Online Supplementary Material

Title: Sex-Specific association between low oral doses of cannabidiol (CBD) and plasma concentration of anandamide (AEA), N-palmitoylethanolamine (PEA) and N-oleoylethanolamine (OEA) in healthy occasional cannabis users

Anita Abboud[1,2], Lucy Chester[1,2], Francois-Olivier Hébert[2], and Didier Jutras-Aswad*[1,2]

[1]Department of Psychiatry and Addictology, Université de Montréal, Montréal, Québec, Canada;

[2]Research Centre, Centre hospitalier de l'Université de Montréal, Montréal, Québec, Canada;

Corresponding author:
Didier Jutras-Aswad
didier.jutras-aswad@umontreal.ca

Table of contents

Supplementary Table S1: Results from GLMM model 1 showing the association between Cmin and CBD doses for each analyte.

AEA					
		Estimate	**SE**	**t value**	**p-value**
(Intercept)		0.099	0.015	6.750	
Dose					0.501
	20 mg	-0.001	0.009	-0.119	
	50 mg	-0.004	0.009	-0.451	
	100 mg	-0.002	0.009	-0.225	
	200 mg	-0.014	0.009	-1.603	
Visit					**0.000 *****
	V2	0.029	0.009	3.258	
	V3	0.029	0.009	3.261	
	V4	0.041	0.009	4.573	
	V5	0.026	0.009	2.895	
Sex		-0.036	0.007	-4.848	**0.000 *****
T0		0.330	0.030	10.947	**< 2.2e-16 *****
PEA					
(Intercept)		0.616	0.065	9.470	
Dose					0.302
	20 mg	-0.036	0.034	-1.066	
	50 mg	-0.058	0.034	-1.709	
	100 mg	-0.040	0.034	-1.169	
	200 mg	-0.070	0.034	-2.037	
Visit					0.458
	V2	0.028	0.035	0.813	
	V3	0.027	0.035	0.787	
	V4	0.054	0.035	1.552	
	V5	-0.003	0.034	-0.084	
Sex		-0.151	0.040	-3.761	**0.000 *****
T0		0.258	0.028	9.330	**<2e-16 *****
OEA					
(Intercept)		0.497	0.051	9.763	
Dose					0.110
	20 mg	-0.048	0.031	-1.570	
	50 mg	-0.082	0.031	-2.664	
	100 mg	-0.045	0.031	-1.476	
	200 mg	-0.058	0.031	-1.879	
Visit					0.382
	V2	0.013	0.031	0.425	
	V3	0.029	0.031	0.920	
	V4	0.058	0.032	1.825	
	V5	0.009	0.031	0.276	

Sex		-0.081	0.029	-2.765	**0.006 ***
T0		0.196	0.024	8.290	**3.481e-15 *****
AEA, anandamide; Cmin, Minimum Concentration; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; SE, Standard Error; T0, Pre-ingestion timepoint *** Signifies p < 0.050; *** Signifies p < 0.001**					

Visit effect: A significant visit effect was observed for AEA Cmin ($p < 0.001$), with post-hoc analyses showing this effect specifically for visit 4 compared to visit 1 (Cohen's $d = -0.7835$, 95% CI [-1.1321; -0.435]).

Supplementary Table S2: *Post-hoc* exploratory subgroup analysis by sex using Model 1.

		Males				Females			
AEA									
		Estimate	**SE**	**t value**	**p-value**	**Estimate**	**SE**	**t value**	**p-value**
(Intercept)		0.088	0.024	3.748		0.070	0.018	3.884	
Dose					0.693				0.502
	20 mg	-0.013	0.014	-0.934		0.010	0.012	0.833	
	50 mg	-0.011	0.014	-0.782		0.004	0.012	0.318	
	100 mg	-0.008	0.014	-0.591		0.003	0.012	0.259	
	200 mg	-0.020	0.014	-1.450		-0.011	0.012	-0.947	
Visit					0.066 .				**0.009 ***
	V2	0.034	0.014	2.417		0.023	0.012	1.953	
	V3	0.028	0.014	2.039		0.029	0.012	2.401	
	V4	0.037	0.014	2.650		0.044	0.012	3.613	
	V5	0.023	0.014	1.657		0.024	0.012	2.027	
T0		0.383	0.053	7.290	**3.097e-13 *** **	0.295	0.037	7.885	**3.135e-15 *** **
PEA									
(Intercept)		0.559	0.093	5.990		0.489	0.085	5.741	
Dose					0.809				0.211
	20 mg	-0.033	0.049	-0.677		-0.031	0.046	-0.662	
	50 mg	-0.027	0.051	-0.522		-0.064	0.047	-1.359	
	100 mg	-0.062	0.051	-1.231		-0.023	0.047	-0.489	
	200 mg	-0.040	0.050	-0.802		-0.104	0.047	-2.199	
Visit					0.504				0.087 .
	V2	0.004	0.051	0.081		0.052	0.047	1.115	
	V3	-0.073	0.050	-1.481		0.101	0.049	2.076	
	V4	-0.002	0.051	-0.031		0.101	0.048	2.101	
	V5	-0.025	0.050	-0.503		0.008	0.048	0.171	
T0		0.312	0.043	7.233	**4.733e-13 *** **	0.228	0.037	6.209	**5.334e-10 *** **
OEA									

(Intercept)		0.461	0.074	6.232		0.421	0.070	6.029	
Dose					0.901				0.085 .
	20 mg	-0.044	0.046	-0.947		-0.047	0.041	-1.138	
	50 mg	-0.038	0.048	-0.788		-0.108	0.042	-2.583	
	100 mg	-0.034	0.047	-0.710		-0.062	0.042	-1.468	
	200 mg	-0.026	0.047	-0.547		-0.094	0.042	-2.235	
Visit					0.721				0.175
	V2	-0.003	0.048	-0.071		0.038	0.042	0.897	
	V3	-0.041	0.046	-0.890		0.083	0.044	1.906	
	V4	0.026	0.048	0.549		0.095	0.044	2.162	
	V5	-0.008	0.047	-0.174		0.031	0.043	0.733	
T0		0.228	0.038	6.041	**1.534e-09 *****	0.184	0.031	5.899	**3.656e-09 *****

AEA, anandamide; Cmin, Minimum Concentration; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; SE, Standard Error; T0, Pre-ingestion timepoint
. Signifies p < 0.100; * Signifies p < 0.050; * Signifies p < 0.001**

Supplementary Table S3: Results from GLMM model 2 showing the association between AUCi and CBD doses for each analyte.

AEA					
		Estimate	**SE**	**t value**	**p-value**
(Intercept)		37.162	4.711	7.888	
Dose					0.810
	20 mg	-2.096	2.816	-0.744	
	50 mg	-2.062	2.845	-0.725	
	100 mg	-0.439	2.828	-0.155	
	200 mg	-3.046	2.845	-1.071	
Visit					0.014 *
	V2	6.675	2.879	2.319	
	V3	5.077	2.859	1.776	
	V4	9.975	2.873	3.472	
	V5	4.972	2.827	1.759	
Sex		-11.813	2.402	-4.918	**8.76e-07 *** **
T0		-187.273	9.675	-19.356	**< 2e-16 *** **
PEA					
(Intercept)		281.659	24.3	11.591	
Dose					**0.019 ***
	20 mg	-29.417	12.485	-2.356	
	50 mg	-37.859	12.588	-3.008	
	100 mg	-19.906	12.523	-1.59	
	200 mg	-34.848	12.584	-2.769	
Visit					0.854
	V2	13.386	12.738	1.051	
	V3	6.295	12.73	0.495	
	V4	11.507	12.8	0.899	
	V5	6.291	12.577	0.5	
Sex		-45.755	15.478	-2.956	**0.003 ***
T0		-221.857	10.299	-21.542	**< 2e-16 *** **
OEA					
(Intercept)		216.780	19.103	11.348	
Dose					**0.014 ***
	20 mg	-23.890	11.073	-2.158	
	50 mg	-38.653	11.163	-3.463	
	100 mg	-20.756	11.116	-1.867	
	200 mg	-25.614	11.155	-2.296	
Visit					0.511
	V2	8.088	11.409	0.709	
	V3	16.192	11.312	1.431	
	V4	18.641	11.525	1.617	
	V5	11.630	11.211	1.037	

Sex		-29.958	11.812	-2.536	**0.011 ***
T0		-237.486	8.821	-26.922	**< 2e-16 *****
AEA, anandamide; AUCi, Area Under the Curve to increase; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; SE, Standard Error; T0, Pre-ingestion timepoint . Signifies p < 0.100; * Signifies p < 0.050; * Signifies p < 0.001**					

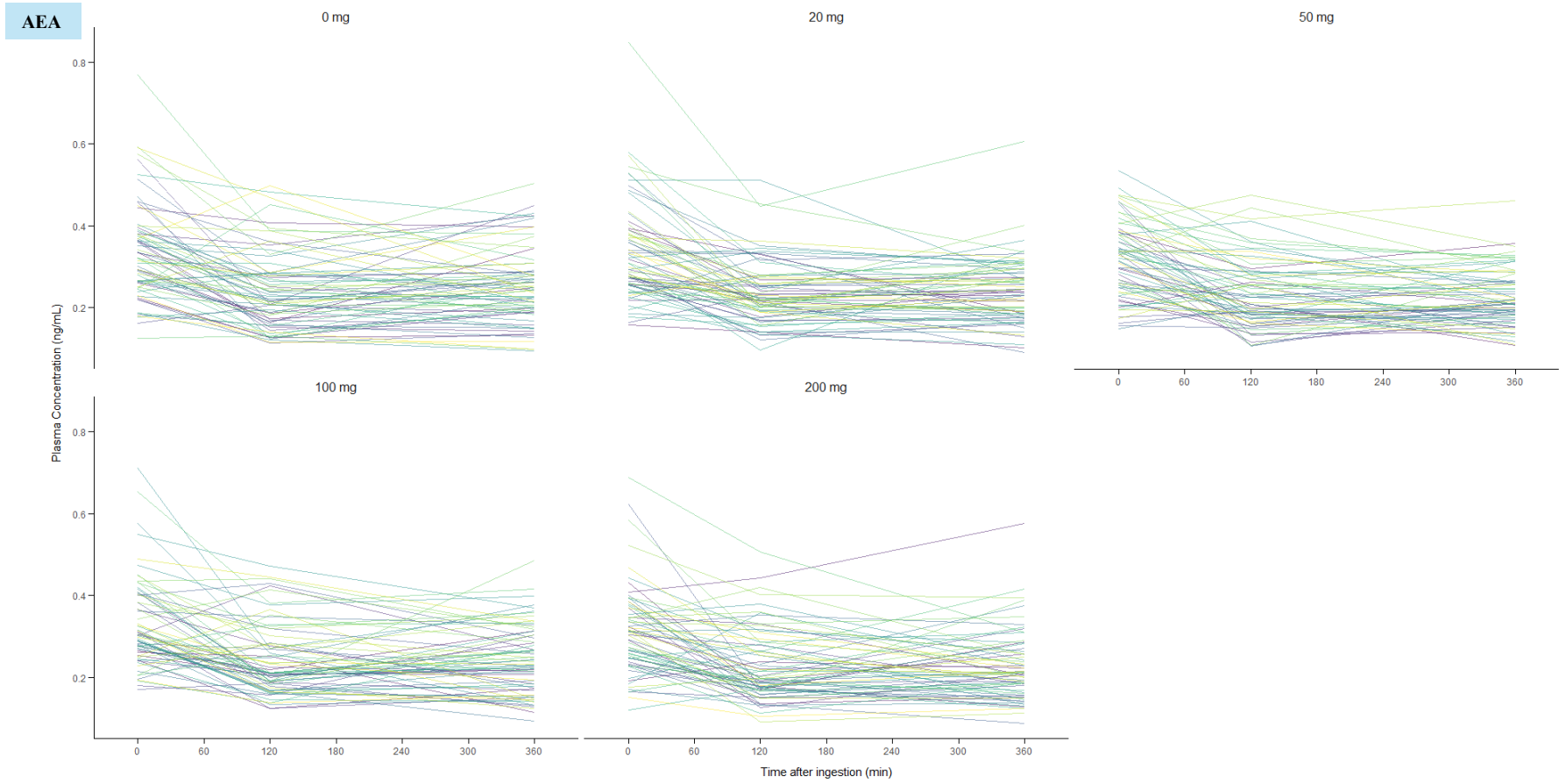
Visit effect: A significant visit effect was observed for AEA AUCi ($p < 0.001$), with post-hoc analyses showing this effect specifically for visit 4 compared to visit 1 (Cohen's $d = -0.585$, 95% CI [-0.9318, -0.2389]).

Supplementary Table S4: *Post-hoc* exploratory subgroup analysis by sex using Model 2.

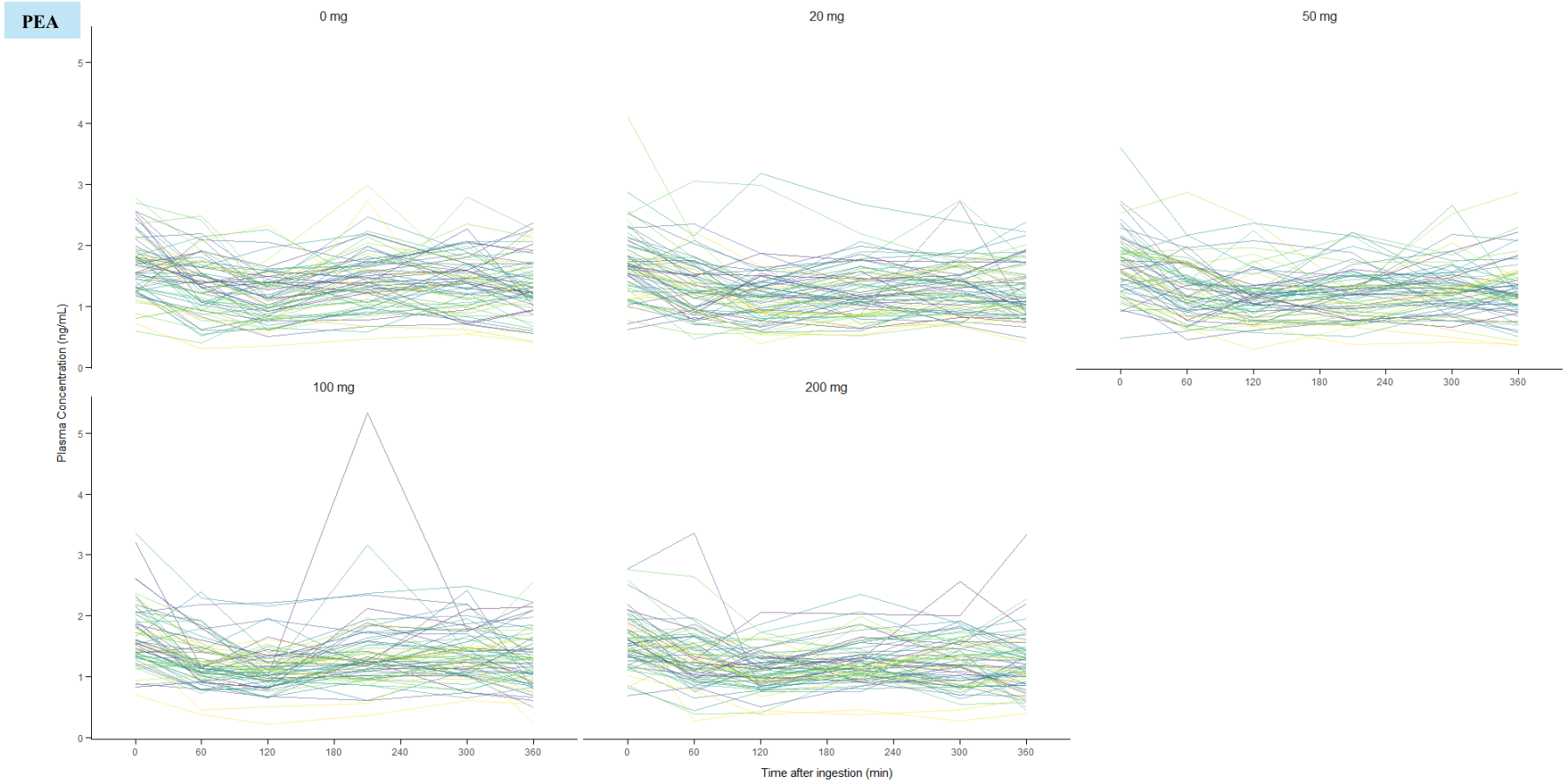
		Males				Females			
AEA									
		Estimate	**SE**	**t value**	**p-value**	**Estimate**	**SE**	**t value**	**p-value**
(Intercept)		31.530	7.234	4.358		28.323	6.029	4.697	
Dose					0.575				0.917
	20 mg	-4.292	4.140	-1.037		0.151	3.918	0.039	
	50 mg	-2.240	4.355	-0.514		-1.108	3.983	-0.278	
	100 mg	2.021	4.251	0.475		-1.959	3.981	-0.492	
	200 mg	-2.907	4.179	-0.696		-3.169	3.992	-0.794	
Visit					0.082 .				0.228
	V2	9.346	4.272	2.188		4.786	4.024	1.189	
	V3	6.037	4.159	1.452		4.559	4.056	1.124	
	V4	11.512	4.248	2.710		9.591	4.059	2.363	
	V5	6.324	4.230	1.495		4.201	4.020	1.045	
T0		-173.897	16.155	-10.765	**< 2e-16 *****	-195.124	12.463	-15.657	**<2e-16 *****
PEA									
(Intercept)		256.967	32.298	7.956		247.416	33.558	7.373	
Dose					0.586				**0.050 .**
	20 mg	-24.797	16.551	-1.498		-30.848	18.408	-1.676	
	50 mg	-21.037	17.282	-1.217		-44.417	18.717	-2.373	
	100 mg	-22.566	16.982	-1.329		-15.463	18.721	-0.826	
	200 mg	-19.515	16.700	-1.169		-48.517	18.689	-2.596	
Visit					0.502				0.652
	V2	8.931	17.304	0.516		19.378	18.667	1.038	
	V3	-17.737	16.661	-1.065		23.262	19.248	1.209	
	V4	4.195	17.187	0.244		18.715	19.106	0.980	
	V5	9.207	16.954	0.543		3.184	18.945	0.168	
T0		-206.866	14.973	-13.816	**<2e-16 *****	-229.778	14.470	-15.880	**< 2e-16 *****
OEA									
(Intercept)		187.325	25.163	7.445		200.050	27.570	7.257	

Dose					0.724				**0.025 ***
	20 mg	-12.610	15.044	-0.838		-31.310	16.100	-1.944	
	50 mg	-21.349	15.707	-1.359		-49.550	16.360	-3.028	
	100 mg	-15.261	15.428	-0.989		-27.930	16.370	-1.706	
	200 mg	-8.340	15.195	-0.549		-43.880	16.360	-2.682	
Visit					0.779				0.315
	V2	5.594	15.713	0.356		14.530	16.590	0.876	
	V3	-8.621	15.102	-0.571		34.210	17.060	2.005	
	V4	11.356	15.560	0.730		28.520	17.160	1.662	
	V5	3.129	15.398	0.203		20.300	16.740	1.213	
T0		-217.183	12.966	-16.751	**<2e-16 *** **	-246.030	12.340	-19.940	**< 2e-16 *** **

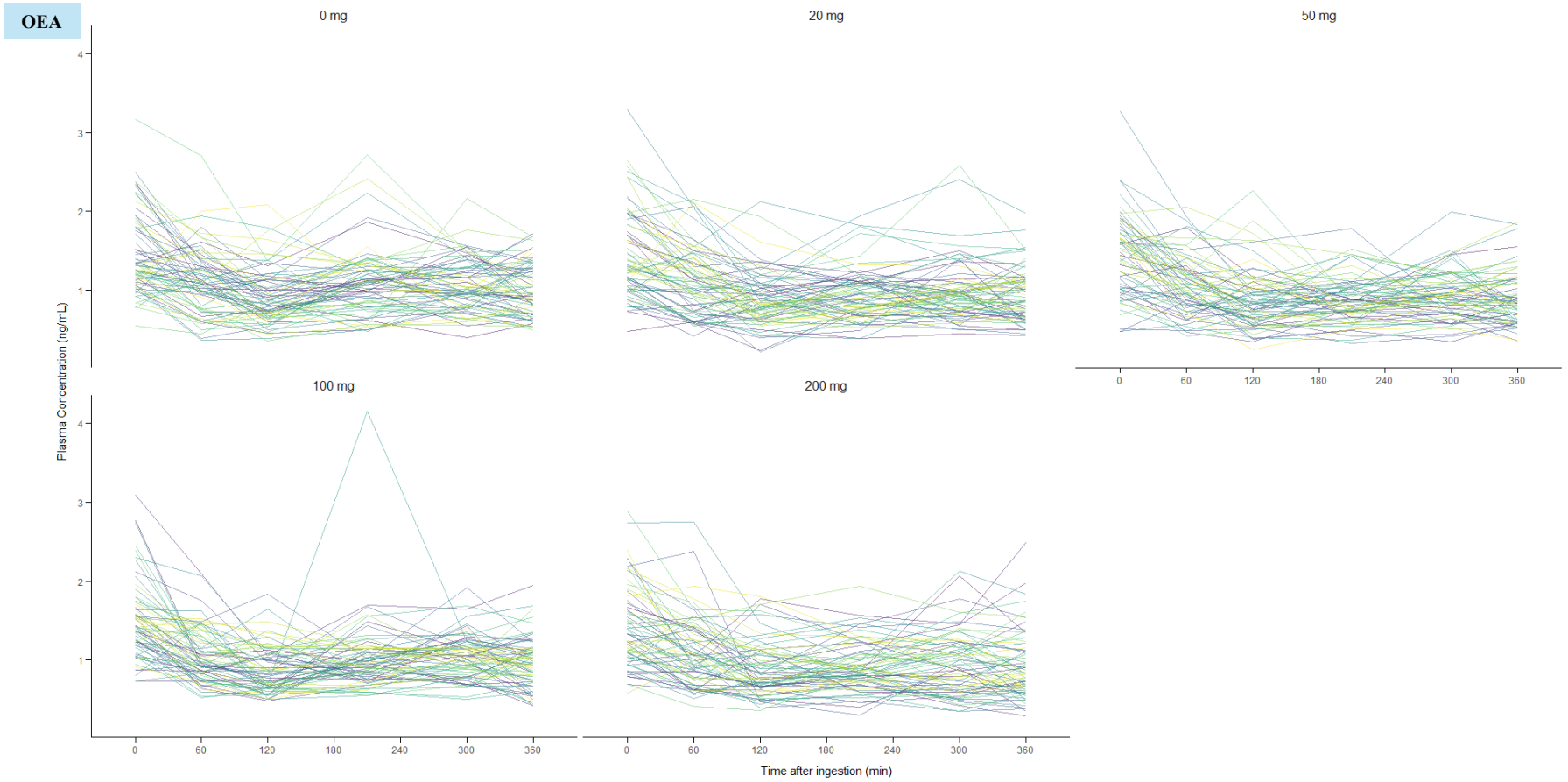
AEA, anandamide; Cmin, Minimum Concentration; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; SE, Standard Error; T0, Pre-ingestion timepoint
. Signifies p < 0.100; * Signifies p < 0.050; * Signifies p < 0.001**



Supplementary Figure S1. Individual Trajectories of Plasma AEA Levels Across Timepoints by CBD Dose.



Supplementary Figure S2. Individual Trajectories of Plasma PEA Levels Across Timepoints by CBD Dose.



Supplementary Figure S3. Individual Trajectories of Plasma OEA Levels Across Timepoints by CBD Dose.

Title: **LC-MS/MS Method for the determination of Arachidonoyl ethanolamide in Human Plasma.**

Project: 2023-10502_CBD-LD_ING

François-Olivier Hébert Ph.D.
Research Associate
Faculty of Medecine
Departement of Psychiatry and Addiction
Université de Montréal

Didier Jutras-Aswad M.D., M.Sc.
Researcher-clinician Associate Professor
Faculty of Medecine
Departement of Psychiatry and Addiction
Université de Montréal

Author:

Fleur Gaudette, M.Sc. Chemist
Supervisor
Pharmacokinetics Core Facility
CRCHUM

Date: 2024-11-26

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

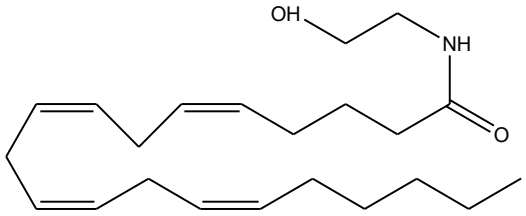
Arachidonoyl ethanolamide in Human Plasma

(*Dr. Didier Jutras-Aswad*)

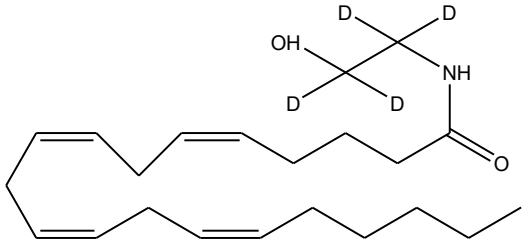
Chemical Composition

The chemical structures, formulae, and molecular weights of arachidonoyl ethanolamide (d_0-AEA) and it's isotopologues d_4-AEA and d_8-AEA are depicted in figure 1.

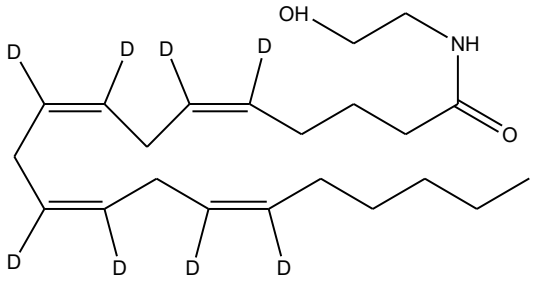
Figure 1: <u>Chemical structures, formulae, and molecular weights of d_0-AEA, d_4-AEA and d_8-AEA.</u>



d_0-AEA (target analyte)
$C_{22}H_{37}NO_2$
Mol. Wt.: 347.53



d_4-AEA (surrogate analyte)
$C_{22}H_{33}D_4NO_2$
Mol. Wt.: 351.56



d_8-AEA (internal standard)
$C_{22}H_{29}D_8NO_2$
Mol. Wt.: 355.58

Analytical Procedure

Quantitation of biomarkers by LC–MS/MS is complicated by the presence of endogenous analytes in the matrix being analyzed. Due to the endogenous nature of d_0-AEA, a surrogate analyte approach will be used. This approach involves the use of two stable-isotope-labeled standards (d_4-AEA and d_8-AEA) to be used as a surrogate analyte and internal standard enabling calibration in the actual biological matrix.

Reagents

d_0-AEA, d_4-AEA and d_8-AEA were purchased from Cayman Chemical (Ann Arbor, MI, USA) and received in ampoules as certified solutions containing 50.0 mg/mL, 5.0 mg/mL and 1.0 mg/mL dissolved in ethanol for d_0-AEA, d_4-AEA and in methyl acetate for d_8-AEA. Human plasma containing EDTA as anticoagulant was purchased from Bioreclamation (Westbury, NY, USA). Other chemicals, including, methanol, acetonitrile, ammonium formate and water were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Sample preparation

Using a protein precipitation as sample preparation technique, d_0-AEA was extracted from human plasma. One thousand microliters of internal standard solution (2.4 ng/mL d_8-AEA in methanol) was added to an aliquot of two hundred and fifty microliters of sample. The sample was vortexed for approximately 5 seconds and let stand for a period of 10 minutes, then centrifuged at 16000 *g* for 10 minutes. The supernatant was transferred into a clean 16 x 100 mm borosilicate tube and evaporated to dryness at 40°C under a gentle stream of nitrogen. The dried extract was resuspended with 80 µL of 50% (v/v) methanol in water solution and transferred to an injection vial for analysis.

Chromatographic conditions

A gradient mobile phase was used with an Agilent Zorbax Eclipse Plus C18 RRHD analytical column (100 x 2.1 mm I.D., 1.8 µm) operating at 40°C. The initial mobile phase condition consisted of acetonitrile containing 0.1% (v/v) formic acid and 10 mM of ammonium formate in type 1 water pH 3.0 at a ratio of 60:40, respectively, and this ratio was maintained for 1 min. From 1 to 5 min a linear gradient was applied up to a ratio of 95:5 and maintained for 1 min. At 6.1 min, the mobile phase composition was reverted to the original conditions and the column was allowed

to equilibrate for 2 min for a total run time of 8 min. The flow rate was fixed at 300 µL/min and d_0-AEA and its isotopically labelled standards (d_4-AEA and d_8-AEA) eluted at 5.3 min. Five microliters of the extracted sample was injected and the total run time was set at 12 min.

Mass spectrometric conditions

The mass spectrometer was interfaced with the UPLC system using a pneumatic assisted heated electrospray ion source. MS detection was performed in positive ion mode, using selected reaction monitoring (SRM). In order to optimize the MS/MS parameters, standard solutions of d_0-AEA, d_4-AEA and d_8-AEA were infused into the mass spectrometer. The following parameters were obtained. Nitrogen was used for the sheath and auxiliary gases and was set at 50 and 15 arbitrary units. The HESI electrode was set to 3500V. The capillary and vaporizer temperatures were set at 350°C and 400°C, respectively. Argon was used as collision gas at a pressure of 2.5 mTorr. The precursor-ion reactions and the collision energy for d_0-AEA, d_4-AEA and d_8-AEA are in table 1. Total cycle time was set at 0.25 seconds. Peak width of Q1 and Q3 were both set at 0.7 FWHM.

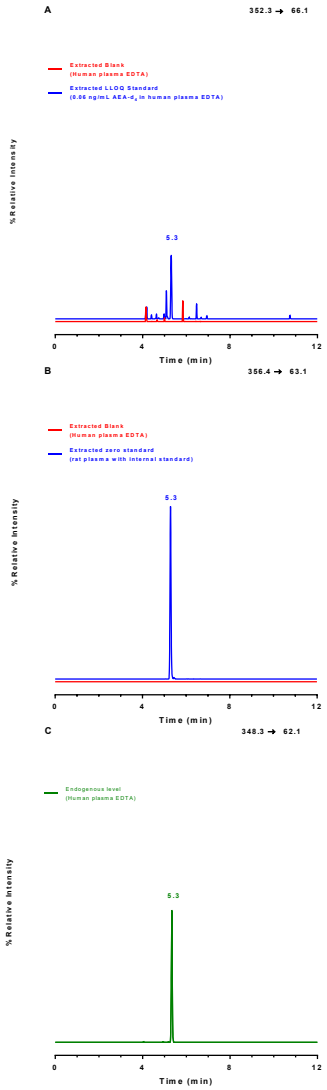
Table 1: Mass spectrometry operating conditions

Compound	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
d_0-AEA	348.3	62.1	15	64
d_4-AEA	352.3	66.1	15	65
d_8-AEA	356.3	63.1	15	66

Chromatograms

Representative chromatograms obtained upon analysis of blank human plasma, a zero standard and an extracted LLOQ standard are shown in figure 2. The overlay of an extracted LLOQ standard (blue line) and an extracted blank human plasma sample (red line) for the SRM transition of d_4-AEA are shown in figure 2a. An overlay of an extracted zero standard (blue line) and an extracted blank human plasma sample (red line) for the SRM transition of d_8-AEA are in figure 2b and the endogenous level of d_0-AEA in the blank human plasma lot extracted are shown in figure 2c.

Figure 2: **Reconstructed ion chromatograms for d_4-AEA m/z 352.3 → 66.1, d_8-AEA (IS) 356.4 → 63.1 and d_0-AEA 348.3 → 62.1**



Title: **LC-MS/MS Method for the determination of Palmitoyl Ethanolamide and Oleoyl Ethanolamide in Human Plasma.**

Project: 2023-10502_CBD-LD_ING

François-Olivier Hébert Ph.D.
Research Associate
Faculty of Medecine
Departement of Psychiatry and Addiction
Université de Montréal

Didier Jutras-Aswad M.D., M.Sc.
Researcher-clinician Associate Professor
Faculty of Medecine
Departement of Psychiatry and Addiction
Université de Montréal

Author:

Fleur Gaudette, M.Sc. Chemist
Supervisor
Pharmacokinetics Core Facility
CRCHUM

Date: 2024-11-26

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

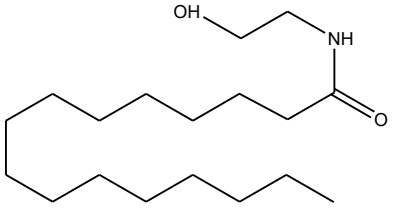
Palmitoyl Ethanolamide and Oleoyl Ethanolamide in Human Plasma

(*Dr. François-Olivier Hébert/Dr. Didier Jutras-Aswad*)

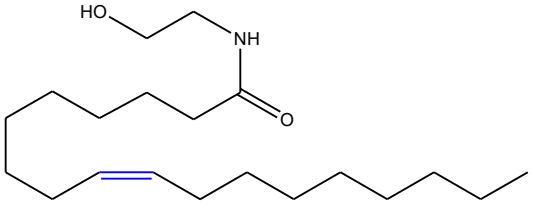
Chemical Composition

The chemical structures, formulae, and molecular weights of palmitoyl ethanolamide (d_0-PEA), oleoyl ethanolamide (d_0-OEA) and the internal standards (d_4-PEA and d_4-OEA), are depicted in Figure 1.

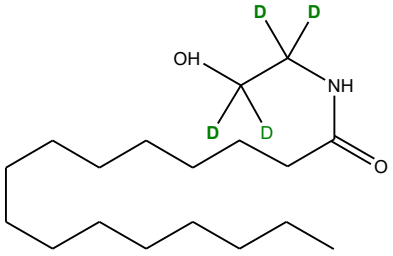
Figure 3: Chemical structures, formulae, and molecular weights of d_0-PEA, d_0-OEA and the internal standards (d_4-PEA & d_4-OEA)



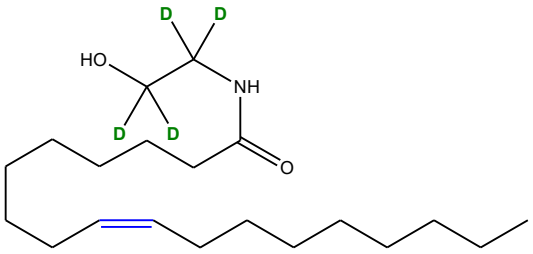
d_0-Palmitoyl Ethanolamide
d_0-PEA (target analyte)
$C_{18}H_{37}NO_2$
Mol. Wt.: 299.49



d_0-Oleoyl Ethanolamide
d_0-OEA (target analyte)
$C_{20}H_{39}NO_2$
Mol. Wt.: 325.53



d_4-Palmitoyl Ethanolamide
d_4-PEA (internal standard)
$C_{18}H_{33}D_4NO_2$
Mol. Wt.: 303.51653



d_4-Oleoyl Ethanolamide
d_4-OEA (internal analyte)
$C_{20}H_{35}D_4NO_2$
Mol. Wt.: 329.55

Analytical Procedure

Quantitation of biomarkers by LC–MS/MS is complicated by the presence of endogenous analytes in the matrix being analyzed. Due to the endogenous nature of d_0-PEA and d_0-OEA, a surrogate matrix approach will be used. This approach involves using an authentic standard spiked into a surrogate matrix devoid of the target analyte.

Reagents

d_0-PEA, d_0-OEA, d_4-PEA, and d_4-OEA were purchased from Cayman Chemical (Ann Arbor, MI, USA) and d_4-PEA and d_4-OEA were received in ampoules as certified solutions containing 1.0 mg/mL dissolved in ethanol. Hexanes, acetonitrile, methanol and water were purchased from EMD Millipore Corporation (Burlington, MA, USA). Isopropyl alcohol (IPA) was purchased from Mat Laboratories (Québec, QC, Canada), Ethanol (EtOH) was purchase from Anachemia through VWR (Mississauga, On, Canada). Other chemicals, including, ammonium formate, and formic acid were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

Sample preparation

Using a liquid-liquid extraction procedure, d_0-PEA and d_0-OEA were extracted from human plasma. Twenty five µL of internal standard solution (72.0 ng/mL of d_4-PEA and d_4-OEA in EtOH) was added to an aliquot of 300 µL of plasma in a 13 x 100 mm borosilicate screw cap tube. Two mL of extraction solvent (90:10 hexanes:IPA) was added to the sample followed by two hundred and fifty µL of water. The tube was capped and gently mixed by rotation for 10 minutes. The sample was then centrifuged at approximately 4500 *g* for 10 min at 5°C and the organic layer was transferred into a clean 13 x 100 mm borosilicate tube and evaporated to dryness at 30°C under a gentle stream of nitrogen (5-10 psi). The dried extract was re-suspended with 75 µL of reconstitution solution (50:50 methanol:H_2O) and transferred to a micro injection vial for analysis.

Chromatographic conditions

A gradient mobile phase was used with an Agilent Zorbax Eclipse Plus C18 RRHD analytical column (100 x 2.1 mm I.D., 1.8 µm) and Zorbax Eclipse Plus C18 (5.0 x 2.1 mm I.D., 1.8 µm) guard operating operating at 40°C. The initial mobile phase condition consisted of acetonitrile containing 0.1 % (v/v) formic acid and 10 mM of ammonium formate in type 1 water pH 3.0 at a

ratio of 60:40, respectively, and this ratio was maintained for 0.5 min. From 0.5 to 14 min a linear gradient was applied up to a ratio of 95:5 and maintained for 1 min. At 15.1 min, the mobile phase composition was reverted to the original conditions and the column was allowed to equilibrate for 5 min for a total run time of 20 min. The flow rate was fixed at 300 µL/min and five microliters of the extracted sample was injected.

Mass spectrometric conditions

The mass spectrometer was interfaced with the UPLC system using a pneumatic assisted heated electrospray ion source. MS detection was performed in positive ion mode, using selected reaction monitoring (SRM). In order to optimize the MS/MS parameters, standard solutions of d_0-PEA, d_0-OEA, d_4-PEA and d_4-OEA were infused into the mass spectrometer. The following parameters were obtained. Nitrogen was used for the sheath and auxiliary gases and was set at 50 and 15 arbitrary units. The HESI electrode was set to 3500V. The capillary and vaporizer temperatures were set at 350°C and 400°C, respectively. Argon was used as collision gas at a pressure of 2.5 mTorr. The precursor-ion reactions and the collision energy for PEA, OEA, d_4-PEA and d_4-OEA are in table 1. Total cycle time was set at 0.25 seconds. Peak width of Q1 and Q3 were both set at 0.7 FWHM.

Table 2: Mass spectrometry operating conditions

Compound	Polarity	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)
d_0-PEA	Positive	300.3	62.1	13.2	59
d_4-PEA	Positive	304.3	66.1	13.2	64
d_0-OEA	Positive	326.3	62.1	14.9	68
d_4-OEA	Positive	330.3	66.1	14.9	64

Chromatograms

Representative chromatograms obtained upon analysis of an extracted blank surrogate matrix, an extracted LLOQ standard prepared at 0.30 ng/mL for d_0-PEA and d_0-OEA in surrogate matrix and an extracted human plasma sample are shown in Figure 2.

Figure 4: **Reconstructed ion chromatograms for d_0-PEA, d_0-OEA,** d_4-PEA and d_4-OEA**. (A) and (C) represent an overlay of an extracted blank surrogate matrix (red line), an LLOQ standard (blue line) and an extracted human plasma sample (green line) for d_0-PEA and d_0-OEA m/z 300 → 62 and 326 → 62, respectively. (B) and (D) represent an overlay of an extracted blank surrogate matrix (red line) and an extracted zero standard (blue line) for** d_4-PEA d_4-OEA **m/z 304 → 66** and **330 → 66, respectively**