

**SUPPLEMENTARY MATERIAL**

## **Statistical Appendix – Treatment of Buprenorphine**

It may be of assistance to the reader to consider the treatment of buprenorphine in the present study in some further detail.

Supplementary Figure 1 shows the comparison of buprenorphine and cannabis to the control group, with all controls considered as a single group conflating tobacco smokers and non-smokers together.

Supplementary Figure 2 does the same exercise but now separating out the two groups.

Supplementary Figure 3 compares the four inhalational using groups to buprenorphine.

From these figures it is clear that the quantitative effect of buprenorphine to apparently accelerate the RA/CA ratio and therefore the rate of cardiovascular aging is quantitatively small.

Supplementary Figure 4 is a similar figure, but also includes methadone for comparison, whose cardiovascular toxicity has been described in a previous report <sup>1</sup>.

One way to do this is by including it as a variable independent of cannabis and then by comparing all the groups to non-smoking controls. This was done first as a cross-sectional linear regression regressing the RA/CA ratio against interactions between CA, BMI and drug category. In this regard a model cubic in age was shown to be the optimal model (RSS linear model 131.84, cubic 126.51,  $F=2.163$ ,  $DF=22$ ,  $P=0.0014$ ). In this model, shown in Supplementary Table 1, interactive terms with the square and cube of CA, BMJI and cannabis use. Terms including buprenorphine then were not significant.

A similar exercise was performed for the longitudinal dataset. In this exercise the RA/CA ratio was regressed in a mixed effects model against the CA, time, BMI and drug category. On this occasion the linear model (Supplementary Table 2) was significantly better than the cubic model, but the linear model accounted for more of the AIC but not significantly so than a model quadratic in CA (AIC = 1071.19, 1061.25,  $DF=27$  and  $24$ ,  $L.Ratio=3.93$ ,  $P=0.2682$ ). The result of the quadratic model is shown in Supplementary Table 3.

The effects of cannabis and buprenorphine were next considered as continuous variables. When the RA/CA ratio was regressed against CA, cannabis duration and cannabis use in a cross-sectional model, the interaction of all three was highly significant ( $P=0.0042$ , Supplementary Table 4).

When the same exercise was repeated over time in the longitudinal dataset in a mixed effects model again terms in cannabis duration and buprenorphine dose were significant, from  $P=0.0057$ , Supplementary Table 5).

No data on the duration for which buprenorphine had been used was available. In clinical practice the use of buprenorphine by many patients is highly variable, with periods of use and periods of non-use commonplace.

Hence in summary it may be said that both buprenorphine and cannabis have an effect on cardiovascular aging but the effect of buprenorphine appears to be small, whilst the effect of

cannabis appears to be quantitatively much larger. In considering the results of the regression studies, the size of the study groups must be borne in mind. The size of the buprenorphine group in the longitudinal study is 1109 and the size of the cannabis groups in this study is 31. To some extent this difference in study group size explains the statistical results described.

### **References**

1. Reece A.S., Hulse G.K. Impact of Opioid Pharmacotherapy on Arterial stiffness and Vascular Ageing: Cross-sectional and Longitudinal Studies. *Cardiovascular Toxicology* 2013;13:254-66.

**Supplementary Table 1 – Linear Regression of VA/CA**

Parameter	Parameter Values				Model Values				
	Estimate	Std. Error	t	P	Adj. R Squ.	F	DF	P	
(Age) <sup>2</sup> : BMI	-0.2350	0.0503	-4.669	<0.0001	0.424	2.61	32, 1130	<0.0001	***
(Age) <sup>3</sup> : BMI	-0.1262	0.0384	-3.282	0.0011					**
(Age) <sup>2</sup> : BMI: Cannabis	144.2000	46.2700	3.117	0.0019					**
Age: Cannabis	184.5000	69.8700	2.641	0.0084					**
(Age) <sup>2</sup> : Cannabis	-222.4000	87.2900	-2.548	0.0110					*
(Age) <sup>3</sup> : Cannabis	50.8700	20.5700	2.473	0.0136					*
BMI: Cannabis	-38.4900	16.2700	-2.366	0.0181					*
BMI	0.2307	0.1008	2.289	0.0223					*

**Supplementary Table 2.: –Linear Regression of VA/CA**

**Final Model Linear in Chronologic Age**

Parameter Values						Model		
Statistical Measure	Value	Std.Error	DF	t-value	P	AIC	BIC	Log.Lik
Age: BMI	-0.0722	0.0157	716	-4.6071	<0.0001	1196.08	1346.524	-571.0399
BMI	0.4888	0.1219	716	4.0112	0.0001			
Days: None	-0.1333	0.0456	716	-2.9241	0.0036			
Age: Days: None	0.0353	0.0122	716	2.8934	0.0039			
Age: BMI: Tobacco	0.1127	0.0394	716	2.8646	0.0043			
BMI: Both	-1.4492	0.5312	716	-2.7284	0.0065			
Both	3.5170	1.3237	716	2.6570	0.0081			
Age: Days: Buprenorphine	0.0291	0.0110	716	2.6518	0.0082			
Days: Buprenorphine	-0.0965	0.0391	716	-2.4668	0.0139			
BMI: Tobacco	-0.6647	0.2975	716	-2.2340	0.0258			
Age: BMI: Buprenorphine	0.0498	0.0241	716	2.0640	0.0394			

**Supplementary Table 3 – Mixed Effects Linear Regression of VA/CA**

**Final (Optimum) Model Quadratic in Chronologic Age**

Parameter Values						Model		
Statistical Measure	Value	Std.Error	DF	t-value	P	AIC	BIC	Log.Lik
Age: BMI	-0.1397	0.0266	719	-5.2511	<0.0001	1174.824	1308.589	-563.4121
BMI	0.1928	0.0576	719	3.3492	0.0009			
Age: BMI: Cannabis	0.1745	0.0675	719	2.5868	0.0099			
(Age) <sup>2</sup> : BMI: Buprenorphine	0.1174	0.0482	719	2.4350	0.0151			
Age: BMI: Tobacco	0.0788	0.0346	719	2.2767	0.0231			
(Age) <sup>2</sup> : Days	0.0466	0.0208	719	2.2347	0.0257			
(Age) <sup>2</sup> : BMI: Tobacco	0.1703	0.0768	719	2.2179	0.0269			
Age: Days: Buprenorphine	0.0265	0.0121	719	2.1886	0.0289			
Age: BMI: Buprenorphine	0.0429	0.0205	719	2.0896	0.0370			

**Supplementary Table 4 – Linear Regression of VA/CA**

**Linear Model, Continuous Variables**

<b>Parameter</b>	<b>Parameter Values</b>				<b>Model Values</b>			
	<b>Estimate</b>	<b>Std. Error</b>	<b>t</b>	<b>P</b>	<b>Adj. R Squ.</b>	<b>F</b>	<b>DF</b>	<b>P</b>
Cannabis Use: Cannabis Duration: Buprenorphine Dose	0.0137	0.0036	3.807	0.0042	0.5744	14.49	1,9	0.0042

\*\*

**Supplementary Table 5 – Mixed Effects Linear Regression of VA/CA**

**Linear Model, Continuous Variables**

Parameter Values						Model		
Statistical Measure	Value	Std.Error	DF	t-value	P	AIC	BIC	Log.Lik
Cannabis Years: Buprenorphine Dose	0.0551	0.0169	14	3.2635	0.0057	23.7869	29.6771	-6.8934
Cannabis Years	-0.3700	0.1339	10	-2.7638	0.0200			



**Supplementary Table 6.: Clinical Laboratory Values**

<b>Parameter</b>	<b>None (534)</b>	<b>Tobacco (506)</b>	<b>THC (11)</b>	<b>Both (112)</b>	<b>THC-None</b>	<b>THC-Tob</b>	<b>THC-Both</b>
					P	P	P
Haemoglobin (g/dl)	143.02 (1.13)	144.53 (0.87)	153.25 (7.19)	144.22 (1.69)	0.1117	0.2348	0.1999
<i><b>Immune</b></i>							
WCC (x10 <sup>9</sup> /L)	7.21 (0.28)	8.25 (0.13)	9.05 (0.98)	8 (0.21)	0.2346	0.4734	0.2310
Platelets (x10 <sup>9</sup> /L)	259.58 (5.75)	265.55 (3.87)	279.75 (33.35)	257.77 (8.09)	0.5305	0.6627	0.5129
Neutrophils (x10 <sup>9</sup> /L)	4.2 (0.24)	4.85 (0.12)	5.675 (0.96)	4.609375 (0.18)	0.2697	0.4198	0.1662
Lymphocytes (x10 <sup>9</sup> /L)	2.21 (0.07)	2.54 (0.05)	2.575 (0.41)	2.53 (0.12)	0.3365	0.9298	0.9302
Monocytes (x10 <sup>9</sup> /L)	0.56 (0.03)	0.61 (0.01)	0.58 (0.09)	0.62 (0.02)	0.9237	0.6988	0.6766
ESR (mm/hr)	9 (0.88)	11.97 (0.9)	10 (2)	11.4 (1.69)	0.8712	0.8133	0.8555
hs-CRP (mg/L)	3.31 (0.73)	6.55 (0.73)	14.75 (13.25)	8.17 (1.64)	0.0320	0.2407	0.4173
<i><b>Electrolytes</b></i>							
Sodium (mmol/l)	140.19 (0.22)	139.99 (0.14)	139.75 (0.25)	139.8 (0.36)	0.2230	0.8378	0.9718
Potassium (mmol/l)	4.36 (0.04)	4.41 (0.03)	4.58 (0.11)	4.37 (0.05)	0.3420	0.4468	0.2759
Chloride (mmol/l)	102.68 (0.21)	101.82 (0.14)	101.75 (1.25)	102.21 (0.36)	0.4446	0.9522	0.7603
Bicarbonate (mmol/l)	28.23 (0.23)	28.46 (0.16)	26.5 (1.85)	27.8 (0.36)	0.1969	0.1692	0.3947
Anion Gap (mmol/l)	13.61 (0.23)	14.16 (0.17)	16.25 (1.18)	14.17 (0.41)	0.0446	0.1547	0.2255
<i><b>Metabolic</b></i>							
Glucose (mmol/l)	5.15 (0.14)	4.98 (0.06)	4.8 (0.4)	5.15 (0.12)	0.6657	0.7200	0.4966
Urea (mmol/l)	4.92 (0.1)	4.94 (0.09)	4.65 (0.74)	4.95 (0.2)	0.6271	0.6964	0.7102
Creatinine (mmol/l)	75.86 (1.56)	79.46 (0.88)	81.75 (4.77)	79.12 (1.7)	0.5066	0.7603	0.7096
Urate (mmol/l)	0.3 (0.01)	0.3 (0.01)	0.27 (0.03)	0.3 (0.01)	0.4271	0.3656	0.3819

Total Bilirubin (mmol/l)	10.86 (0.63)	8.8 (0.35)	6.25 (1.7)	8.77 (0.79)	0.1991	0.4005	0.4386
Alkaline Phosphatase	74.76 (2.86)	82.68 (1.7)	81 (14.02)	77.92 (2.86)	0.7006	0.9086	0.7996
ALT (IU/L)	34.75 (2.54)	57.48 (4.38)	23.5 (3.2)	47.14 (11.82)	0.4319	0.3659	0.6264
AST (IU/L)	28.99 (1.75)	43.82 (2.81)	24.75 (6.32)	32.79 (3.41)	0.6684	0.4286	0.5685
LDH (IU/L)	173.13 (3.46)	189.88 (3.05)	168.5 (8.18)	183.68 (5.64)	0.8117	0.4137	0.5140
GGT (IU/L)	36.11 (6.64)	44.16 (3.84)	40.25 (18.63)	27.61 (2.71)	0.9121	0.9055	0.2882
Total Protein (g/dl)	73.32 (0.42)	74.4 (0.33)	75.25 (4.66)	74.18 (0.69)	0.7074	0.7684	0.7214
Albumin (g/dl)	43.34 (0.27)	42.72 (0.18)	43.25 (2.69)	43 (0.41)	0.9523	0.7257	0.8873
Globulin (g/dl)	29.98 (0.4)	31.69 (0.29)	32 (2.48)	31.18 (0.64)	0.3715	0.9014	0.7590
<b><i>Lipid</i></b>							
Cholesterol (mmol/l)	5.03 (0.09)	4.61 (0.06)	4.575 (0.37)	4.47 (0.12)	0.3754	0.9465	0.8303
Triglycerides (mmol/l)	1.45 (0.09)	1.41 (0.05)	1.825 (0.38)	1.35 (0.09)	0.4899	0.3052	0.2216
LDL (mmol/l)	3.11 (0.12)	2.57 (0.08)	2.02 ()	2.51 (0.14)	0.2395	0.5282	0.5706
HDL (mmol/l)	1.26 (0.05)	1.22 (0.03)	1.77 ()	1.12 (0.04)	0.1895	0.1137	0.0090
<b><i>Infectious Agents</i></b>							
HBsAg	22 (42%)	57 (23%)	0 (0%)	12 (23%)	0.0000	0.0000	0.0016
HBsAb	281.07 (74.26)	264.02 (31.82)	500 (500)	313.06 (74.36)	0.5068	0.4157	0.5739
HBcAb	7 (14%)	68 (28%)	0 (0%)	9 (16%)	0.5008	0.0000	0.4593
HCVAb	25 (57%)	190 (81%)	3 (100%)	35 (67%)	0.0000	0.0000	0.0000
HIV	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0.1372	0.7160	0.6845

**Supplementary Table 7.: Clinical Laboratory Values**

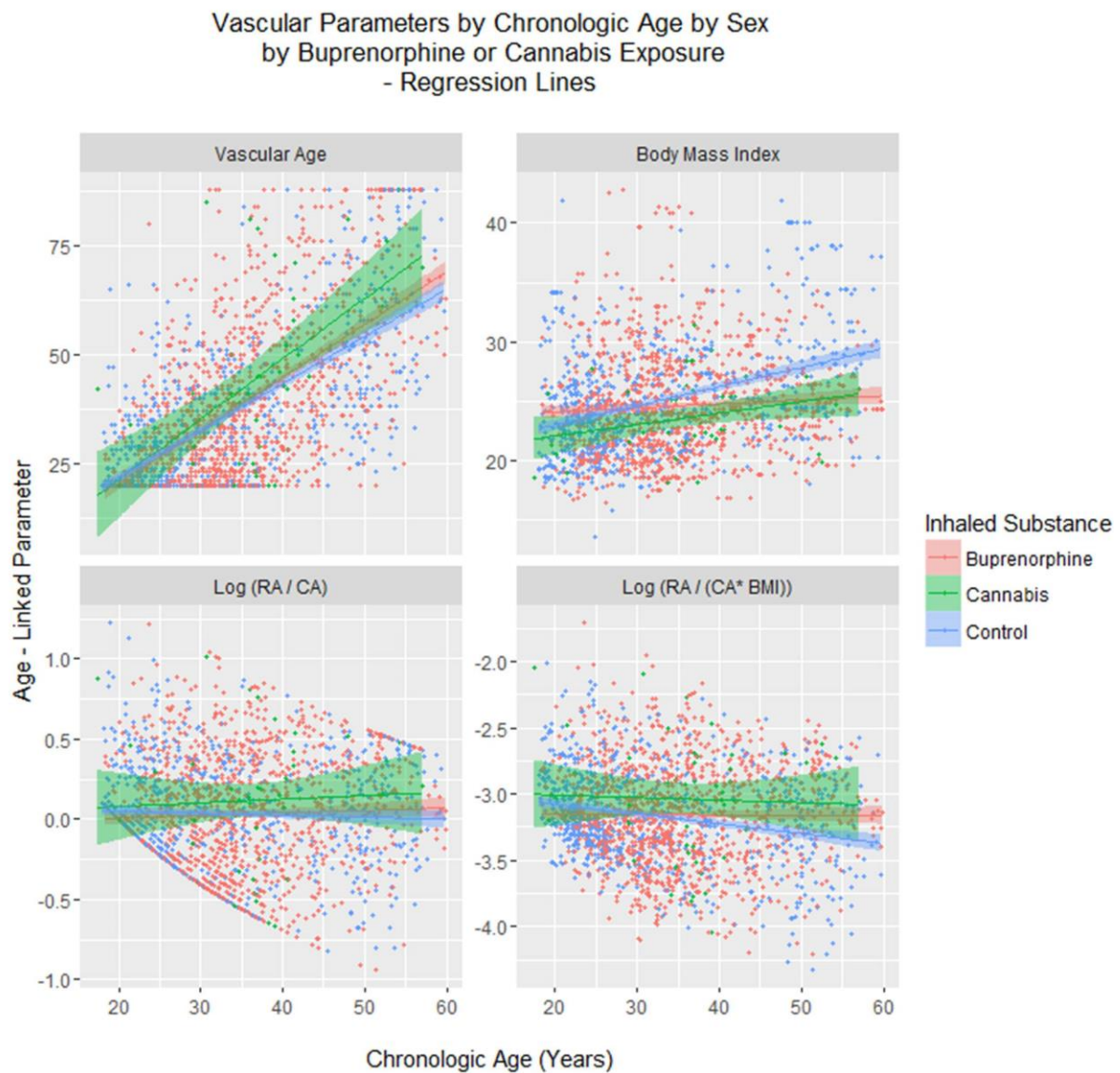
<b>Parameter</b>	<b>None (534)</b>	<b>Tobacco (506)</b>	<b>THC (11)</b>	<b>Both (112)</b>	<b>THC- None P</b>	<b>THC- Tob P</b>	<b>THC- Both P</b>
CA	30.47 (0.48)	33.33 (0.40)	40.36 (2.44)	34.56 (0.88)	0.0032	0.0100	0.0491
<i>Operator Index</i>	88.67 (0.26)	88.66 (0.28)	91 (2.22)	88.76 (0.62)	0.2119	0.2181	0.2882
<i>Timing</i>							
HR	69.6 (0.5)	70.28 (0.53)	64 (2.34)	68.49 (1.06)	0.1088	0.0836	0.1983
ED	324.67 (0.9)	322.29 (0.94)	333.45 (4.31)	323.89 (1.9)	0.1656	0.0827	0.1264
CalcED	324.67 (0.9)	322.29 (0.94)	333.45 (4.31)	323.89 (1.9)	0.1656	0.0827	0.1264
C_SVI	143.8 (1.3)	142.79 (1.3)	152.45 (6.93)	146.16 (2.82)	0.3430	0.2782	0.4982
C_TTI	2166.13 (16.69)	2176.83 (17.65)	2130.45 (132.93)	2102.48 (32.27)	0.7622	0.7023	0.8012
C_DTI	3019.64 (17.81)	3014.41 (17.55)	3197.82 (200.36)	2995.42 (40.85)	0.3962	0.3830	0.1626
C_PERIOD	887.53 (6.34)	880.42 (6.72)	950.45 (32.67)	902.04 (14.1)	0.1574	0.1275	0.2972
C_DD	562.93 (5.78)	558.17 (6.09)	617.18 (30.43)	578.22 (12.93)	0.1809	0.1559	0.3603
C_ED_PERIOD	37.43 (0.21)	37.46 (0.21)	35.64 (1.17)	36.78 (0.46)	0.2222	0.2100	0.4491
C_DD_PERIOD	62.73 (0.21)	62.73 (0.21)	64.55 (1.19)	63.37 (0.46)	0.2192	0.2132	0.4352
<i>Pressure</i>							
SP	123.53 (0.53)	123.42 (0.56)	122.91 (4.98)	122.09 (1.14)	0.8682	0.8946	0.8358
DP	68.96 (0.41)	68.22 (0.44)	69.45 (4.63)	66.27 (0.91)	0.9181	0.6892	0.3249
P_MEANP	86.53 (0.44)	86.59 (0.46)	88.82 (5.13)	85.1 (0.9)	0.6659	0.6748	0.4902
C_SP	105.42 (0.51)	106.05 (0.51)	111 (6.19)	105.56 (0.96)	0.3899	0.4434	0.4047
C_DP	70.31 (0.42)	69.82 (0.45)	70.82 (4.68)	67.5 (0.93)	0.8653	0.7482	0.3172
C_ESP	93.1 (0.52)	93.67 (0.51)	98.82 (5.99)	93.23 (1.03)	0.3633	0.4113	0.3782
C_MPS	96.57 (0.45)	96.81 (0.46)	100.09 (5.5)	95.73 (0.89)	0.5381	0.5660	0.4516
C_MPD	80.51 (0.42)	80.5 (0.45)	82.45 (5)	78.9 (0.92)	0.7067	0.7058	0.4998
C_MEANP	86.53 (0.44)	86.59 (0.46)	88.82 (5.13)	85.1 (0.9)	0.6656	0.6748	0.4902

**Supplementary Table 8.:**

**Linear Regression Table of Comparative Cannabis and Opioid Exposure**

Parameter Values						Model		
Statistical Measure	Value	Std.Error	DF	t-value	P	AIC	BIC	Log.Lik
Cannabis.Duration	-2.1785	0.6936	20	-3.1409	0.0051	59.1548	71.1268	-21.5774
Cannabis.Use: Cannabis.Duration	0.5565	0.1847	13	3.0126	0.0100			
Cannabis.Use	-1.6725	0.5581	13	-2.997	0.0103			
Opioid.Use: Opioid Duration: Cannabis.Duration	0.0375	0.0141	13	2.6715	0.0192			
Opioid.Use: Opioid Duration: Cannabis.Use: Cannabis.Duration	-0.0100	0.0041	13	-2.4135	0.0313			

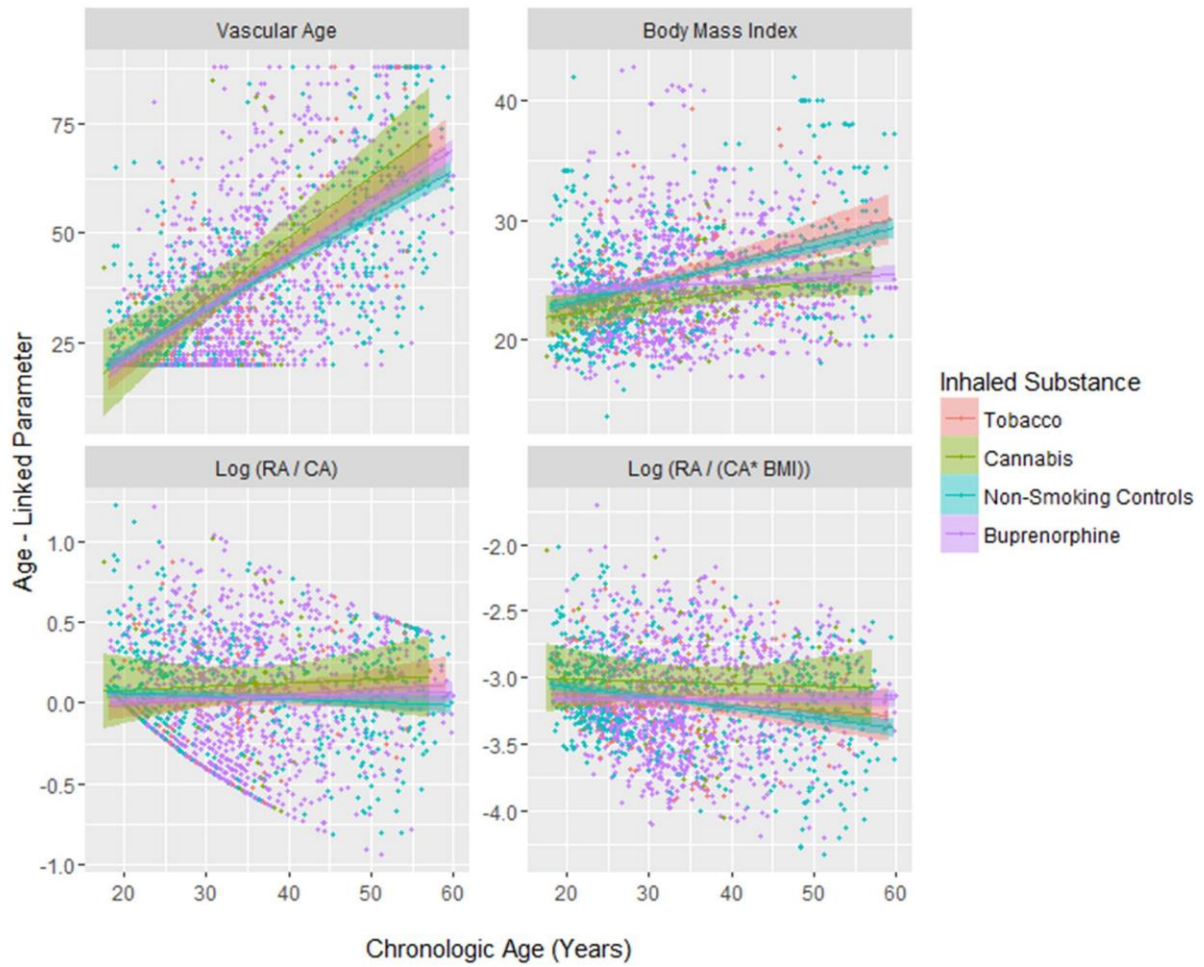
# Supplementary Figure 1



Abbreviations as in Figure 2

# Supplementary Figure 2

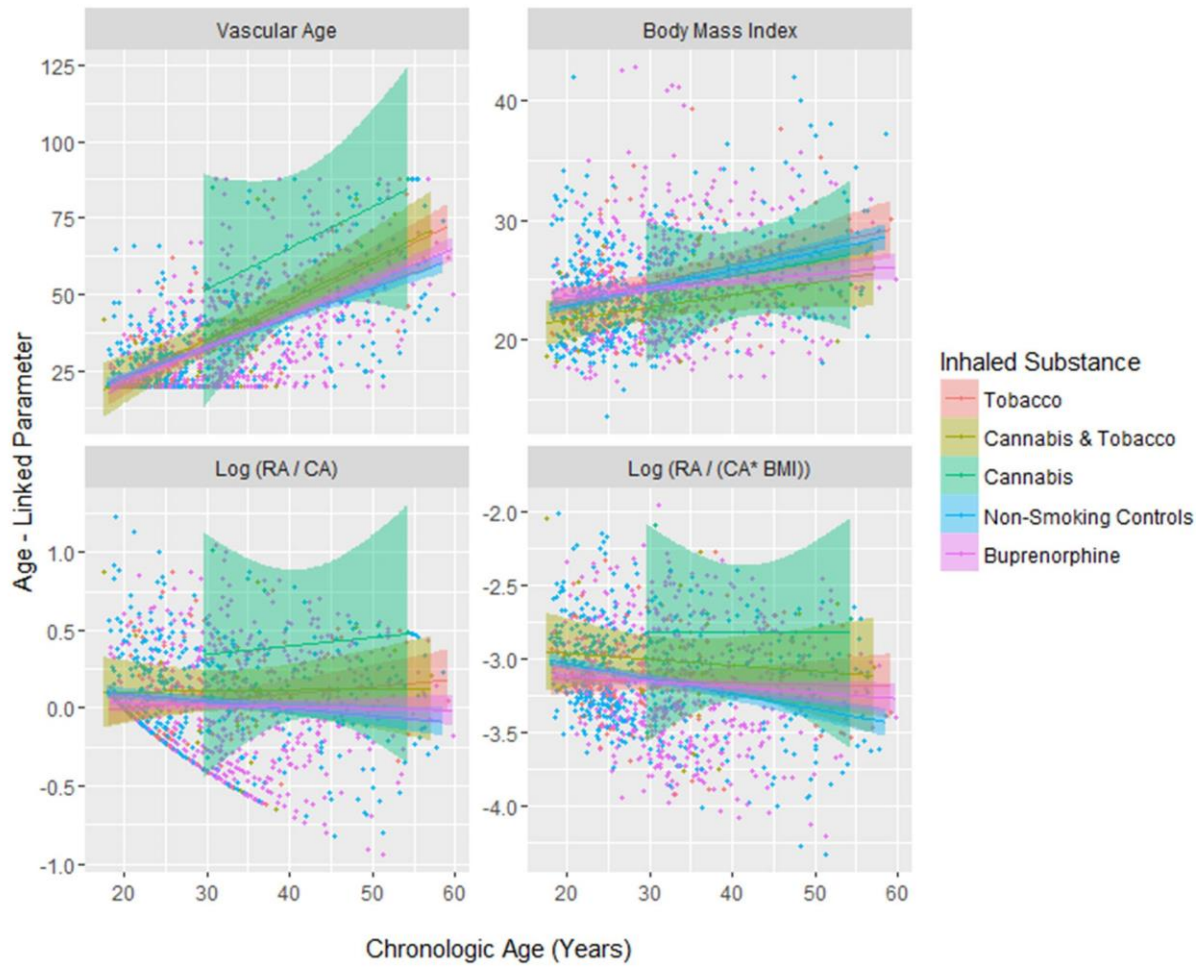
Vascular Parameters by Chronologic Age by Sex  
by Tobacco and / or Cannabis Exposure  
- Regression Lines



Abbreviations as in Figure 2

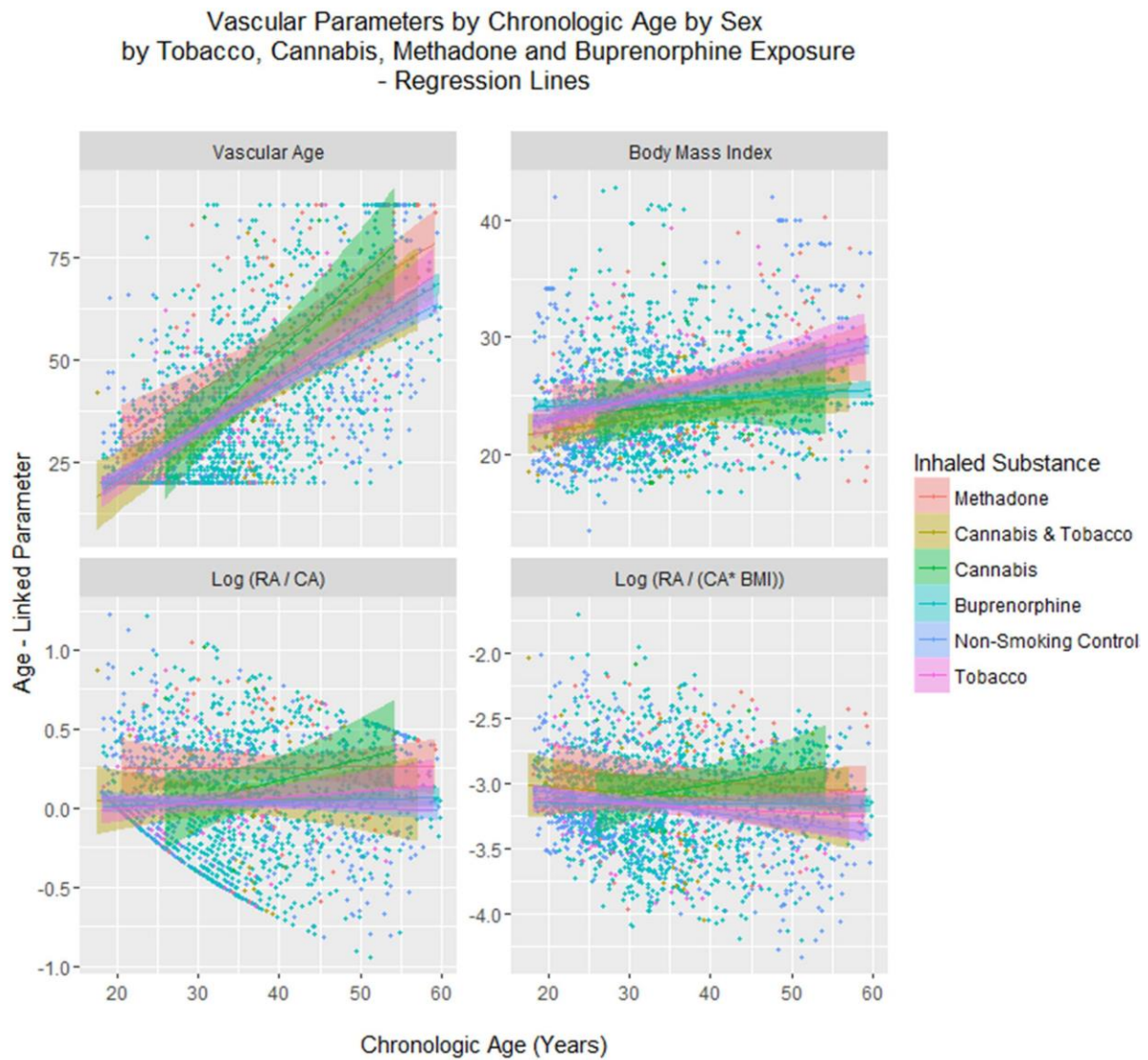
# Supplementary Figure 3

Vascular Parameters by Chronologic Age by Sex  
by Tobacco, Cannabis and Buprenorphine Exposure  
- Regression Lines



Abbreviations as in Figure 2

# Supplementary Figure 4

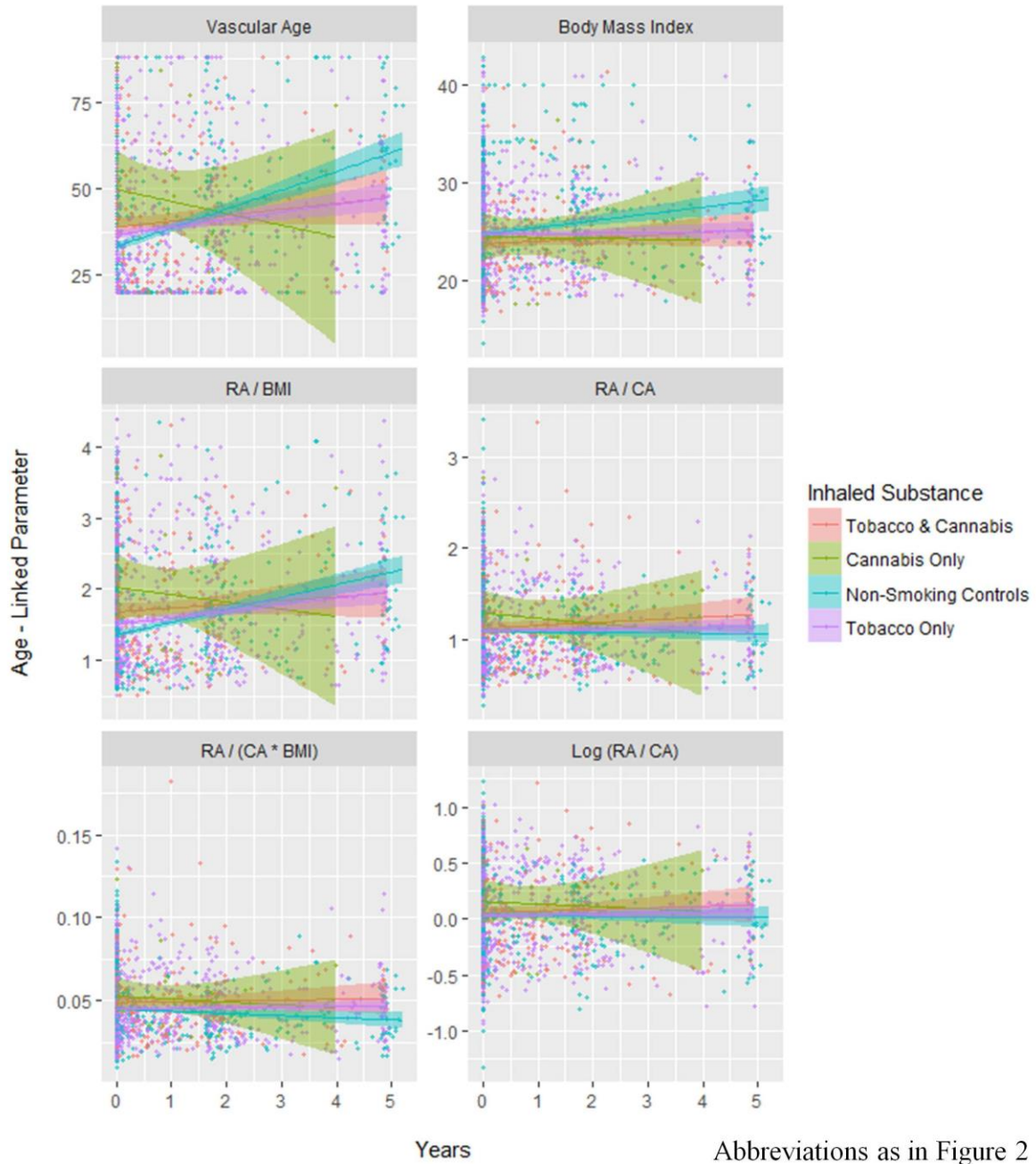


Abbreviations as in Figure 2



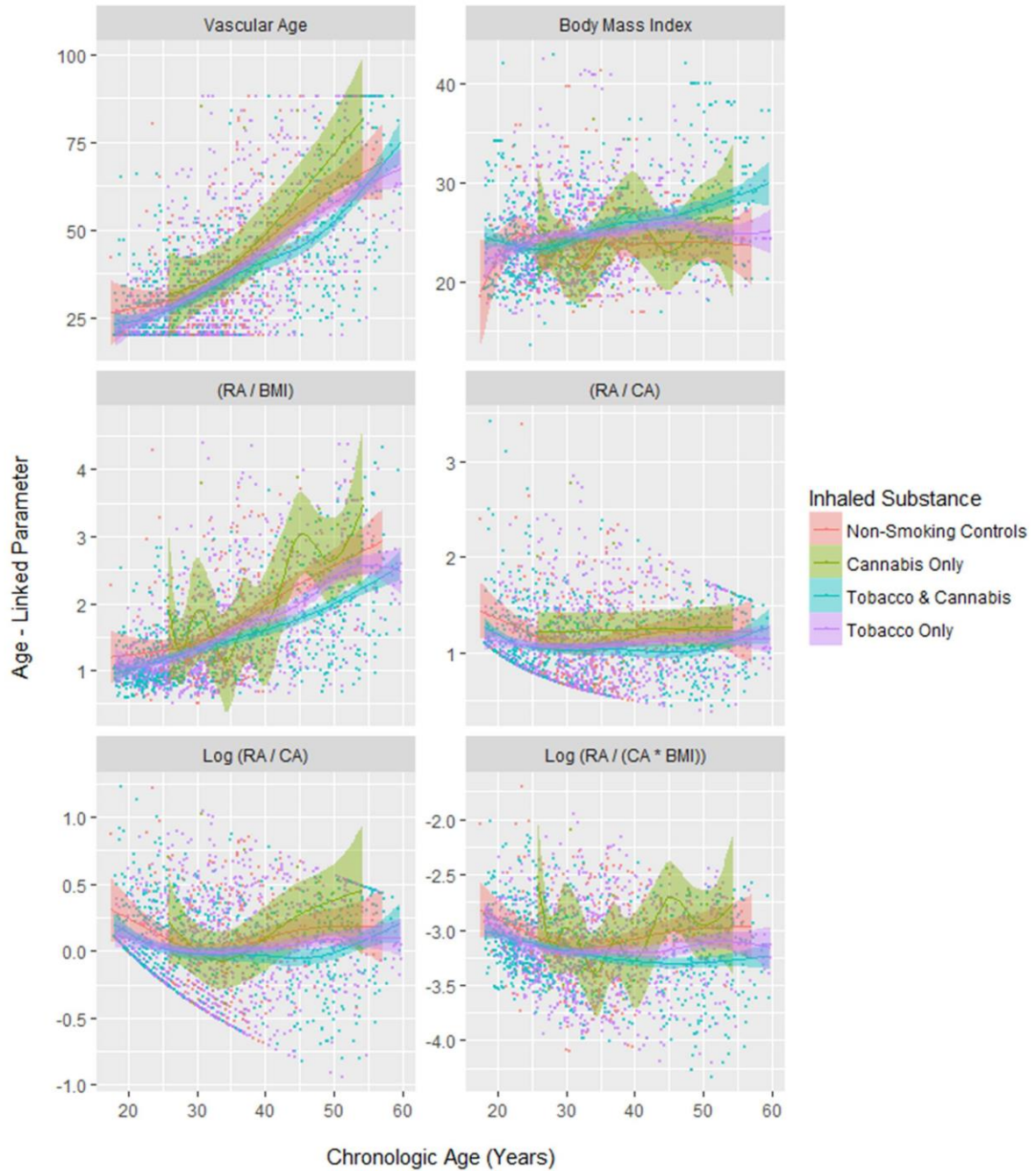
# Supplementary Figure 5

Vascular Parameters by Time  
by Tobacco and / or Cannabis Exposure  
- Regression Lines



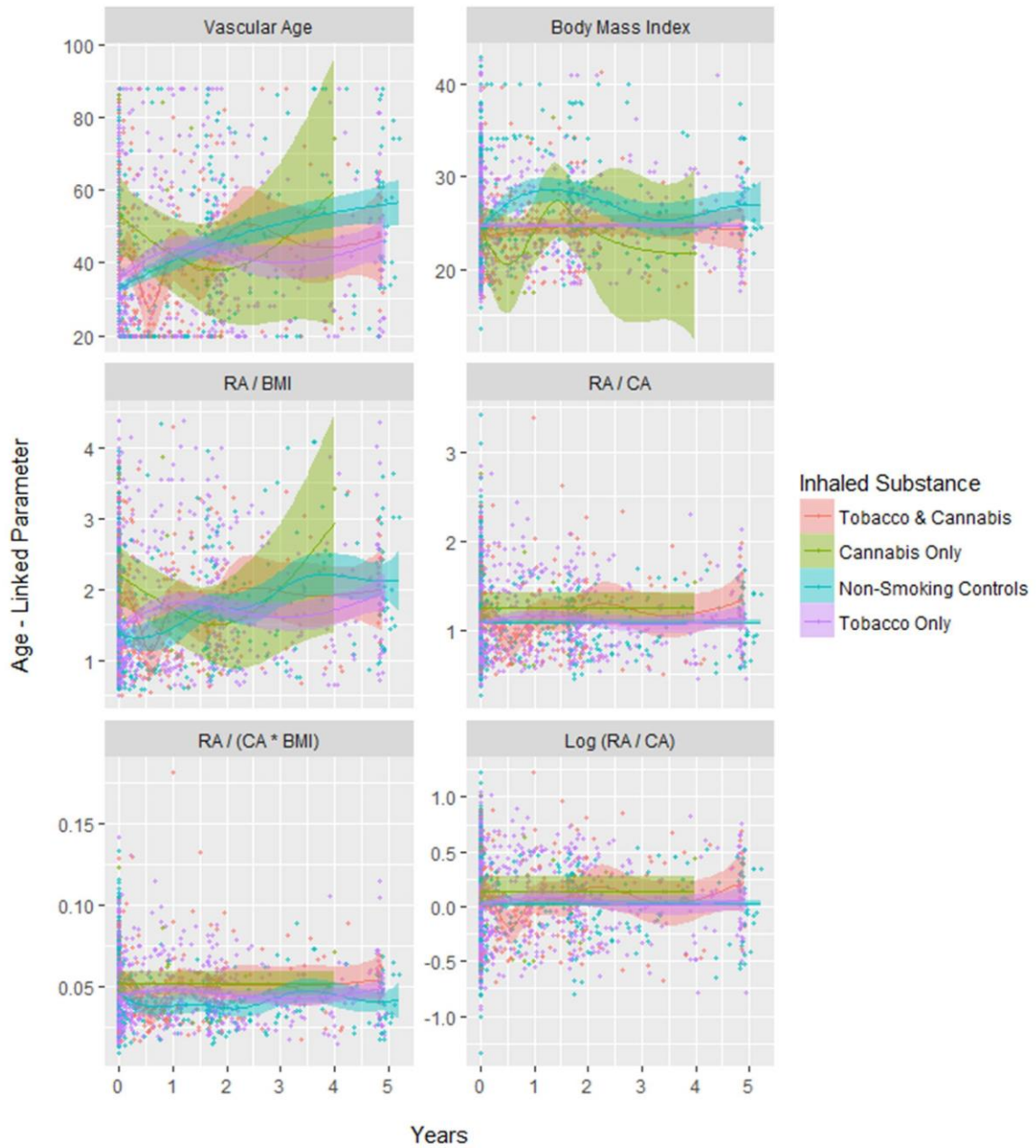
# Supplementary Figure 6

Vascular Parameters by Chronologic Age  
by Tobacco and / or Cannabis Exposure  
- Loess Curves



# Supplementary Figure 7

Vascular Parameters by Time  
by Tobacco and / or Cannabis Exposure  
- Loess Curves



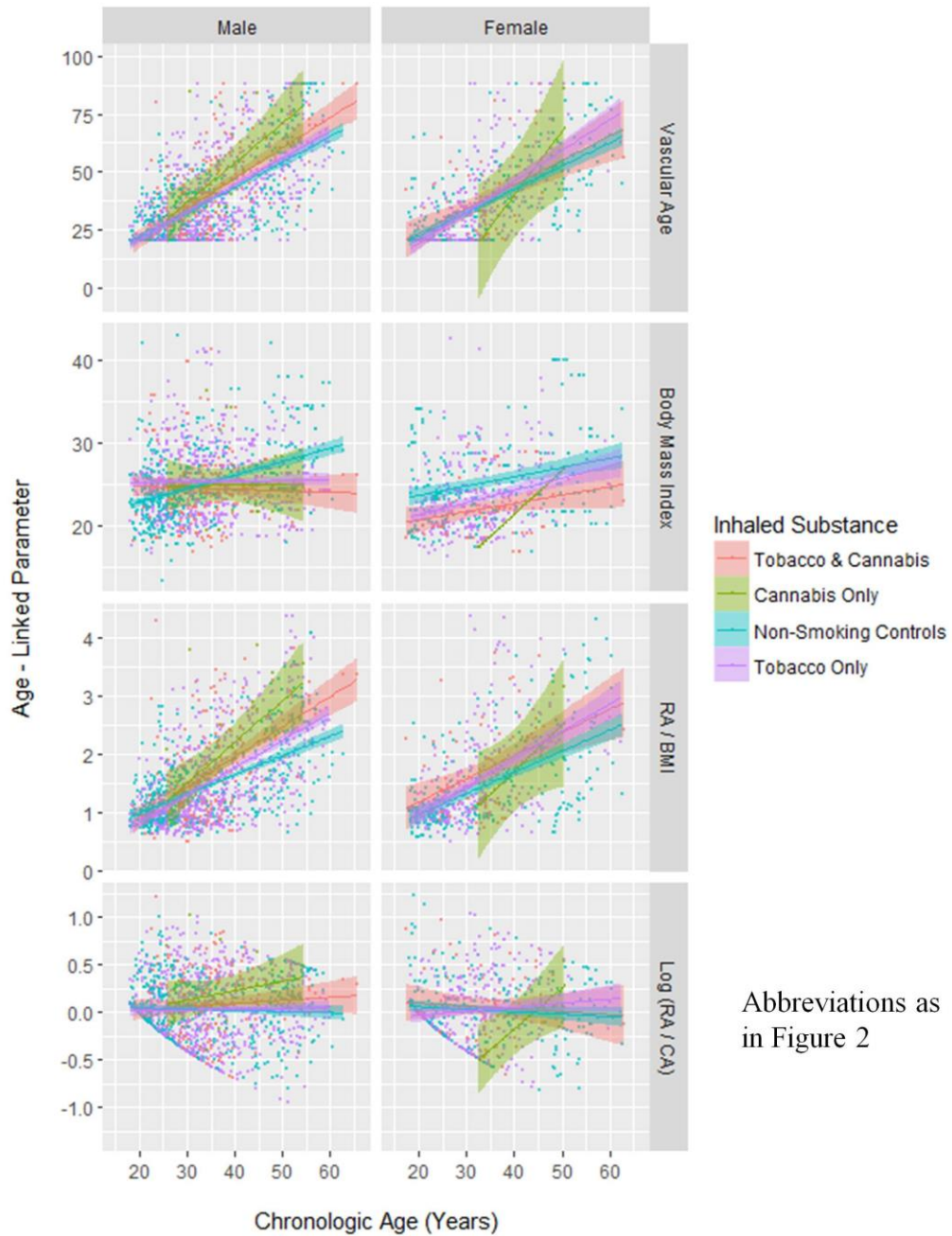
# Comments on Supplementary Figure 7

Abbreviations as in Figure 2

Loess curves = LOcally WEighted Scatterplot Smoother

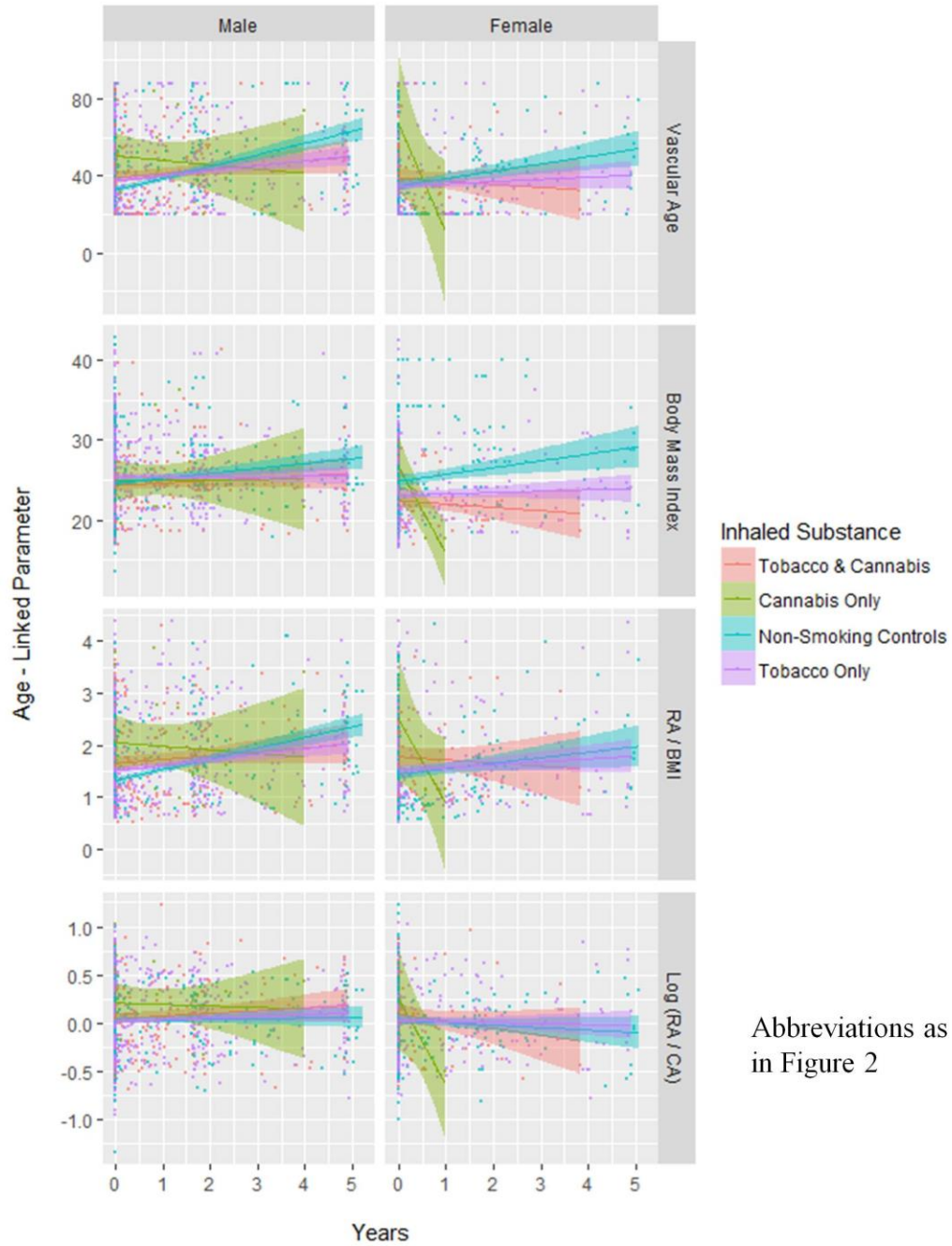
# Supplementary Figure 8

Vascular Parameters by Chronologic Age by Sex  
by Tobacco and / or Cannabis Exposure  
- Regression Lines



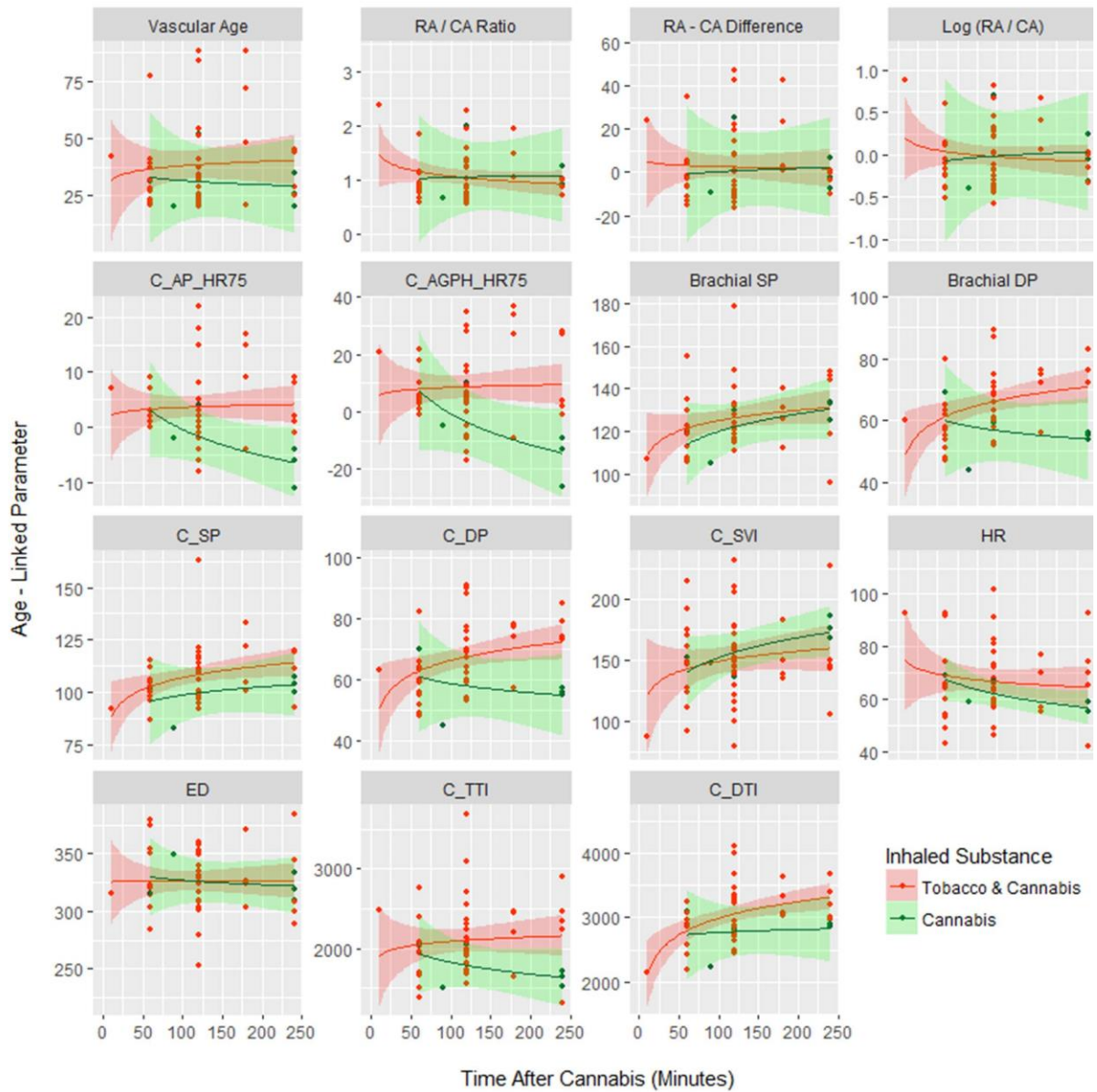
# Supplementary Figure 9

Vascular Parameters by Time by Sex  
by Tobacco and / or Cannabis Exposure  
- Regression Lines



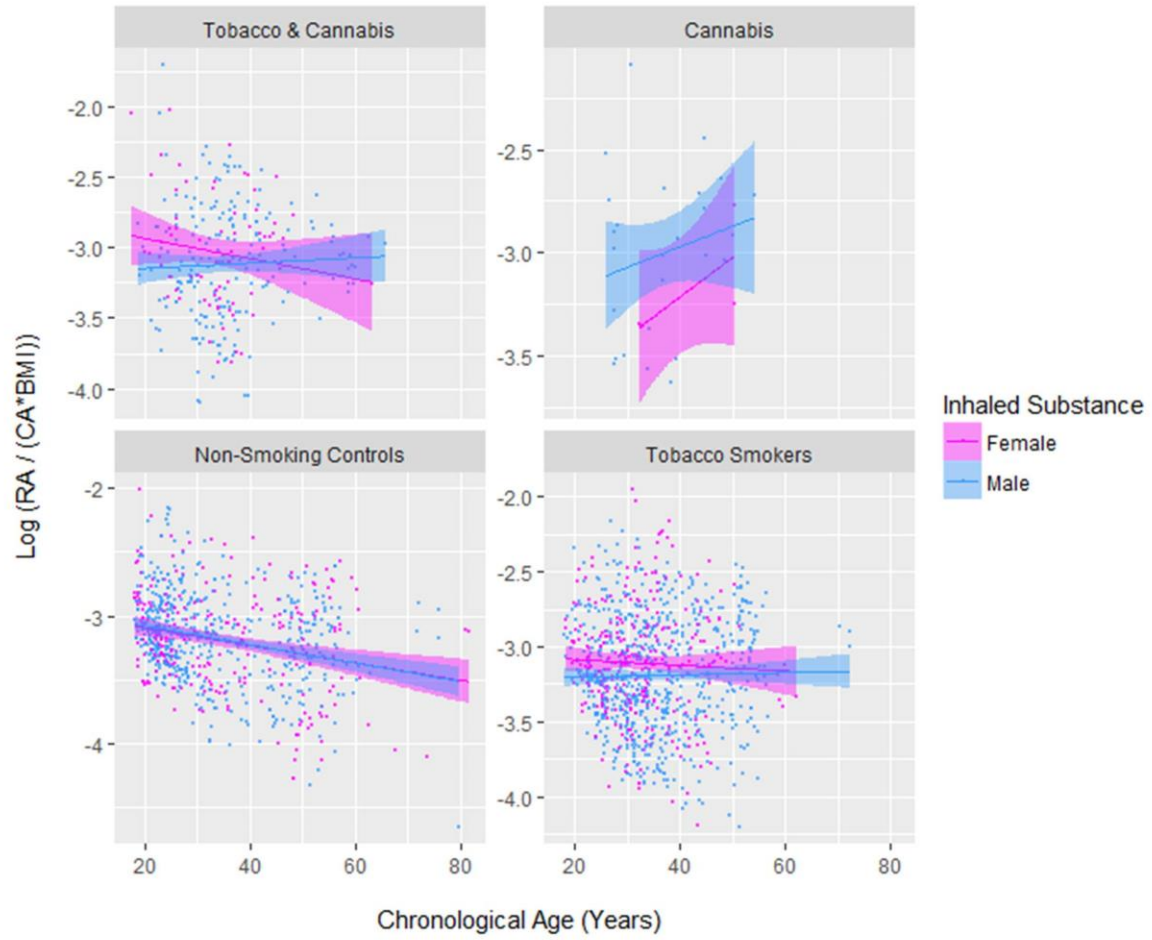
# Supplementary Figure 10

Cardiovascular Parameters in First Four Hours After Cannabis Exposure  
by Tobacco and / or Cannabis Exposure  
- Logarithmic Smoothers



# Supplementary Figure 11

Log (RA / (CA\*BMI)) by Chronologic Age by Sex  
by Tobacco and / or Cannabis Exposure  
- Regression Lines





# Supplementary Figure 12

Log (RA / (CA\*BMI)) by Time by Sex  
by Tobacco and / or Cannabis Exposure  
- Regression Lines

