Cannabis Use, Psychotic-like Experiences, and Vascular Risk in Young Adults

by

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ABSTRACT

There is a robust association between psychosis and cannabis use, but the mechanisms underlying this relation are poorly understood. Because both psychosis and cannabis use have been linked to cardiovascular problems, it is possible that cannabis use exacerbates an underlying vascular vulnerability in individuals prone to psychosis. To investigate microvascular differences in individuals with psychotic symptoms and cannabis use, the current study tested associations between psychotic-like experiences, cannabis use, and retinal vessel diameter in 101 young adults (mean age=19.37 years [SD=1.93]). Retinal venular diameter did not differ between participants with (M=218.08, SD=15.09) and without psychotic-like experiences (M=216.61, SD=16.18) (F(1, 97)=0.01, p=.93) or between cannabis users (M=218.41, SD=14.31) and non-users (M=216.95, SD=16.26) (F(1, 97)=0.37, p=.54). Likewise, mean retinal arteriolar diameter did not differ between participants with (M=157.07, SD=10.96) and without psychotic-like experiences (M=154.88, SD=9.03) (F(1, 97)=0.00, p=.97). However, cannabis users had statistically significantly wider retinal arterioles (M=159.10, SD=9.94) than did non-users (M=154.29, SD=10.20) (F(1, 97)=5.99, p=.016), and this effect was robust to control for covariates. There was no evidence of an interaction between psychotic-like experiences and cannabis use in predicting retinal vessel diameter. These results indicate that cannabis use is associated with microvascular differences in young adulthood. Given current trends toward legalization of recreational cannabis use, future research should explore these differences and their potential consequences for cardiovascular health.
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Introduction

There is a well-established link between cannabis use and psychosis, but the mechanisms underlying the relation remain unclear (Arseneault et al., 2002; Fergusson, Horwood, & Swain-Campbell, 2003; Henquet et al., 2005 Stefanis et al., 2004; van Os et al., 2002; Weiser, Knobler, Noy, & Kaplan, 2002; Zammit, Allebeck, Andreasson, Lundberg, & Lewis, 2002). Considerable evidence suggests that cannabis use may exacerbate an underlying vulnerability to psychosis (Caspi et al., 2005; Hall & Degenhardt, 2014; Malone, Hill, & Rubino, 2010; van Winkel & Kuepper, 2014). I hypothesize that one way cannabis use may trigger psychosis is by exacerbating underlying vascular risk. Accumulating evidence suggests that individuals with psychosis have an underlying vascular vulnerability (Andreassen et al., 2013; Hanson & Gottesman, 2005; Harris et al., 2008; Malaspina, 2013; Meier et al., 2015; Meier et al., 2013; Schmidt-Kastner, van Os, Esquivel, Steinbusch, & Rutten, 2012), and cannabis has known effects on the vasculature (Hall & Degenhardt, 2014; Jacobus et al., 2012; Jouanjus, Lapeyre-Mestre, Micalef, & French Assoc Regional Abuse, 2014; Thomas, Kloner, & Rezkalla, 2014; Wolff et al., 2014). The present study aims to test whether vascular risk is detectable in individuals with subclinical symptoms of psychosis, and whether cannabis use accentuates that vascular risk.

There are at least four lines of evidence suggesting that individuals with psychosis may have a vascular vulnerability that predisposes them to psychotic illness. The first line of evidence relates to the robust finding that individuals with schizophrenia are more likely than members of the general population to develop cardiovascular diseases (Hennekens, Hennekens, Hollar, & Casey, 2005; McCreadie, 2003). Results from a
A population-based study spanning two decades showed that adults with schizophrenia were approximately two times more likely to die of cardiovascular disease than members of the general population (Osby, Correia, Brandt, Ekbom, & Sparen, 2000). This increased risk has been only partially attributed to lifestyle factors, such as tobacco use and poor diet, and antipsychotic medication (Fan, Wu, Shen, Ji, & Zhan, 2013; Osborn et al., 2007). Studies of antipsychotic-naive, first-episode psychosis patients suggest that cardiovascular risk may be related to the initiating vulnerability to schizophrenia. For example, first-episode psychosis patients and their unaffected relatives have increased rates of cardiovascular risks (Mitchell et al., 2013; Ryan, Collins, & Thakore, 2003; Spelman, Walsh, Sharifi, Collins, & Thakore, 2007). A recent study also showed that several genetic loci associated with cardiovascular risks are also associated with psychosis (Andreassen et al., 2013), consistent with the implication that cardiovascular risk is not simply a consequence of illness. These findings suggest that individuals with schizophrenia may have an underlying vascular vulnerability that is unrelated to treatment and lifestyle factors.

The second line of evidence implicating vascular risk in the pathogenesis of psychosis is recent research documenting retinal microvascular abnormalities, an indicator of subclinical cardiovascular risk, in individuals with schizophrenia (Meier et al., 2013). Because the microvessels in the retina are similar in structure and function to microvessels in the brain and heart, they provide a proxy measure of the health of cerebral and coronary microvessels (Patton et al., 2005). In a population-based longitudinal study, Meier and colleagues (2013) compared the diameter of the retinal venules (small veins) in individuals with schizophrenia to medical and psychiatric
controls. Individuals with schizophrenia had substantially wider venules than did individuals with pre-diabetes/diabetes, high blood pressure, persistent tobacco dependence, and persistent depression. This finding is important because prior research has shown that wider retinal venules predict cardiovascular disease, stroke, and dementia, even after controlling for other cardiovascular risk factors including smoking, hypertension, high cholesterol, diabetes, and obesity (Ikram et al., 2006; Ikram, Ong, Cheung, & Wong, 2013; McGeechan et al., 2009; Sun, Wang, Mackey, & Wong, 2009). Findings could not be explained by antipsychotic medication, as effect sizes were similar among the subgroup of individuals with schizophrenia who had not taken antipsychotic medication in the prior year. The study also showed that children with psychosis symptoms had wider venules as adults, suggesting that the pathological mechanisms underlying wider venules in schizophrenia may begin in childhood.

As a follow-up, Meier and colleagues (2015) used retinal imaging to examine retinal venular diameter in adolescent and young adult twins discordant for psychotic-like experiences. As in the earlier study, individuals who reported psychotic-like experiences had wider venules than controls. Interestingly, the unaffected co-twins of individuals with psychotic-like experiences had retinal venular diameters that were intermediate between probands and controls. These differences in the venules of young adults with subthreshold psychosis symptoms, along with a similar but attenuated pattern in their unaffected co-twins, suggest that wide venules may be a marker of underlying vascular risk rather than a product of illness and treatment (Meier et al., 2015).

The third line of evidence comes from research demonstrating that patients with schizophrenia show altered patterns of cerebral blood flow and blood volume, which
have been hypothesized to arise from vascular abnormalities (Bachneff, 1996; Cohen, Yurgelun-Todd, & Renshaw, 1995). The fourth line of evidence is that some individuals with schizophrenia, and their unaffected relatives, demonstrate a minimal or absent response to niacin skin flush challenges, suggesting an impairment in vasodilation (Hudson, Lin, Cogan, Cashman, & Warsh, 1997; Lien et al., 2013; Ward, Sutherland, Glen, & Glen, 1998). Taken together, these four pieces of evidence suggest an underlying vascular vulnerability in individuals with psychosis.

Such a vascular vulnerability may be exacerbated by cannabis use. Cannabinoid receptors are widely distributed in vascular and endothelial cells (Liu et al., 2000; Rajesh et al., 2007; Sugiura et al., 1998). A recent review of in-vitro animal and human studies found that various cannabinoids, including tetrahydrocannabinol (THC, the primary psychoactive ingredient in cannabis), have both vasorelaxant and vasoconstrictive effects, depending on the type and location of the receptor targeted (Stanley & O'Sullivan, 2014). Human studies of acute vascular effects of cannabis consumption generally find increases in heart rate and cardiac output and modest increases in blood pressure immediately following cannabis inhalation (Aryana & Williams, 2007; Jones, 2002). Additionally, studies report acute increases in postural hypotension, or a decrease in blood pressure when moving from a sitting to a standing position, which can result in dizziness and fainting (Jones, 2002; Sidney, 2002). Several early studies also demonstrated increased cerebral blood flow (CBF) (Mathew et al., 1999; Mathew, Wilson, Coleman, Turington, & DeGrado, 1997; Mathew, Wilson, & Tant, 1989) and glucose metabolism (Volkow et al., 1996) in regular cannabis users immediately following cannabis consumption. These findings suggest an acute effect of cannabis use on the vasculature.
Furthermore, neuroimaging studies suggest a potential effect of chronic cannabis use on the cerebral vasculature. Regular cannabis users have lower resting levels of cerebral blood flow (CBF) compared with controls (Lundqvist, Jonsson, & Warkentin, 2001). Moreover, patterns of blood flow immediately following cannabis intake differ among experienced and inexperienced cannabis users, suggesting that chronic cannabis use alters vascular function. Experienced users show dose-related increases in CBF immediately following cannabis administration (Mathew, Wilson, Humphreys, Lowe, & Weithe, 1993; Mathew et al., 1989), whereas inexperienced users show a reduction in CBF (Mathew et al., 1989). Experienced users also show a decrease in CBF in the period immediately following cessation of cannabis use, perhaps suggesting a withdrawal effect on CBF (Lundqvist et al., 2001; Herning, Better, Tate, & Cadet, 2005; Jacobus et al., 2012; Tunving, Thulin, Risberg, & Warkentin, 1986). Whether differences persist after prolonged abstinence is currently unclear, however. One study found that differences in CBF between cannabis users and non-users persisted after prolonged abstinence (Herning et al., 2005) whereas two others did not (Jacobus et al., 2012; Tunving et al., 1986).

Cannabis use has also been associated with cardiovascular events in case reports and observational studies. Recent reviews have concluded that cannabis users have an increased risk of myocardial infarction (i.e., heart attack) and stroke (Hall & Degenhardt, 2014; Thomas et al., 2014; Volkow, Baler, Compton, & Weiss, 2014). The association between cannabis use and myocardial infarction appears to be attributable to the acute effects of cannabis (Thomas et al., 2014), with one study reporting that risk of myocardial infarction was 4.8 times greater within one hour of smoking (Mittleman, Lewis, Maclure, Sherwood, & Muller, 2001). Recent reports have indicated that cannabis
use can induce myocardial infarction and stroke in otherwise healthy young cannabis users with limited cardiovascular risk factors (Jouanjus et al., 2014). Furthermore, a study demonstrating myocardial infarction following consumption of the synthetic cannabinoid K2, which contains an analogue of THC, indicates that cardiovascular events may be caused by psychoactive cannabinoids rather than one of the 500 other chemical components of the marijuana plant (Mir, Obafemi, Young, & Kane, 2011).

In light of these findings, I submit that cannabis use may exacerbate vascular problems in individuals prone to psychosis. Because cannabis may have deleterious effects on the vasculature, chronic use could result in repeated “hits” to an already vulnerable vascular system (Malone et al., 2010). Such a “hit” process could subtly but chronically deprive the brain of oxygen and nutrients and lead to the development of psychosis symptoms in those with pre-existing vascular vulnerability (Hanson & Gottesman, 2005). Thus, the present study aimed to test whether cannabis use exacerbates retinal vessel abnormalities in young adults with psychotic-like experiences. I tested four hypotheses. First, I tested whether individuals with psychotic-like experiences have wider retinal venules than individuals without psychotic-like experiences, thereby replicating previous findings (Meier et al., 2015; Meier et al., 2013). Second, I tested whether cannabis users have wider retinal venules than non-users, given previous findings linking cannabis and neurovascular alterations (Herning et al., 2005; Jacobus et al., 2012). Third, I tested whether individuals with psychotic-like experiences who also use cannabis regularly have the widest retinal venules, based upon a “hit” model by which cannabis use accentuates vascular abnormalities related to psychosis (Malone et al., 2010). Fourth, I tested whether the associations between wider venules,
cannabis use, and psychotic symptoms held after controlling for other risk factors such as tobacco and alcohol use, BMI, blood pressure, and depression and anxiety symptoms.

Method

Participants

Participants were 108 college students from a large southwestern university. Students enrolled in introductory psychology courses were screened for psychotic-like experiences using the Yale University PRIME Screening Test (Miller, Cicchetti, Markovich, McGlashan, & Woods, 2004). Students who scored in the top 10% of the distribution of this screen (corresponding to scores roughly 1.5 standard deviations above the mean) were recruited, as were students who scored in the bottom 10% of the distribution. Students then underwent a phone screening to assess cannabis use. Students who reported less than 20 lifetime cannabis uses and no use in the past month were recruited as non-users. Students who reported at least 40 lifetime cannabis uses and use in the past month were recruited as cannabis users. Both users and non-users were then invited to the laboratory to complete questionnaires and undergo retinal imaging. Of the 108 participants, 7 were excluded from analyses as one participant had ungradable retinal images and 6 participants reported low-moderate cannabis use, preventing them from being classified as users or non-users.

Of the 101 participants included in analyses, 45.54% (n=46) were women and 54.46% (n=55) were men. Participants were a mean age of 19.37 years (SD=1.93), and 73.27% of participants were White, 13.86% were Hispanic, 4.95% were African-American, 3.96% were Asian, and 3.96% identified as “Other.”

Measures
Psychotic-like Experiences (PLEs).

The Yale PRIME screen is a 12-item self-report questionnaire that asks participants to report on whether they have had unusual subjective experiences (Miller et al., 2004). Participants rate whether they have experienced each item on a 0-6 Likert scale (0=definitely disagree; 6=definitely agree). Example items are: “I think that I may hear my own thoughts being said out loud,” “I think that I might be able to predict the future,” and “I wonder if people may be planning to hurt me or even may be about to hurt me.” The total score is computed by summing the items. The Yale PRIME screen has demonstrated good convergent and discriminant validity as well as high reliability in a college student sample (Kline et al., 2012). For the current study, I categorized participants with a total score of 40 or higher as positive for psychotic-like experiences because individuals with these scores represented the top 10% of the screening-sample distribution. Participants with a total score of less than 6 were classified as negative for psychotic-like experiences because these scores represented the bottom 10% of the screening-sample distribution.

Cannabis Use.

Participants self-reported their lifetime frequency of cannabis use. Response options were: 0 = never, 1 = one or two times, 2 = three to nine times, 3 = 10 to 19 times, 4 = 20 to 39 times, 5 = 40 to 99 times, 6 = 100 to 199 times, 7 = 200 to 500 times, and 8 = more than 500 times. Participants also reported whether they used cannabis in the 24 hours prior to coming to the laboratory. The prevalence of past 24-hour cannabis use in the sample was 21.78%.
Participants were classified as cannabis users if they had used cannabis 40 or more times and as non-users if they had used fewer than 20 times, based on the original recruitment process that aimed to capture users and non-users. However, I conducted sensitivity analyses using several different methods for categorizing cannabis users and non-users, and results were unchanged (see Supplement 1). In addition, I ran analyses controlling for past 24-hour cannabis use to test whether the acute effects of cannabis might explain my findings, and results were unchanged (see Supplement 2).

**Retinal Imaging.**

Digital fundus photographs were taken at our laboratory after 5 minutes of dark adaptation. The same camera (Canon CR-2 with an EOS 60D backing) was used for all photographs, and each participant had both eyes photographed. I report results for measurements of the right eye, as measurements for the right and left eye are highly correlated (Leung et al., 2003). In the case of one participant whose right eye image was ungradable, the left eye image was used instead. Retinal photographs were graded at the Singapore Eye Research Institute, National University of Singapore by trained graders who were blind to participant characteristics. Graders used semi-automated computer software (Singapore “I” Vessel Assessment [SIVA], version 3.0) to measure retinal vessel diameters, following a standardized protocol with high intergrader reliability (Cheung et al., 2010). Measurements were made for arterioles and venules where they pass through a region located 0.50–2.00 disk diameters from the optic disk margin (Cheung et al., 2010). Vessel diameters refer to the internal space of the vessels and were based on the six largest arterioles and venules passing through this region. These diameters were summarized using the revised Knudtson-Parr-Hubbard formula as central
retinal artery equivalent (CRAE) and central retinal vein equivalent (CRVE) (Knudtson et al., 2003). Retinal vessel diameters are reported in micrometers (μm). The mean venular diameter for the sample was 217.56 μm (SD=15.42) and the mean arteriolar diameter was 156.29 μm (SD=10.32). The correlation between venules and arterioles was 0.54. Mean venular and arteriolar diameter in the current sample were generally similar to corresponding mean diameters reported in population-based samples (Ikram et al., 2004; Kim et al., 2011; Meier et al., 2014; Meier et al., 2013; Wong et al., 2006). See **Supplemental Table 1** for mean arteriolar and venular diameters in each of these previous studies.

**Covariates**

Blood pressure, body mass index, tobacco use, alcohol use, depression symptoms, and anxiety symptoms were considered as covariates in analyses because these factors have been shown to be associated with psychotic-like experiences (Degenhardt, Hall, & Lynskey, 2001; Foley et al., 2013; Johns et al., 2004; Kelleher & Cannon, 2011; Morgan et al., 2014), cannabis use (Badiani et al., 2015; Wechsler & McFadden, 1979), and retinal vessel diameter (Ikram, Ong, et al., 2013; Klag et al., 1989; Meier et al., 2014; Meier et al., 2015; Meier et al., 2013; Sun et al., 2009).

**Blood Pressure.**

Blood pressure was assessed using an Omron HEM 705 CP auto-inflate blood pressure monitor. I created a dichotomous variable for high blood pressure that included individuals with hypertension and prehypertension based on American Heart Association standards (Chobanian et al., 2003). First, I took the average of three successive blood pressure measurements for systolic and diastolic pressure. Then, I classified participants
as having high blood pressure if they had a mean systolic blood pressure of ≥ 120, or if
they had a mean diastolic blood pressure of ≥ 80. Fourteen participants (13.86% of the
sample) were classified as having high blood pressure.

**Body Mass Index.**

I used participants’ self-reported height and weight to calculate body mass index
(BMI). Using a subsample of 66 participants, I validated these estimates of BMI against
estimates from a non-invasive, leg-to-leg bioelectrical impedance analysis (BIA) monitor
(Tanita SC331S). The BIA monitor measures body mass index by determining
impedance to a small electrical current sent through the body. Accuracy of the monitor
for weight is ±0.2lb, and ± 2% for impedance measurement. The correlation between
estimates of BMI was r= .98. Mean BMI for the sample was 23.85 (SD=5.26).

**Tobacco Use.**

Participants reported on their tobacco use. Participants were classified as tobacco
smokers if they currently smoked cigarettes or if they had ever smoked cigarettes
regularly. The prevalence of tobacco smoking in the sample was 23.47%.

**Alcohol Use.**

Participants self-reported lifetime alcohol use by responding to the question,
“During your life, on how many days have you had at least one drink of alcohol?” Of
participants in the sample, 6.93% reported never having used alcohol, 7.92% reported one
or two days of use, 14.85% reported three to nine days of use, 9.90% reported 10 to 19
days of use, 12.87% reported 20 to 39 days of use, 13.83% reported 40 to 99 days of use,
and 30.69% reported 100 or more days of use.

**Depression Symptoms.**
Depression symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D), a 20-item scale with total scores ranging from 0-60 developed for use in non-clinical populations (Radloff, 1977). The CES-D has been found to be reliable across ethnic groups (Roberts, 1980) and has good sensitivity and specificity in college student samples (Shean & Baldwin, 2008). Mean total CES-D score for the sample was 20.67 (SD=13.11).

**Anxiety Symptoms.**

Anxiety symptoms were assessed using the anxiety subscale from the Depression and Anxiety Stress Scale (DASS) (Lovibond & Lovibond, 1995). The subscale is composed of 14 items, with each item rated on a 4-point Likert scale, yielding total scores ranging from 0-42. The DASS shows excellent reliability and adequate convergent and discriminant validity (Crawford & Henry, 2003). The mean DASS anxiety score for the sample was 4.30 (SD=5.68).

**Statistical Analyses**

I used analysis of variance (ANOVA) to test differences in retinal arteriolar and venular diameter across four groups: a group with no psychosis symptoms and no cannabis use (PLE-/CAN-; n=27); a group with cannabis use but no psychosis symptoms (PLE-/CAN+; n=9); a group with psychosis symptoms but no cannabis use (PLE+/CAN-; n=32); and a group with both psychosis symptoms and cannabis use (PLE+/CAN+; n=33).

To test whether cannabis use was associated with wider retinal venules, I tested the main effect of cannabis use on venular diameter. That is, I estimated mean venular diameter for the cannabis-using groups (i.e., the mean pooled across PLE-/CAN+ and
PLE+/CAN+ groups) and compared this mean to the mean for the non-using groups (i.e., the mean pooled across the PLE-/CAN- and PLE+/CAN- groups). To test whether psychotic-like experiences were associated with wider venules, I tested the main effect of psychotic-like experiences on retinal venular diameter. That is, I estimated mean venular diameter for the groups with psychotic-like experiences (PLE+/CAN- and PLE+/CAN+) and compared this mean to the mean for the groups without psychotic-like experiences (PLE-/CAN- and PLE-/CAN+). Finally, I tested for an interaction between cannabis use and psychotic-like experiences. That is, I tested whether cannabis use had a greater effect on mean venular diameter for people with psychotic-like experiences than for people without psychotic-like experiences (i.e., whether the difference between the means of PLE-/CAN- and PLE-/CAN+ was smaller than the difference between the means of PLE+/CAN- and PLE+/CAN+). I then repeated all analyses for arteriolar diameter.

Next, I used analysis of covariance (ANCOVA) to test whether the observed effects were robust to control for covariates. To determine which of the theoretically-relevant variables should be included as covariates in the ANCOVAs, I examined the correlations between covariates and independent and dependent variables. Potential covariates that were associated with both the independent variables and the dependent variable were incorporated into analyses. I used Pearson product moment correlations to assess the associations between pairs of continuous variables, Pearson point-biserial correlations to assess the associations between continuous and dichotomous variables, and chi-square tests to assess the associations between pairs of dichotomous variables. As shown in Table 2, none of the potential covariates were related to both the dependent variable and an independent variable, indicating that these variables could not have
confounded the results of the original ANOVAs. However, for thoroughness, I included each of these variables in a series of ANCOVAs to ensure that results did not change.

Prior to running all analyses, I conducted diagnostic tests to determine whether the ANOVA assumptions of homogeneity of variance and normal distribution of dependent variable scores for each group had been met. The Levene’s test of homogeneity of variance was not significant for venules (F=0.28, p=.84) or for arterioles (F=1.39, p=.25), indicating that the homogeneity of variance assumption was met for both dependent variables. To examine the distributions of the dependent variables for each group, I examined box-plot graphics and histograms. There were no obvious outliers on venular diameter or arteriolar diameter (i.e., there were no observed scores that differed from the mean by more than three standard deviations). However, one observation for arteriolar diameter and one observation for venular diameter were nearly 3 standard deviations above each respective mean. To ensure that these observations did not unduly influence results, I repeated all analyses twice: once with these scores excluded and once with these scores recoded to be equal to the next highest observed score. Results remained unchanged and details are presented in Supplement 3. Skew and kurtosis of venular and arteriolar diameter scores were between -1 and 1 within each group (with the exception of the PLE-/CAN+, for which kurtosis of arteriolar diameter was -1.39). The Kolmogorov-Smirnov test revealed that the skew and kurtosis of retinal venular and arteriolar diameter were not outside acceptable ranges within each group (p>.15 on both variables for every group).

I also performed diagnostic tests to determine whether the ANCOVA assumption of homogeneity of regression was met. Because this assumption requires the same
association between the covariate and the dependent variable for all groups, I tested for interactions between each covariate and cannabis use status and for interactions between each covariate and psychotic-like experiences group status in predicting each dependent variable. There were no significant interactions, indicating that the homogeneity of regression assumption was satisfied for all covariates.

**Results**

Descriptive statistics for all study variables are presented for each group in Table 1. This table shows that, as intended, psychotic-like experiences scores were higher for the groups classified as having psychotic-like experiences than for the groups classified as not having psychotic-like experiences. Likewise, means for lifetime cannabis use were higher for the cannabis-using groups than for the groups that did not use cannabis.

To test my hypotheses, I conducted a two-factor ANOVA predicting venular diameter from cannabis use and psychotic-like experiences. I included the main effects of psychotic-like experiences and cannabis use and the interaction between psychotic-like experiences and cannabis use in the model. The interaction term was not statistically significant (F(1, 97)=0.46, p=.50), indicating that there was not a statistical interaction between cannabis use and psychotic-like experiences in predicting venular diameter. Therefore, I interpreted the main effects of psychotic-like experiences and cannabis use on venular diameter. Figure 1 shows that there was no statistically significant difference in venular diameter between groups with (M=218.08, SD=15.09) versus without psychotic-like experiences (M=216.61, SD=16.18) (F(1, 97)=0.01, p=.93). Figure 2 shows that there was no statistically significant difference in venular diameter for cannabis users (M=218.41, SD=14.31) versus non-users (M=216.95, SD=16.26) (F(1,
Because there were no significant effects of psychotic-like experiences, cannabis use, or their interaction on venular diameter, I did not test models including covariates.

I next performed a two-factor ANOVA predicting arteriolar diameter from psychotic-like experiences and cannabis use. I included the main effects of psychotic-like experiences and cannabis use and the interaction between psychotic-like experiences and cannabis use in the model. The interaction term was not statistically significant (F(1, 97)=1.33, p=.25), which indicated that there was little evidence of a statistical interaction between psychotic-like experiences and cannabis use in predicting arteriolar diameter. Because the interaction was not statistically significant, I interpreted the main effects of psychotic-like experiences and cannabis use on arteriolar diameter. Figure 3 shows that there was no statistically significant difference in arteriolar diameter between groups with (M=157.07, SD=10.96) versus without (M=154.88, SD=9.03) psychotic-like experiences (F(1, 97)=0.00, p=.97). Figure 4 shows that cannabis users had statistically significantly greater mean arteriolar diameter (M=159.10, SD=9.94) than did non-users (M=154.29, SD=10.20) (F(1, 97)=5.99, p=.016). There was a mean difference of 0.47 standard deviation units on arteriolar diameter between cannabis users and non-users, which is commensurate with a medium effect size (Cohen, 1988).

I next conducted a series of analyses of covariance to determine whether the association between cannabis use and arteriolar diameter was robust to control for potential confounds. The main effect of cannabis use was robust to controls for sex, psychotic-like experiences, tobacco use, alcohol use, BMI and blood pressure, and depression and anxiety symptoms. The final ANCOVA with all covariates included
indicated statistically significant mean differences in arteriolar diameter between cannabis users and non-users \((F(1, 86) = 11.30, p = .001)\), such that cannabis users had wider retinal arterioles than did non-users. **Figure 5** shows the adjusted means for each ANCOVA, with each set of additional covariates included.

Finally, to most rigorously control for Type I Errors, I applied a Bonferroni correction to the alpha level of the significant finding that cannabis users had wider arterioles than did non-users. My full set of analyses included six tests (testing for differences between cannabis use groups, psychotic-like experiences groups, and their interaction for both arterioles and venules). After correcting for these multiple tests, the new threshold for statistical significance was \(p<.008\). Using this more stringent threshold, the difference in mean arteriolar diameter between cannabis users and non-users with all covariates included in the model remained statistically significant \((p=.001)\).

**Discussion**

The current study aimed to test the hypotheses that psychotic-like experiences and cannabis use are associated with wider retinal vessels, and that individuals with psychotic-like experiences who also use cannabis have the widest retinal vessels. In this college student sample, there were no significant differences in venular or arteriolar diameter between participants who endorsed psychotic-like experiences and those who did not. This is inconsistent with past research that has found wider retinal venules in individuals with schizophrenia and in individuals with psychotic symptoms, as compared with healthy controls (Meier et al., 2015; Meier et al., 2013). However, my results may not be directly comparable with these past findings due to methodological differences between the studies. For example, the current study sampled participants from a college
student population, whereas one previous study involved adults diagnosed with schizophrenia (Meier et al., 2013). Because of the greater level of psychopathology in the previous study, as well as the older age of the participants (38 years), microvascular abnormalities may only be apparent in chronic, severe schizophrenia. This might suggest that microvascular abnormalities in schizophrenia are a result of the disease process rather than indicative of an underlying liability to psychosis.

However, this interpretation is at odds with the results of a second study showing wider retinal venules among young adults with psychotic-like symptoms and their unaffected relatives as compared with healthy controls (Meier et al., 2015). An alternate explanation, therefore, is that the psychosis screening measure used in the current study failed to identify individuals with severe psychotic-like experiences. Whereas the current study employed a brief self-report measure of psychotic-like experiences, Meier et al. (2013) used more thorough assessments. Their assessment, which counted only hallucinations and delusions as psychotic symptoms, may have captured more severe levels of psychopathology than did the screener used in the current study. Indeed, the cut-off used to recruit high risk individuals into the current study (a Yale PRIME score in the top 10% of the screening distribution) may have captured many participants with relatively normative experiences.

Additionally, the current study used a college student sample with demographic characteristics that may not be representative of a community sample. For example, nearly 80% of the current sample was from upper middle class families. Given these characteristics, the results may not be directly comparable with those of studies using samples with greater heterogeneity in health, socioeconomic status, and other
characteristics associated with well-being (Meier et al., 2015; Meier et al., 2013). Because the participants in the current study were all enrolled in college courses, it is also reasonable to believe that they may have been, on average, higher-functioning and experiencing less severe psychopathology than participants with psychotic-like experiences drawn from a community sample. Moreover, severe functional impairment may have caused individuals at the highest risk of developing a psychotic disorder to drop out of school, preventing them from participating in the study.

In examining the association between cannabis use and retinal vessel diameter, I found that participants who used cannabis had wider retinal arterioles than did participants who did not use cannabis. This result is similar to findings in older adults of wider retinal arterioles in tobacco smokers as compared with non-smokers (Daien et al., 2013; Ikram et al., 2004; Kifley et al., 2007; Klein, Klein, Knudtson, Wong, & Tsai, 2006; Wong et al., 2006). However, these studies also tend to find similar or stronger associations of tobacco use with wider venules (Ikram et al., 2004; Kifley et al., 2007). It is unclear why I found that cannabis use is associated with wider arterioles but not wider venules. One explanation may be that cannabis and tobacco have slightly different effects on the retinal microcirculation. An alternative explanation is that most research on retinal vessels and tobacco use has been conducted in older adult populations with greater exposure to tobacco. The participants in our study were young adults, with relatively low exposure to cannabis. The finding of wider arterioles in cannabis users in the current sample of young, otherwise healthy adults is in line with the hypothesis that wide arterioles reflect the earliest stages of pathology, and that, with increases in exposure to cannabis, wider venules may also become apparent.
In the current study, the difference in arteriolar diameter between cannabis users and non-users remained significant even when controlling for potential confounds, including tobacco use. Furthermore, the relative youth and low prevalence of cigarette smoking (23.47% of participants smoked regularly at some point in their lives), high blood pressure (13.86% of participants had hypertension or prehypertension), and obesity (9.90% of participants) in this sample minimize disease-related explanations for arteriolar widening. Thus, the current evidence suggests that wide retinal arterioles may be related specifically to cannabis use rather than comorbid tobacco use, hypertension, or obesity. Although the cross-sectional nature of the current study precludes causal inferences, this finding of wider arterioles among cannabis users is consistent with existing evidence that cannabis use may affect vascular function (Herning et al., 2005; Jacobus et al., 2012; Lundqvist et al., 2001; Mathew et al., 1993; Mathew et al., 1989; Tunving et al., 1986).

This finding advances scientific understanding as the first evidence, to my knowledge, of a microvascular difference between cannabis users and non-users. This difference may be especially important because wide arterioles have been linked to heart failure, diabetes, and retinopathy (Kifley et al., 2007; Nguyen et al., 2008; Phan et al., 2015; Rogers et al., 2008; Yau et al., 2012). Although the mechanism underlying these relations remains uncertain, evidence suggests that arteriolar widening reflects impaired autoregulation of the arterioles, or problems maintaining blood flow in response to changes in perfusion pressure (Alibrahim et al., 2006; Cheung et al., 2008; Gardiner, Archer, Curtis, & Stitt, 2007). In normal retinae, the arterioles constrict in response to increased blood pressure and dilate in response to decreased blood pressure (Ikram,
Cheung, et al., 2013). In some individuals, however, this response is impaired, and such impairment predicts future widening of the arterioles (Lorenzi et al., 2010).

Given this relation, wide arterioles may be a sign of impaired autoregulation that can permit abnormally high or low blood flow to organs including the brain. In the context of high pressure, impaired autoregulation could lead to hyperperfusion, resulting in leakage and rupture of the capillary walls (Alibrahim et al., 2006; Gardiner et al., 2007; Ikram, Cheung, et al., 2013; Keel, Koklanis, Vukicevic, Itsiopoulos, & Brazionis, 2014). In the context of low pressure, impaired autoregulation could result in hypoperfusion and hypoxia, or an inadequate supply of oxygen and nutrients due to insufficient blood flow. Thus, wide arteriolar diameter in cannabis users may suggest one mechanism by which chronic cannabis use results in damage to the vasculature.

However, this potential mechanism remains speculative, and experimental studies are needed to determine whether the relations between cannabis exposure, arteriolar widening, and cardiovascular consequences are indeed causal.

The current findings must be interpreted in the context of a number of limitations of this study. First, the psychosis screening measure may have classified healthy individuals as positive for psychotic-like experiences, resulting in an attenuated effect size for associations between psychotic-like experiences and retinal vessel diameter. Relatedly, the current sample size was relatively small, and very small effects may not have been detectable. Second, young adults in college may differ in important ways from young adults not in college, limiting the generalizability of these findings. Future research may rectify these problems by testing these associations in larger and more
diverse samples, and by using more rigorous assessments of psychosis symptoms such as structured clinical interviews.

Third, the cross-sectional nature of the current study precludes causal inference about the relation between cannabis use and arteriolar widening. One possibility is that arteriolar widening may be a consequence of cannabis use. However, another possibility is that wide arteriolar diameter is characteristic of individuals who are predisposed to substance abuse, such that wide arteriolar diameter precedes the onset of heavy cannabis use. Thus, longitudinal evidence is needed to establish the temporal precedence of cannabis use or arteriolar diameter to determine the direction of the effect observed in this study. Finally, although I controlled for several potential confounds, there remains a possibility that unmeasured variables related to both cannabis use and arteriolar diameter may be driving the association between them.

Despite these limitations, the current findings suggest a number of promising directions for future research on the relation of cannabis use and cardiovascular health. Given the association between cannabis use and wide arterioles observed in this study, other designs can be used to provide further evidence for a causal role of cannabis use in arteriolar widening. For example, future studies might assess retinal vessel diameter in childhood, before the onset of substance use, to rule out the possibility that wide arterioles precede cannabis use. To provide further evidence of a causal relationship, twin studies could also be used to separate the risk posed by cannabis use from other factors. Comparing arteriolar diameter in monozygotic twins discordant for cannabis use would allow researchers to rule out shared genetic and environmental influences, strengthening
the theory that chronic cannabis use is causally related to changes in the microvasculature.

In addition to longitudinal and twin studies, future research might test a dose-response relation between cannabis use and arteriolar diameter. The current study provides evidence for mean differences in arteriolar diameter between relatively heavy cannabis users and non-users. Future studies including light, moderate, and heavy cannabis users could test a linear relation of increasing cannabis consumption and arteriolar widening within the cannabis-using group. Evidence for such a dose-response relation could provide further support for the theory that cannabis use is causally related to arteriolar widening (Hill, 1965).

Finally, experimental designs could be used to provide the strongest evidence for a causal relation. For example, intervention studies could test whether cannabis users who quit in treatment show subsequent arteriolar narrowing. Such a design could provide evidence for a reversible effect of cannabis on the microvasculature, although null findings could not rule out the possibility of permanent effects of cannabis use. To test the effect of cannabis initiation on arteriolar diameter, researchers could use animal studies to manipulate cannabis exposure. In addition to testing a causal effect, such studies have the added potential to clarify the mechanism by which the effect occurs. For example, one recent study found that rats exposed to second-hand cannabis smoke showed acute impairment of endothelial function, a form of arterial autoregulation (Wang et al., 2016). Such experimental designs could be used to test chronic effects of cannabis exposure on arteriolar auto-regulation, future arteriolar widening, and subsequent pathological states.
In conclusion, the current study provides preliminary evidence of damage to the microvasculature in young adult cannabis users. Given recent changes in public policy that have increased the accessibility of cannabis for young adults, it is important to consider the potential public health consequences of widespread cannabis use. The current findings indicate that cannabis use may be a modifiable risk factor for poor cardiovascular health and that future research is needed to determine the long-term consequences of recreational use.
References


with Psychosis from the General Population, by Age and Gender. *Plos One, 8*(12). doi:10.1371/journal.pone.0082606


Table 1. Descriptive Statistics for all study variables

<table>
<thead>
<tr>
<th></th>
<th>PLE-/CAN-</th>
<th>PLE-/CAN+</th>
<th>PLE+/CAN-</th>
<th>PLE+/CAN+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 27</td>
<td>N = 9</td>
<td>N = 32</td>
<td>N = 33</td>
</tr>
<tr>
<td>Gender: Male</td>
<td>44.44%</td>
<td>77.78%</td>
<td>43.75%</td>
<td>66.67%</td>
</tr>
<tr>
<td>Ethnicity: White</td>
<td>70.37%</td>
<td>66.67%</td>
<td>65.63%</td>
<td>84.85%</td>
</tr>
<tr>
<td>Psychotic-like Experiences</td>
<td>2.44</td>
<td>2.11</td>
<td>48.16</td>
<td>48.30</td>
</tr>
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<td></td>
<td>1.72</td>
<td>1.36</td>
<td>6.74</td>
<td>6.85</td>
</tr>
<tr>
<td>Lifetime Cannabis Use</td>
<td>0.63</td>
<td>7.00</td>
<td>1.00</td>
<td>6.94</td>
</tr>
<tr>
<td></td>
<td>1.04</td>
<td>1.22</td>
<td>1.11</td>
<td>1.06</td>
</tr>
<tr>
<td>Smoker</td>
<td>0.00%</td>
<td>44.44%</td>
<td>19.35%</td>
<td>41.94%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lifetime Alcohol Use</td>
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<td>5.33</td>
<td>2.88</td>
<td>5.48</td>
</tr>
<tr>
<td></td>
<td>1.67</td>
<td>1.00</td>
<td>1.90</td>
<td>0.80</td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td>14.81%</td>
<td>33.33%</td>
<td>12.50%</td>
<td>9.09%</td>
</tr>
<tr>
<td></td>
<td>4.62</td>
<td>3.09</td>
<td>7.25</td>
<td>3.78</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>22.98</td>
<td>23.46</td>
<td>25.40</td>
<td>23.27</td>
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<tr>
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<td>4.62</td>
<td>3.09</td>
<td>7.25</td>
<td>3.78</td>
</tr>
<tr>
<td>Depression</td>
<td>12.07</td>
<td>14.00</td>
<td>25.22</td>
<td>25.12</td>
</tr>
<tr>
<td></td>
<td>7.57</td>
<td>4.42</td>
<td>12.84</td>
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<tr>
<td>Anxiety</td>
<td>1.81</td>
<td>1.56</td>
<td>5.59</td>
<td>5.82</td>
</tr>
<tr>
<td></td>
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<td>1.88</td>
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<td>Retinal Arteriolar Diameter</td>
<td>152.79</td>
<td>161.13</td>
<td>155.55</td>
<td>158.55</td>
</tr>
<tr>
<td></td>
<td>7.98</td>
<td>9.56</td>
<td>11.74</td>
<td>10.11</td>
</tr>
<tr>
<td>Retinal Venular Diameter</td>
<td>215.46</td>
<td>220.07</td>
<td>218.21</td>
<td>217.96</td>
</tr>
<tr>
<td></td>
<td>16.79</td>
<td>14.54</td>
<td>15.96</td>
<td>14.45</td>
</tr>
</tbody>
</table>

Note. PLE+ indicates groups with psychotic-like experiences and PLE- indicates groups without psychotic-like experiences. CAN+ indicates cannabis user groups and CAN- indicates cannabis non-user groups.

Note. Lifetime Cannabis Use is scored such that 0 = never used, 1 = used one or two times, 2 = used three to nine times, 3 = used 10 to 19 times, 4 = used 20 to 39 times, 5 = used 40 to 99 times, 6 = used 100 to 199 times, 7 = used 200 to 500 times, and 8 = used more than 500 times.

Note. For Lifetime Alcohol Use, participants indicated on how many days they had at least 1 alcoholic beverage, with 0 = never, 1 = 1 to 2 days, 2 = 3 to 9 days, 3 = 10 to 19 days, 4 = 20 to 39 days, 5 = 40 to 99 days, and 6 = 100 days or more.
### Table 2. Associations of covariates with each independent and dependent variable.

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Arteriolar Diameter</th>
<th>Venular Diameter</th>
<th>PLE- vs. PLE+</th>
<th>CAN- vs. CAN+</th>
</tr>
</thead>
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<tr>
<td>Sex</td>
<td>r = -0.10</td>
<td>r = -0.004</td>
<td>φ = 0.03</td>
<td>φ = 0.25</td>
</tr>
<tr>
<td></td>
<td>p = .33</td>
<td>p = .97</td>
<td>p = .80</td>
<td>p = .013</td>
</tr>
<tr>
<td>Race (White vs. Non-white)</td>
<td>r = 0.11</td>
<td>r = -0.10</td>
<td>φ = 0.06</td>
<td>φ = 0.15</td>
</tr>
<tr>
<td></td>
<td>p = .28</td>
<td>p = .30</td>
<td>p = .52</td>
<td>p = .14</td>
</tr>
<tr>
<td>Smoker</td>
<td>r = -0.03</td>
<td>r = 0.05</td>
<td>φ = 0.22</td>
<td>φ = 0.37</td>
</tr>
<tr>
<td></td>
<td>p = .80</td>
<td>p = .64</td>
<td>p = .028</td>
<td>p &lt; .001</td>
</tr>
<tr>
<td>Lifetime Alcohol Use</td>
<td>r = 0.05</td>
<td>r = -0.09</td>
<td>r = 0.22</td>
<td>r = 0.68</td>
</tr>
<tr>
<td></td>
<td>p = .63</td>
<td>p = .36</td>
<td>p = .024</td>
<td>p &lt; .001</td>
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<tr>
<td>High blood pressure</td>
<td>r = <strong>-0.23</strong></td>
<td>r = -0.08</td>
<td>φ = -0.12</td>
<td>φ = 0.01</td>
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<tr>
<td></td>
<td>p = <strong>.024</strong></td>
<td>p = .43</td>
<td>p = .23</td>
<td>p = .92</td>
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<tr>
<td>BMI</td>
<td>r = <strong>-0.32</strong></td>
<td>r = -0.05</td>
<td>r = 0.11</td>
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<tr>
<td></td>
<td>p &lt; .001</td>
<td>p = .62</td>
<td>p = .29</td>
<td>p = .38</td>
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<tr>
<td>Depression</td>
<td>r = -0.03</td>
<td>r = -0.02</td>
<td>r = <strong>0.46</strong></td>
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<tr>
<td></td>
<td>p = .75</td>
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<td>p &lt; .001</td>
<td>p = .18</td>
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<tr>
<td>Anxiety</td>
<td>r = -0.03</td>
<td>r = -0.07</td>
<td>r = <strong>0.34</strong></td>
<td>r = 0.09</td>
</tr>
<tr>
<td></td>
<td>p = .74</td>
<td>p = .47</td>
<td>p &lt; .001</td>
<td>p = .37</td>
</tr>
</tbody>
</table>

**Note.** Significant correlations at alpha <.05 level are in bold.

**Note.** Phi coefficients are reported for associations between dichotomous variables.

**Note.** Male sex, white race, high blood pressure, smoker status, PLE+, and CAN+ are coded as 1.
Figure 1. Mean venular diameter by PLE group.

Note. Error bars indicate standard errors.
Figure 2. Mean venular diameter by cannabis group.

Note. Error bars indicate standard errors.
Figure 3. Mean arteriolar diameter by PLE group

Note. Error bars indicate standard errors.
Figure 4. Mean arteriolar diameter by cannabis group.

Note. The standardized mean difference between the groups is 0.47, which is a medium effect size. Error bars indicate standard errors.
Figure 5. Mean arteriolar diameter by cannabis group, with and without adjustment for covariates.

Note. All group differences remained significant when controlling for covariates. Error bars indicate standard errors.
APPENDIX A
SUPPLEMENTAL ANALYSES
Supplement 1

To recruit cannabis users and non-users for this study, participants were originally screened for cannabis use based on the following criteria: individuals who had used cannabis 40 or more times during their lives and had used cannabis in the past month were recruited as users, and individuals who had used cannabis 20 or fewer times and had not used in the past month were recruited as non-users. Thus, for the primary analyses, I categorized participants who had used cannabis 40 or more times as users and those who had used 20 or fewer times as non-users.

However, there is currently no gold standard for classifying cannabis users versus non-users, and cut-offs on frequency of use to define users in the cannabis literature are variable. Because some participants in the current sample reported intermediate levels of cannabis use despite screening that aimed to capture heavy users and non-users, I repeated the ANOVAs for each dependent variable using two additional methods for classifying cannabis use groups.

First, I dichotomized the variable indexing number of lifetime uses of cannabis. I classified participants as cannabis users if they had used cannabis 40 or more times based on recruitment procedures, and as non-users if they had used fewer than 40 times. Using these cut-offs, all 107 participants with gradable retinal images from the original sample were included in analyses. I then tested associations of PLEs, cannabis use, and their interaction with venular and arteriolar diameter in this full sample.

Results were unchanged after reclassifying cannabis users. Mean retinal venular and arteriolar calibers for groups with and without PLEs and groups with and without cannabis use are presented in Supplemental Table 2. There was no significant difference
in venular diameter between groups with and without PLEs (F(1, 103)=0.00, p=.97). Likewise, there was no significant difference in venular diameter between cannabis users and non-users (F(1, 103)=0.34, p=.56). There was also no significant effect of an interaction between PLEs and cannabis use in predicting venular diameter (F(1, 103)=0.30, p=.58).

For arteriolar diameter, there was no significant difference between groups with versus without psychotic-like experiences (F(1, 103)=0.02, p=.90). However, cannabis users had significantly greater mean arteriolar diameter than did non-users (F(1, 103)=6.27, p=.014). There was no significant interaction between PLEs and cannabis use in predicting arteriolar diameter (F(1, 103)=1.00, p=.32).

Second, I created cannabis use groups that captured the extreme ends of the distribution of the lifetime frequency of cannabis use variable. I classified participants as cannabis users if they had used cannabis 200 or more times and as non-users if they had used fewer than 10 times, which resulted in a sample size of 84 participants. I then tested associations of PLEs, cannabis use, and their interaction with venular and arteriolar diameter in this sub-sample of participants.

Using this classification method, results remained unchanged. Mean retinal venular and arteriolar calibers for each group are presented in Supplemental Table 3. There was no significant difference in venular diameter between groups with PLEs and groups without PLEs (F(1, 80)=0.26, p=.61), or for cannabis users versus non-users (F(1, 80)=0.18, p=.67). There was also no significant interaction between PLEs and cannabis use in predicting venular diameter (F(1, 80)=0.31, p=.58).
For arteriolar diameter, there was no significant difference between groups with versus without psychotic-like experiences (F(1, 80)=0.00, p=.96), although there was a difference between cannabis users and non-users such that users had significantly greater mean arteriolar diameter than did non-users (F(1, 80)=6.04, p=.016). The interaction between PLEs and cannabis use in predicting arteriolar diameter was not significant (F(1, 80)=2.22, p=.14).

**Supplement 2**

To test whether acute effects of cannabis use could account for the finding that cannabis users had greater mean arteriolar diameter than did non-users, I also conducted an ANCOVA with past 24-hour cannabis use included as a covariate. For this analysis, I used my original cannabis use classification method and tested for mean differences in arteriolar diameter between cannabis users and non-users, controlling for past 24-hour cannabis use. Results were unchanged: there was no statistically significant difference in arteriolar diameter between groups with and without psychotic-like experiences (F(1, 96)=0.00, p=.98), cannabis users had significantly wider arterioles than did non-users (F(1, 96)=5.45, p=.022), and there was no statistically significant interaction between psychotic-like experiences and cannabis use (F(1, 96)=1.36, p=.25). Furthermore, past 24-hour use was not a statistically significant predictor of arteriolar diameter (F(1, 96)=0.37, p=.54).

**Supplement 3**

Finally, to ensure that my results were not unduly influenced by potential outliers, I re-ran the original two-factor ANOVA predicting arteriolar diameter in two ways. First, I conducted the ANOVA with the observation approaching the cut-off of three standard
deviations above the mean excluded. Second, I conducted the ANOVA with this observation recoded to be equal to the nearest observed score.

Results remained unchanged using each method. With the observation excluded, there was no statistically significant difference in mean arteriolar diameter between groups with and without psychotic-like experiences \((F(1, 96)=0.02, p=.88)\), cannabis users had significantly wider arterioles than did non-users \((F(1, 96)=5.47, p=.021)\), and the interaction between psychotic-like experiences and cannabis use was not statistically significant \((F(1, 96)=1.90, p=.17)\). With the extreme observation recoded, there was no statistically significant difference in mean arteriolar diameter between groups with and without psychotic-like experiences \((F(1, 97)=0.00, p=.98)\), cannabis users had significantly wider arterioles than did non-users \((F(1, 97)=5.94, p=.017)\), and the interaction between psychotic-like experiences and cannabis use was not statistically significant \((F(1, 97)=1.55, p=.22)\).

In sum, analyses using various methods to classify participants as cannabis users and non-users produced results that were consistent with initial analyses. Moreover, these results could not be accounted for by acute effects of cannabis use or by undue influence from extreme observations. The main effect of cannabis use on arteriolar diameter was significant in all supplemental analyses, and all other effects remained non-significant.
**Supplemental Table 1.** Means and standard deviations for arteriolar and venular diameter in population-based samples.

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Sample</th>
<th>N</th>
<th>Mean Age or Age Range</th>
<th>Mean Arteriolar Diameter in µm (SD)</th>
<th>Mean Venular Diameter in µm (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Study</td>
<td>College Student Health and Behavior</td>
<td>101</td>
<td>19.37</td>
<td>156.29 (10.32)</td>
<td>217.56 (15.42)</td>
</tr>
<tr>
<td>Ikram et al. (2004)</td>
<td>Rotterdam Study</td>
<td>5674</td>
<td>≥55</td>
<td>146.9 (14.4)</td>
<td>222.0 (20.9)</td>
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<tr>
<td>Kim et al. (2011)</td>
<td>Cardiovascular Health Study</td>
<td>1744</td>
<td>78</td>
<td>166 (19)</td>
<td>191 (18)</td>
</tr>
<tr>
<td></td>
<td>Dunedin Multidisciplinary Health and Development Study</td>
<td>922</td>
<td>38</td>
<td>137.3 (10.9)</td>
<td>196.2 (14.8)</td>
</tr>
<tr>
<td>Meier et al. (2014)</td>
<td>Brisbane Longitudinal Twins Study / Twins Eye Study of Tasmania</td>
<td>865</td>
<td>19.07</td>
<td>165.3 (12.9)</td>
<td>249.5 (18.0)</td>
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<td>Brisbane Longitudinal Twins Study / Twins Eye Study of Tasmania</td>
<td>531</td>
<td>20.6</td>
<td>163.88 (12.5)</td>
<td>249.7 (17.2)</td>
</tr>
<tr>
<td>Wong et al. (2006)</td>
<td>Multi-Ethnic Study of Atherosclerosis</td>
<td>5979</td>
<td>45-84</td>
<td>144.1 (14.4)</td>
<td>214.0 (22.2)</td>
</tr>
</tbody>
</table>
**Supplemental Table 2.** Mean venular and arteriolar diameters by group. After re-defining cannabis users as individuals using 40+ times and non-users as individuals using < 40 times, results were unchanged.

<table>
<thead>
<tr>
<th></th>
<th>Groups with PLEs</th>
<th>Groups without PLEs</th>
<th>Cannabis Users</th>
<th>Cannabis Non-users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=68</td>
<td>N=39</td>
<td>N=42</td>
<td>N=65</td>
</tr>
<tr>
<td><strong>Mean Venular Diameter</strong></td>
<td>217.90 (SD=15.08)</td>
<td>216.96 (SD=16.64)</td>
<td>218.41 (SD=14.31)</td>
<td>217.01 (SD=16.45)</td>
</tr>
<tr>
<td><strong>Mean Arteriolar Diameter</strong></td>
<td>156.78 (SD=11.07)</td>
<td>154.97 (SD=8.94)</td>
<td>159.10 (SD=9.94)*</td>
<td>154.19 (SD=10.20)*</td>
</tr>
</tbody>
</table>

*significant difference between group means
**Supplemental Table 3.** Mean venular and arteriolar diameters by group. After re-defining cannabis users as individuals using 200+ times and non-users as individuals using < 10 times, results were unchanged.

<table>
<thead>
<tr>
<th></th>
<th>Groups with PLEs</th>
<th>Groups without PLEs</th>
<th>Cannabis Users</th>
<th>Cannabis Non-users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=53</td>
<td>N=31</td>
<td>N=31</td>
<td>N=53</td>
</tr>
<tr>
<td>Mean Venular Diameter</td>
<td>218.15 (SD=15.70)</td>
<td>214.98 (SD=15.37)</td>
<td>217.91 (SD=15.56)</td>
<td>216.44 (SD=15.69)</td>
</tr>
<tr>
<td>Mean Arteriolar Diameter</td>
<td>157.05 (SD=11.27)</td>
<td>154.22 (SD=8.94)</td>
<td>159.28 (SD=10.75)*</td>
<td>154.09 (SD=9.96)*</td>
</tr>
</tbody>
</table>

*significant difference between group means
APPENDIX B

INSTITUTIONAL REVIEW BOARD HUMAN SUBJECTS APPROVAL
Dear Madeline Meier:

On 6/20/2016 the ASU IRB reviewed the following protocol:

<table>
<thead>
<tr>
<th>Type of Review:</th>
<th>Continuing Review</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title:</td>
<td>College Student Health and Behavior Study</td>
</tr>
<tr>
<td>Investigator:</td>
<td>Madeline Meier</td>
</tr>
<tr>
<td>IRB ID:</td>
<td>STUDY00001231</td>
</tr>
<tr>
<td>Category of review:</td>
<td>(8)(c) Data analysis</td>
</tr>
<tr>
<td>Funding:</td>
<td>None</td>
</tr>
<tr>
<td>Grant Title:</td>
<td>None</td>
</tr>
<tr>
<td>Grant ID:</td>
<td>None</td>
</tr>
</tbody>
</table>

The IRB approved the protocol from 6/20/2016 to 7/15/2017 inclusive. Three weeks before 7/15/2017 you are to submit a completed Continuing Review application and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 7/15/2017 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the “Documents” tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator
cc:

Makita White
Connor Jones
Melanie Hill
Lucia Carbajal
Madeline Meier
Amanda Bruening