.010**

Abbreviations: AVLT, Rey Auditory Verbal Learning Test; CHR, clinical high-risk group; EMM, Estimated Marginal Mean; HC, healthy control group; TMT, Trail Making Task; VERFL, Verbal Fluency Test; WAIS-III, shortened Wechsler Adult Intelligence Scale-III.
TMT scores have been reversed so that higher EMM differences = better performance of the HC group.
Statistically significant rows ($p<0.05$) are presented in bold.
Models adjusted for alcohol, tobacco, other substance use, age, gender, and socioeconomic status, and site as a random effect.

Supplementary Table 3-4. Sensitivity analysis: cognitive assessment scores in CHR lifetime cannabis users and non-users

Cognitive assessment		EMM difference, NU-CU (95% CI)	P value
WAIS-III	IQ	-4.2 (-9.8, 1.5)	0.145
TMT	Trail A	-3.62 (-8.01, 0.78)	0.106
	Trail B	-12.29 (-22.85, 1.74)	**0.023**
	Trail B/A Ratio	-0.156 (-0.481, 0.169)	0.346
AVLT	Immediate Recall	-0.89 (-3.96, 2.17)	0.567
	Delayed Recall	0.45 (-0.56, 1.46)	0.378
VERFL	Phonetic Fluency	-2.57 (-6.30, 1.15)	0.175
	Semantic Fluency	-1.15 (-3.04, 0.74)	0.232

Abbreviations: AVLT, Rey Auditory Verbal Learning Test; CU, cannabis-using CHR group; EMM, Estimated Marginal Mean; NU; non-cannabis-using CHR group; TMT, Trail Making Task; VERFL, Verbal Fluency Test; WAIS-III, shortened Wechsler Adult Intelligence Scale-III.
TMT scores have been reversed so that lower EMM differences = better performance of the CU group.
Statistically significant rows ($p<0.05$) are presented in bold.
Models adjusted for alcohol, tobacco, other substance use, age, gender, and socioeconomic status, and site as a random effect.

Supplementary Table 3-5. Sociodemographic and clinical features stratified by cannabis use status

	Never (n=79)	Past (n=158)	Current (n=89)	P value
Age, years	20.8 (4.8)	22.9 (4.5)[a]	23.3 (5.5)[a]	**0.001**
Male gender	34 (43.0%)	81 (51.3%)	57 (64.0%)[a]	**0.021**
Ethnicity	--	--	--	0.236
White	55 (69.6%)	113 (71.5%)	66 (74.2%)	
Black	5 (6.3%)	21 (13.3%)	7 (7.9 %)	
Other	19 (24.1%)	24 (15.2%)	16 (18.0%)	
SES	--	--	--	**0.029**
Salariat	20 (28.6%)	50 (36.5%)	28 (35.0%)	
Intermediate	19 (27.1%)	56 (40.9%)	26 (32.5%)	
Working class	31 (44.3%)	31 (22.6%)[a]	26 (32.5%)	
Years in education	13.8 (3.1)	14.9 (3.1)[a]	14.1 (3.0)	**0.023**
Antipsychotic use	7 (10.0%)	15 (9.9%)	9 (11.0%)	0.967
Tobacco use, daily *	1.0 (3.7)	7.9 (9.3)[a]	10.7 (9.9) [a, b]	**<0.001**
Alcohol use, weekly *	1.7 (5.3)	6.4 (12.3)[a]	7.8 (9.2)[a]	**<0.001**
Other substance use	2 (2.6%)	51 (34.9%)[a]	44 (55.7%)[a, b]	**<0.001**
GAF disability *	56.3 (14.8)	55.9 (12.1)	53.7 (10.7)	0.267
CAARMS positive symptoms	34.1 (19.8)	37.2 (20.8)	38.5 (17.6)	0.338
CAARMS negative symptoms	27.7 (20.2)	30.3 (17.8)	29.4 (17.8)	0.596
Cannabis use frequency		--	--	**<0.001**
Only once or twice		44 (29.5%)	5 (5.8%)[b]	
> twice each year		28 (18.8%)	17 (19.8%)	
> twice each month		14 (9.4%)	17 (19.8%)[b]	
At least once a week		23 (15.4%)	10 (11.6%)	
Every day		40 (26.8%)	37 (43.0%)[b]	
Age first cannabis use		16.2 (3.0)	15.3 (2.5)	**0.015**
Cannabis dependence		11 (8.3%)	25 (36.8%)[b]	**<0.001**

Abbreviations: CAARMS, Comprehensive Assessment of At-Risk Mental State; GAF, Global Assessment of Functioning; SES, socio-economic status.

P values for χ^2 test, one-way ANOVA (Never vs Past vs Current) or t-test (Past vs Current), or Welsch test (denoted by *) if assumption of homogeneity of variance violated. Data as mean (SD) or n (%). Significant (<0.05) p values in bold.

Superscript letters denote subsets whose column proportions (t-test of z-score) or means (Tukey HSD) differ significantly from each other at the .05 level after Bonferroni correction for multiple comparisons.

[a] Significantly different from Never used cannabis group.

[b] Significantly different from Past user cannabis group.

Supplementary Table 3-6. Sociodemographic and clinical features stratified by frequency of cannabis use

	Occasional use (n=125)	Weekly use (n=33)	Daily use (n=77)	P value
Age, years	23.0 (4.7)	21.6 (3.8)	23.9 (5.5)	0.064
Male gender	59 (47.2%)	22 (66.7%)	49 (63.6%)	**0.027**
Ethnicity	--	--	--	0.214
White	91 (72.8%)	21 (63.6%)	57 (74.0%)	
Black	12 (9.6%)	8 (24.2%)	8 (10.4%)	
Other	22 (17.6%)	4 (12.1%)	12 (15.6%)	
SES	--	--	--	0.325
Salariat	43 (37.4%)	10 (33.3%)	21 (31.3%)	
Intermediate	44 (38.3%)	15 (50.0%)	23 (34.3%)	
Working class	28 (24.3%)	5 (16.7%)	23 (34.3%)	
Years in education	14.9 (2.9)	14.6 (3.2)	14.3 (3.3)	0.530
Antipsychotic use	10 (8.5%)	3 (9.7%)	7 (9.5%)	0.968
Tobacco use, daily	7.0 (8.7)	8.2 (12.1)	12.0 (9.0)[a]	**0.001**
Alcohol use, weekly	6.4 (12.3)	7.0 (11.6)	7.7 (9.8)	0.751
Other substance use	43 (37.4%)	15 (48.4%)	33 (48.5%)	0.262
GAF disability	56.6 (12.2)	58.2 (9.9)	52.1 (10.7)[a, b]	**0.009**
CAARMS positive symptoms	38.8 (19.7)	37.8 (23.9)	36.5 (18.7)	0.750
CAARMS negative symptoms	31.5 (17.4)	28.8 (18.4)	26.6 (17.6)	0.168
Age first cannabis use	16.4 (2.7)	16.0 (2.6)	15.0 (3.1)[a]	**0.002**
Cannabis dependence	0 (0.0%)	5 (17.2%)[a]	29 (48.3%)[a, b]	**<0.001**

Abbreviations: CAARMS, Comprehensive Assessment of At-Risk Mental State; GAF, Global Assessment of Functioning; SES, socio-economic status.

P values for χ^2 or one-way ANOVA tests. Data as mean (SD) or n (%). Significant (<0.05) p values in bold. Superscript letters denote subsets whose column proportions (t-test of z-score) or means (Tukey HSD) differ significantly from each other at the .05 level after Bonferroni correction for multiple comparisons.

[a] Significantly different from Occasional use cannabis group.

[b] Significantly different from Weekly use cannabis group.

Supplementary Table 3-7. Cognitive assessment scores in CHR current and past cannabis users versus non-users

			Initial model[a]		Sensitivity analysis[b]	
Outcome	Cannabis use status	Raw cognitive assessment score, mean (SD)	EMM difference NU-CU group (95% CI)	P value	EMM difference NU-CU group (95% CI)	P value
IQ	NU	97.5 (19.1)				
	Former CU	99.5 (17.5)	-3.9 (-9.2, 1.4)	0.247	-4.8 (-10.8, 1.03)	0.175
	Current CU	98.1 (15.1)	-2.4 (-8.3, 3.5)	0.617	-2.6 (-9.46, 4.33)	0.659
TMT-A	NU	32.62 (14.43)[c]				
	Former CU	28.46 (10.64)[c]	**-4.61 (-8.57, -0.66)**	**0.040**	-4.30 (-8.84, 0.24)	0.107
	Current CU	31.58 (13.28)[c]	-2.21 (-6.60, 2.19)	0.491	-1.88 (-7.20, 3.43)	0.683
TMT-B	NU	79.60 (35.21)[c]				
	Former CU	70.16 (28.40)[c]	-10.26 (-19.93, -0.59)	0.066	-11.89 (-22.83, -0.94)	0.058
	Current CU	72.20 (29.39)[c]	-10.90 (-21.65, -0.15)	0.082	-13.34 (-26.13, -0.55)	0.071
TMT-B/A Ratio	NU	2.543 (0.910)[c]				
	Former CU	2.590 (0.849)[c]	0.039 (-0.261, 0.339)	0.949	-0.103 (-0.438, 0.233)	0.748
	Current CU	2.480 (1.055)[c]	-0.111 (-0.447, 0.224)	0.721	-0.291 (-0.684, 0.101)	0.233
AVLT-IR	NU	50.43 (10.52)				
	Former CU	51.48 (9.47)	-0.67 (-3.48, 2.14)	0.841	-0.97 (-4.15, 2.20)	0.749
	Current CU	50.76 (10.92)	-0.49 (-3.60, 2.62)	0.927	-0.68 (-4.40, 3.05)	0.900
AVLT-DR	NU	10.78 (2.74)				
	Former CU	10.53 (3.02)	0.23 (-0.68, 1.15)	0.821	0.36 (-0.68, 1.41)	0.693
	Current CU	10.05 (3.34)	0.43 (-0.58, 1.44)	0.588	0.67 (-0.55, 1.89)	0.425
VERFL-PF	NU	33.85 (13.84)				
	Former CU	34.31 (11.20)	-1.38 (-4.93, 2.17)	0.640	-1.90 (-5.76, 1.95)	0.494
	Current CU	38.39 (13.92)	-3.97 (-7.91, -0.04)	0.083	-4.27 (-8.77, 0.24)	0.107
VERFL-SF	NU	20.63 (6.47)				
	Former CU	21.73 (5.43)	-1.13 (-2.86, 0.61)	0.322	-1.22 (-3.18, 0.74)	0.345
	Current CU	21.49 (6.69)	-0.94 (-2.87, 0.98)	0.507	-0.98 (-3.27, 1.31)	0.582

P values adjusted for multiple comparisons using the multivariate t method for 2 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

[c] Raw Trail Making Test scores not reversed, higher score = poorer performance.

Supplementary Table 3-8. Cognitive assessment scores in CHR occasional, weekly, and daily cannabis users versus non-users

			Initial model[a]		Sensitivity analysis[b]	
Outcome	Cannabis use frequency	Raw cognitive assessment score, mean (SD)	EMM difference NU-CU group (95% CI)	P value	EMM difference NU-CU group (95% CI)	P value
IQ	NU	97.5 (19.1)				
	Occasional CU	102.8 (16.8)	**-6.6 (-11.9, -1.3)**	**0.038**	**-6.98 (-12.80, -1.16)**	**0.049**
	> Weekly CU	100.7 (14.0)	-4.4 (-11.8, 3.0)	0.510	-2.83 (-11.21, 5.56)	0.838
	Daily CU	91.8 (15.3)	3.6 (-2.3, 9.6)	0.487	3.39 (-3.57, 10.36)	0.643
TMT-A	NU	32.62 (14.43)[c]				
	Occasional CU	28.42 (10.71)[c]	-4.76 (-8.83, -0.68)	0.059	-4.71 (-9.328, -0.10)	0.113
	> Weekly CU	30.96 (10.66)[c]	-1.60 (-7.50, 4.29)	0.910	0.24 (-6.62, 7.10)	1.000
	Daily CU	31.25 (13.86)[c]	-2.39 (-6.97, 2.19)	0.606	-1.33 (-6.88, 4.21)	0.932
TMT-B	NU	79.60 (35.21)[c]				
	Occasional CU	67.87 (29.47)[c]	**-12.88 (-22.82, -2.94)**	**0.030**	**-14.87 (-26.01, -3.73)**	**0.025**
	> Weekly CU	73.92 (31.75)[c]	-5.70 (-19.94, 8.55)	0.769	-4.24 (-20.60, 12.13)	0.917
	Daily CU	74.85 (26.20)[c]	-7.45 (-18.60, 3.70)	0.412	-7.36 (-20.61, 5.89)	0.550
TMT-B/A Ratio	NU	2.543 (0.910)[c]				
	Occasional CU	2.519 (0.922)[c]	-0.034 (-0.344, 0.276)	0.993	-0.167 (-0.512, 0.178)	0.648
	> Weekly CU	2.542 (1.099)[c]	-0.028 (-0.471, 0.416)	0.999	-0.203 (-0.710, 0.303)	0.759
	Daily CU	2.597 (0.879)[c]	0.024 (-0.325, 0.372)	0.998	-0.146 (-0.556, 0.264)	0.816
AVLT-IR	NU	50.43 (10.52)				
	Occasional CU	52.46 (10.24)	-1.87 (-4.71, 0.97)	0.425	-2.09 (-5.27, 1.09)	0.418
	> Weekly CU	52.22 (8.42)	-0.63 (-4.72, 3.46)	0.981	-0.38 (-5.12, 4.37)	0.997
	Daily CU	48.42 (10.04)	1.69 (-1.52, 4.91)	0.598	-2.28 (-1.57, 6.13)	0.501
AVLT-DR	NU	10.78 (2.74)				
	Occasional CU	10.45 (3.21)	0.23 (-0.70, 1.16)	0.931	0.35 (-0.72, 1.41)	0.853
	> Weekly CU	10.81 (2.53)	0.09 (-1.25, 1.43)	0.999	0.39 (-1.19, 1.98)	0.926
	Daily CU	9.83 (3.33)	0.63 (-0.44, 1.70)	0.518	0.95 (-0.36, 2.26)	0.340
VERFL-PF	NU	33.85 (13.84)				
	Occasional CU	35.90 (11.81)	-3.13 (-6.70, 0.44)	0.205	-3.31 (-7.17, 0.54)	0.213
	> Weekly CU	37.64 (12.51)	-4.67 (-9.73, 0.39)	0.171	-4.07 (-9.74, 1.60)	0.346
	Daily CU	34.38 (13.13)	-0.48 (-4.50, 3.55)	0.991	-1.24 (-5.88, 3.41)	0.910

P values adjusted for multiple comparisons using the multivariate t method for 3 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

[c] Raw Trail Making Test scores not reversed, higher score = poorer performance.

Supplementary Table 3-8 continued. *Cognitive assessment scores in CHR occasional, weekly, and daily cannabis users versus non-users*

			Initial model[a]		Sensitivity analysis[b]	
Outcome	Cannabis use frequency	Raw cognitive assessment score, mean (SD)	EMM difference NU-CU group (95% CI)	P value	EMM difference NU-CU group (95% CI)	P value
VERFL-SF	NU	20.63 (6.47)				
	Occasional CU	22.08 (5.67)	-1.60 (-3.37, 0.17)	0.185	-1.74 (-3.72, 0.23)	0.196
	> Weekly CU	22.43 (6.06)	-1.97 (-4.47, 0.54)	0.283	-1.40 (-4.31, 1.52)	0.653
	Daily CU	20.47 (6.24)	0.20 (-1.80, 2.19)	0.995	-0.47 (-1.92, 2.85)	0.960

P values adjusted for multiple comparisons using the multivariate t method for 3 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

Supplementary Table 3-9. Cognitive assessment scores in CHR current CUs, subdivided as occasional, weekly, and daily users, versus non-users

			Initial model[a]		**Sensitivity analysis[b]**	
Outcome	Cannabis use frequency	Raw cognitive assessment score, mean (SD)	EMM difference NU-CU group (95% CI)	P value	EMM difference NU-CU group (95% CI)	P value
IQ	NU	97.5 (19.1)				
	Occasional CU	104.5 (14.2)	-7.9 (-15.0, -0.8)	0.081	-9.0 (-18.1, 0.1)	0.132
	> Weekly CU	95.9 (8.2)	0.5 (-11.3, 12.3)	1.000	1.0 (-12.7, 14.6)	0.998
	Daily CU	91.8 (15.1)	4.5 (-3.1, 12.0)	0.547	2.5 (-7.6, 12.6)	0.931
TMT-A	NU	32.62 (14.43)[c]				
	Occasional CU	28.09 (9.67)[c]	-6.36 (-12.40, -0.31)	0.109	-6.49 (-14.14, 1.17)	0.235
	> Weekly CU	37.00 (10.86)[c]	1.91 (-9.14, 12.97)	0.978	4.02 (-8.13, 16.17)	0.859
	Daily CU	34.20 (16.43)[c]	-0.38 (-6.77, 6.02)	0.999	0.37 (-8.24, 8.98)	1.000
TMT-B	NU	79.60 (35.21)[c]				
	Occasional CU	66.84 (32.20)[c]	-15.99 (-30.45, 1.54)	0.085	-20.95 (-38.49, -3.42)	0.053
	> Weekly CU	86.14 (32.04)[c]	-0.65 (-26.86, 25.57)	1.000	0.40 (-27.09, 27.88)	1.000
	Daily CU	75.14 (25.20)[c]	-10.48 (-25.89, 4.93)	0.429	-14.27 (-33.86, 5.31)	0.349
TMT-B/A Ratio	NU	2.543 (0.910)[c]				
	Occasional CU	2.545 (1.197)[c]	0.010 (-0.445, 0.465)	1.000	-0.239 (-0.816, 0.338)	0.757
	> Weekly CU	2.447 (1.217)[c]	-0.181 (-1.014, 0.652)	0.958	-0.458 (-1.362, 0.446)	0.633
	Daily CU	2.433 (0.885)[c]	-0.184 (-0.677, 0.310)	0.827	-0.523 (-1.169, 0.123)	0.266
AVLT-IR	NU	50.43 (10.52)				
	Occasional CU	53.34 (10.01)	-4.09 (-7.97, -0.21)	0.107	**-6.06 (-10.82, -1.30)**	**0.036**
	> Weekly CU	51.33 (9.26)	0.06 (-6.23, 6.35)	1.000	-2.90 (-10.16, 4.37)	0.776
	Daily CU	47.78 (11.86)	2.83 (-1.19, 6.84)	0.400	0.69 (-4.67, 6.06)	0.989
AVLT-DR	NU	10.78 (2.74)				
	Occasional CU	10.56 (3.00)	-0.17 (-1.38, 1.04)	0.988	-0.53 (-2.01, 0.94)	0.822
	> Weekly CU	10.56 (2.88)	0.10 (-1.87, 2.07)	0.999	-0.18 (-2.42, 2.06)	0.997
	Daily CU	9.31 (3.73)	1.07 (-0.19, 2.32)	0.247	0.78 (-0.87, 2.44)	0.679
VERFL-PF	NU	97.5 (19.1)				
	Occasional CU	104.5 (14.2)	-7.9 (-15.0, -0.8)	0.081	-9.0 (-18.1, 0.1)	0.132
	> Weekly CU	95.9 (8.2)	0.5 (-11.3, 12.3)	1.000	1.0 (-12.7, 14.6)	0.998
	Daily CU	91.8 (15.1)	4.5 (-3.1, 12.0)	0.547	2.5 (-7.6, 12.6)	0.931

P values adjusted for multiple comparisons using the multivariate t method for 3 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

[c] Raw Trail Making Test scores not reversed, higher score = poorer performance.

Supplementary Table 3-9 continued. *Cognitive assessment scores in CHR current CUs, subdivided as occasional, weekly, and daily cannabis users, versus non-users*

			Initial model[a]		Sensitivity analysis[b]	
Outcome	Cannabis use frequency	Raw cognitive assessment score, mean (SD)	EMM difference NU-CU group (95% CI)	P value	EMM difference NU-CU group (95% CI)	P value
VERFL-SF	NU	20.63 (6.47)				
	Occasional CU	22.77 (6.47)	-2.40 (-5.01, 0.21)	0.190	-2.01 (-5.24, 1.23)	0.478
	> Weekly CU	20.78 (7.43)	-0.70 (-5.09, 3.69)	0.983	-0.12 (-5.21, 4.97)	1.000
	Daily CU	20.16 (6.74)	0.74 (-2.07, 3.55)	0.931	0.95 (-2.77, 4.66)	0.926

P values adjusted for multiple comparisons using the multivariate t method for 3 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

Supplementary Table 3-10. Cognitive assessment scores in CHR early and late cannabis user initiators versus non-users

			Initial model[a]		Sensitivity analysis[b]	
Outcome	Cannabis use status	Raw cognitive assessment score, mean (SD)	EMM difference NU-CU group (95% CI)	P value	EMM difference NU-CU group (95% CI)	P value
IQ	NU	97.5 (19.1)				
	Later age first use	101.1 (17.9)	-5.1 (-10.5, 0.3)	0.111	-5.5 (-11.5, 0.5)	0.118
	Early age first use	96.5 (14.8)	-1.0 (-6.6, 4.6)	0.912	-2.1 (-8.5, 4.4)	0.734
TMT-A	NU	32.62 (14.43)[c]				
	Later age first use	29.89 (10.66)[c]	-3.55 (-7.63, 0.52)	0.146	-3.54 (-8.20, 1.13)	0.220
	Early age first use	29.21 (13.11)[c]	-3.97 (-8.22, 0.29)	0.114	-3.74 (-8.81, 1.33)	0.236
TMT-B	NU	79.60 (35.21)[c]				
	Later age first use	73.10 (29.78)[c]	-8.48 (-18.39, 1.43)	0.155	-11.32 (-22.53, -0.12)	0.081
	Early age first use	68.34 (27.60)[c]	**-12.62 (-22.94, 2.30)**	**0.030**	**-13.83 (-25.96, -1.39)**	**0.045**
TMT-B/A Ratio	NU	2.543 (0.910)[c]				
	Later age first use	2.582 (0.908)[c]	0.017 (-0.292, 0.327)	0.990	-0.140 (-0.484, 0.205)	0.611
	Early age first use	2.522 (0.963)[c]	-0.038 (-0.359, 0.284)	0.957	-0.181 (-0.555, 0.192)	0.503
AVLT-IR	NU	50.43 (10.52)				
	Later age first use	51.26 (10.14)	-0.80 (-3.69, 2.09)	0.790	-0.98 (-4.26, 2.29)	0.755
	Early age first use	51.13 (9.92)	-0.28 (-3.24, 2.68)	0.972	-0.76 (-4.26, 2.74)	0.862
AVLT-DR	NU	10.78 (2.74)				
	Later age first use	10.12 (3.08)	0.48 (-0.46, 1.43)	0.477	0.67 (-0.42, 1.75)	0.351
	Early age first use	10.57 (3.22)	0.15 (-0.81, 1.10)	0.931	0.16 (-0.98, 1.31)	0.935
VERFL-PF	NU	33.85 (13.84)				
	Later age first use	36.80 (12.17)	-2.82 (-6.48, 0.85)	0.214	-2.54 (-6.52, 1.44)	0.328
	Early age first use	34.79 (12.64)	-1.64 (-5.39, 2.11)	0.572	-2.62 (-6.88, 1.63)	0.349
VERFL-SF	NU	20.63 (6.47)				
	Later age first use	21.76 (5.92)	-1.03 (-2.82, 0.76)	0.398	-0.91 (-2.92, 1.11)	0.552
	Early age first use	21.47 (5.95)	-1.03 (-2.87, 0.80)	0.410	-1.51 (-3.66, 0.65)	0.268

P values adjusted for multiple comparisons using the multivariate t method for 2 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

[c] Raw Trail Making Test scores not reversed, higher score = poorer performance.

Supplementary Table 3-11. Cognitive assessment scores in CHR non-dependent and dependent cannabis users versus non-users

Outcome	Cannabis use status	Raw cognitive assessment score, mean (SD)	Initial model[a] EMM difference NU-CU group (95% CI)	Initial model[a] P value	Sensitivity analysis[b] EMM difference NU-CU group (95% CI)	Sensitivity analysis[b] P value
IQ	NU	97.5 (19.1)				
	Non-dependent CU	100.2 (16.2)	-3.6 (-8.8, 1.6)	0.286	-4.93 (-10.69, 0.84)	0.161
	Dependent CU	93.9 (15.1)	3.3 (-3.9, 10.6)	0.565	3.37 (-5.38, 12.11)	0.656
TMT-A	NU	32.62 (14.43)[c]				
	Non-dependent CU	28.87 (10.88)[c]	-4.35 (-8.43, 0.27)	0.067	-4.34 (-9.00, 0.31)	0.118
	Dependent CU	34.70 (15.94)[c]	1.97 (-3.76, 7.71)	0.720	2.53 (-4.43, 9.49)	0.686
TMT-B	NU	79.60 (35.21)[c]				
	Non-dependent CU	69.49 (29.16)[c]	**-12.74 (-22.59, -2.89)**	**0.022**	**-14.59 (-25.62, -3.57)**	**0.018**
	Dependent CU	72.03 (27.13)[c]	-10.32 (-24.21, 3.58)	0.247	-10.84 (-27.30, 5.61)	0.319
TMT-B/A Ratio	NU	2.543 (0.910)[c]				
	Non-dependent CU	2.541 (0.912)[c]	-0.068 (-0.370, 0.235)	0.868	-0.18 (-0.53, 0.17)	0.476
	Dependent CU	2.305 (1.060)[c]	-0.360 (-0.788, 0.067)	0.171	-0.50 (-1.02, 0.02)	0.100
AVLT-IR	NU	50.43 (10.52)				
	Non-dependent CU	51.96 (10.17)	-1.49 (-4.25, 1.26)	0.457	-1.76 (-4.81, 1.29)	0.405
	Dependent CU	49.21 (9.75)	2.55 (-1.21, 6.32)	0.304	4.04 (-0.52, 8.59)	0.141
AVLT-DR	NU	10.78 (2.74)				
	Non-dependent CU	10.35 (3.13)	0.10 (-0.81, 1.01)	0.965	0.19 (-0.84, 1.21)	0.908
	Dependent CU	10.06 (3.33)	0.67 (-0.59, 1.93)	0.473	1.36 (-0.19, 2.91)	0.146
VERFL-PF	NU	33.85 (13.84)				
	Non-dependent CU	35.16 (12.45)	-2.27 (-5.83, 1.28)	0.345	-2.91 (-6.82, 1.00)	0.239
	Dependent CU	38.42 (12.05)	-3.50 (-8.45, 1.45)	0.278	-4.00 (-9.89, 1.89)	0.299
VERFL-SF	NU	20.63 (6.47)				
	Non-dependent CU	21.88 (5.81)	-1.30 (-3.08, 0.48)	0.255	-1.42 (-3.42, 0.57)	0.267
	Dependent CU	21.45 (6.93)	-0.31 (-2.79, 2.16)	0.955	-0.05 (-2.96, 3.06)	0.999

P values adjusted for multiple comparisons using the multivariate t method for 2 comparisons. Statistically significant rows ($p<0.05$) are presented in bold.

[a] Initial models adjusted for age, gender, and SES, and site as a random effect.

[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

[c] Raw Trail Making Test scores not reversed, higher score = poorer performance.

Supplementary Table 3-12. Cognitive assessment scores between healthy controls and CHR current, occasional cannabis users

Cognitive assessment		**Initial model[a]** EMM difference, HC-CHR (95% CI)	**Sensitivity analysis[b]** EMM difference, HC-CHR (95% CI)
WAIS-III	IQ	7.1 (-0.3, 14.6)	7.5 (-1.3, 16.3)
TMT	Trail A	-1.25 (-6.96, 4.46)	-2.29 (-8.99, 4.41)
	Trail B	9.45 (-3.04, 21.95)	7.71 (-4.72, 20.13)
	Trail B/A Ratio	0.396 (-0.054, 0.847)	0.420 (-0.100, 0.939)
AVLT	Immediate Recall	0.80 (-3.47, 5.08)	0.21 (-4.62, 5.04)
	Delayed Recall	0.13 (-1.50, 1.76)	0.23 (-1.71, 2.17)
VERFL	Phonetic Fluency	4.80 (-1.25, 10.84)	7.77 (0.55, 14.99)
	Semantic Fluency	0.86 (-3.39, 3.56)	0.35 (-3.75, 4.43)

Abbreviations: AVLT, Rey Auditory Verbal Learning Test; CHR, clinical high-risk current, occasional cannabis using group; EMM, Estimated Marginal Mean; HC, healthy control group; TMT, Trail Making Task; VERFL, Verbal Fluency Test; WAIS-III, shortened Wechsler Adult Intelligence Scale-III.
TMT scores have been reversed so that positive EMM differences = better performance of the HC group and negative EMM differences = better performance of the CHR group.
Statistically significant rows ($p<0.05$) are presented in bold.
[a] Initial models adjusted for age, gender, and SES, and site as a random effect.
[b] Sensitivity analysis fully adjusted for alcohol, tobacco, other substance use, age, gender, and SES, and site as a random effect.

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CHAPTER 4 - EFFECTS OF CANNABIDIOL AND DELTA-9-TETRAHYDROCANNABINOL ON PLASMA ENDOCANNABINOID LEVELS IN HEALTHY VOLUNTEERS

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Effects of Cannabidiol and Delta-9-Tetrahydrocannabinol on Plasma Endocannabinoid Levels in Healthy Volunteers: A Randomized Double-Blind Four-Arm Crossover Study

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4.2 ABSTRACT

4.2.1 BACKGROUND

The effects of cannabis are thought to be mediated by interactions between its constituents and the endocannabinoid system. Delta-9-tetrahydrocannabinol (THC) binds to central cannabinoid receptors, while cannabidiol (CBD) may influence endocannabinoid function without directly acting on cannabinoid receptors. We examined the effects of THC co-administered with different doses of CBD on plasma levels of endocannabinoids in healthy volunteers.

4.2.2 METHODS

In a randomised, double-blind, four-arm cross-over study, healthy volunteers (n=46) inhaled cannabis vapour containing 10mg THC plus either 0, 10, 20 or 30mg CBD, in four experimental sessions. The median time between sessions was 14 days (IQR=20). Blood samples were taken pre-cannabis inhalation and at 0-, 5-, 15- and 90-min post-inhalation. Plasma concentrations of THC, CBD, anandamide, 2-arachidonoylglycerol (2-AG) and related non-cannabinoid lipids were measured using liquid chromatography-mass spectrometry.

4.2.3 RESULTS

Administration of cannabis induced acute increases in plasma concentrations of anandamide (+18.0%, 0.042ng/ml [95%CI: 0.023–0.062]), and the non-cannabinoid ethanolamides, docosatetraenylethanolamide (DEA) (+35.8%, 0.012ng/ml [95%CI: 0.008–0.016]), oleoylethanolamide (OEA) (+16.1%, 0.184ng/ml [95%CI: 0.076–0.293]), and N-arachidonoyl-L-serine (ARA-S) (+25.1%, 0.011ng/ml [95%CI: 0.004–0.017]) ($p<0.05$). CBD had no significant effect on the plasma concentration of anandamide, 2-AG or related non-cannabinoid lipids at any of three doses used. Over the four sessions, there were progressive decreases in the pre-inhalation concentrations of anandamide and DEA, from 0.254ng/ml [95%CI: 0.223–0.286] to 0.194ng/ml [95%CI: 0.163–0.226], and from 0.039ng/ml [95%CI: 0.032–0.045] to 0.027ng/ml [95%CI: 0.020–0.034] ($p<0.05$), respectively.

4.2.4 DISCUSSION

THC induced acute increases in plasma levels of anandamide and non-cannabinoid ethanolamides, but there was no evidence that these effects were influenced by the co-administration of CBD. It is possible that such effects may be evident with higher doses of CBD or following chronic administration. The progressive reduction in pre-treatment anandamide and DEA levels across sessions may be related to repeated exposure to THC or participants becoming less anxious about the testing procedure and requires further investigation.

Key Words: THC; CBD; endocannabinoids; anandamide; 2-arachidonoylglycerol; cannabis

4.3 INTRODUCTION

Cannabis is the world's most used illicit drug,[1] and regular use is associated with adverse effects on mental health and cognition.[2–6] On the other hand, one of its constituents, cannabidiol (CBD) is a novel candidate treatment in psychiatry.[7–10]

The main psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC), is a partial agonist at G-protein-coupled cannabinoid receptors type-1 and type-2 (CB_1 and CB_2).[11,12] THC is responsible for the 'high' from cannabis use as well as its adverse effects. CBD is the second most abundant phytocannabinoid in cannabis and has relatively low affinity for the orthosteric binding sites of CB_1 and CB_2.[13] The endogenous ligands for these receptors are endocannabinoids such as anandamide [AEA] and 2-arachidonoyl glycerol [2-AG]).[12,14] Both AEA and 2-AG are high affinity CB_1 receptor agonists, while AEA has lower affinity for CB_2.[15] The endocannabinoid system has been implicated in the regulation of brain development, synaptic plasticity and neuronal signalling.[14,16,17]

The mechanism by which CBD exerts its effects is unclear. In preclinical studies CBD can act as a negative allosteric modulator at the CB_1 receptor, but it does not alter the subjective effects of cannabis associated with THC binding to CB_1 receptors.[13,18–21] One hypothesis is that CBD inhibits AEA metabolism, leading to an upregulation in AEA signalling.[22] In vitro experimentation has shown that CBD can reduce AEA degradation by inhibiting both its cellular reuptake via the anandamide membrane transporter and its hydrolysis by the intracellular enzyme fatty acid amide hydrolase (FAAH).[23] Other putative mechanisms of action of CBD include inhibiting the metabolism and/or inducing the synthesis of N-acylethanolamines (NAEs).[24,25] Members of the NAE family include AEA, docosatetraenylethanolamide (DEA), oleoylethanolamide (OEA) and stearoylethanolamide (SEA). While non-endocannabinoid NAEs such as DEA, OEA and SEA either do not or weakly exert direct action via CB_1 or CB_2, they do have endocannabinoid-like properties.[26,27]

Acute intravenous administration of THC has been shown to transiently increase plasma levels of AEA and 2-AG, through unclear mechanisms.[28] In contrast, cross-sectional studies suggest that chronic cannabis use can downregulate AEA and possibly upregulate 2-AG signalling.[29–31] However, the acute dose-effects of inhaled THC and CBD in quantities naturally present in cannabis on circulating endocannabinoids have yet to be established.

The aim of the present study was to examine the effects of THC and CBD on plasma endocannabinoid levels and related non-cannabinoid lipids. Four preparations of cannabis were used, each containing a fixed dose of THC, but a different dose of CBD. We hypothesised that i) Administration of THC would lead to a transient increase in plasma AEA and 2-AG, and that ii) these effects would be modulated by co-administered CBD in a dose-dependent manner.

4.4 MATERIALS AND METHODS

4.4.1 STUDY DESIGN

Randomised, double-blind, four-arm cross-over study. Healthy volunteers were studied on four occasions. In each session they received a dose of cannabis vapour containing 10mg THC plus CBD at a dose of either 0, 10, 20 or 30mg. These doses were designed to reflect the doses of THC and CBD typically found in recreational cannabis.[32]

4.4.2 ETHICS

The study was approved by the KCL Research Ethics Committee (RESCMR-16/17-4163). Written informed consent was obtained from each participant. The study was conducted in compliance with the principles of Good Clinical Practice and the Declaration of Helsinki (1996) and registered on Open Science Framework (https://osf.io/kt3f7) and clinicaltrials.gov (NCT05170217).

4.4.3 STUDY DRUGS

Raw cannabis plant material was provided by Bedrocan BV, Netherlands. Bedrocan (batch release specifications: 0.1% CBD, 22.6% THC), Bedrolite (7.5% CBD, 0.3% THC) and placebo (<0.1% cannabinoids) were prepared in order to administer CBD:THC in 4 different ratios: 0:1, 1:1, 2:1 and 3:1. In all 4 preparations, the dose of THC was 10mg (two standard THC units)[33], whereas the dose of CBD was 0mg (0:1), 10mg (1:1), 20mg (2:1), and 30mg (3:1), respectively. Placebo cannabis was used to equalise the weight of each preparation (**Table** 4-1).

Table 4-1. Depiction of Cannabis Preparations

CBD:THC ratio	0:1	1:1	2:1	3:1
THC dose (mg)	10	10	10	10
CBD dose (mg)	0	10	20	30
Bedrocan cannabis (mg)	44.2	42.5	40.7	38.9
Bedrolite cannabis (mg)	0.0	132.8	266.1	399.5
Placebo cannabis (mg)	394.2	263.1	131.6	0.0
Batch specifications of cannabis products: Bedrocan - 22.6% THC, 0.1% CBD; Bedrolite -0.3% THC, 7.5% CBD; placebo - <0.1% THC, <0.1% CBD.				

4.4.4 PARTICIPANTS

Participants were aged 21-50 years, had used cannabis at least once previously, had used cannabis < once weekly on average over the last 12 months, were not taking medications (excluding contraceptives), and had no psychiatric or medical history. Details of recruitment and full inclusion/exclusion criteria are listed in the **4.12.1 Supplementary Methods** (page 187).

4.4.5 PROCEDURE

The study was conducted at the NIHR Wellcome Trust Clinical Research Facility at King's College Hospital. Each participant attended a screening visit at which a physical and mental health examination and assessment for study eligibility were undertaken by a physician. Participants also practiced the vapour inhalation technique with an air-filled balloon.

Experimental Visits

Each participant attended four experimental visits, with a minimum 7-day wash-out between visits. Participants were asked to abstain from illicit drugs for the duration of the study, and from alcohol, tobacco and vaping 24 hours before each visit, verified by a urine drug screen, alcohol breath test (BAC=0) and carbon monoxide breath test (CO<10ppm). Experiments began at either 10:00 or 12:00. An intravenous cannula was inserted, and the baseline blood sample was drawn 30 minutes (95%CI: 29–33) prior to drug administration.

The order that participants received the four cannabis preparations (CBD:THC ratios) was randomised. Drug was administered by inhalation using a Volcano Medic Vaporizer (Storz & Bickel, Germany), following the protocol from Lawn et al., 2016.[21] Cannabis was vaporized at 210°C into a covered polythene balloon with a valve mouthpiece, which prevented loss of cannabinoids between inhalations. The same balloon was filled twice using the same cannabis to ensure the full dose was administered. A standardised inhalation procedure was repeated until both balloons had been emptied. During the study visit participants also completed cognitive and psychological assessments; see **Supplementary Figure 4-1** (page 191).

4.4.6 BLOOD COLLECTION AND ANALYSIS

Venous blood samples were collected into lithium-heparin tubes 30 minutes pre-cannabis inhalation, immediately after the final inhalation (0-min), and at 5-min, 15-min, and 90-min post-inhalation. Samples were centrifuged at 4^{o}C, divided into two cryovials, stored at -20^{o}C until all samples from that day had been collected, then moved to a -80^{o}C freezer.

Plasma concentrations of CBD and THC were determined using High Performance Liquid Chromatography–Mass Spectrometry (LC/MS) at the Mass Spectrometry Facility, KCL.[34] Plasma concentrations of AEA and 2-AG, their precursor arachidonic acid (AA), and six biologically-related endogenous fatty acid ethanolamides: N-arachidonoyl-L-serine (ARA-S), DEA, OEA, SEA, alpha-linolenoylethanolamide (aLEA) and gamma-linolenoylethanolamide (gLEA) (eFigure 1) were quantified using a validated Ultra-High Pressure Liquid Chromatography (UHPLC)-MS method (Dickens et al., 2020)[35] at the Turku Metabolomics Centre (Turku Bioscience, Finland). As it was not possible to separate 1-AG and 2-AG in plasma due to rapid isomerisation,[36] the quantity was reported as total AG (henceforth described as '2-AG').

4.4.7 STATISTICAL ANALYSIS

All analyses were completed using R, version 3.3.2.[37] Missing values were imputed using multiple imputation chain equations (MICE; mice package version 3.13.0)[38] after confirming no detected of deviation from missing completely at random (MCAR) based on Little's MCAR test. All analyses were completed using linear mixed models (lme4 package version 1.1-26).[39] Power and sensitivity calculations can be found in **4.12.1 Supplementary Methods**.

The primary outcome of the effects of different CBD:THC ratios on plasma analyte level was measured as peak effects (Model 1) and area under the curve (AUC; Model 2) of mean plasma concentrations. Peak effects (i.e., estimated Cmax) were determined as the plasma concentrations at the timepoint at which they were at the highest (estimated Tmax). AUC values were calculated after baseline correction using the spline method (DescTools package).[40] The CBD:THC ratios (0:1, 1:1, 2:1, 3:1) were coded as a categorical variable. Participant ID was coded as a categorical variable and included as a random effect to account for dependency between repeated measures. Estimated marginal mean (EMM; emmeans

package version 1.5.2-1)[41] differences were calculated for all 6 contrasts (0:1 vs 1:1, 0:1 vs 2:1 etc). Models 1 and 2 were fully adjusted by including pre-inhalation plasma concentration (continuous variable) and visit number (categorical variable; visit 1, 2, 3, 4), to account for within-subject differences, as well as the number of days between each of the four experimental visits (continuous variable) to account for the possible carry-over effect of repeated exposure to THC.[30,31] For time between experimental visits, one outlier value was identified using Rosner's generalised extreme Studentised deviate test (GEST; EnvStats package version 2.7.0)[42] and excluded.

The secondary outcome of the effects of THC on plasma analyte levels was assessed by Model 3. The effect of THC alone was determined by analysing plasma levels following administration with THC only (0:1 CBD:THC ratio), excluding all other visits (Model 3a). Mean plasma concentrations at each of the timepoints (categorical variable; pre-inhalation, 0min, 5min, 15min and 90min) were compared, including participant ID as a random effect. EMM differences were calculated for all 10 contrasts (pre-inhalation vs 0min etc.) The fully adjusted Model 3a included the visit number and time since last visit variables. To maximise statistical power, the analysis was then repeated to include all experimental visits (Model 3b). The fully adjusted Model 3b included the CBD:THC ratio, visit number and time since last visit variables.

Exploratory analyses assessed changes in plasma analyte levels over the experimental visits (Model 4). Model 4a compared pre-inhalation concentrations of the analytes between the 4 visits, with participant ID as a random effect. EMM differences were calculated for all 6 contrasts (visit 1 vs visit 2 etc). In post-hoc analyses, we assessed whether any identified effects were influenced by CBD. Pre-inhalation levels of analytes at visits 2, 3 and 4 (Models 4b, 4c and 4d, respectively) were compared with total CBD dose from previous visits (categorical variable). Models 4a, 4b, 4c and 4d were fully adjusted by including the time since last experimental visit variable.

Post-hoc analyses to explore sex differences in endocannabinoid responses to THC and/or CBD were performed by adding sex (categorial variable) as an interaction term to the predictor variable in each model.

EMM differences were corrected for multiple comparisons using the Tukey adjustment method and are presented along with p-values and 95% confidence intervals.

4.5 RESULTS

64 potential participants were randomised, of whom 46 completed all four experimental sessions and contributed data. Demographics and physical characteristics are shown in **Table *4-2***. Median inhalation time was 17 minutes (IQR=11). The median time between experimental visits was 14 days (IQR=20).

Table 4-2*. Demographics of participants at baseline*

Variables	N (%)	Mean (SD)
Gender		
Male	25 (54.3)	
Female	21 (45.7)	
Age		26.62 (4.94)
Ethnicity		
White	21 (45.7)	
Asian	10 (21.7)	
Mixed	3 (6.5)	
Black	1 (2.2)	
Other	11 (23.9)	
BMI (kg/m^2)		23.72 (2.57)
Body Fat (%)- Male		15.56 (5.50)
Body Fat (%)- Female		25.50 (6.33)
Days since last use of alcohol		4.17 (4.62)
Alcohol use/month (days)		8.02 (4.86)
eCigarette use (ever)	12 (26.1)	
Daily eCigarette user	1 (2.2)	
Tobacco use (ever; separate from cannabis)	34 (73.9)	
Daily tobacco user (separate from cannabis)	3 (6.5)	
Use tobacco with cannabis	36 (78.3)	
Age of first cannabis use		17.67 (2.46)
Years of cannabis use		6.63 (4.68)
Cannabis use/year		8.91 (12.67)

4.5.1 PLASMA CBD & THC CONCENTRATIONS

Figure 1 shows the mean plasma concentrations of the endocannabinoids, plus CBD and THC for comparison, versus time, stratified by CBD:THC ratio. The peak and AUC THC concentration remained similar across the four conditions ($p>0.05$), and there was a dose-dependent increase in peak and AUC plasma CBD as the CBD:THC ratio increased ($p<0.001$, **Supplementary Table** 4-1).

4.5.2 COMPARISON OF CBD:THC RATIOS

There were no significant differences in either peak or AUC plasma concentrations for any of the endocannabinoids or related non-cannabinoid lipids between CBD:THC ratios (**Figure 4-1**, **Supplementary Figure 4-1**, **Supplementary Table** 4-1). The estimated Tmax was 0min for AEA, aLEA, ARA-S, DEA, OEA and SEA, 5min for AA and gLEA, and 90min for 2-AG. For gLEA, the lowest plasma level was selected since levels decreased post-inhalation.

4.5.3 EFFECT OF DRUG ADMINISTRATION

THC alone

When limiting data to the visits where cannabis containing only THC was administered (0:1 CBD:THC ratio), mean DEA concentration rose by 37.8% (0.013ng/ml [95%CI:0.005–0.020], t(180)=3.273, p=0.011) at 0min post-inhalation, before falling to pre-inhalation levels by 5min (Figure 2). While the mean AEA concentration was greater at 0min than at 5min, 15min or 90min ($p<0.05$), it was not significantly higher than pre-inhalation (+17.0%, 0.040ng/ml [95%CI:0.010–0.070], t(180)=2.633, p=0.069) (**Figure 4-2**). There were no significant changes in plasma levels of any of the other the endocannabinoids or related non-cannabinoid lipids (**Supplementary Table** 4-2, **Supplementary Figure 4-3**).

Overall effect of THC

The above analysis was extended to include all experimental visits (i.e., including those in which THC was co-administered with CBD). Plasma levels of AEA, DEA, OEA and ARA-S increased significantly post-cannabis inhalation (**Supplementary Figure 4-4**). Mean AEA concentration rose by 18.0% (0.042ng/ml [95%CI:0.023–0.062], t(858)=4.298, $p<0.001$), mean DEA concentration rose 35.8% (0.012ng/ml [95%CI:0.008–0.016], t(858)=5.797, $p<0.0001$), mean OEA concentration rose 16.1% (0.184ng/ml [95%CI:0.076–0.293], t(858)=3.332, p=0.008), and mean ARA-S concentration increased 25.1% (0.011ng/ml [95%CI:0.004–0.017], t(858)=3.326, p=0.008) immediately post-inhalation, before falling to pre-inhalation levels by 5min. There were no significant changes in plasma levels of any of the other analytes (**Supplementary Table** 4-3).

Figure 4-1. Plasma concentration-time graphs, stratified by CBD:THC ratio.

A. delta-9-tetrahydrocannabinol (THC), B. cannabidiol (CBD), C. anandamide (AEA), D. 2-arachidonoylglycerol (2-AG), reported as total AG.
Circles show individual data points, larger shapes show mean values and boxplots show median and interquartile range.

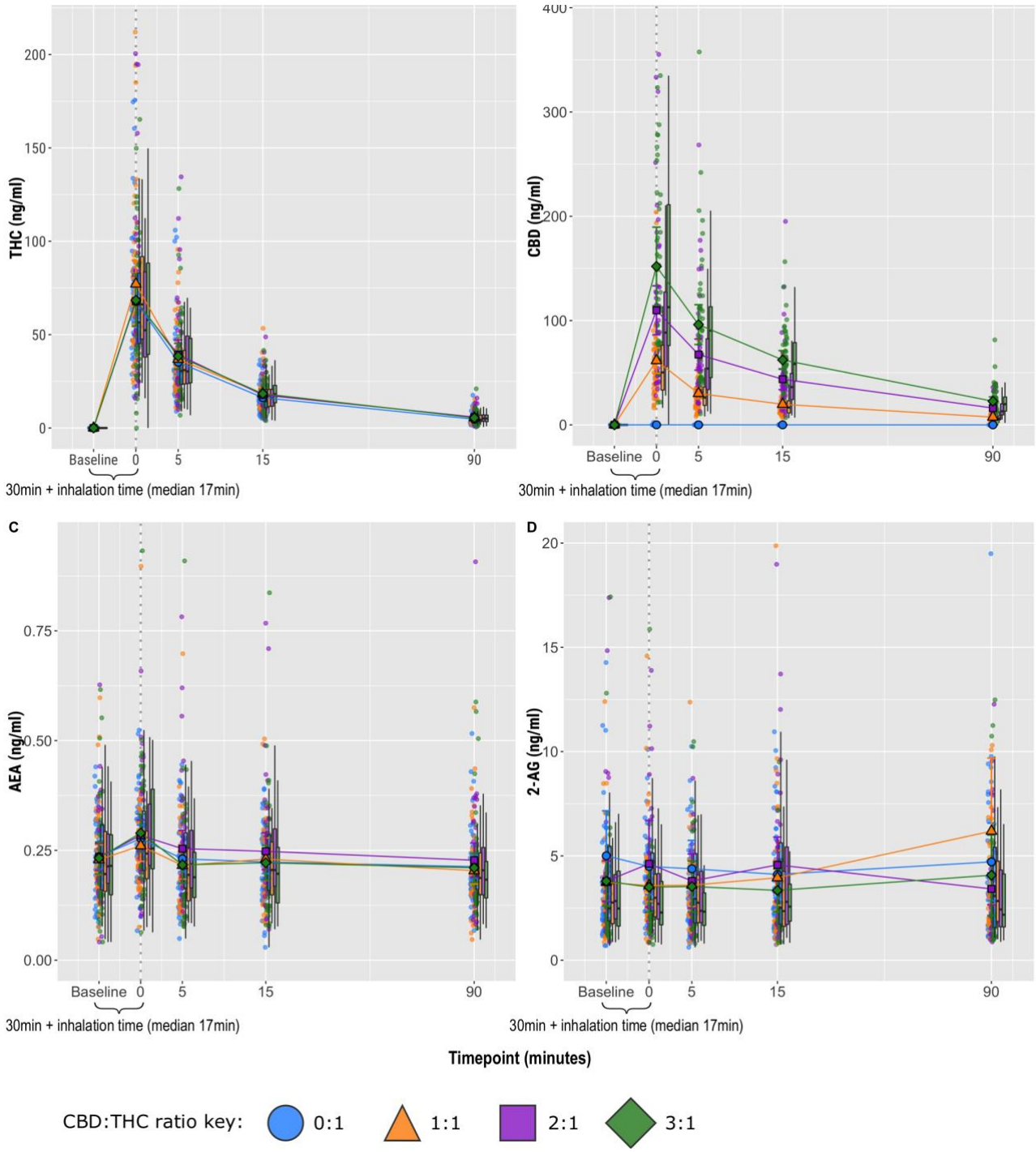
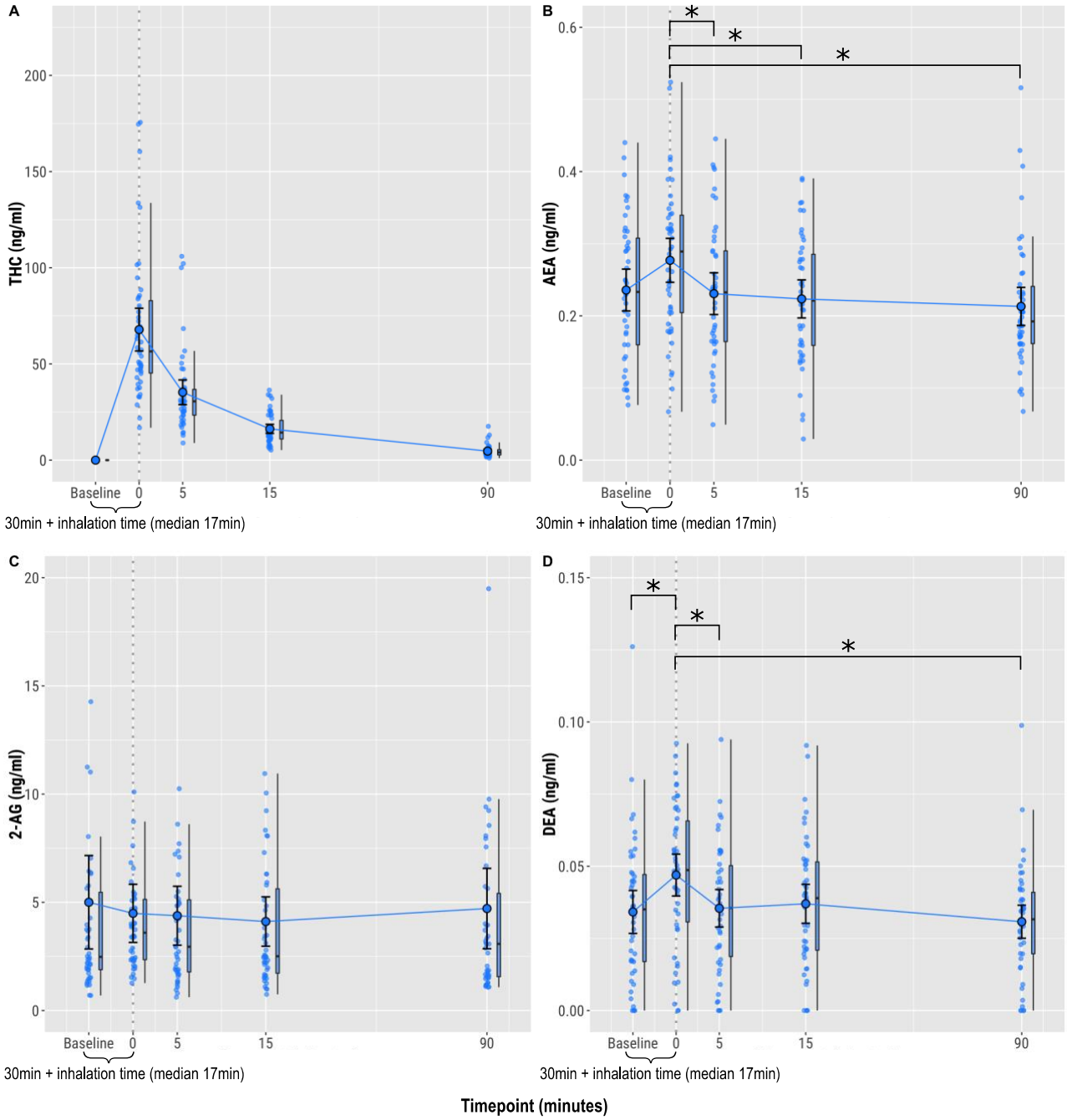


Figure 4-2. Plasma concentrations following administration of 10mg THC, 0mg CBD (0:1 ratio).

A. delta-9-tetrahydrocannabinol (THC), B. anandamide (AEA), C. 2-arachidonoylglycerol (2-AG) reported as total AG, D. docosatetraenylethanolamide (DEA).
Circles show individual data points, larger circles show mean values and boxplots show median and interquartile range.



4.5.4 EFFECT OF VISIT ORDER ON ENDOCANNABINOID LEVELS

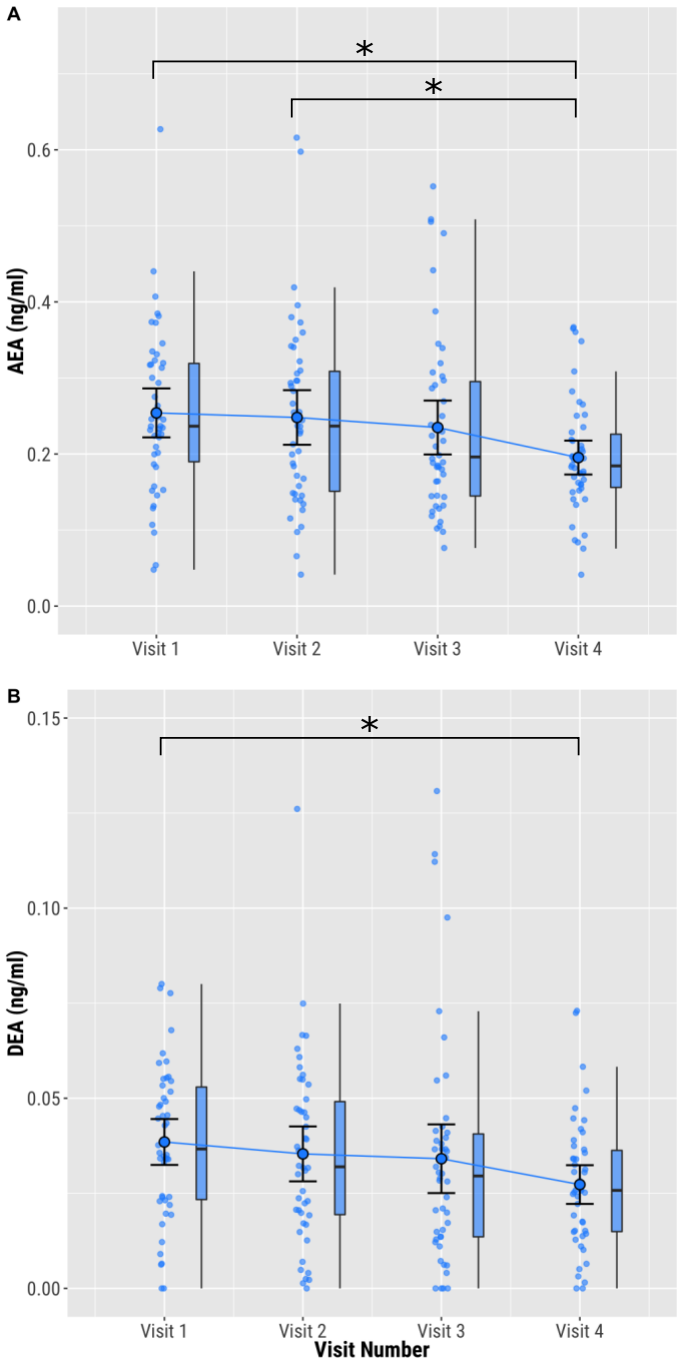
Between visit 1 and visit 4 the mean pre-inhalation AEA concentration fell by 23.6% (0.060ng/ml [95%CI:0.024–0.096]), t(135)=3.278, p=0.007), and the mean pre-inhalation DEA concentration fell by 29.1% (0.011ng/ml [95%CI:0.003–0.019], t(135)=2.779, p=0.031) (**Figure 4-3**). After adjusting for time between visits, the decrease in baseline DEA no longer reached statistical significance (p=0.086) (**Supplementary Table** 4-4). Post-hoc analyses showed that none of pre-inhalation concentrations of AEA and DEA at visits 2, 3 and 4 were associated with the total dose of CBD received at the previous visits (p>0.05) (**Supplementary Table** 4-5). There were no significant changes in pre-inhalation plasma levels of any of the other analytes across experimental visits (**Supplementary Table** 4-4).

4.5.5 SEX DIFFERENCES

There were no significant sex differences between the endocannabinoids or related non-cannabinoid lipid responses to THC or CBD, with the exception of Models 3b and 4a for SEA. However, these results were found to be caused by two outliers, identified using Rosner's generalised extreme Studentised deviate test, and were no longer significant when these outliers were removed; see **4.12.2 Supplementary Results** (page 238).

Figure 4-3. Pre-inhalation plasma concentrations vs. visit number.

A. anandamide (AEA), B. docosatetraenylethanolamide (DEA).
Circles show individual data points, larger circles show mean values and boxplots show median and interquartile range.
** = p<0.05*



4.6 DISCUSSION

To our knowledge, this is the first study to investigate the acute effects of co-administered THC and CBD on plasma endocannabinoid concentrations. Its strengths include the use of a double-blind, within-subjects design, which mitigated against potential placebo effects related to CBD, as well as inter-individual differences in response to THC and CBD. Restricting participation to infrequent cannabis users reduced the risk of prior cannabis use impacting circulating endocannabinoid levels.

We did not detect an effect of the CBD:THC ratio in cannabis on the plasma concentration of any of the tested endocannabinoids or related lipid compounds. Previous research has indicated that CBD may enhance AEA signalling. Leweke et al.[22] reported that treatment with 800mg of oral CBD for 14 days led to an increase in AEA and OEA in patients with psychosis, with AEA serum levels increasing 1pmol/ml (equivalent to 0.348ng/ml) after 28 days. However, another study found that 200mg of CBD daily for 13 weeks had no effect on plasma levels of AEA, 2-AG or OEA in patients with type-2 diabetes .[43] The absence of an effect on plasma endocannabinoids in our study may have been due to the administration of single doses of CBD at relatively low dosages. Comparing doses between oral and vaporised CBD is difficult due to the differences in pharmacokinetics between formulations; CBD undergoes significant first-pass metabolism,[44] and its absorption and elimination is slower when taken orally versus inhalation.[45] Nevertheless, an oral dose of 800mg CBD will produce much greater systemic availability of the drug than our maximum inhaled dose of 30mg CBD.[45] The doses of THC and CBD that we used were designed to reflect those typically found in recreational cannabis.[32] As typical 'joint' contains between 300-350mg of cannabis material,[46] it would not be possible for cannabis used recreationally to provide quantities of CBD equivalent to an 800mg oral dose.

The inhalation of vaporised cannabis containing 10mg THC led to transient increases in plasma levels of AEA and the endocannabinoid-like lipids DEA, OEA and ARA-S. These findings are consistent with those of Thieme et al.,[28] who found that plasma AEA increased by 0.060ng/ml 30min after an IV dose of 0.1mg/kg IV THC. However, we did not detect the increase in plasma 2-AG reported by Thieme et al. Walter et al.[47] found that 20mg THC given orally (as dronabinol) produced higher concentrations of AEA, OEA and 2-AG after 2

and 3 hours compared to placebo. In contrast, Kearney-Ramos et al.[48] did not detect any changes in either plasma AEA or 2-AG after the inhalation of an estimated 30mg THC in 26 near-daily cannabis users. This may be explained by frequent cannabis use leading to compensatory adaptations in the ECS, examples including reductions in circulating endocannabinoids and CB_1 receptor availability.[49–52]

The increase in AEA, DEA, OEA and ARA-S plasma concentrations immediately post-drug administration could be due to a direct effect of THC on either their synthesis or degradation. It's also possible that THC indirectly increased endocannabinoid levels via enhanced catecholaminergic and glucocorticoid signalling, which are known to cause significant increases in plasma endocannabinoid concentrations.[53–57] THC may also have simply displaced the endogenous ligands which have a similar protein binding profile, particularly ligands of the GPR55 receptor which include AEA, OEA and ARA-S.[58–60]

Pre-inhalation levels of AEA and DEA decreased in a stepwise fashion between the first and final experimental visit. Differences in CBD dose between sessions did not alter these results, suggesting that CBD was not a factor. However, repeated doses of THC have been shown to downregulate AEA and 2-AG signalling in the rat striatum.[31] Similarly, in humans, frequent cannabis users have lower cerebrospinal fluid (CSF) concentrations of AEA than infrequent users.[30] Our results are unlikely to be due to a direct pharmacological action of THC on the synthesis or degradation of AEA, as adjusting the model for time between experimental sessions (minimum 7 days) had no significant impact, and pre-inhalation plasma samples taken at each visit consistently found no measurable THC or CBD post-washout. Another possible explanation is that as participants became increasingly familiar with the experimental sessions, there may have been a reduction in the stress associated with the procedure. Stress can induce glucocorticoid and catecholamine responses that can increase AEA release.[53,54] Future studies may wish to explore if the gradual decrease of baseline AEA represents a conditioned response to the experimental setting.

Certain limitations should be considered in the interpretation of the data. CSF levels of AEA are not correlated with those in peripheral blood, so plasma levels of endocannabinoids do not necessarily reflect those present in brain.[61] The duration of cannabis inhalation varied

significantly between participants and between experiments, with a median duration of 17 minutes. Future studies should consider methods to standardise duration of inhalation. Because the absorption of cannabinoids will have started before the end of the inhalation period, referring to the first timepoint as "0min" is not strictly accurate. This also limits our ability to compare the sampling timelines of the present study with those of Thieme et al. or Walter et al., as the routes and durations of administration were different.[44,62] It is possible that food consumption could have impacted levels of endocannabinoids.[63,64] Our participants were asked to eat their usual breakfast, but it's timing and content were not controlled. The study did not include a placebo THC condition, so we cannot exclude the possibility that the inhalation procedure itself, rather than THC administration, produced changes in AEA, DEA, OEA and/or ARA-S.

4.6.1 CONCLUSIONS

Inhalation of vapourised cannabis increased levels of plasma AEA and several endocannabinoid-like lipids, but there was no evidence that CBD influenced any of these effects. It is possible that the doses of CBD were either too low to have measurable influence, and/or that CBD affected central but not peripheral endocannabinoids. There was a progressive reduction in the plasma concentrations of AEA and DEA across successive experimental sessions, which could reflect a downregulation of endocannabinoid signalling with repeated THC administration, or habituation with the testing procedure.

4.7 AUTHOR DISCLOSURE STATEMENT

A.E. has received speakers' honoraria from GW Pharmaceuticals. A.E.'s position is funded by, and L.C. and J.S. are supported by, the National Institute for Health Research (NIHR) Biomedical Research Centre for Mental Health at South London and Maudsley NHS Foundation Trust and King's College London. R.M.M. has received speakers' honoraria from Janssen, Lundbeck, Otsuka, and Sunovian. All remaining authors report no conflicting interests.

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4.9 ABBREVIATIONS USED

2-AG, 2-arachidonoylglycerol; AA, arachidonic acid; AEA, anandamide; aLEA, alpha-linolenoylethanolamide; ARA-S, N-arachidonoyl-L-serine; CBD, cannabidiol; CSF, cerebrospinal fluid; DEA, docosatetraenylethanolamide; ECS, endocannabinoid system; FAAH, fatty acid amide hydrolase; gLEA, gamma-linolenoylethanolamide; NAE, N-acylethanolamine; OEA, oleoylethanolamide; SEA, stearoylethanolamide; THC, delta-9-tetrahydrocannabinol.

4.10 AUTHOR CONTRIBUTION STATEMENT

L.A.C. contributed to conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; validation; visualization; writing—original draft, review and editing.

A.E. assisted with conceptualization; data curation; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; visualization;

writing—review and editing. E.C. and D.O. contributed to conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; validation; visualization; writing—review and editing. J.W. performed conceptualization; data curation; investigation; methodology; project administration; resources; writing—review and editing. S.S. performed data curation; investigation; project administration; resources; writing— review and editing. A.M.D. and T.L. contributed to data curation; investigation; resources; writing—review and editing. M.O. assisted with investigation; resources; writing—review and editing. J.H. performed formal analysis; methodology; software; visualization; writing— review and editing. A.M. contributed to writing—review and editing. J.S. assisted with conceptualization; method- ology; writing—review and editing. R.M.M. contributed to conceptualization; funding acquisition; writing— review and editing. T.P.F. performed conceptualization; funding acquisition; methodology; project administration; visualisation; writing – review & editing. P.M. contributed to conceptualization; funding acquisition; methodology; project administration; supervision; writing—review and editing.

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4.12 SUPPLEMENTARY MATERIAL

4.12.1 Supplementary Methods

Supplementary Figure 4-1. Timeline of baseline and experimental sessions.

Supplementary Figure 4-2. Molecular structure of plant cannabinoids, endocannabinoids. and biologically related compounds analysed from plasma samples.

Supplementary Figure 4-3. Plasma concentration-time graphs, stratified by CBD:THC ratio.

Supplementary Figure 4-4. Pre-inhalation plasma concentrations vs. visit number.

Supplementary Figure 4-5. Plasma concentrations following 10mg THC, 0mg CBD (0:1 ratio).

Supplementary Table 4-1. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.

Supplementary Table 4-2. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.

Supplementary Table 4-3. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.

Supplementary Table 4-4. Pre-inhalation plasma concentrations vs. visit number – Model 4a.

Supplementary Table 4-5. Pre-inhalation plasma concentrations of AEA and DEA vs. total CBD dose from previous visits – Models 4b, 4c and 4d.

4.12.2 Supplementary Results

4.12.3 Supplementary References

4.12.1 SUPPLEMENTARY METHODS

Study Drugs

Standardised cannabis plant material is produced according to Good Manufacturing Practice (GMP) and meet the European Medicines Agency's contaminant levels for products used in the respiratory tract. The products are regulated by the Dutch government's Office of Medicinal Cannabis at the Dutch Ministry of Health, Welfare and Sport.

Participants

Recruitment

Email advertisements were sent to staff and students at King's College London. After completing an initial phone screening, participants were invited to attend a baseline session at King's College Hospital.

Inclusion/Exclusion criteria

Inclusion criteria:

i) Be aged between 21-50 years old
ii) Have used cannabis at least once in the past
iii) Willing and able to provide written informed consent
iv) Willing to provide blood samples
v) Be a fluent English speaker

Exclusion criteria:

i) cannabis use more than one day per week on average over the last 12 months;
ii) any past or present major mental illness;
iii) a past or present major physical illness;
iv) a score of 5 and above on the Michigan Alcoholism Screening Test;[1]
v) a score of 5 and above on the Fagerstrom Test for Nicotine Dependence;[2]
vi) a score of 5 and above on the Drug Abuse Screening Test (DAST-20);[3]
vii) past or present use of anti-psychotic or anti-depressant medication;
viii) a first-degree relative with a psychotic disorder;
ix) currently use of psychotropic medication;

x) BMI classified as obese or underweight;
xi) having taken part in any drug study within the last 30 days or taking part in another study over the course of the trial;
xii) a known drug sensitivity/allergy towards cannabis or lorazepam; and
xiii) pregnancy (current or planned) or lactation in women.

Urine pregnancy tests were performed in all female participants at the start of each study visit with drug administration only performed upon a negative result. In addition, participants were asked not to use recreational/illicit drugs at least 7 days and alcohol and tobacco for 24 hours before each visit, confirmed by a urine drug test, alcohol breath test (BAC=0) and carbon monoxide breath test (CO <10 ppm) at each study visit, respectively. Urine drug testing kits tested for amphetamines, benzodiazepines, buprenorphine, cocaine, cannabis, methadone, methamphetamines, morphine, and opiates. Failure to pass tests resulted in rescheduling of the study visit.

Screening Visit

A study physician was present at screening visits to complete a general medical history and brief physical examination, to exclude subjects with medical conditions. Participants were given the opportunity to discuss the patient information sheet and ask questions before completing consent forms. Psychological scales were completed before the participant was discharged.

Randomisation and masking

The study design was a 4-phase cross-over with each phase corresponding to one of the 4 CBD:THC dosing ratios: 0:1, 1:1, 2:1, 3:1. Each participant was required to complete all phases corresponding to all ratios of cannabis preparations. For 4 conditions there are 24 possible permutations of sequential order, e.g., Visit 1, 2:1 – Visit 2, 0:1 – Visit 3, 1:1 – Visit 4, 3:1. Randomised sequences were generated in blocks with the first 24 participants allocated each of the 24 possible orders of ratios, as were the next 24 and so on. Where there were fewer participants than possible order sequences, each participant received a random selection from the 24, sampled without replacement. The randomisation list was generated by

a statistician not involved with the study, using a customised randomisation script generated in R software v 3.1 (available on request).

The randomisation was double blinded to both researchers and participants. The randomisation list was passed from the independent statistician to the Maudsley Pharmacy who prepared the cannabis preparations. The pharmacy dispensed the study drug to a blinded researcher. The cannabis preparation was then loaded into the filling chamber and vaporised by a research nurse at the Clinical Research Facility (CRF) who was not involved with any other study procedures. Once filled, this was encased with an opaque bag to ensure the blind was maintained (a higher CBD:THC ratio produces a denser vapour). Upon completion of data collection and entry the randomisation schedule was revealed to the research team prior to data analysis.

Experimental visit

Experimental sessions were scheduled to begin at either 10AM or 12PM. Participants were asked to have a normal (by their own standards) amount of sleep and eat a normal breakfast before arriving. An intravenous cannula was inserted into the participants' arm before the administration of vaporised cannabis.

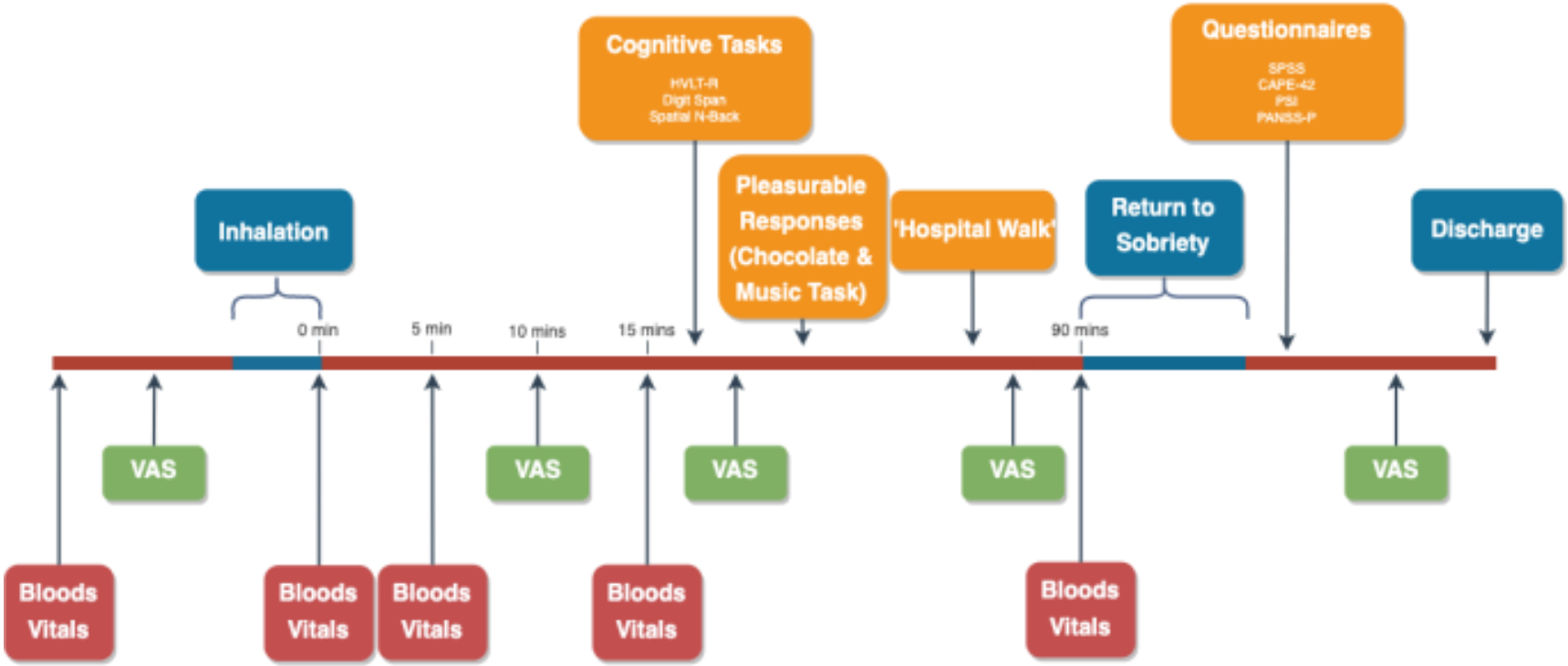
Rescue medication (oral Lorazepam, 1-2mg prn) was available on request of the participant should they become overly distressed by the effects of the drug but was never required. The participant remained in the CRF until the effects of the drug had worn off (roughly 3 hours after cannabis inhalation). Participants were discharged after completing a field sobriety test and vital signs, and told to avoid driving, operating heavy machinery, or cycling for the next 24 hours. The researchers ensured the participant had contact details to the study physician and knew to contact them with any concern relating to participation in the study or side-effects.

Supplementary Figure 4-1. Timeline of baseline and experimental sessions.

Bloods, blood sample; Vitals, vital signs (HR, BP, O_2 sat.); CAPE-42, Community assessment of Psychic Experiences – state;[4] Digit Span, Forward and reverse digit span; HVLT-R, Hopkins verbal learning task – Revised;[5] PANSS-P, Positive and negative syndrome scale – positive subscale;[6] PSI, Psychotomimetic states inventory;[7] Spatial N-Back task;[8] SPSS, State social paranoia scale;[9] VAS, Visual Analogue Scale.

The timings of the blood draws were based on the known pharmacokinetic profiles of inhaled THC and CBD[10,11]

See study protocol for full description of timeline and tasks: https://osf.io/kt3f7



Inhalation procedure

Participants were instructed to inhale and hold their breath for 8 seconds before exhaling fully and resting for 8 seconds. Participants inhaled the first balloon standing upright and the second lying on a clinical bed in preparation of blood sampling.

Vaporization of was chosen as the method of drug preparation replicates the bioavailability characteristics of with smoking, while avoiding toxic chemicals from burnt cannabis. A cup of hot lemon and honey water was provided to help with the abrasiveness of cannabis inhalation.

Sample storage and transport

Plasma samples were stored at −80 °C for 329 days (95%CI: 310 to 348) at King's College Hospital, before being transported via a temperature-controlled courier to Turku Metabolomics Centre (Turku Bioscience, Finland).

Sensitivity and power calculations

The eCBD study was powered *a priori* based on a cognitive outcome measure, change in delayed verbal recall. From an estimated effect size of d=0.5 based on a previous study,[12] a power calculation indicated that a sample size of n = 45 would provide 80% power to detect differences between the cannabis preparations with largest differences in CBD:THC ratio (0:1 to 3:1) at $\alpha = 0.008$ (paired t-test; adjusted for multiple comparisons).

Post hoc sensitivity and power calculations were conducted for the current study using G*Power version 3.1.9.4.[13]

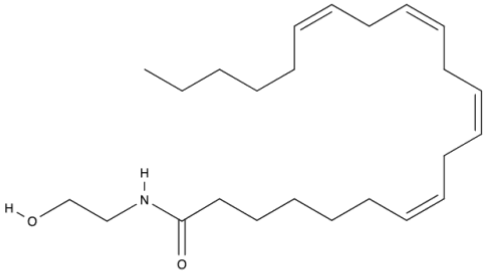
<u>Power</u>

We calculated the power of the primary outcome from this study, difference in peak AEA concentrations between the cannabis preparations with largest differences in CBD:THC ratio (0:1 to 3:1), Model 1. With an effect size (standardised EMM difference) of 0.139, a significance criterion of $\alpha = 0.008$ (paired t-test; adjusted for multiple comparisons), and sample size of n = 46, the calculated power = 0.152.

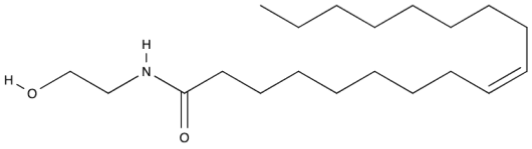
Sensitivity

With a significance criterion of $\alpha = 0.008$, power = 0.80, and sample size of n = 46, the minimum detectable effect size (dz) in for this study was 0.536, considered to be a medium effect size using Cohen's (1988) criteria.

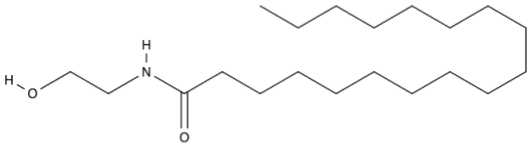
Supplementary Figure 4-2. Molecular structure of plant cannabinoids, endocannabinoids and biologically related compounds analysed from plasma samples



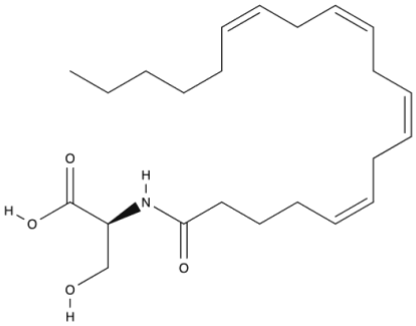
Docosatetraenylethanolamide - DEA



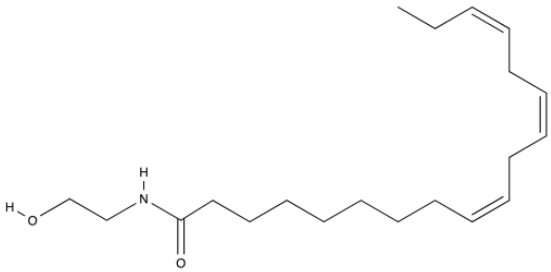
Oleoylethanolamide - OEA



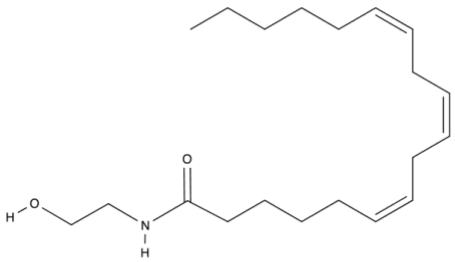
Stearoylethanolamide - SEA



N-arachidonyl-L-serine - ARA-S

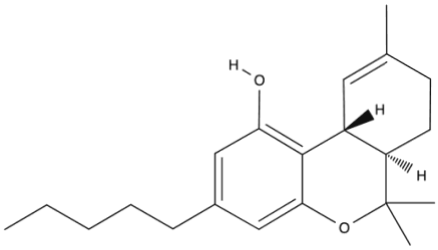


Alpha-linolenoylethanolamide - aLEA

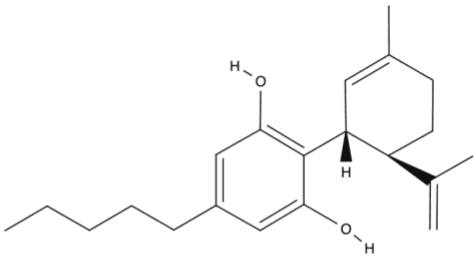


Gamma-linolenoylethanolamide - gLEA

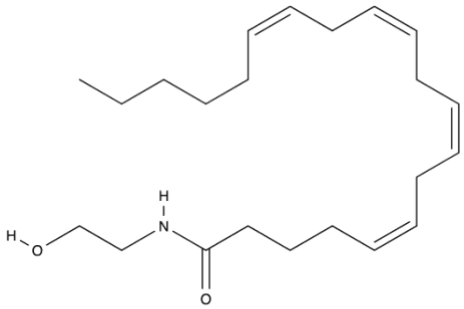
***Supplementary Figure 4-2 continued**. Molecular structure of plant cannabinoids, endocannabinoids and biologically related compounds analysed from plasma samples*



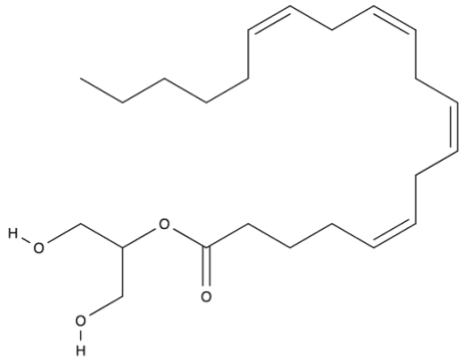
Delta-9-tetrahydrocanabinol - THC



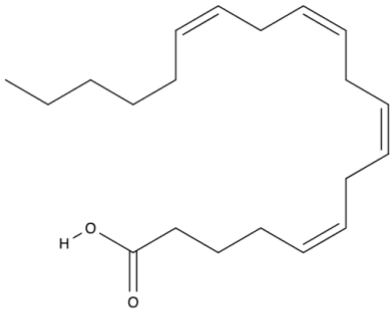
Cannabidiol - CBD



Anandamide - AEA



2-Arachidonoylglycerol - 2-AG



Arachidonic acid - AA

Supplementary Figure 4-3. Plasma concentration-time graphs, stratified by CBD:THC ratio.

***A**. Docosatetraenylethanolamide (DEA), **B**. oleoylethanolamide (OEA), **C**. stearoylethanolamide (SEA), **D**. arachidonic acid (AA).*
Circles show individual data points, larger shapes show mean values and boxplots show median and interquartile range.
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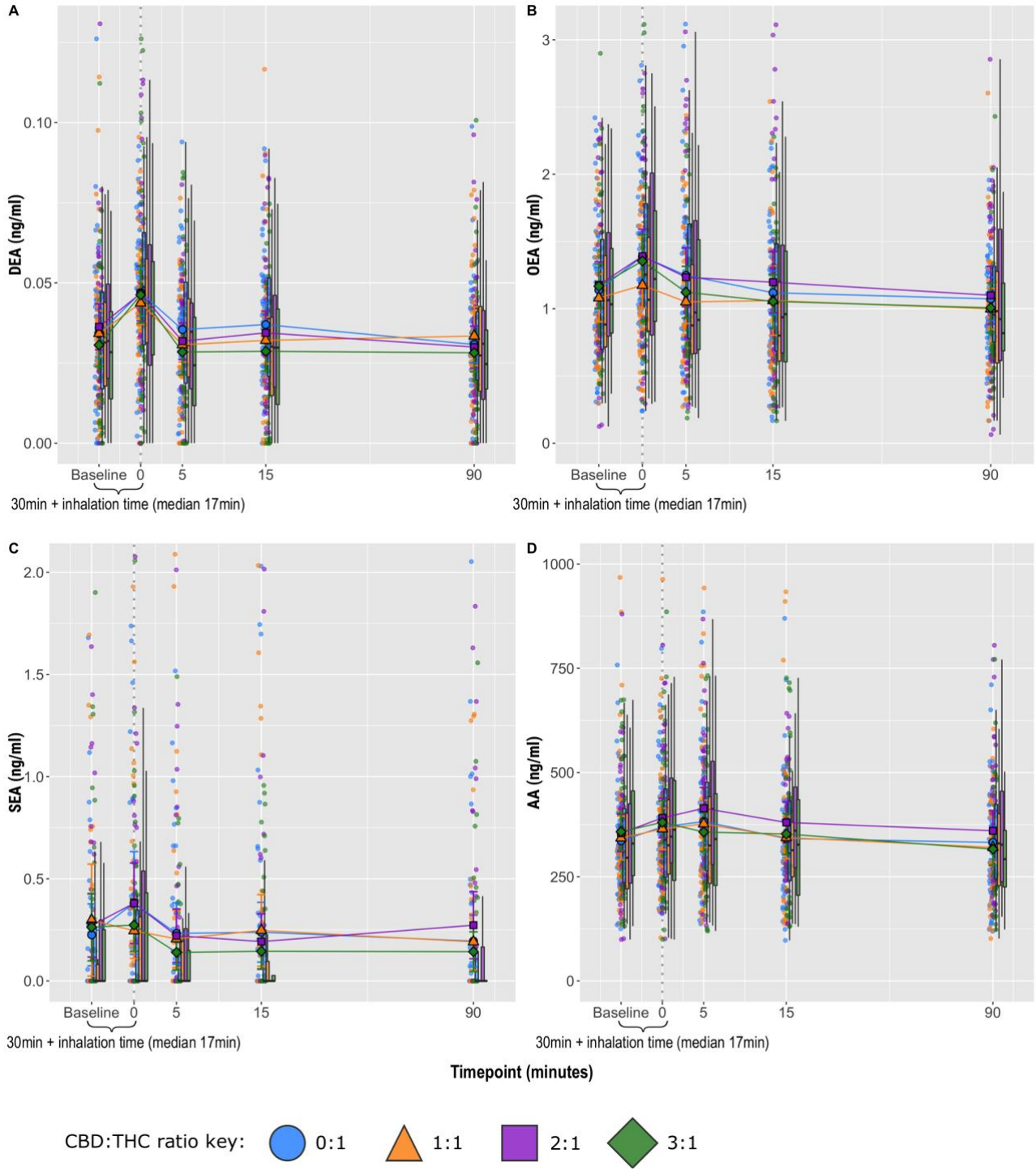
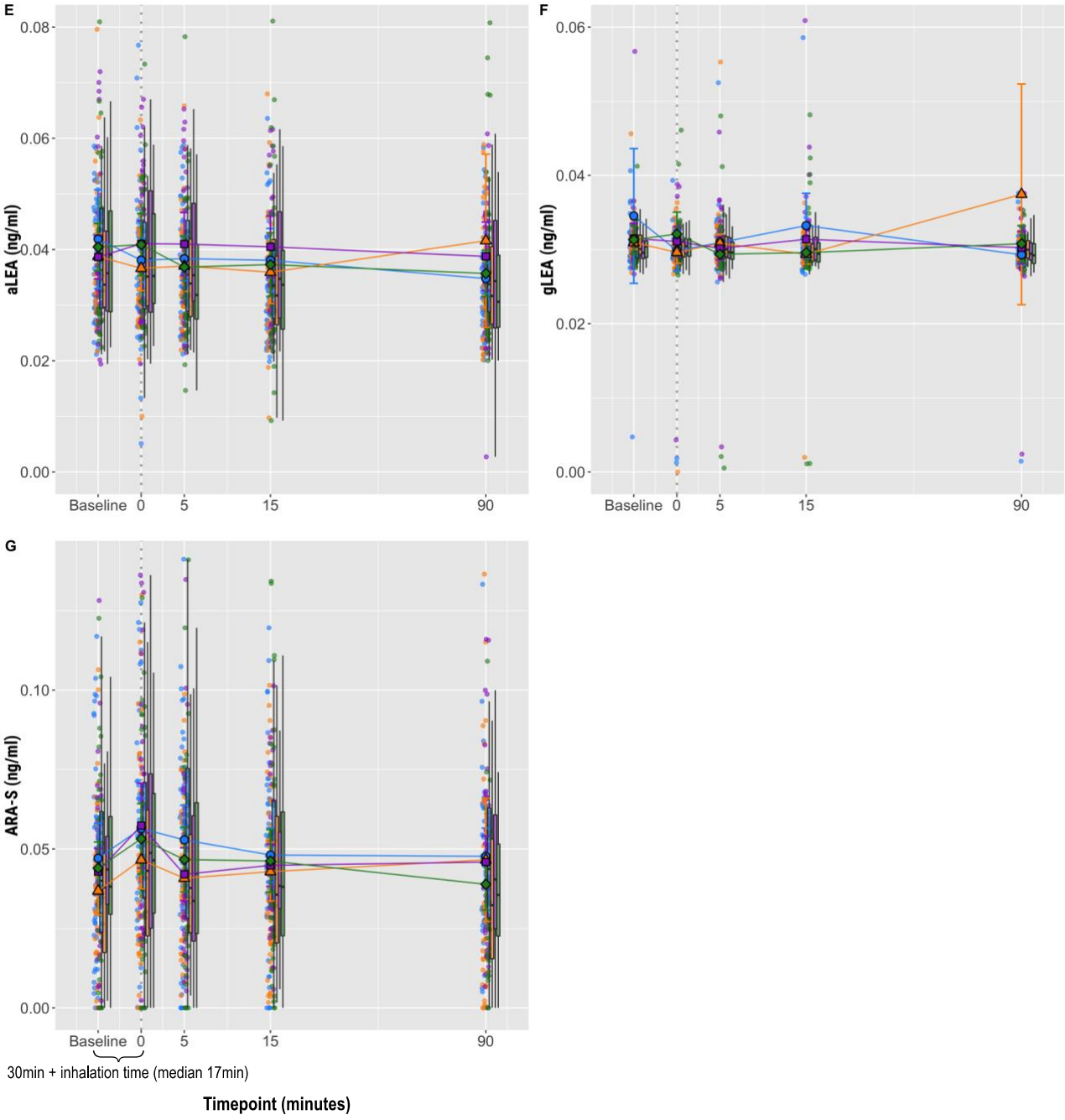


***Supplementary Figure 4-3 continued**. Plasma concentration-time graphs, stratified by CBD:THC ratio.*

***E.** alpha-linolenoylethanolamide (aLEA), **F**. gamma-linolenoylethanolamide (gLEA), **G**. N-arachidonoyl-L-serine (ARA-S).*
Circles show individual data points, larger shapes show mean values and boxplots show median and interquartile range.



Supplementary Figure 4-4. Plasma concentrations following 10mg THC, 0mg CBD (0:1 ratio).

***A**. oleoylethanolamide (OEA), **B**. stearoylethanolamide (SEA), **C**. arachidonic acid (AA).*
Circles show individual data points, diamonds show mean values and boxplots show median and interquartile range.
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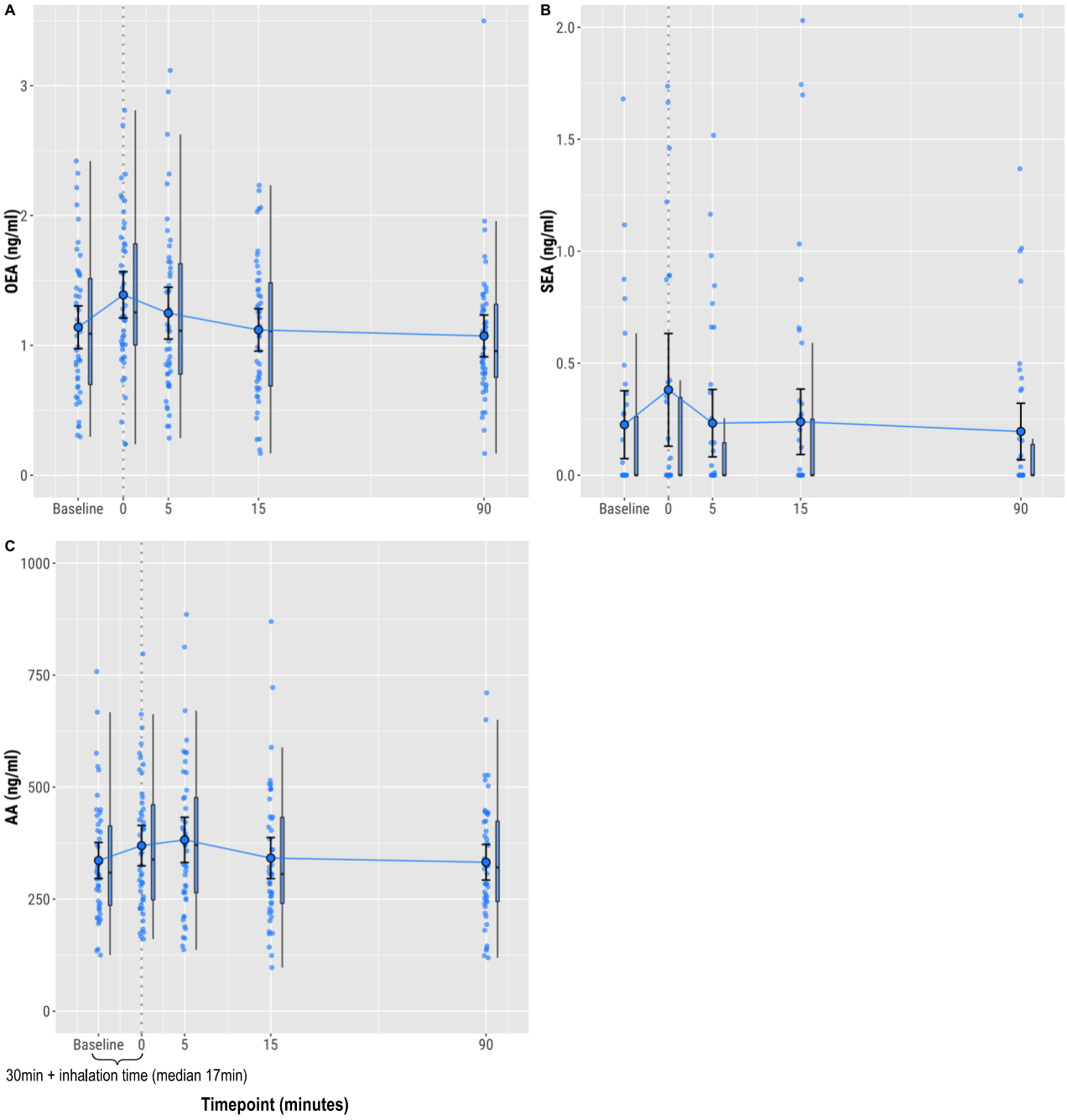
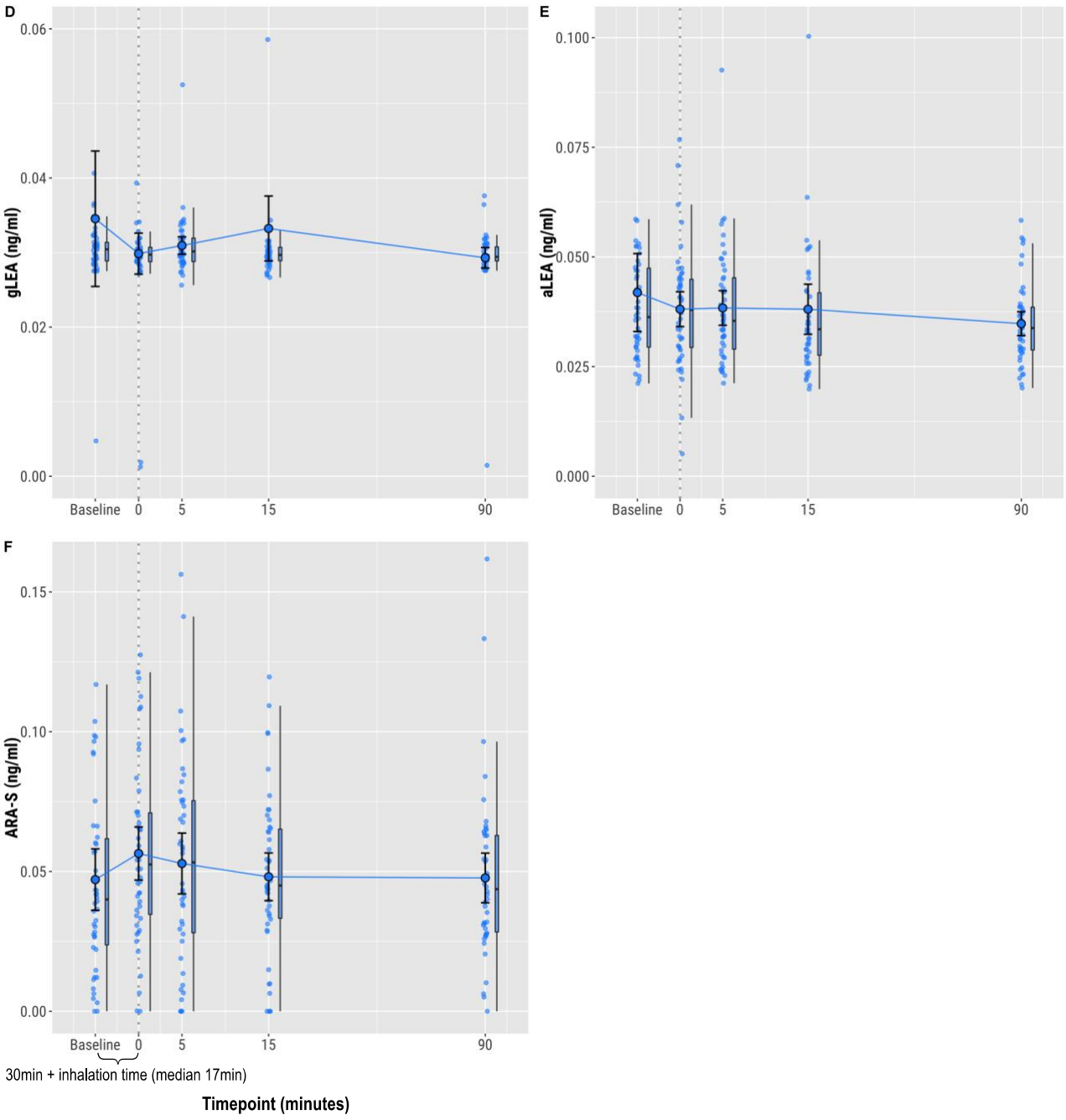
***Supplementary Figure 4-4 continued**. Plasma concentrations following administration of 10mg THC, 0mg CBD (0:1 ratio).*



***E**. gamma-linolenoylethanolamide (gLEA), **F.** alpha-linolenoylethanolamide (aLEA), **G**. N-arachidonoyl-L-serine (ARA-S).*
Circles show individual data points, diamonds show mean values and boxplots show median and interquartile range.



Supplementary Figure 4-5. Pre-inhalation plasma concentrations vs. visit number.

***A**. 2-arachidonoylglycerol (2-AG), **B**. arachidonic acid (AA), **C**. oleoylethanolamide (OEA), **D**. stearoylethanolamide (SEA).*
Circles show individual data points, diamonds show mean values and boxplots show median and interquartile range.
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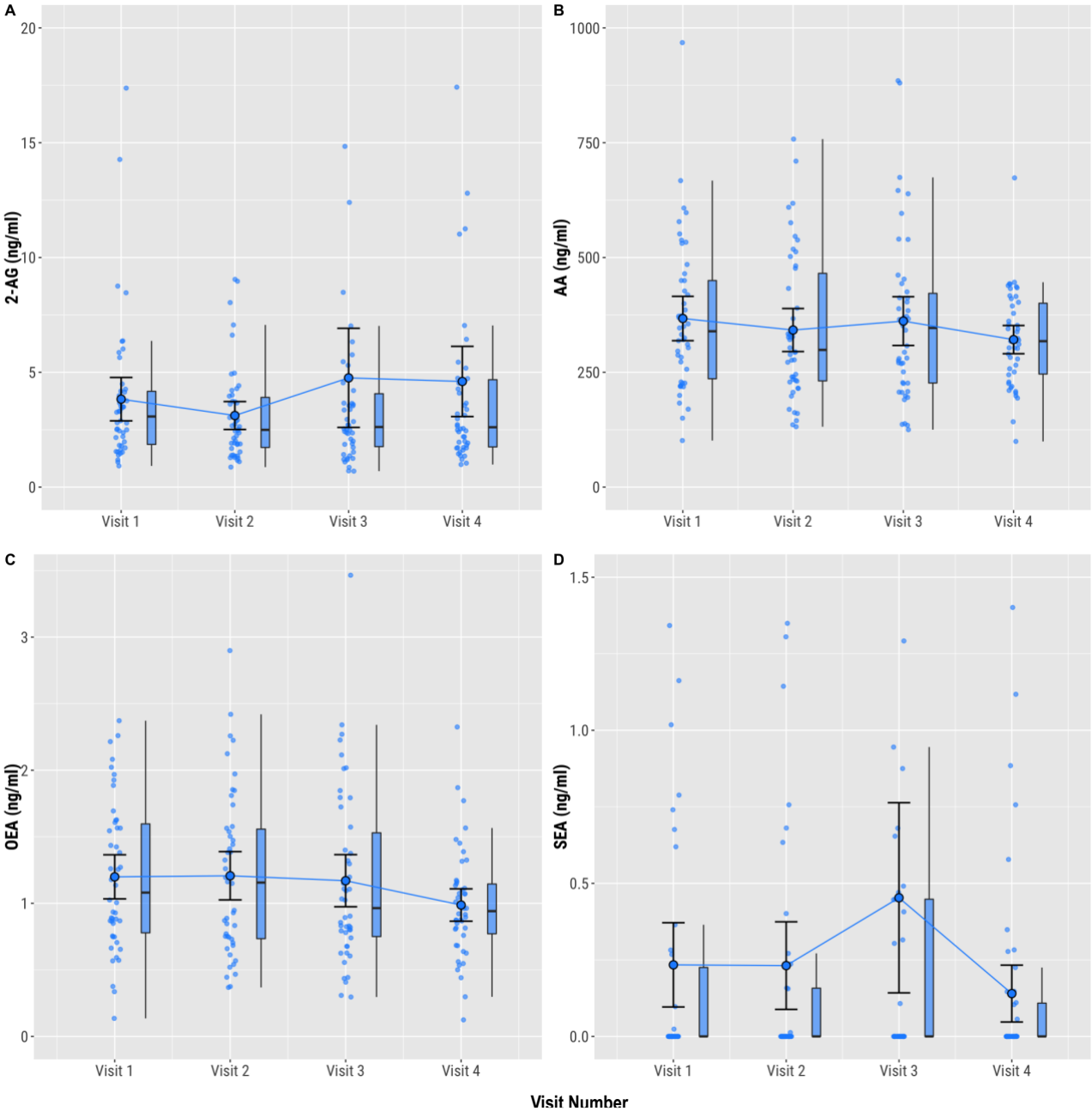
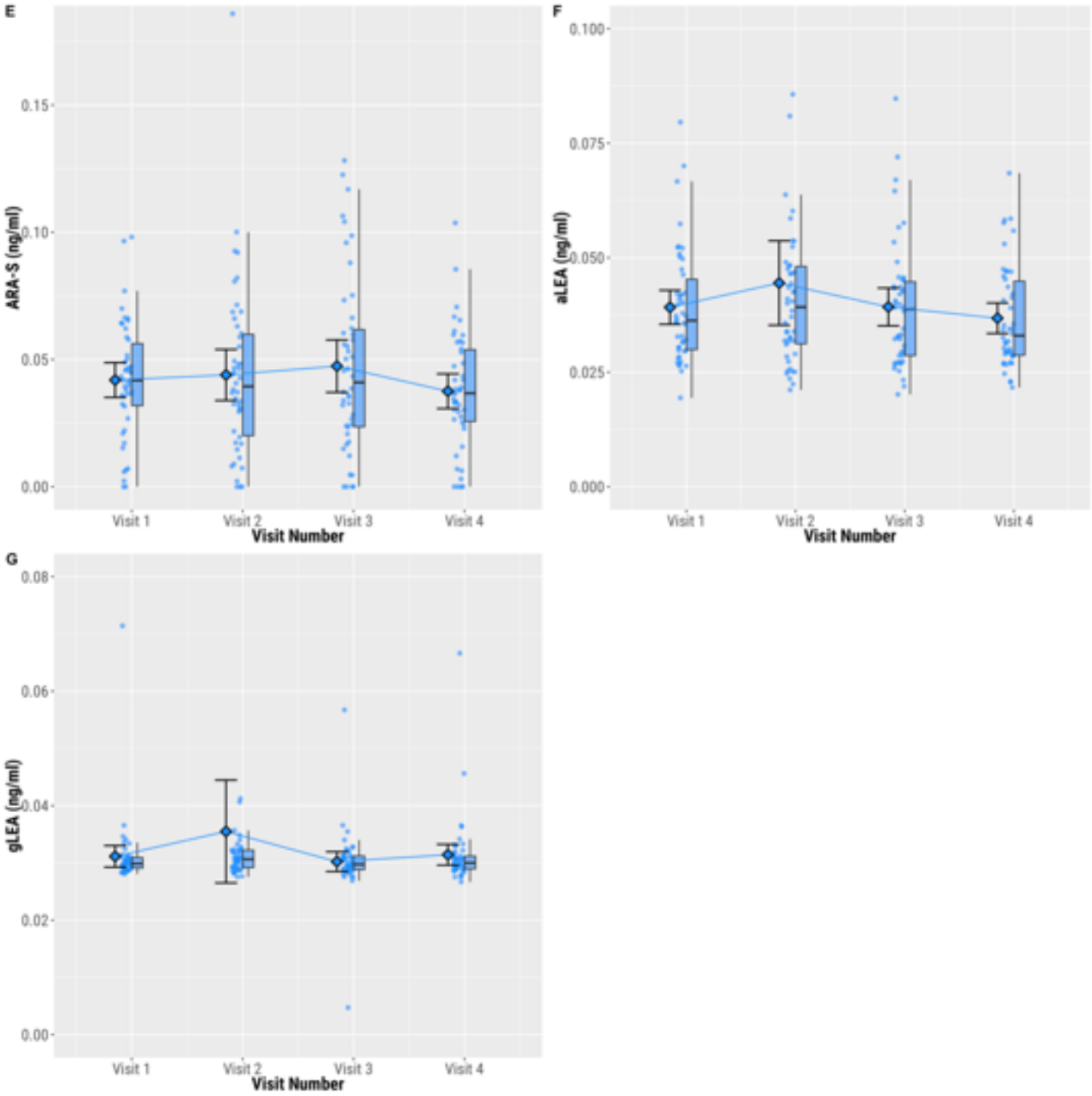


***Supplementary Figure 4-5 continued**. Pre-inhalation plasma concentrations vs. visit number.*

***E**. N-arachidonoyl-L-serine (ARA-S), **F.** alpha-linolenoylethanolamide (aLEA), **G**. gamma-linolenoylethanolamide (gLEA).*
Circles show individual data points, diamonds show mean values and boxplots show median and interquartile range.



Supplementary Table 4-1. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.

Statistically significant rows are presented in bold.

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	THC AUC			
0:1 - 1:1	-15.336	-32.679	2.008	0.287
0:1 - 2:1	-6.549	-23.893	10.794	0.872
0:1 - 3:1	-7.412	-24.756	9.931	0.825
1:1 - 2:1	8.786	-8.557	26.130	0.738
1:1 - 3:1	7.923	-9.420	25.267	0.794
2:1 - 3:1	-0.863	-18.207	16.480	1.000
	THC AUC (adjusted for visit number)			
0:1 - 1:1	-16.038	-33.514	1.437	0.256
0:1 - 2:1	-7.112	-24.571	10.347	0.845
0:1 - 3:1	-6.943	-24.495	10.609	0.856
1:1 - 2:1	8.927	-8.524	26.377	0.732
1:1 - 3:1	9.095	-8.499	26.689	0.726
2:1 - 3:1	0.169	-17.475	17.813	1.000
	THC Peak			
0:1 - 1:1	-9.255	-21.379	2.869	0.418
0:1 - 2:1	-0.085	-12.209	12.039	1.000
0:1 - 3:1	-1.013	-13.137	11.111	0.998
1:1 - 2:1	9.170	-2.954	21.294	0.426
1:1 - 3:1	8.242	-3.882	20.366	0.521
2:1 - 3:1	-0.928	-13.052	11.196	0.999
	THC Peak (adjusted for visit number)			
0:1 - 1:1	-9.255	-21.843	2.631	0.418
0:1 - 2:1	-0.085	-12.508	11.944	1.000
0:1 - 3:1	-1.013	-13.449	11.132	0.998
1:1 - 2:1	9.170	-2.896	21.544	0.426
1:1 - 3:1	8.242	-3.872	20.768	0.521
2:1 - 3:1	-0.876	-13.232	11.479	0.999

***Supplementary Table 4-1 continued**. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	CBD AUC			
0:1 - 1:1	**-123.709**	**-168.682**	**-78.736**	**9.088×10^{-7}**
0:1 - 2:1	**-244.322**	**-289.295**	**-199.349**	**1.565×10^{-14}**
0:1 - 3:1	**-345.036**	**-390.009**	**-300.063**	**$< 2.22 \times 10^{-16}$**
1:1 - 2:1	**-120.613**	**-165.586**	**-75.640**	**1.722×10^{-6}**
1:1 - 3:1	**-221.327**	**-266.300**	**-176.354**	**2.975×10^{-14}**
2:1 - 3:1	**-100.714**	**-145.687**	**-55.741**	**8.148×10^{-5}**
	CBD AUC (adjusted for visit number)			
0:1 - 1:1	**-124.426**	**-169.361**	**-79.492**	**7.738×10^{-7}**
0:1 - 2:1	**-245.398**	**-290.354**	**-200.442**	**8.549×10^{-15}**
0:1 - 3:1	**-342.526**	**-387.656**	**-297.397**	**$< 2.22 \times 10^{-16}$**
1:1 - 2:1	**-120.972**	**-165.893**	**-76.050**	**1.570×10^{-6}**
1:1 - 3:1	**-218.100**	**-263.368**	**-172.833**	**2.431×10^{-14}**
2:1 - 3:1	**-97.129**	**-142.478**	**-51.779**	**1.798×10^{-4}**
	CBD Peak			
0:1 - 1:1	**-61.543**	**-87.712**	**-35.374**	**3.197×10^{-5}**
0:1 - 2:1	**-109.768**	**-135.936**	**-83.599**	**2.828×10^{-13}**
0:1 - 3:1	**-153.819**	**-179.988**	**-127.650**	**1.110×10^{-16}**
1:1 - 2:1	**-48.225**	**-74.394**	**-22.056**	**0.002**
1:1 - 3:1	**-92.277**	**-118.445**	**-66.108**	**3.820×10^{-10}**
2:1 - 3:1	**-44.052**	**-70.221**	**-17.883**	**0.005**
	CBD Peak (adjusted for visit number)			
0:1 - 1:1	**-61.743**	**-87.989**	**-35.496**	**3.200×10^{-5}**
0:1 - 2:1	**-110.067**	**-136.327**	**-83.808**	**2.964×10^{-13}**
0:1 - 3:1	**-153.120**	**-179.480**	**-126.759**	**$< 2.22 \times 10^{-16}$**
1:1 - 2:1	**-48.325**	**-74.564**	**-22.086**	**0.002**
1:1 - 3:1	**-91.377**	**-117.818**	**-64.936**	**8.214×10^{-10}**
2:1 - 3:1	**-43.052**	**-69.541**	**-16.563**	**0.007**

***Supplementary Table 4-1 continued**. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Anandamide AUC			
0:1 - 1:1	0.007	-0.136	0.150	1.000
0:1 - 2:1	-0.062	-0.206	0.081	0.825
0:1 - 3:1	-0.015	-0.158	0.128	0.997
1:1 - 2:1	-0.069	-0.213	0.074	0.774
1:1 - 3:1	-0.022	-0.165	0.121	0.991
2:1 - 3:1	0.048	-0.096	0.191	0.913
	Anandamide AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.018	-0.098	0.134	0.990
0:1 - 2:1	-0.066	-0.182	0.050	0.673
0:1 - 3:1	0.007	-0.110	0.125	0.999
1:1 - 2:1	-0.084	-0.200	0.033	0.487
1:1 - 3:1	-0.011	-0.128	0.107	0.998
2:1 - 3:1	0.073	-0.046	0.192	0.617
	Anandamide Peak			
0:1 - 1:1	0.014	-0.028	0.057	0.912
0:1 - 2:1	-0.009	-0.052	0.033	0.971
0:1 - 3:1	-0.012	-0.055	0.030	0.942
1:1 - 2:1	-0.024	-0.066	0.019	0.690
1:1 - 3:1	-0.026	-0.069	0.016	0.612
2:1 - 3:1	-0.003	-0.045	0.040	0.999
	Anandamide Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.013	-0.029	0.055	0.927
0:1 - 2:1	-0.010	-0.052	0.031	0.962
0:1 - 3:1	-0.005	-0.047	0.037	0.995
1:1 - 2:1	-0.023	-0.065	0.019	0.692
1:1 - 3:1	-0.018	-0.060	0.024	0.828
2:1 - 3:1	0.005	-0.038	0.048	0.996

***Supplementary Table 4-1 continued**. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	2-AG AUC			
0:1 - 1:1	-3.214	-11.042	4.613	0.849
0:1 - 2:1	-4.005	-11.833	3.822	0.743
0:1 - 3:1	-1.337	-9.164	6.491	0.987
1:1 - 2:1	-0.791	-8.619	7.037	0.997
1:1 - 3:1	1.878	-5.950	9.705	0.965
2:1 - 3:1	2.669	-5.159	10.496	0.907
	2-AG AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	1.068	-2.526	4.662	0.936
0:1 - 2:1	0.189	-3.391	3.770	1.000
0:1 - 3:1	3.068	-0.567	6.702	0.344
1:1 - 2:1	-0.879	-4.467	2.709	0.962
1:1 - 3:1	1.999	-1.611	5.610	0.693
2:1 - 3:1	2.879	-0.784	6.541	0.408
	2-AG Peak			
0:1 - 1:1	-1.656	-4.555	1.243	0.672
0:1 - 2:1	1.120	-1.778	4.018	0.870
0:1 - 3:1	0.463	-2.435	3.361	0.989
1:1 - 2:1	2.776	-0.108	5.661	0.231
1:1 - 3:1	2.119	-0.765	5.004	0.469
2:1 - 3:1	-0.657	-3.541	2.227	0.969
	2-AG Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	-1.711	-4.620	1.199	0.651
0:1 - 2:1	1.141	-1.758	4.041	0.864
0:1 - 3:1	0.180	-2.762	3.123	0.999
1:1 - 2:1	2.852	-0.053	5.757	0.216
1:1 - 3:1	1.891	-1.033	4.815	0.578
2:1 - 3:1	-0.961	-3.923	2.000	0.918

***Supplementary Table 4-1 continued**. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Arachidonic acid AUC			
0:1 - 1:1	36.986	-148.669	222.642	0.979
0:1 - 2:1	-42.727	-228.383	142.929	0.969
0:1 - 3:1	83.001	-102.655	268.657	0.813
1:1 - 2:1	-79.713	-265.369	105.942	0.831
1:1 - 3:1	46.015	-139.641	231.670	0.961
2:1 - 3:1	125.728	-59.928	311.384	0.540
	Arachidonic acid AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	17.069	-132.965	167.104	0.996
0:1 - 2:1	-68.190	-217.688	81.309	0.804
0:1 - 3:1	29.306	-123.244	181.856	0.981
1:1 - 2:1	-85.259	-235.767	65.250	0.677
1:1 - 3:1	12.237	-139.517	163.990	0.999
2:1 - 3:1	97.495	-56.136	251.127	0.593
	Arachidonic acid Peak			
0:1 - 1:1	9.139	-48.117	66.395	0.989
0:1 - 2:1	-21.807	-79.118	35.503	0.875
0:1 - 3:1	36.819	-20.516	94.154	0.584
1:1 - 2:1	-30.946	-88.219	26.327	0.709
1:1 - 3:1	27.680	-29.609	84.970	0.775
2:1 - 3:1	58.626	1.377	115.876	0.184
	Arachidonic acid Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	8.911	-48.980	66.802	0.990
0:1 - 2:1	-19.579	-77.275	38.118	0.908
0:1 - 3:1	33.525	-25.289	92.339	0.673
1:1 - 2:1	-28.489	-86.536	29.557	0.766
1:1 - 3:1	24.614	-33.931	83.160	0.839
2:1 - 3:1	53.103	-6.114	112.321	0.291

Supplementary Table 4-1 continued. *Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	DEA AUC			
0:1 - 1:1	0.013	-0.020	0.045	0.864
0:1 - 2:1	0.014	-0.018	0.047	0.814
0:1 - 3:1	0.005	-0.027	0.037	0.990
1:1 - 2:1	0.002	-0.031	0.034	1.000
1:1 - 3:1	-0.008	-0.040	0.025	0.965
2:1 - 3:1	-0.009	-0.042	0.023	0.938
	DEA AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.013	-0.006	0.031	0.535
0:1 - 2:1	0.009	-0.010	0.027	0.785
0:1 - 3:1	0.015	-0.004	0.034	0.393
1:1 - 2:1	-0.004	-0.023	0.015	0.976
1:1 - 3:1	0.002	-0.016	0.021	0.994
2:1 - 3:1	0.006	-0.013	0.025	0.915
	DEA Peak			
0:1 - 1:1	0.003	-0.006	0.012	0.894
0:1 - 2:1	0.001	-0.008	0.010	0.998
0:1 - 3:1	0.000	-0.009	0.009	1.000
1:1 - 2:1	-0.002	-0.011	0.007	0.952
1:1 - 3:1	-0.004	-0.013	0.006	0.867
2:1 - 3:1	-0.001	-0.010	0.008	0.995
	DEA Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.004	-0.005	0.013	0.848
0:1 - 2:1	0.001	-0.008	0.010	0.998
0:1 - 3:1	0.002	-0.008	0.011	0.987
1:1 - 2:1	-0.003	-0.012	0.006	0.926
1:1 - 3:1	-0.002	-0.011	0.007	0.966
2:1 - 3:1	0.001	-0.009	0.010	0.999

***Supplementary Table 4-1 continued**. Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	OEA AUC			
0:1 - 1:1	0.312	-0.469	1.093	0.859
0:1 - 2:1	0.048	-0.733	0.829	0.999
0:1 - 3:1	0.357	-0.424	1.138	0.802
1:1 - 2:1	-0.264	-1.045	0.517	0.908
1:1 - 3:1	0.045	-0.736	0.826	0.999
2:1 - 3:1	0.310	-0.471	1.091	0.861
	OEA AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.412	-0.242	1.066	0.599
0:1 - 2:1	-0.008	-0.659	0.643	1.000
0:1 - 3:1	0.264	-0.398	0.927	0.859
1:1 - 2:1	-0.420	-1.078	0.237	0.587
1:1 - 3:1	-0.148	-0.809	0.514	0.971
2:1 - 3:1	0.273	-0.396	0.941	0.851
	OEA Peak			
0:1 - 1:1	0.188	-0.028	0.405	0.315
0:1 - 2:1	0.015	-0.201	0.231	0.999
0:1 - 3:1	0.047	-0.169	0.263	0.974
1:1 - 2:1	-0.174	-0.390	0.043	0.390
1:1 - 3:1	-0.142	-0.358	0.075	0.568
2:1 - 3:1	0.032	-0.184	0.248	0.991
	OEA Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.192	-0.028	0.412	0.312
0:1 - 2:1	0.019	-0.200	0.237	0.998
0:1 - 3:1	0.059	-0.163	0.281	0.954
1:1 - 2:1	-0.173	-0.394	0.048	0.410
1:1 - 3:1	-0.133	-0.355	0.089	0.635
2:1 - 3:1	0.040	-0.184	0.264	0.985

Supplementary Table 4-1 continued. *Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	SEA AUC			
0:1 - 1:1	0.431	-0.583	1.445	0.835
0:1 - 2:1	0.198	-0.815	1.212	0.980
0:1 - 3:1	0.472	-0.541	1.486	0.793
1:1 - 2:1	-0.233	-1.246	0.781	0.969
1:1 - 3:1	0.041	-0.972	1.055	1.000
2:1 - 3:1	0.274	-0.740	1.288	0.951
	SEA AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.164	-0.252	0.581	0.863
0:1 - 2:1	0.056	-0.358	0.471	0.993
0:1 - 3:1	0.295	-0.125	0.716	0.509
1:1 - 2:1	-0.108	-0.525	0.309	0.956
1:1 - 3:1	0.131	-0.289	0.551	0.927
2:1 - 3:1	0.239	-0.186	0.664	0.682
	SEA Peak			
0:1 - 1:1	0.139	-0.114	0.392	0.700
0:1 - 2:1	0.003	-0.250	0.256	1.000
0:1 - 3:1	0.109	-0.144	0.363	0.828
1:1 - 2:1	-0.136	-0.389	0.118	0.715
1:1 - 3:1	-0.029	-0.282	0.224	0.996
2:1 - 3:1	0.106	-0.147	0.359	0.840
	SEA Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.142	-0.115	0.398	0.694
0:1 - 2:1	0.013	-0.242	0.268	1.000
0:1 - 3:1	0.103	-0.156	0.362	0.861
1:1 - 2:1	-0.129	-0.386	0.128	0.754
1:1 - 3:1	-0.039	-0.298	0.220	0.991
2:1 - 3:1	0.090	-0.172	0.351	0.905

Supplementary Table 4-1 continued. *Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	ARA-S AUC			
0:1 - 1:1	-0.008	-0.053	0.036	0.983
0:1 - 2:1	-0.002	-0.046	0.043	1.000
0:1 - 3:1	0.004	-0.041	0.048	0.998
1:1 - 2:1	0.007	-0.038	0.051	0.991
1:1 - 3:1	0.012	-0.032	0.056	0.951
2:1 - 3:1	0.005	-0.039	0.050	0.995
	ARA-S AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.023	-0.004	0.050	0.352
0:1 - 2:1	0.011	-0.016	0.038	0.838
0:1 - 3:1	0.014	-0.013	0.042	0.723
1:1 - 2:1	-0.011	-0.039	0.016	0.839
1:1 - 3:1	-0.008	-0.036	0.019	0.932
2:1 - 3:1	0.003	-0.025	0.031	0.996
	ARA-S Peak			
0:1 - 1:1	0.007	-0.007	0.021	0.758
0:1 - 2:1	-0.002	-0.016	0.012	0.991
0:1 - 3:1	0.002	-0.012	0.016	0.987
1:1 - 2:1	-0.009	-0.023	0.005	0.575
1:1 - 3:1	-0.005	-0.019	0.009	0.913
2:1 - 3:1	0.004	-0.010	0.018	0.923
	ARA-S Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.007	-0.007	0.021	0.764
0:1 - 2:1	-0.002	-0.017	0.012	0.986
0:1 - 3:1	0.004	-0.011	0.018	0.957
1:1 - 2:1	-0.009	-0.024	0.005	0.554
1:1 - 3:1	-0.003	-0.018	0.011	0.968
2:1 - 3:1	0.006	-0.008	0.021	0.836

Supplementary Table 4-1 continued. *Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	aLEA AUC			
0:1 - 1:1	-0.009	-0.038	0.021	0.939
0:1 - 2:1	-0.022	-0.051	0.007	0.457
0:1 - 3:1	-0.006	-0.036	0.023	0.972
1:1 - 2:1	-0.013	-0.043	0.016	0.808
1:1 - 3:1	0.002	-0.027	0.031	0.999
2:1 - 3:1	0.015	-0.014	0.045	0.728
	aLEA AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.001	-0.017	0.019	1.000
0:1 - 2:1	-0.012	-0.029	0.006	0.554
0:1 - 3:1	-0.003	-0.021	0.015	0.985
1:1 - 2:1	-0.013	-0.030	0.005	0.492
1:1 - 3:1	-0.004	-0.022	0.014	0.968
2:1 - 3:1	0.009	-0.010	0.027	0.787
	aLEA Peak			
0:1 - 1:1	0.001	-0.005	0.007	0.990
0:1 - 2:1	-0.004	-0.009	0.002	0.626
0:1 - 3:1	-0.003	-0.009	0.003	0.726
1:1 - 2:1	-0.004	-0.010	0.001	0.432
1:1 - 3:1	-0.004	-0.010	0.002	0.535
2:1 - 3:1	0.000	-0.005	0.006	0.998
	aLEA Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.001	-0.005	0.007	0.982
0:1 - 2:1	-0.003	-0.009	0.002	0.652
0:1 - 3:1	-0.003	-0.009	0.003	0.765
1:1 - 2:1	-0.005	-0.011	0.001	0.424
1:1 - 3:1	-0.004	-0.010	0.002	0.537
2:1 - 3:1	0.001	-0.006	0.007	0.998

Supplementary Table 4-1 continued. *Pharmacokinetics for each contrast between CBD:THC ratios – Models 1 and 2.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	gLEA AUC			
0:1 - 1:1	-0.011	-0.037	0.014	0.810
0:1 - 2:1	-0.010	-0.035	0.015	0.866
0:1 - 3:1	-0.009	-0.034	0.016	0.892
1:1 - 2:1	0.001	-0.024	0.027	0.999
1:1 - 3:1	0.002	-0.023	0.028	0.998
2:1 - 3:1	0.001	-0.024	0.026	1.000
	gLEA AUC (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.002	-0.006	0.009	0.963
0:1 - 2:1	0.001	-0.006	0.009	0.979
0:1 - 3:1	0.002	-0.006	0.009	0.974
1:1 - 2:1	0.000	-0.008	0.007	1.000
1:1 - 3:1	0.000	-0.008	0.007	1.000
2:1 - 3:1	0.000	-0.008	0.008	1.000
	gLEA Peak			
0:1 - 1:1	0.000	-0.002	0.002	1.000
0:1 - 2:1	0.001	-0.001	0.003	0.886
0:1 - 3:1	0.002	-0.001	0.004	0.452
1:1 - 2:1	0.001	-0.001	0.003	0.907
1:1 - 3:1	0.001	-0.001	0.004	0.483
2:1 - 3:1	0.001	-0.001	0.003	0.874
	gLEA Peak (adjusted for baseline concentration, visit number and time between visits)			
0:1 - 1:1	0.000	-0.002	0.002	0.998
0:1 - 2:1	0.001	-0.001	0.003	0.900
0:1 - 3:1	0.002	0.000	0.004	0.246
1:1 - 2:1	0.001	-0.002	0.003	0.957
1:1 - 3:1	0.002	0.000	0.004	0.327
2:1 - 3:1	0.001	-0.001	0.003	0.640

Supplementary Table 4-2. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.

Statistically significant rows are presented in bold.
Pre = pre-inhalation plasma sample.

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Anandamide (ng/ml)			
Pre – 0min	-0.040	-0.070	-0.010	0.069
Pre – 5min	0.003	-0.027	0.033	1.000
Pre – 15min	0.011	-0.019	0.041	0.949
Pre – 90min	0.019	-0.011	0.049	0.735
0min – 5min	**0.043**	**0.013**	**0.073**	**0.040**
0min – 15min	**0.051**	**0.021**	**0.081**	**0.008**
0min – 90min	**0.059**	**0.029**	**0.089**	**0.001**
5min – 15min	0.008	-0.022	0.038	0.985
5min – 90min	0.016	-0.014	0.046	0.845
15min – 90min	0.008	-0.022	0.038	0.988
	Anandamide (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	-0.040	-0.070	-0.010	0.069
Pre – 5min	0.003	-0.027	0.033	1.000
Pre – 15min	0.011	-0.019	0.041	0.949
Pre – 90min	0.019	-0.011	0.049	0.735
0min – 5min	**0.043**	**0.013**	**0.073**	**0.040**
0min – 15min	**0.051**	**0.021**	**0.081**	**0.008**
0min – 90min	**0.059**	**0.029**	**0.089**	**0.001**
5min – 15min	0.008	-0.022	0.038	0.985
5min – 90min	0.016	-0.014	0.046	0.845
15min – 90min	0.008	-0.022	0.038	0.988

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	2-AG (ng/ml)			
Pre – 0min	0.513	-1.451	2.478	0.986
Pre – 5min	0.621	-1.343	2.586	0.971
Pre – 15min	0.891	-1.073	2.855	0.898
Pre – 90min	0.291	-1.673	2.255	0.998
0min – 5min	0.108	-1.856	2.072	1.000
0min – 15min	0.378	-1.587	2.342	0.996
0min – 90min	-0.222	-2.187	1.742	0.999
5min – 15min	0.270	-1.695	2.234	0.999
5min – 90min	-0.330	-2.295	1.634	0.997
15min – 90min	-0.600	-2.564	1.364	0.975
	2-AG (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	0.513	-1.451	2.478	0.986
Pre – 5min	0.621	-1.343	2.586	0.971
Pre – 15min	0.891	-1.073	2.855	0.898
Pre – 90min	0.291	-1.673	2.255	0.998
0min – 5min	0.108	-1.856	2.072	1.000
0min – 15min	0.378	-1.587	2.342	0.996
0min – 90min	-0.222	-2.187	1.742	0.999
5min – 15min	0.270	-1.695	2.234	0.999
5min – 90min	-0.330	-2.295	1.634	0.997
15min – 90min	-0.600	-2.564	1.364	0.975

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Arachidonic acid (ng/ml)			
Pre – 0min	-33.180	-74.904	8.543	0.519
Pre – 5min	-46.043	-87.767	-4.320	0.193
Pre – 15min	-5.436	-47.159	36.287	0.999
Pre – 90min	3.859	-37.865	45.582	1.000
0min – 5min	-12.863	-54.586	28.860	0.974
0min – 15min	27.744	-13.979	69.468	0.684
0min – 90min	37.039	-4.684	78.762	0.405
5min – 15min	40.607	-1.116	82.331	0.310
5min – 90min	49.902	8.179	91.626	0.131
15min – 90min	9.295	-32.429	51.018	0.992
	Arachidonic acid (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	-33.180	-74.904	8.543	0.519
Pre – 5min	-46.043	-87.767	-4.320	0.193
Pre – 15min	-5.436	-47.159	36.287	0.999
Pre – 90min	3.859	-37.865	45.582	1.000
0min – 5min	-12.863	-54.586	28.860	0.974
0min – 15min	27.744	-13.979	69.468	0.684
0min – 90min	37.039	-4.684	78.762	0.405
5min – 15min	40.607	-1.116	82.331	0.310
5min – 90min	49.902	8.179	91.626	0.131
15min – 90min	9.295	-32.429	51.018	0.992

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	DEA (ng/ml)			
Pre – 0min	**-0.013**	**-0.020**	**-0.005**	**0.011**
Pre – 5min	-0.001	-0.009	0.006	0.998
Pre – 15min	-0.003	-0.011	0.005	0.952
Pre – 90min	0.003	-0.004	0.011	0.907
0min – 5min	**0.012**	**0.004**	**0.019**	**0.029**
0min – 15min	0.010	0.002	0.018	0.084
0min – 90min	**0.016**	**0.008**	**0.024**	**0.000**
5min – 15min	-0.002	-0.009	0.006	0.995
5min – 90min	0.005	-0.003	0.012	0.756
15min – 90min	0.006	-0.001	0.014	0.504
	DEA (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	**-0.013**	**-0.020**	**-0.005**	**0.011**
Pre – 5min	-0.001	-0.009	0.006	0.998
Pre – 15min	-0.003	-0.011	0.005	0.952
Pre – 90min	0.003	-0.004	0.011	0.907
0min – 5min	**0.012**	**0.004**	**0.019**	**0.029**
0min – 15min	0.010	0.002	0.018	0.084
0min – 90min	**0.016**	**0.008**	**0.024**	**0.000**
5min – 15min	-0.002	-0.009	0.006	0.995
5min – 90min	0.005	-0.003	0.012	0.756
15min – 90min	0.006	-0.001	0.014	0.504

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	OEA (ng/ml)			
Pre – 0min	-0.250	-0.432	-0.068	0.057
Pre – 5min	-0.109	-0.291	0.073	0.760
Pre – 15min	0.020	-0.162	0.202	0.999
Pre – 90min	0.066	-0.116	0.248	0.952
0min – 5min	0.141	-0.041	0.322	0.548
0min – 15min	**0.270**	**0.088**	**0.452**	**0.031**
0min – 90min	**0.316**	**0.134**	**0.498**	**0.007**
5min – 15min	0.129	-0.053	0.311	0.627
5min – 90min	0.175	-0.007	0.357	0.320
15min – 90min	0.046	-0.136	0.228	0.987
	OEA (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	-0.250	-0.432	-0.068	0.057
Pre – 5min	-0.109	-0.291	0.073	0.760
Pre – 15min	0.020	-0.162	0.202	0.999
Pre – 90min	0.066	-0.116	0.248	0.952
0min – 5min	0.141	-0.041	0.322	0.548
0min – 15min	**0.270**	**0.088**	**0.452**	**0.031**
0min – 90min	**0.316**	**0.134**	**0.498**	**0.007**
5min – 15min	0.129	-0.053	0.311	0.627
5min – 90min	0.175	-0.007	0.357	0.320
15min – 90min	0.046	-0.136	0.228	0.987

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	SEA (ng/ml)			
Pre – 0min	-0.156	-0.387	0.075	0.673
Pre – 5min	-0.007	-0.238	0.224	1.000
Pre – 15min	-0.013	-0.244	0.218	1.000
Pre – 90min	0.030	-0.201	0.261	0.999
0min – 5min	0.149	-0.082	0.380	0.709
0min – 15min	0.143	-0.088	0.374	0.741
0min – 90min	0.186	-0.045	0.417	0.507
5min – 15min	-0.006	-0.237	0.225	1.000
5min – 90min	0.037	-0.194	0.268	0.998
15min – 90min	0.043	-0.188	0.274	0.996
	SEA (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	-0.156	-0.387	0.075	0.673
Pre – 5min	-0.007	-0.238	0.224	1.000
Pre – 15min	-0.013	-0.244	0.218	1.000
Pre – 90min	0.030	-0.201	0.261	0.999
0min – 5min	0.149	-0.082	0.380	0.709
0min – 15min	0.143	-0.088	0.374	0.741
0min – 90min	0.186	-0.045	0.417	0.507
5min – 15min	-0.006	-0.237	0.225	1.000
5min – 90min	0.037	-0.194	0.268	0.998
15min – 90min	0.043	-0.188	0.274	0.996

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	ARA-S (ng/ml)			
Pre – 0min	-0.009	-0.021	0.003	0.552
Pre – 5min	-0.006	-0.018	0.006	0.883
Pre – 15min	-0.001	-0.013	0.011	1.000
Pre – 90min	-0.001	-0.013	0.012	1.000
0min – 5min	0.004	-0.009	0.016	0.978
0min – 15min	0.008	-0.004	0.020	0.657
0min – 90min	0.009	-0.003	0.021	0.614
5min – 15min	0.005	-0.007	0.017	0.938
5min – 90min	0.005	-0.007	0.017	0.918
15min – 90min	0.000	-0.012	0.013	1.000
	ARA-S (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	-0.009	-0.021	0.003	0.552
Pre – 5min	-0.006	-0.018	0.006	0.883
Pre – 15min	-0.001	-0.013	0.011	1.000
Pre – 90min	-0.001	-0.013	0.012	1.000
0min – 5min	0.004	-0.009	0.016	0.978
0min – 15min	0.008	-0.004	0.020	0.657
0min – 90min	0.009	-0.003	0.021	0.614
5min – 15min	0.005	-0.007	0.017	0.938
5min – 90min	0.005	-0.007	0.017	0.918
15min – 90min	0.000	-0.012	0.013	1.000

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	aLEA (ng/ml)			
Pre – 0min	0.004	-0.003	0.011	0.820
Pre – 5min	0.004	-0.003	0.010	0.857
Pre – 15min	0.004	-0.003	0.011	0.817
Pre – 90min	0.007	0.000	0.014	0.265
0min – 5min	0.000	-0.007	0.007	1.000
0min – 15min	0.000	-0.007	0.007	1.000
0min – 90min	0.003	-0.004	0.010	0.882
5min – 15min	0.000	-0.007	0.007	1.000
5min – 90min	0.004	-0.003	0.011	0.849
15min – 90min	0.003	-0.004	0.010	0.884
	aLEA (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	0.004	-0.003	0.011	0.820
Pre – 5min	0.004	-0.003	0.010	0.857
Pre – 15min	0.004	-0.003	0.011	0.817
Pre – 90min	0.007	0.000	0.014	0.265
0min – 5min	0.000	-0.007	0.007	1.000
0min – 15min	0.000	-0.007	0.007	1.000
0min – 90min	0.003	-0.004	0.010	0.882
5min – 15min	0.000	-0.007	0.007	1.000
5min – 90min	0.004	-0.003	0.011	0.849
15min – 90min	0.003	-0.004	0.010	0.884

***Supplementary Table 4-2 continued**. Plasma concentrations vs. time following 10mg THC, 0mg CBD (0:1 ratio) – Model 3a.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	gLEA (ng/ml)			
Pre – 0min	0.005	-0.002	0.011	0.625
Pre – 5min	0.004	-0.003	0.010	0.817
Pre – 15min	0.001	-0.005	0.008	0.995
Pre – 90min	0.005	-0.001	0.012	0.516
0min – 5min	-0.001	-0.008	0.005	0.998
0min – 15min	-0.003	-0.010	0.003	0.849
0min – 90min	0.001	-0.006	0.007	1.000
5min – 15min	-0.002	-0.009	0.004	0.959
5min – 90min	0.002	-0.005	0.008	0.988
15min – 90min	0.004	-0.003	0.010	0.762
	gLEA (ng/ml) (adjusted for visit number and time between visits)			
Pre – 0min	0.005	-0.002	0.011	0.627
Pre – 5min	0.004	-0.003	0.010	0.818
Pre – 15min	0.001	-0.005	0.008	0.995
Pre – 90min	0.005	-0.001	0.012	0.518
0min – 5min	-0.001	-0.008	0.005	0.998
0min – 15min	-0.003	-0.010	0.003	0.850
0min – 90min	0.001	-0.006	0.007	1.000
5min – 15min	-0.002	-0.009	0.004	0.959
5min – 90min	0.002	-0.005	0.008	0.988
15min – 90min	0.004	-0.003	0.011	0.764

Supplementary Table 4-3. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.

Statistically significant rows are presented in bold.
Pre = pre-inhalation plasma sample.

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Anandamide (ng/ml)			
Pre – 0min	**-0.044**	**-0.064**	**-0.025**	**0.000**
Pre – 5min	0.003	-0.017	0.022	0.999
Pre – 15min	0.001	-0.019	0.020	1.000
Pre – 90min	0.017	-0.002	0.037	0.420
0min – 5min	**0.047**	**0.028**	**0.067**	**0.000**
0min – 15min	**0.045**	**0.025**	**0.065**	**0.000**
0min – 90min	**0.061**	**0.042**	**0.081**	**0.000**
5min – 15min	-0.002	-0.022	0.017	1.000
5min – 90min	0.014	-0.005	0.034	0.604
15min – 90min	0.016	-0.003	0.036	0.465
	Anandamide (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	**-0.042**	**-0.062**	**-0.023**	**0.000**
Pre – 5min	0.003	-0.016	0.022	0.998
Pre – 15min	0.002	-0.018	0.021	1.000
Pre – 90min	0.018	-0.001	0.037	0.349
0min – 5min	**0.045**	**0.026**	**0.065**	**0.000**
0min – 15min	**0.044**	**0.025**	**0.063**	**0.000**
0min – 90min	**0.060**	**0.041**	**0.080**	**0.000**
5min – 15min	-0.001	-0.021	0.018	1.000
5min – 90min	0.015	-0.004	0.034	0.547
15min – 90min	0.016	-0.003	0.036	0.457

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	2-AG (ng/ml)			
Pre – 0min	0.030	-0.986	1.046	1.000
Pre – 5min	0.251	-0.765	1.267	0.989
Pre – 15min	0.083	-0.933	1.099	1.000
Pre – 90min	-0.521	-1.537	0.496	0.853
0min – 5min	0.221	-0.796	1.237	0.993
0min – 15min	0.053	-0.963	1.069	1.000
0min – 90min	-0.551	-1.567	0.465	0.825
5min – 15min	-0.168	-1.184	0.848	0.998
5min – 90min	-0.771	-1.787	0.245	0.569
15min – 90min	-0.603	-1.619	0.413	0.771
	2-AG (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	0.030	-0.985	1.045	1.000
Pre – 5min	0.251	-0.764	1.265	0.989
Pre – 15min	0.083	-0.932	1.098	1.000
Pre – 90min	-0.521	-1.535	0.494	0.852
0min – 5min	0.221	-0.794	1.235	0.993
0min – 15min	0.053	-0.962	1.067	1.000
0min – 90min	-0.551	-1.565	0.464	0.824
5min – 15min	-0.168	-1.183	0.847	0.998
5min – 90min	-0.771	-1.786	0.244	0.568
15min – 90min	-0.603	-1.618	0.411	0.770

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Arachidonic acid (ng/ml)			
Pre – 0min	-28.446	-56.267	-0.625	0.263
Pre – 5min	-34.638	-62.458	-6.817	0.105
Pre – 15min	-6.587	-34.408	21.233	0.990
Pre – 90min	15.642	-12.179	43.463	0.805
0min – 5min	-6.192	-34.012	21.629	0.992
0min – 15min	21.859	-5.962	49.679	0.535
0min – 90min	**44.088**	**16.267**	**71.909**	**0.016**
5min – 15min	28.050	0.229	55.871	0.277
5min – 90min	**50.279**	**22.459**	**78.100**	**0.004**
15min – 90min	22.229	-5.592	50.050	0.518
	Arachidonic acid (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	-27.334	-54.956	0.287	0.296
Pre – 5min	-34.263	-61.885	-6.641	0.107
Pre – 15min	-5.838	-33.460	21.784	0.994
Pre – 90min	16.478	-11.144	44.100	0.768
0min – 5min	-6.929	-34.550	20.693	0.988
0min – 15min	21.496	-6.126	49.118	0.545
0min – 90min	**43.812**	**16.191**	**71.434**	**0.016**
5min – 15min	28.425	0.803	56.047	0.257
5min – 90min	**50.741**	**23.119**	**78.363**	**0.003**
15min – 90min	22.316	-5.306	49.938	0.507

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	DEA (ng/ml)			
Pre – 0min	**-0.012**	**-0.016**	**-0.008**	**0.000**
Pre – 5min	0.002	-0.002	0.006	0.805
Pre – 15min	0.001	-0.003	0.005	0.995
Pre – 90min	0.003	-0.001	0.007	0.497
0min – 5min	**0.014**	**0.010**	**0.018**	**0.000**
0min – 15min	**0.013**	**0.009**	**0.017**	**0.000**
0min – 90min	**0.015**	**0.011**	**0.019**	**0.000**
5min – 15min	-0.001	-0.005	0.003	0.954
5min – 90min	0.001	-0.003	0.005	0.988
15min – 90min	0.002	-0.002	0.006	0.748
	DEA (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	**-0.012**	**-0.016**	**-0.008**	**0.000**
Pre – 5min	0.002	-0.002	0.006	0.795
Pre – 15min	0.001	-0.003	0.005	0.995
Pre – 90min	0.003	0.000	0.007	0.412
0min – 5min	**0.014**	**0.010**	**0.018**	**0.000**
0min – 15min	**0.012**	**0.008**	**0.016**	**0.000**
0min – 90min	**0.015**	**0.011**	**0.019**	**0.000**
5min – 15min	-0.001	-0.005	0.002	0.952
5min – 90min	0.001	-0.003	0.005	0.973
15min – 90min	0.003	-0.001	0.007	0.669

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	OEA (ng/ml)			
Pre – 0min	**-0.186**	**-0.296**	**-0.077**	**0.008**
Pre – 5min	-0.023	-0.133	0.086	0.994
Pre – 15min	0.033	-0.076	0.142	0.976
Pre – 90min	0.096	-0.013	0.206	0.418
0min – 5min	**0.163**	**0.054**	**0.272**	**0.029**
0min – 15min	**0.219**	**0.110**	**0.329**	**0.001**
0min – 90min	**0.282**	**0.173**	**0.392**	**0.000**
5min – 15min	0.056	-0.053	0.166	0.850
5min – 90min	0.119	0.010	0.229	0.203
15min – 90min	0.063	-0.046	0.172	0.790
	OEA (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	**-0.184**	**-0.293**	**-0.076**	**0.008**
Pre – 5min	-0.024	-0.133	0.084	0.992
Pre – 15min	0.030	-0.079	0.138	0.984
Pre – 90min	0.095	-0.013	0.204	0.420
0min – 5min	**0.160**	**0.051**	**0.269**	**0.032**
0min – 15min	**0.214**	**0.105**	**0.323**	**0.001**
0min – 90min	**0.280**	**0.171**	**0.388**	**0.000**
5min – 15min	0.054	-0.055	0.163	0.866
5min – 90min	0.120	0.011	0.228	0.194
15min – 90min	0.066	-0.043	0.174	0.757

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	SEA (ng/ml)			
Pre – 0min	-0.056	-0.163	0.052	0.847
Pre – 5min	0.065	-0.043	0.172	0.762
Pre – 15min	0.058	-0.049	0.166	0.822
Pre – 90min	0.064	-0.043	0.171	0.770
0min – 5min	0.120	0.013	0.227	0.181
0min – 15min	0.114	0.007	0.221	0.226
0min – 90min	0.119	0.012	0.227	0.186
5min – 15min	-0.006	-0.113	0.101	1.000
5min – 90min	-0.001	-0.108	0.106	1.000
15min – 90min	0.005	-0.102	0.113	1.000
	SEA (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	-0.056	-0.168	0.046	0.846
Pre – 5min	0.065	-0.052	0.162	0.761
Pre – 15min	0.058	-0.059	0.155	0.821
Pre – 90min	0.064	-0.053	0.161	0.769
0min – 5min	0.120	0.010	0.224	0.179
0min – 15min	0.114	0.003	0.217	0.224
0min – 90min	0.119	0.008	0.222	0.185
5min – 15min	-0.006	-0.114	0.100	1.000
5min – 90min	-0.001	-0.109	0.105	1.000
15min – 90min	0.005	-0.102	0.112	1.000

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference ARA-S (ng/ml) (adjusted for CBD:THC ratio and visit number)	Lower 95% CI	Upper 95% CI	P value
Pre – 0min	**-0.011**	**-0.017**	**-0.004**	**0.007**
Pre – 5min	-0.003	-0.009	0.003	0.895
Pre – 15min	-0.003	-0.009	0.003	0.903
Pre – 90min	-0.002	-0.008	0.004	0.967
0min – 5min	0.008	0.002	0.014	0.104
0min – 15min	0.008	0.002	0.014	0.098
0min – 90min	0.009	0.002	0.015	0.054
5min – 15min	0.000	-0.006	0.006	1.000
5min – 90min	0.001	-0.005	0.007	0.999
15min – 90min	0.001	-0.006	0.007	0.999
	ARA-S (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	**-0.011**	**-0.017**	**-0.004**	**0.008**
Pre – 5min	-0.003	-0.009	0.003	0.890
Pre – 15min	-0.002	-0.008	0.004	0.964
Pre – 90min	-0.002	-0.008	0.004	0.977
0min – 5min	0.008	0.001	0.014	0.114
0min – 15min	0.008	0.002	0.015	0.060
0min – 90min	0.009	0.002	0.015	0.050
5min – 15min	0.001	-0.005	0.007	0.999
5min – 90min	0.001	-0.005	0.007	0.998
15min – 90min	0.000	-0.006	0.006	1.000

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	aLEA (ng/ml)			
Pre – 0min	0.001	-0.003	0.005	0.995
Pre – 5min	0.002	-0.002	0.005	0.918
Pre – 15min	0.002	-0.002	0.006	0.843
Pre – 90min	0.002	-0.002	0.006	0.782
0min – 5min	0.001	-0.003	0.005	0.992
0min – 15min	0.001	-0.003	0.005	0.971
0min – 90min	0.001	-0.002	0.005	0.945
5min – 15min	0.000	-0.003	0.004	1.000
5min – 90min	0.001	-0.003	0.004	0.998
15min – 90min	0.000	-0.004	0.004	1.000
	aLEA (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	0.001	-0.003	0.005	0.993
Pre – 5min	0.002	-0.002	0.005	0.921
Pre – 15min	0.002	-0.002	0.006	0.861
Pre – 90min	0.002	-0.002	0.006	0.779
0min – 5min	0.001	-0.003	0.005	0.995
0min – 15min	0.001	-0.003	0.005	0.981
0min – 90min	0.001	-0.002	0.005	0.952
5min – 15min	0.000	-0.004	0.004	1.000
5min – 90min	0.001	-0.003	0.004	0.998
15min – 90min	0.000	-0.004	0.004	1.000

***Supplementary Table 4-3 continued**. Plasma concentrations vs. time following 10mg THC (all CBD:THC ratios) – Model 3b.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	gLEA (ng/ml)			
Pre – 0min	0.001	-0.002	0.004	0.891
Pre – 5min	0.002	-0.001	0.005	0.788
Pre – 15min	0.001	-0.002	0.004	0.940
Pre – 90min	0.000	-0.003	0.003	1.000
0min – 5min	0.000	-0.003	0.003	0.999
0min – 15min	0.000	-0.003	0.003	1.000
0min – 90min	-0.001	-0.004	0.002	0.920
5min – 15min	-0.001	-0.004	0.002	0.996
5min – 90min	-0.002	-0.005	0.001	0.831
15min – 90min	-0.001	-0.004	0.002	0.960
	gLEA (ng/ml) (adjusted for CBD:THC ratio, visit number and time between visits)			
Pre – 0min	0.001	-0.002	0.004	0.892
Pre – 5min	0.002	-0.001	0.005	0.784
Pre – 15min	0.001	-0.002	0.004	0.965
Pre – 90min	0.000	-0.003	0.003	1.000
0min – 5min	0.000	-0.003	0.003	0.999
0min – 15min	0.000	-0.003	0.003	0.999
0min – 90min	-0.001	-0.004	0.002	0.918
5min – 15min	-0.001	-0.004	0.002	0.989
5min – 90min	-0.002	-0.005	0.001	0.821
15min – 90min	-0.001	-0.004	0.002	0.977

Supplementary Table 4-4. Pre-inhalation plasma concentrations vs. visit number – Model 4a.

Statistically significant rows are presented in bold.

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Anandamide (ng/ml)			
Visit 1 – Visit 2	0.006	-0.030	0.042	0.988
1 – 3	0.021	-0.015	0.057	0.651
1 – 4	**0.060**	**0.024**	**0.096**	**0.007**
2 – 3	0.015	-0.021	0.051	0.838
2 – 4	**0.054**	**0.018**	**0.090**	**0.019**
3 – 4	0.039	0.003	0.075	0.154
	Anandamide (ng/ml) (adjusted for time between visits)			
1 – 2	0.006	-0.035	0.048	0.990
1 – 3	0.025	-0.015	0.065	0.606
1 – 4	**0.063**	**0.024**	**0.103**	**0.010**
2 – 3	0.019	-0.018	0.055	0.745
2 – 4	**0.057**	**0.021**	**0.093**	**0.013**
3 – 4	0.038	0.002	0.074	0.156
	2-AG (ng/ml)			
1 – 2	0.713	-1.171	2.596	0.877
1 – 3	-0.927	-2.810	0.957	0.765
1 – 4	-0.771	-2.654	1.113	0.850
2 – 3	-1.639	-3.523	0.244	0.317
2 – 4	-1.483	-3.367	0.400	0.407
3 – 4	0.156	-1.727	2.040	0.998
	2-AG (ng/ml) (adjusted for time between visits)			
1 – 2	1.070	-0.990	3.130	0.734
1 – 3	-0.452	-2.447	1.543	0.970
1 – 4	-0.327	-2.301	1.646	0.988
2 – 3	-1.522	-3.358	0.314	0.360
2 – 4	-1.397	-3.237	0.443	0.439
3 – 4	0.125	-1.696	1.946	0.999

***Supplementary Table 4-4 continued**. Pre-inhalation plasma concentrations vs. visit number – Model 4a*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Arachidonic acid (ng/ml)			
Visit 1 – Visit 2	25.080	-30.462	80.623	0.808
1 – 3	5.628	-49.914	61.171	0.997
1 – 4	45.946	-9.596	101.489	0.362
2 – 3	-19.452	-74.995	36.090	0.900
2 – 4	20.866	-34.677	76.408	0.880
3 – 4	40.318	-15.225	95.860	0.480
	Arachidonic acid (ng/ml) (adjusted for time between visits)			
1 – 2	24.413	-38.909	87.735	0.871
1 – 3	8.438	-52.837	69.712	0.993
1 – 4	48.571	-12.031	109.174	0.391
2 – 3	-15.975	-72.233	40.283	0.943
2 – 4	24.158	-32.240	80.557	0.832
3 – 4	40.134	-15.653	95.920	0.487
	DEA (ng/ml)			
1 – 2	0.003	-0.005	0.011	0.866
1 – 3	0.004	-0.004	0.012	0.694
1 – 4	**0.011**	**0.003**	**0.019**	**0.031**
2 – 3	0.001	-0.007	0.009	0.989
2 – 4	0.008	0.000	0.016	0.192
3 – 4	0.007	-0.001	0.015	0.335
	DEA (ng/ml) (adjusted for time between visits)			
1 – 2	0.002	-0.008	0.011	0.988
1 – 3	0.004	-0.005	0.012	0.848
1 – 4	0.010	0.002	0.019	0.086
2 – 3	0.002	-0.006	0.010	0.954
2 – 4	0.009	0.001	0.017	0.130
3 – 4	0.007	-0.001	0.015	0.329

Supplementary Table 4-4 continued. *Pre-inhalation plasma concentrations vs. visit number – Model 4a*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	OEA (ng/ml)			
Visit 1 – Visit 2	-0.008	-0.205	0.189	1.000
1 – 3	0.029	-0.168	0.226	0.991
1 – 4	0.212	0.014	0.409	0.151
2 – 3	0.037	-0.160	0.234	0.982
2 – 4	0.220	0.022	0.417	0.128
3 – 4	0.183	-0.015	0.380	0.263
	OEA (ng/ml) (adjusted for time between visits)			
1 – 2	-0.083	-0.310	0.143	0.886
1 – 3	-0.036	-0.254	0.183	0.988
1 – 4	0.151	-0.065	0.368	0.512
2 – 3	0.048	-0.153	0.248	0.965
2 – 4	0.235	0.034	0.435	0.100
3 – 4	0.187	-0.012	0.385	0.249
	SEA (ng/ml)			
1 – 2	0.002	-0.256	0.260	1.000
1 – 3	-0.219	-0.477	0.039	0.339
1 – 4	0.094	-0.164	0.352	0.889
2 – 3	-0.222	-0.480	0.037	0.329
2 – 4	0.091	-0.167	0.349	0.897
3 – 4	0.313	0.055	0.571	0.082
	SEA (ng/ml) (adjusted for time between visits)			
1 – 2	-0.007	-0.290	0.276	1.000
1 – 3	-0.260	-0.534	0.015	0.246
1 – 4	0.056	-0.216	0.328	0.977
2 – 3	-0.253	-0.508	0.002	0.209
2 – 4	0.063	-0.193	0.319	0.962
3 – 4	0.316	0.062	0.569	0.070

Supplementary Table 4-4 continued. *Pre-inhalation plasma concentrations vs. visit number – Model 4a*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	ARA-S (ng/ml)			
Visit 1 – Visit 2	-0.002	-0.012	0.008	0.981
1 – 3	-0.005	-0.016	0.005	0.726
1 – 4	0.004	-0.006	0.015	0.840
2 – 3	-0.003	-0.014	0.007	0.913
2 – 4	0.006	-0.004	0.017	0.620
3 – 4	0.010	-0.001	0.020	0.246
	ARA-S (ng/ml) (adjusted for time between visits)			
1 – 2	-0.001	-0.013	0.011	0.998
1 – 3	-0.004	-0.015	0.007	0.902
1 – 4	0.006	-0.006	0.017	0.747
2 – 3	-0.003	-0.013	0.008	0.949
2 – 4	0.007	-0.004	0.017	0.570
3 – 4	0.010	-0.001	0.020	0.255
	aLEA (ng/ml)			
1 – 2	-0.005	-0.013	0.002	0.480
1 – 3	0.000	-0.007	0.007	1.000
1 – 4	0.002	-0.005	0.010	0.918
2 – 3	0.005	-0.002	0.013	0.494
2 – 4	0.008	0.000	0.015	0.165
3 – 4	0.002	-0.005	0.010	0.910
	aLEA (ng/ml) (adjusted for time between visits)			
1 – 2	-0.007	-0.015	0.001	0.314
1 – 3	-0.001	-0.009	0.007	0.987
1 – 4	0.001	-0.007	0.009	0.991
2 – 3	0.006	-0.002	0.013	0.403
2 – 4	0.008	0.001	0.016	0.118
3 – 4	0.003	-0.005	0.010	0.902

Supplementary Table 4-4 continued. *Pre-inhalation plasma concentrations vs. visit number – Model 4a*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	gLEA (ng/ml)			
Visit 1 – Visit 2	-0.004	-0.011	0.002	0.563
1 – 3	0.001	-0.006	0.008	0.993
1 – 4	0.000	-0.007	0.006	1.000
2 – 3	0.005	-0.001	0.012	0.395
2 – 4	0.004	-0.003	0.011	0.618
3 – 4	-0.001	-0.008	0.005	0.984
	gLEA (ng/ml) (adjusted for time between visits)			
1 – 2	-0.004	-0.012	0.003	0.625
1 – 3	0.001	-0.006	0.008	0.995
1 – 4	0.000	-0.007	0.007	1.000
2 – 3	0.005	-0.001	0.012	0.388
2 – 4	0.004	-0.003	0.011	0.609
3 – 4	-0.001	-0.008	0.005	0.984

Supplementary Table 4-5. Pre-inhalation plasma concentrations of AEA and DEA vs. total CBD dose from previous visits – Models 4b, 4c and 4d.

Possible previous doses of CBD at beginning of:
Visit 2: 0, 10, 20 or 30mg
Visit 3: 10, 20, 30, 40 or 50mg
Visit 4: 30, 40, 50 or 60mg

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Visit 2 - Anandamide (ng/ml) (adjusted for time between visits)			
0mg – 10mg	0.007	-0.093	0.108	0.999
0mg – 20mg	0.028	-0.076	0.131	0.949
0mg – 30mg	0.044	-0.076	0.164	0.881
10mg – 20mg	0.020	-0.079	0.119	0.976
10mg – 30mg	0.036	-0.079	0.151	0.918
20mg – 30mg	0.016	-0.103	0.136	0.993
	Visit 3 – Anandamide (ng/ml) (adjusted for time between visits)			
30mg – 40mg	-0.020	-0.146	0.105	0.998
30mg – 50mg	0.004	-0.105	0.113	1.000
30mg – 60mg	0.018	-0.115	0.151	0.999
30mg – 70mg	0.086	-0.049	0.221	0.700
40mg – 50mg	0.024	-0.081	0.129	0.990
40mg – 60mg	0.038	-0.087	0.164	0.972
40mg – 70mg	0.106	-0.031	0.243	0.530
50mg – 60mg	0.014	-0.100	0.128	0.999
50mg – 70mg	0.082	-0.040	0.203	0.656
60mg – 70mg	0.068	-0.076	0.211	0.875
	Visit 4 – Anandamide (ng/ml) (adjusted for time between visits)			
30mg – 40mg	0.038	-0.080	0.053	0.972
30mg – 50mg	0.024	-0.070	0.054	0.990
30mg – 60mg	0.086	-0.075	0.053	0.700
40mg – 50mg	0.018	-0.066	0.078	0.999
40mg – 60mg	0.004	-0.070	0.076	1.000
50mg – 60mg	-0.020	-0.072	0.067	0.998

Supplementary Table 4-5 continued. *Pre-inhalation plasma concentrations of AEA and DEA vs. total CBD dose from previous visits – Models 4b, 4c and 4d.*

Contrast	Estimated marginal mean difference	Lower 95% CI	Upper 95% CI	P value
	Visit 2 - DEA (ng/ml) (adjusted for time between visits)			
0mg – 10mg	0.014	-0.005	0.033	0.470
0mg – 20mg	0.017	-0.003	0.037	0.351
0mg – 30mg	-0.001	-0.024	0.022	1.000
10mg – 20mg	0.003	-0.017	0.022	0.993
10mg – 30mg	-0.015	-0.037	0.007	0.525
20mg – 30mg	-0.018	-0.041	0.005	0.422
	Visit 3 – DEA (ng/ml) (adjusted for time between visits)			
30mg – 40mg	-0.003	-0.034	0.028	1.000
30mg – 50mg	0.000	-0.027	0.026	1.000
30mg – 60mg	0.013	-0.019	0.044	0.931
30mg – 70mg	0.022	-0.010	0.055	0.634
40mg – 50mg	0.003	-0.024	0.029	1.000
40mg – 60mg	0.016	-0.015	0.047	0.844
40mg – 70mg	0.025	-0.008	0.059	0.549
50mg – 60mg	0.013	-0.014	0.040	0.871
50mg – 70mg	0.023	-0.006	0.052	0.517
60mg – 70mg	0.010	-0.025	0.044	0.978
	Visit 4 – DEA (ng/ml) (adjusted for time between visits)			
30mg – 40mg	-0.001	-0.015	0.014	1.000
30mg – 50mg	-0.002	-0.016	0.012	0.994
30mg – 60mg	0.007	-0.007	0.021	0.767
40mg – 50mg	-0.001	-0.017	0.015	0.998
40mg – 60mg	0.007	-0.009	0.024	0.798
50mg – 60mg	0.009	-0.007	0.024	0.675

4.12.2 SUPPLEMENTARY RESULTS

Sex differences

There were no significant sex differences between the endocannabinoids or related non-cannabinoid lipid responses to THC or CBD, with the exception of Model 3b (effect of drug administration: all CBD:THC ratios) and Model 4a (effect of visit number on plasma concentrations) for SEA:

Overall effect of THC

Mean SEA concentration in men rose by 127.2% (0.220ng/ml [95%CI: 0.075–0.364], t(854)=2.981, p=0.025) immediately post-inhalation, before falling to pre-inhalation levels by 5min. In women, there was no significant change in SEA concentration across the sampling timepoints (p>0.05). However, an outlier was identified using Rosner's generalised extreme Studentised deviate test in a male participant (0 min, value= 4.527ng/ml, 55.8% higher than next highest value for that timepoint). When this outlier value was removed, there was no longer a significant change in mean SEA concentration in men (p=0.060).

Effect of visit order on endocannabinoid levels

Over the four experimental visits, pre-inhalation plasma levels of SEA did not significantly change in men (p>0.05) but did alter in women. Between visits 1 and 3, mean SEA concentrations rose by 315.6% (0.584ng/ml [95%CI: 0.192–0.975], t(140)=2.949, p=0.019), before falling again by visit 4 (-76.3%, 0.594ng/ml [95%CI: 0.221–0.967], t(131)=3.153, p=0.011). However, an outlier was identified using Rosner's generalised extreme Studentised deviate test in a female participant (visit 3, value= 5.830ng/ml, 115.6% higher than next highest value for that visit). When this outlier value was removed, there was no longer a significant change in pre-inhalation SEA between visits in women (p≥0.128).

Effect of peak THC concentration

There was a negative correlation between peak THC plasma concentration and change in DEA plasma concentration immediately post-inhalation (R= -0.162 [95%CI: -0.018 to -0.300], t(182)= -2.2181, p=0.028). This was no longer significant, however, when the model was fully adjusted for pre-inhalation concentration of DEA (p=0.279).

There was no correlation between peak THC concentration and change in any of the other analytes ($p>0.05$).

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CHAPTER 5 - GENERAL DISCUSSION

The aim of this thesis was to examine risks associated with cannabis use in relation to psychosis. My first two studies explored cognition, functioning, remission, and transition rates in people at CHR for psychosis. My third study investigated the effects of THC and CBD on the endocannabinoid system. In this chapter I summarise the main findings, discuss their implications, and review the methodological strengths and limitations of the studies that comprise the thesis.

5.1 SUMMARY OF RESULTS

The first study (**Chapter 2**, **Paper 1**) aimed to assess the role of cannabis in the onset of psychotic disorders. I used data from a prospective study of a large cohort of CHR subjects. The main finding was that there was no evidence that cannabis use in CHR participants predicted later transition to psychosis. Similarly, cannabis use was not associated with persistence of the CHR state or a poor functional outcome. These data suggest that, in the CHR population, cannabis use is not a significant risk factor for adverse clinical outcomes.

I also found that CHR participants were both more likely to have started and more likely to have then stopped cannabis use than HCs. This suggests that the CHR state was associated with lifetime cannabis use, but that CHR participants often stopped using cannabis before presenting to early-intervention mental health teams. This study is one of the largest of its kind to evaluate cannabis use and outcomes in CHR, and the first to distinguish between the use of high strength cannabis strains with greater THC content and lower potency strains.

Chapter 3 examined cognitive functioning in the same CHR cohort. CHR participants showed significant impairments in IQ, executive function, verbal working memory, and verbal fluency, and trend level impairment in episodic working memory, when compared to HCs. Within the CHR sample, having ever used cannabis was associated with a less severe impairment on executive function. In an exploratory analysis, this surprising association with relatively better cognitive performance was particularly evident in participants who were occasional cannabis users at the time of assessment.

The study in **Chapter 4** demonstrated that in healthy volunteers, THC in experimentally administered cannabis increased in concentrations of the endocannabinoid AEA and the biologically related lipids DEA, OEA, and ARA-S. However, there was no evidence that this effect was influenced by the dose of CBD in cannabis (0, 10, 20 or 30mg). Pre-inhalation plasma concentrations of AEA and DEA declined in a stepwise fashion at each subsequent experimental visit. These findings have contributed new knowledge of how THC and CBD in ratios commonly found in recreational cannabis influence the peripheral endocannabinoid system.

Table *5-1* presents the thesis hypotheses and a summary of the results.

Table 5-1. *Thesis hypotheses and results*

Hypothesis	Supported?	Detailed Results
Cannabis use would be associated with an increased incidence of psychosis in CHR subjects. (**Chapter 2**)	No.	– No measure of cannabis use (never used vs past user vs current user, use before age 16 vs use after age 16, daily user vs weekly user vs occasional user, use of high potency cannabis vs low potency, cannabis dependent vs non-dependent) was associated with the incidence of psychosis in the CHR cohort.
Cannabis use would be associated with non-remittance from the CHR state (persistence of symptoms). (**Chapter 2)**	No.	– No measure of cannabis use was associated with the incidence of non-remittance from the CHR state.
Cannabis use would be associated with poor functional outcome for CHR subjects. (**Chapter 2**)	No.	– No measure of cannabis use was associated with GAF disability score at follow-up in the CHR cohort.
CHR subjects would show cognitive impairments when compared to HC participants. (**Chapter 3**)	Yes.	– CHR participants performed significantly worse than HC participants in the WAIS-III estimated IQ, trail making task test B, trail making task B/A ratio, verbal working memory and verbal phonetic and semantic fluency assessments.
Having ever used cannabis would be associated with better performance in cognitive assessments in CHR subjects. (**Chapter 3**)	Yes.	– Trail making task test B times were significantly lower in the cannabis-using CHR than non-users. In exploratory analyses: – Past cannabis users performed better than non-users at trail making task test A. – Less-than-weekly cannabis users performed better than non-users in IQ and trail making B tests. – In current cannabis users only, less-than-weekly users showed phonetic fluency than non-users. – Cannabis users who initiated cannabis use before age 16 performed better than non-users at trail making B. – Cannabis users who were not cannabis dependent performed better than non-users at trail making B. – No differences were found for the other cognitive measures between cannabis-users and non-users in the CHR cohort.
Administration of THC would lead to a transient increase in plasma AEA and 2-AG. (**Chapter 4**)	Partially supported.	– Inhalation of cannabis vapour containing 10mg THC led to transient increases in AEA, DEA, OEA and ARA-S. – No changes were observed in plasma levels of 2-AG, AA, SEA, aLEA or gLEA.
Co-administration of CBD would modulate the effect of THC on plasma AEA in a dose-dependent manner. (**Chapter 4**)	No.	– The dose of CBD administered (0, 10, 20 or 30mg) was not associated with either peak concentration or AUC of any plasma endocannabinoids or related lipids.

5.2 CANNABIS USE AND THE RISK OF PSYCHOSIS

Predicting and preventing psychosis are key aims in current research and healthcare,[1,2] and associations between cannabis use and psychosis are well established.[3–10] As cannabis use is modifiable, it is prudent to find out if delaying or avoiding expose to cannabis could reduce the incidence of psychotic disorders, particularly in vulnerable populations.

A key challenge in this field is establishing causality. Cannabis use is neither necessary or sufficient to cause psychosis, but is more likely a causal component which, in combination with other environmental and genetic components, could then become sufficient for the development of the disorder.[11] As with any other environmental risk factor, it is difficult to untangle cannabis' relationship with psychosis due to the large number of potentially mediating and confounding factors. Much of the evidence of linking cannabis use to the risk of psychotic disorders comes from cross-sectional studies of patients who have already developed the disorder, compared them to matched healthy controls groups.[12–17] As a result, these analyses depend on retrospective estimates of cannabis use prior to psychosis onset.[8]

Prospective studies address this issue by establishing temporal correlations between cannabis use and later disease outcomes. A few longitudinal studies have attempted to assess cannabis use in general population samples as a predictor of later diagnoses of psychotic disorders and have reported positive associations.[18–20] However, because the incidence of psychosis in the general population is relatively low, very large samples are required to collect enough subjects who later become psychotic.[21,22] In addition, because the number of subjects is so large, it is difficult to assess cannabis use and clinical features in detail.

Studying people at CHR permits the prospective study of the development of psychosis in a population that is already enriched in terms of psychosis risk.[23] As a result, samples can be more modestly sized, allowing for a more detailed assessment of cannabis use, psychopathology, and clinical outcomes.[24]

5.2.1 PATTERNS OF CANNABIS USE IN THE CHR POPULATION

I found that 74.3% of the CHR sample that I studied had ever used cannabis. This is higher than the average rate of lifetime cannabis use (48.7%, 95% CI = 42.8–54.6%) reported in a meta-analysis of CHR cohorts by Farris and colleagues,[25] possibly due to the high rates of cannabis use in the locations where the EU-GEI study recruited from, e.g., Amsterdam and London. However, the rates of current use (25.8%) and cannabis dependence (14.9%) in the EU-GEI sample and in the meta-analysis were similar.

While the rate of current cannabis use was comparable between HC and CHR subjects (26.9% for both), there were significantly more CHR subjects reporting to be past users only than HC subjects (47.3% vs. 34.3%), a finding is echoed by Buchy and colleagues.[26] One explanation is that CHR patients may be more likely to experience psychotic effects from cannabis than the general population, and therefore choose to stop using the drug.[27,28] This is consistent with evidence that CHR subjects have relatively high levels of insight.[29] On the other hand, CHR subjects were more likely to report daily use of cannabis than controls (33.1% vs. 7.7%), and to prefer high potency (estimated >10% THC) strains (76.2% vs. 43.8%). These findings are in line with results by Farris and colleagues and Buchy and colleagues.[25,26] As the risk of developing psychotic symptoms from cannabis use is thought to be strengthened by dose.[10,12–14,30,31] these results suggest that a subgroup of CHR subjects are vulnerable to its psychotogenic effects and may not have entered the prodromal stage of psychosis if not for their heavy cannabis use. This would be consistent with the finding of McHugh and colleagues[32] that a history of cannabis-induced attenuated psychotic symptoms (APS) was linked to a higher rate transition to psychosis in a CHR sample.

5.2.2 DIFFERENCES BETWEEN CANNABIS USERS AND NON-USERS WITHIN THE CHR SAMPLE

At baseline, cannabis users did not differ from non-users in terms of level of functioning or the severity of positive or negative symptoms. This was true for all measures of cannabis use except frequency of use. Daily users had a lower level of functioning than both weekly and occasional users, as well as non-users. A meta-analysis from Carney and colleagues[33] also found no differences in positive or negative symptom scores between cannabis-using and

non-using CHR subjects, and a previous study reported no difference in level of functioning between CHR participants with or without cannabis use disorder.[34] As these data were cross-sectional, it is not possible to determine the direction of the association between daily cannabis use and a low level of functioning. However, longitudinal follow up of the CHR sample did not indicate that daily use was inked to poor functional outcomes.

Cognition and cannabis use

I found that cognitive deficits in CHR subjects were less pronounced in cannabis users, particularly in occasional users, and particularly on the TMT-B task. This task requires visuomotor speed and executive function in the form of sustained attention and task-switching.[35] The absence of differences on the TMT-A task, which involves visuomotor speed but does not engage executive function,[35] suggests that this difference was related to an effect on executive visuomotor speed.

Previous studies in CHR subjects have not found any significant relationship between cannabis and cognition but may have lacked statistical power due to small sample sizes.[36,37] In contrast, in patients with psychotic disorders, five of seven meta-analyses found that patients who used cannabis use or met criteria for cannabis abuse performed better than non-cannabis-users.[38–42] These studies indicated that having ever used cannabis was associated with less severe impairments in general cognitive ability, attention, memory, and executive function. **Table *5-2*** compares the results of one such meta-analysis, by Rabin and colleagues,[40] with the results in **Chapter 3**. Although I could not produce Cohen's d effect sizes from these multilevel models, the EMM z-score differences suggest that the strength of the association between cannabis and cognitive function in the CHR sample was not as strong as that in patients with psychotic disorders.

There has been much debate as to whether cannabis use has a lasting effect on cognition.[43] Several epidemiological studies report an association between adolescent cannabis use and poorer educational attainment,[44–49] and a systematic review of over 40 studies concluded that persistent cannabis use can alter the structure and function of the brain.[50] One might therefore expect cannabis use to be linked to an exacerbation of cognitive deficits in the CHR state. While it has been suggested that cannabis may act differently on

***Table 5-2**. Effect of lifetime cannabis use on cognition domains in patients with psychosis versus results from Chapter 3*

Cognitive Domain	Schizophrenia patients, effect size (SD)[a]	Clinical high-risk patients, effect size (95% CI)[b]
General Intelligence	0.48 (0.51)	0.18 (-0.09, 0.44)[c]
Selective, sustained, and divided attention	0.35 (0.23)	0.27 (0.00, 0.53)[d]
Executive Functioning	0.14 (0.49)	0.31 (0.04, 0.57)[e] / 0.02 (-0.30, 0.33)[f]
Retrieval and recognition	0.12 (0.50)	0.06 (-0.20, 0.32)[g]
Working Memory	0.07 (0.40)	-0.11 (-0.43, 0.20)[h]
Receptive and expressive language abilities	0.06 (0.30)	0.18 (-0.08, 0.43)[i] / 0.17 (-0.09, 0.42)[j]

Positive effects sizes indicate better performance in cannabis-using patients with schizophrenia / individuals at CHR compared to non-users, negative effect sizes indicate better performance in non-users. A value of -0.20 to -0.50 corresponds to small effect sizes, -0.50 to -0.80 to medium, and a value less than -0.80 to large effect sizes.

[a] Data adapted from Rabin et al. 2011 [40]. Effect size as Cohen's d.

[b] Data adapted from Chapter 3 (Paper 2). Effect sizes as EMM z-score differences (calculated based on non-cannabis-using group).

[c] As measured by the Wechsler Adult Intelligence Scale-III.

[d] As measured by the Trail Making Task test A.

[e] As measured by the Trail Making Task test B.

[f] As measured by the Trail Making Task test B/A ratio.

[g] As measured by the Rey Auditory Verbal Learning Test- delayed recall.

[h] As measured by the Rey Auditory Verbal Learning Test- immediate recall.

[i] As measured by a test of phonetic fluency.

[j] As measured by a test of semantic fluency.

'vulnerable' brains, such as those with or at risk of psychosis, than it does on 'healthy' brains,[51] evidence suggests that patients with psychosis are instead *more* sensitive to the acute cognitive deficits cause by THC, rather than less.[52] One explanation for the opposite finding in the present thesis could be that in a subgroup of CHR subjects, cannabis use triggers psychotic symptoms that would have otherwise not have occurred. These individuals might have a relatively low neurobiological vulnerability to psychosis compared to CHR subjects whose symptoms emerge in the absence of cannabis use.[53] This could explain why participants who used cannabis before the age of 16, a risk factor for psychotic disorder,[20,30,54] showed better performance in the TMT-B assessment compared to non-users while older initiators of the drug did not. However, it was not possible to explore this in the present study, as the extent to which CHR symptoms were linked to cannabis use was not explicitly assessed. Another potential explanation is that the ability to source illicit cannabis requires a level of cognitive functioning, and that cannabis use is a proxy marker for CHR

subjects with less marked cognitive impairments. In any event, it should be emphasised that cannabis use was not associated with *enhanced* cognitive performance, but with a less severe deficit. Thus, even the least impaired cannabis user subgroup (current, occasional users) did not perform better than healthy controls.

5.2.3 CANNABIS USE AND CLINICAL OUTCOMES

No measure of cannabis use was significantly associated with the subsequent incidence of psychosis. This finding is in line with the data from the majority of previous studies of cannabis use in CHR samples.[25,55,56] Some studies have reported associations between transition to psychosis and particular measures of use, such as cannabis dependence, younger age at first use, or daily and/or weekly use.[55,57] However, these findings have not been replicated in other studies,[26,32] and there was no evidence of association with any of these metrics in the present study.

Overall, the mainly negative findings from studies in CHR samples contrast with data from studies in the general population and in patients with psychosis, which point to a link between cannabis use and increased psychosis risk. There are several possible explanations for this difference. One is that cannabis use in the CHR phase, in which individuals are typically aged between 18-25 years, may not be at a time of development that can influence the likelihood of later psychosis.[58] However, cannabis use during the proposed critical period of early adolescence (i.e., before age 15 years) was not associated with clinical outcomes in my analysis. Another consideration is that many of the findings from studies in patients with psychosis and community samples are confounded by the fact that the assessment of cannabis use prior to psychosis were retrospective and could thus be influenced by recall bias.[59] Without establishing a temporal correlation between cannabis use and later development of psychosis, it cannot infer causation.[60] For example, genetic studies have demonstrated a relationship between schizophrenia risk genes and cannabis use[61,62] that may be stronger than the link between cannabis use and the later development of schizophrenia.[62]

In the present thesis, there was also no association between cannabis use in CHR subjects and either the persistence of symptoms or a poor functional outcome. These results are in line with three other studies in CHR cohorts.[34,63,64] Again, these findings contrast with data from studies in patients with psychosis, which consistently report associations between cannabis

use and adverse outcomes, including relapse and more intensive psychiatric treatment, and a poor level of functioning,[6,65–68] These contrasting findings could be related to differences in the level of insight between CHR subjects and patients with psychosis: CHR subjects have a significantly higher level of insight,[29] and may therefore be more likely to avoid cannabis use if they find that it exacerbates their symptoms or functional capacity.

5.2.4 STRENGTHS AND LIMITATIONS

A strength for both **Chapter 2** (**Paper 1**) and **Chapter 3** (**Paper 2**) is the size of the cohort; the EU-GEI study recruited one of the largest samples of CHR individuals to date, collecting extensive data on demographics, cognitive functioning, psychopathology, and clinical outcomes. This was achieved with a multi-site design that also increased the generalisability of the results globally. In addition, the EU-GEI study utilised a comprehensive assessment of cannabis to consider multiple aspects of its use, reflecting variations in the recency, frequency, age of initiation and dependence of cannabis use that cannot be captured in studies employing only a single index of cannabis use.[69] Finally, a main strength of the study featured in **Chapter 2** is the prospective design. The use of a CHR cohort allows for better examination of whether cannabis use has a causal effect on psychosis. This avoids problem of retrospective assessments of cannabis use, and confounding effects of previous illness and treatment.

One of the main limitations of both of these studies is the difficulty in interpreting negative findings. Despite the comparatively large size of the CHR cohort as compared to some previous studies, the power to construct statistical models of was limited by the number of participants who fell into each cannabis use subgroup, and in **Chapter 2** by the number of individuals who transitioned to psychosis or achieved symptomatic remission. Thus, it is still possible that these studies lacked the statistical power to detect the effects of cannabis on cognition and clinical outcomes. Power calculations for **Chapters 2** and **3** (pages 98 and 139) show that the power levels of the primary outcomes for both studies were less than 80%. This limitation could only be addressed by conducting even larger scale studies, but these are logistically demanding and expensive.

A further limitation lies in the matching of groups, in particular the comparatively small sample size of the healthy control group. The primary aim of the EU-GEI project was the

study of gene-environment interactions and their clinical impacts within the CHR population, and less resources were assigned to the recruitment of healthy controls. The control sample recruited from only four of the eleven study sites; London, Amsterdam, Den Haag, and Melbourne. While controls were matched to the CHR sample at the group level by age and gender, they were not matched in terms of education, ethnicity, socio-economic status, or other important socio-demographic characteristics that may have influenced their use of substances and their abilities in neurocognitive assessments. When considered together with the limited number of controls recruited (67 vs. 344 CHR in total), the comparisons between the two study groups must be examined with caution.

There were also baseline differences between the CHR cannabis use groups which may have contributed to the measured outcomes. Cannabis users were older, more likely to be male, more likely to be working class, and used more tobacco, alcohol, and other substances than non-using CHR participants (**Table 3-2**). Socio-demographic differences were also apparent between participants with difference cannabis-use patterns (**eTable 5**, **eTable 6**). Age, sex, and socio-economic status were controlled for in the in the statistical models of **Chapter 3**, and sensitivity analyses also controlled for tobacco, alcohol, and other substance use. However, in **Chapter 2**, potential confounders were included in the model only if they improved the model fit. It is possible that associations between cannabis use and clinical and cognitive outcomes may have been related to, or masked by, interacting environmental and genetic factors.

The selection of participants may have also introduced a source of bias. As mentioned previously, CHR participants by definition had to be help-seeking in order to present to health care professionals and receive the CHR diagnosis. Also mentioned previously, the primary aim of the study was the investigation of gene-environment interactions, which required participants to provide a blood or saliva sample for DNA analysis. Individuals who were unwilling to provide samples, which could theoretically be linked to symptoms of paranoia, were excluded. It should be noted however, that while individuals are excluded from the CHR diagnosis should their psychotic symptoms be attributable only to substance misuse, this rule applied only the substance *other than* alcohol and cannabis.

Finally, whilst the cognitive battery did assess a number of key aspects of cognition, it was not comprehensive enough to include all of the cognitive domains. For example, the EU-GEI

study had no measure of problem solving or visual memory, which are known to be impaired in CHR individuals.[70] It was also not possible to examine cognitive data longitudinally due to attrition during follow-up.

The CHR construct

As well as the methodological considerations discussed in the above section, it is important to reflect on the strengths and limitations of research using CHR cohorts in general.

Health services specifically for those at high-risk of psychosis were originally designed for the purpose of reducing the rate of psychosis, ameliorating symptoms, and minimising the duration of untreated psychosis in those who do transition.[71,72] An additional benefit is the use of this population to study the prodromal phase that occurs prior to a first episode of psychosis, in order to establish potential risk and protective factors as well as the aetiology of psychotic disorders.[73] However, the CHR construct is not without criticism, and many characteristics of the CHR population do not appear generalisable to the population of patients with frank psychotic disorders.[74–76]

It would be wrong to describe the CHR population as being in the prodrome of psychosis, as the majority of those categorised as CHR will never develop a psychotic disorder.[77] In addition, the vast majority of patients presenting with FEP will not have had any previous contact with CHR-targeted health services.[78] This is reflected in the distinct sociodemographic characteristics of people attending CHR services and patients presenting with FEP.[79] People with psychosis who were previously identified as being at CHR are more likely to be employed,[79] more likely to live with a partner or family,[78] and less likely to be migrants [78,79] compared with FEP with no previous contact with early-intervention centres. Many have argued that those who attend CHR clinical services are thus an atypical subgroup of pre-psychotic individuals, as it is necessary for them to have some level of knowledge or insight into their symptoms and seek medical help in order to be referred to these services.[75] This may explain why the results of **Chapter 2** and other CHR cohort studies investigating the relationship between cannabis use and the development of psychosis [25] differ to such an extent from similar studies conducted using individuals with established psychotic disorders.

Despite these limitations, it could also be argued that researching those identified as at risk of psychosis is still the best method of examining pre-psychotic individuals, due to the comparatively small incidence rate in the general population.[22] Even if the results of investigations are not generalisable to the wider population, predicting and preventing poor outcomes in the CHR population is still a worthy cause. Unfortunately, no intervention has yet proved to be effective in preventing transition.[80] The impact and generalisability of work in this field could be improved by expanding access to high-risk clinical services, for example by automating screening of patients in primary care for the CHR state.[81]

5.2.5 CANNABIS, CHR AND PSYCHOSIS – WHERE DO WE GO FROM HERE?

Considering cannabis as a cause of psychosis

Despite claims that cannabis use is linked to a 5-fold increased risk of developing a psychotic disorder,[5] millions of people use cannabis every year without serious consequences to their mental health.[82] Considering the model of disease causation put forward in 1976 by Kenneth Rothman,[11] cannabis use can be considered neither a necessary nor sufficient cause of psychosis, but rather a possible causal component that requires other causal components to be present to produce sufficient cause. The prevalence of other causal components may differ between populations,[11] which may explain in part the vast differences in patient attributable factor between geographical regions,[13] Future research should be done to identify the required accompanying causal components, such as genetic factors[31] and the presence of cannabis-induced psychotic symptoms.[32] and psychosocial factors such as readiness to change.[83]

Examining cannabis use in CHR cohorts

Future research should include more detailed and accurate measurements of cannabis use. First, cannabis use in CHR subjects should be monitored at regular intervals, especially during the first 12 months of patient presentation when transition is most likely.[77] A paper by Corcoran and colleagues[84] reported that cannabis use is temporally associated with psychotic symptoms and anxiety in individuals at CHR. In addition, a number of studies have suggested that progression to daily cannabis use in individuals in the prodromal stage of

psychosis may linked to elevated psychotic symptoms.[85] Thus, in CHR subjects, the change in cannabis use over time may be an important factor for clinical outcomes.

The use of drug testing as well as confirming levels of drug use with family and friends could help validate the results of cannabis use questionnaires such as the CEQ (cannabis experience questionnaire). The type of biological sampling, either hair, blood, urine, or mouth swabs, differ in terms of accuracy, precision, and window of detection.[86] For example, oral fluid testing may also be useful for ensuring sobriety prior to cognitive testing. The use of a timeline follow-back questionnaire for all participants will also help to establish the effects of abstinence from cannabis use, rather than simply categorising individuals as past or current users.[59]

As the potency of cannabis is considered an important influence on its potential harms, samples of participants' cannabis could be used to measure levels of THC and CBD, and possibly to corroborate the assigned potency levels estimated from national reports. Studies including cognitive assessments should also ensure that both premorbid IQ and social functioning are measured, both to include as covariates and in order to investigate the theory of cannabis use as a marker for better premorbid intelligence and social cognition.[87]

5.3 HOW DOES USING CANNABIS AFFECT THE ENDOCANNABINOID SYSTEM?

The effects of cannabis are thought to be due to its modulation of the endocannabinoid system. The main psychoactive constituent of cannabis, THC, is a partial CB1R agonist.[88] CBD, the second most abundant cannabinoid in the cannabis plant, has a number of proposed mechanisms of action, including non-competitive inverse agonism at CB1R[89,90] and up-regulation of the endocannabinoid lipid signalling molecule AEA.[91] Very few studies have investigated the acute effects of THC or CBD on endocannabinoids levels in humans,[92–95] despite the fact that cannabis has been associated with long term alterations in ECS signalling.[96] The key findings from my study (**Chapter 4**, **Paper 3**) were that the inhalation of 10mg THC transiently increased levels of the N-acylethanolamines (NAEs) AEA, DEA, and OEA, as well as the N-acyl amide ARA-S, and that baseline, pre-inhalation levels of AEA and DEA fell in a stepwise fashion after each study visit. These suggest that THC use can cause both acute and long-term modifications to peripheral endocannabinoid levels, suggesting a possible mechanism for the development of tolerance to cannabis and the cognitive impairing and psychogenic effects of chronic cannabis use.

The increases in AEA and OEA I observed following the administration of THC are in line with the results of two previous studies in humans.[92,93] However, Kearney-Ramos and colleagues[94] reported no change in either AEA or 2-AG after THC administration. This difference may be related to Kearney-Ramos and colleagues studying long-term, frequent cannabis users rather than the young, infrequent users that I examined. Regular use of cannabis has been shown to downregulate AEA in the CNS and 2-AG in the peripheral circulation.[94,97] The clinical impact of the aforementioned effects of THC on NAE and ARA-S levels on human health are difficult to discern, partly due to the complex and poorly understood mechanisms by which these lipids act,[98,99] and also due to the brevity of the effects observed. However, it is notable that some of the key functions of AEA and OEA are in the regulation of anxiety, appetite, pain, and memory,[100,101] all of which are also affected by acute cannabis intoxication,[102–105]

In **Chapter 4**, AEA and DEA plasma concentrations were found to become progressively lower before cannabis inhalation within subjects across successive sessions. While it is possible that this was due to the repeated exposure to cannabinoids, the finding was

independent of the length time between experiments (which varied between 7 and 398 days). The progressive reduction in AEA and DEA levels may have been due to habituation to the study procedure, resulting in less stress-induced endocannabinoid release with each session.[106] The endocannabinoid system is thought to modulate the stress response by responding to and regulating the activity of the hypothalamic–pituitary–adrenal (HPA) axis. Acute stress leads to the release AEA, which in turn curbs the release of glucocorticoids and feelings of anxiety.[106,107]

Although CBD has previously been reported to increase levels of AEA in the CNS,[91] in my thesis it had no effect on the concentration of AEA or other endocannabinoids in plasma. This could be because 30mg of CBD is too small a dose to have an acute effect on peripheral endocannabinoid release or metabolism. Although the dose ratio of CBD:THC in illicit cannabis is not typically higher than 3:1, these results suggest that even higher doses of CBD may be necessary to alter the effects of co-administered THC on endocannabinoid levels.

5.3.1 STRENGTHS AND LIMITATIONS

To my knowledge, this is the first published study to investigate the acute effects of THC both with and without the presence of CBD on plasma endocannabinoid concentrations. The main advantages of such a design are the ability to segregate the effects of CBD and THC in a controlled way, while creating more ecologically valid conditions than when compared to previous studies of THC or CBD alone. Cannabis as it is used recreationally is generally inhaled after smoking or vaporising, as opposed to IV or oral administration used in other studies,[92,93] and contains a combination of THC, CBD, and trace cannabinoids and terpenes.[108] Importantly, commercially available cannabis products (either illegal or legal) will contain far less CBD (e.g., maximum 100mg) than administered in previous similar experiments.[91,109] Administering vaporised plant material containing naturalistic dosages of THC and CBD thus allows us to recreate the use of recreational cannabis products, while maintaining a controlled, experimental design.

While the ‘naturalistic’ design of this study can be seen as a strength, it does limit the interpretation of results in some ways. The dose of CBD may have been too low to observe any acute effects, even though it was comparable to that in illicit cannabis.[110] Levels of terpenes, flavonoids and phytocannabinoids other than THC and CBD were not accurately

measured and could theoretically influence the ECS, a result known as the 'entourage effect'. However, evidence for the existence of this effect is poor.[111] The creation of precise dose-profiles of THC and CBD would require a much more consistent mode of dosing than inhalation, with less inter- and intra-subject variation in duration of administration and absorption of the drugs. The significant within and between-subject variation in inhalation times (ranging from 6 min to 138 min) is reflected in the large variation of plasma THC and CBD concentrations at time 0 min, as seen in **Figure 4-1** of **Chapter 4**, page 170. In particular, subjects reported more throat irritation and coughing with increasing concentrations of CBD, which further confounded inhalation time.[112] This study was primarily designed to identify differences in the psychological effects of CBD:THC ratios, while plasma endocannabinoids were a secondary aim. Because of this, fewer plasma samples were taken than if endocannabinoid levels were the primary endpoint. There was also no placebo condition, which might have added value to the study in terms of establishing the effects of THC versus the inhalation procedure alone.

Finally, the study design only allows one to draw conclusions on the acute effects of cannabis and not long-term exposure. As the ECS adapts to persistent cannabis use- changes which can reverse with abstinence[96]- the effect of cumulative dosing with different CBD:THC ratios may be much more apparent than in acute dosing. since the risk of mental health harms with cannabis use is dependent on the cumulative dose,[113] the results of **Chapter 4** may be less relevant than those of longer-term studies.

5.3.2 FUTURE RESEARCH

What is the dose-effect profile of cannabis on endocannabinoids over time?

Future experimental studies should consider the inclusion of a placebo group, despite the risk of unblinding for that session, in order to confirm a causal effect of THC on endocannabinoid levels. A standardised administration procedure with minimal variation in absorption and dosing times will aid in the construction of accurate dose-effect profiles. The effects of persistent cannabis use over a set period of time should be studied in a controlled environment, preferably followed by a monitored wash-out period to assess the length of abstinence required to reverse any changes. An alternative approach could be an at-home study, similar to the acute study by Morgan and colleagues in 2010,[114] where cannabis is provided to or bought by participants and then tested for cannabinoid levels, and use is

monitored as fastidiously as is possible over a period of days or weeks. In either case, the ethical and legal implications of prescribing a possibly harmful drug may be the chief barrier carrying out such a study and must be considered carefully.

What dose of CBD does affect endocannabinoid levels?

While Leweke and colleagues[91] showed that 800mg of CBD daily for 14 days increases AEA signalling, a key issue which has yet to be addressed is the minimum size and number of daily doses required to produce these effects. A dose-ranging study could address this issue. Plasma endocannabinoid levels could be measured alongside CSF endocannabinoid levels and psychological measurements such as anxiety or positive and negative symptoms in psychosis patients, in order to better inform future clinical trials.

How does CBD work as an antipsychotic/neuroprotectant?

The mechanism by which CBD can reduce or prevent psychotic and psychotomimetic symptoms remains elusive but could aid the discovery of new drug targets. Clinical trials of CBD in people who are regular cannabis users, at CHR, or diagnosed with FEP or treatment-resistant psychosis should consider monitoring endocannabinoid levels and CB1R expression in patients. The use of test compounds known with known mechanisms of action, e.g., the FAAH inhibitor PF-04457845,[115] could be used in drug target confirmation.

5.4 THE CURRENT CONTEXT OF CANNABIS USE

Cannabis is by far the world's most popular illicit drug, with the number of users increasing by around 23% over the past decade.[82] While both supply and possession remain illegal in most of the world, cannabis for recreational use has been legalised in Canada, Georgia, Malta, Mexico, South Africa, Thailand, Uruguay and 23 US states, and decriminalised in many more. Cannabis is also available for medical purposes in dozens other countries and territories.[116] Even in the UK, where it is considered a Class B controlled substance, 16.2% of young people aged 16 to 24 used cannabis in year 2022 alone.[117]

The focus of public policy on cannabis has shifted in recent years from enforcing abstinence to harm reduction. In Canada, the first country to provide legal and regulated access to cannabis for non-medical purposes, for example, guidelines have been put in place to encourage what is considered 'less harmful' use of cannabis.[118,119] These recommendations take into consideration on the individual user, such as their age, personal and family history of mental illness, and personal preferences. Focus is also placed on the type of cannabis and mode of administration; the preferential use of low-THC, high-CBD strains is promoted, and users are encouraged to consider different doses for ingested and inhaled products to prevent over-intoxication. Interestingly, despite it now being widely available at government sanctioned stores and dispensaries, up to 52% of users in Canada still do not purchase cannabis solely from legal, licensed sources.[120]

While public attitudes towards cannabis have changed and movements aimed at legalising have grown, there is concern among researchers and policy makers that repealing anti-cannabis legislation will lead to increased incidence of cannabis-related harms, in particular cannabis-induced psychosis.[116,121,122] Of particular concern is the proliferation of high-efficiency indoor growing and breeding techniques which have led to an influx high-THC cannabis products (see section 1.1.1 of the Introduction). In the US and Canada, the 'Green Rush'[a] has brought to the market dried cannabis buds with up to 35% THC content[123,124] and concentrates such as butane hash oil with strengths of 60-90%.[125,126] However, this trend is

[a] A nod to the Gold Rush of 1800s North America, the term 'Green Rush' refers to the sudden and large growth in the cannabis market in the USA and Canada after the legalisation of cannabis in the US state of Washington in December 2012.

also present in US states where non-medical cannabis use remains illegal,[127] as well as in Europe.[128]

Initial data shows that while perceptions of cannabis harms have decreased across North America,[82,129] there has been an increase in the number of cannabis-related hospitalisations in Colorado,[130,131] California[132] and Canada[133] since legalisation. This is likely due to a combination of factors, however, including the increased use of cannabis across the population, the availability of high-THC products, the unintentional ingestion of cannabis products by children as more palatable edible products are introduced to the market, and a greater inclination to seek medical assistance due to the changed legal status.[129]

The evidence for an association between cannabis liberalisation and incidence of psychosis in the US and Canada is limited and mixed. For example, in the state of Colorado, a study by George Wang and colleagues[134] found a positive association between the number recreational dispensaries per capita and the rate of emergency department visits for psychosis from 2013 to 2018 (non-medical cannabis use was legalised in 2012 in Colorado, and the first recreational dispensaries began opening in 2014). Studies across the USA as a whole have found mixed results.[135,136] A limited number of other studies have found increases in the number of hospitalisations for cannabis-induced psychosis in Portugal and Canada following changes in national policies on cannabis,[137,138] however it should be noted that this trend was already taking place in Canada prior to legalisation of non-medical cannabis use in 2018.[139]

It is almost certainly too soon to determine the effects more liberal cannabis policies have had and will have for public health. Whilst the burden of cannabis use disorders and cannabis-induced psychosis has risen across Europe and North America in recent decades,[82,140] the there is a need to consider the tangible effectiveness of anti-cannabis legislation in curbing these problems, balanced with the societal and public health effects of criminalisation.[126,141–143] As cannabis policies continue to evolve, it is becoming clear that the widespread use and increased cultural acceptance of cannabis indicate its enduring presence in society, underscoring the necessity of comprehensive research and evidence-based policies to promote harm reduction and safeguard the wellbeing of the population.

5.5 CONCLUSIONS

I found no evidence that cannabis use in people at high risk was associated with an increased risk of subsequently developing psychosis, or other adverse clinical outcomes. This is at odds with evidence from other studies linking cannabis use with an increased risk of psychosis.

A second counter-intuitive finding was that cannabis use in CHR subjects was associated with less severe impairments in cognitive performance, an association particularly evident in occasional cannabis users.

Finally, I found that manipulating the dose of CBD in cannabis did not alter the effect of THC on the concentration of endocannabinoids and non-cannabinoid biologically related lipids in plasma.

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APPENDICES

APPENDIX A: EU-GEI STUDY METHODS

PARTICIPANTS

344 CHR participants were recruited from 11 Early Detection and Intervention Centres- 9 in Europe (London, Amsterdam, The Hague, Vienna, Basel, Cologne, Copenhagen, Paris, Barcelona), one in Brazil (Saõ Paulo), and one in Australia (Melbourne). 67 HCs matched for age, gender, ethnicity, and socio-economic status were recruited from the London, Melbourne, and The Hague sites. Participants were recruited between July 2010 and September 2017.

CHR participants had to meet for at least one of the three subgroups as assessed by the CAARMS:[1]

1) Trait risk factors, such as schizotypal personality disorder or a first-degree family member with psychosis, with a significant deterioration in functioning (Genetic Risk and Deterioration Syndrome: GRD);
2) Attenuated, sub-threshold positive psychotic symptoms (APS); or
3) Brief limited intermittent psychotic (BLIP) symptoms that last less than 1 week and resolve without treatment.

For all participants, exclusion criteria were:

- Previous diagnosis of a psychotic disorder according to the CAARMS[1] and Structural Interviews for DSM-IV Axis I and II disorders;[2]
- Symptoms relevant for inclusion are explained by an organic disorder or drug or alcohol dependency;
- Passing the 'Psychosis Threshold' as assessed by the CAARMS (i.e., severity and frequency score threshold met for longer than one week);
- Passing the 'Antipsychotic Treatment Threshold' as assessed by the CAARMS (i.e., patients had received antipsychotic medication for one week or longer);

- IQ of less than 60 as estimated by the Wechsler Adult Intelligence Scale – third version (WAIS-III);[3]
- Any past episode of frank psychosis lasting more than 7 days;
- Unwilling to give a blood or saliva sample for genetic analysis;
- Subject is unable to fully comprehend the purpose of the study or make a rational decision whether or not to participate.

In addition, HCs could not meet criteria for the CHR state as defined by CAARMS.[1] A previous history of other non-psychotic mental illnesses, such as episodes of depression or drug and alcohol misuse, was not an exclusion criterion. Participants were required to be proficient in the language local to each site and to provide written informed consent (participant assent and parental/legal guardian informed written consent was required of participants below the age of 18). Age was not restricted for CHR participants, as centres varied in the age of persons accepted for clinical services. HCs were age-matched to the extent that they were required to be over 18 years of age.

MEASURES

Individuals at CHR were assessed at 4 time points – baseline, 12 months, and 24 months after baseline. If a subject transitioned to psychosis, they were assessed as soon as possible after the point of transition.

A range of assessment measures were used to determine sociodemographic variables, medical and psychiatric history, severity of psychiatric symptoms, substance use, and psychosocial functioning. Only those which I assessed as part of my thesis are listed here:

- Sociodemographic data, including age, sex, ethnicity, education, and social class (based on father's occupation at the time of the participants' birth) were measured at baseline using the Medical Research Council Sociodemographic Schedule;[4]
- A modified version of the Cannabis Experience Questionnaire (CEQ)[5] was given at baseline and follow-up;
- Use of illicit drugs, including amphetamines, cocaine, crack, hallucinogens, inhalants, ketamine, opioids, sedatives, and other illicit substances, was measured at baseline and follow-up using the CEQ;

- Use of alcohol and tobacco products was measured at baseline and follow-up using the Composite International Diagnostic Interview;[6]
- Current and past treatment with psychoactive medication, such as antidepressants, anxiolytics, and antipsychotics, was assessed throughout the study;
- Cognition was assessed at baseline and follow-up using a shortened version of the WAIS-III[7] (baseline only), the Verbal Fluency test,[8] the Rey Auditory Verbal Learning task,[9] and the Trail Making Test;[10]
- Psychopathology was measured at baseline, 12, and 24 months using the CAARMS,[1] Structural Interviews for DSM-IV Axis I and II disorders,[2] Clinical Global Impression[11] and GAF disability scales.[12]

All assessments were conducted by trained psychiatrists, psychologists, or research assistants. Training was conducted using a web-based teaching environment, and only researchers that succeeded in passing interrater reliability checks every 12 months were permitted to assess participants.

APPENDIX B: WORK PUBLISHED DURING PHD

1. **Chester LA**, Chesney E, Oliver D, Wilson J, Englund A. How experimental cannabinoid studies will inform the standardized THC unit. *Addiction*. 2020;Early View. doi:10.1111/add.14959

2. Yoganathan P, Claridge H, **Chester LA**, Englund A, Kalk NJ, Copeland CS. Synthetic cannabinoid-related deaths in England, 2012-2019. *Cannabis Cannabinoid Res*. 2021;58(2):457-486.

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In Revision:

1. **Chester LA**, Valmaggia LR, Kempton MJ, et al. Influence of cannabis use on incidence of psychosis in people at clinical high risk. *Psychiatry Clin Neurosci.* 2022

In Preparation:

1. **Chester LA**, Kempton MJ, Tognin S et al. Effects of cannabis use on cognition in people at clinical high risk for psychosis. *Neuropsychopharmacology* 2022

2. Oliver D, Englund A, Chesney E, et al. Cannabidiol does not attenuate acute delta-9-tetrahydrocannabinol-induced attentional bias in healthy volunteers: A randomised, double-blind, crossover study. *Addiction.* 2022

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