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CANNABIS SMOKING AND SERUM C-REACTIVE PROTEIN: A QUANTILE REGRESSIONS APPROACH BASED ON NHANES 2005–2010*

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Abstract

Background—Pre-clinical studies link cannabinoid-1 receptor activation to inflammation and atherosclerotic effects; anti-inflammation and immunosuppression seem to be mediated by cannabinoid-2 receptor activation. In this epidemiological study, we aim to present estimates on suspected cannabis-attributable immunomodulation as manifest in serum C-reactive protein (CRP) levels as non-specific inflammatory markers with interpretable clinical values. With strength of data from recent large nationally representative community sample surveys, the research approach illustrates value of a quantile regressions approach in lieu of the commonly used but relatively arbitrary cutpoints for CRP values.

Methods—The study population encompasses 20–59 year old participants from the National Health and Nutrition Examination Surveys, 2005–2010 ($n = 1115$ recently active cannabis smokers and 8041 non-smokers, identified via confidential Audio Computer Assisted Self-Interviews). Age, sex, race, education, income-poverty ratio, alcohol consumption, and tobacco smoking also were measured, together with body mass index (BMI), which actually might be on a mediational path. Quantile regressions, with bootstrapping for variance estimation, made it possible to hold these covariates constant while estimating cannabis-CRP associations.

Results—Evidence suggesting possible cannabis-attributable immunomodulation emerges at CRP levels below the median ($p < 0.05$). Whereas BMI might help explain a cannabis link with serum CRP, but BMI-stratified analyses disclosed no appreciable variation of the cannabis-CRP relationship across BMI subgroups.

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Contributors:

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No conflict declared

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Conclusions—Extending pre-clinical research on cannabis-attributable immunomodulation, this study's CRP evidence points toward possible anti-inflammatory effects of cannabis smoking. More definitive evidence can be derived by combining pre-clinical research, studies of patients, and epidemiological research approaches.

Keywords

NHANES; CRP; Cannabis smoking; Inflammation; Immunomodulation

1. INTRODUCTION

Several studies provide evidence that inflammation plays a key role in the development and the progression of chronic diseases such as cardiovascular disease and diabetes mellitus (CVD; DM; Calle and Fernandez, 2012; Pearson et al., 2003). Some inflammatory markers now qualify suspected causes of cardiometabolic illnesses (Goff et al., 2014; Pradhan et al., 2001). It is in this respect that the acute phase reactant C-reactive protein (CRP) is of special interest. Acute phase responses are induced by cytokines released from jeopardized tissue, which stimulate liver synthesis of acute phase proteins including CRP (Steel and Whitehead, 1994). Circulating levels of CRP are clinically useful in prediction of the occurrence of cardiovascular events as well as for therapeutic purposes. For example, individuals with CRP levels >3 mg/L represent a high-risk group for CVD deserving special attention (Hage, 2014).

The first evidence that cannabinoids might modulate cytokine production was found in the mid-1980s (Blanchard et al., 1986). Consequences of such cannabinoid immunomodulation are not fully understood (Klein, 2005). On one hand, pre-clinical studies link cannabinoid-1 receptor activation to inflammation and atherosclerotic effects (Dol-Gleizes et al., 2009). On the other hand, activation of cannabinoid-2 receptors primarily is found to mediate anti-inflammation and immunosuppression (Klein and Cabral, 2006; Pacher, 2009; Ribeiro et al., 2012). Much of this work is pre-clinical, but there has been a steady increase in evidence from human studies.

The aim of the current study is to present new epidemiological estimates on suspected cannabis smoking effects on circulating levels of CRP. There has been little epidemiological research on this interesting topic (Costello et al., 2013; Keen et al., 2014; Muniyappa et al., 2013). Due to the large nationally representative sampling and strong CRP assays, of most importance might be recent work based on the United States National Health and Nutrition Examination Survey data gathered between 1988 and 1994 (US NHANES, 1988–1994), completed by Rajavashisth and colleagues (2012). Even so, the main findings from NHANES data were somewhat perplexing in that past but not current cannabis smoking (CS) was associated with having serum CRP levels below 0.5 mg/dl (never smokers served as the referent group for both former and current cannabis smokers). The interpretation is that lower inflammation values are seen among former cannabis smokers, not among active smokers.

Seeking to re-visit these issues, in the present study we turn to independent samples from more recently completed NHANES cycles (2005–2010), and we replace the somewhat

arbitrary CRP cutpoint approach used in prior research, substituting a quantile regressions approach that makes more complete use of the full range of CRP values. Quantile regression (QR) can be used to model any percentile, not just the mean or median. By launching a QR line of CPR research, we hope that QR might replace or possibly complement the use of arbitrary cutpoints that have clear utility when making judgments about individual patients, but that might be less useful in epidemiological workup of suspected exposure-effect relationships. Nonetheless, in order to help confirm or disconfirm what Rajavashisth and colleagues found with a cutpoint, after completing the quantile regressions approach, we imposed their CRP cutpoint (0.5 mg/dl; CRP 85th percentile in the current study), and tried to complete a more or less exact replication of that work, using the newer NHANES data.

Two methodological issues should be noted. First, CRP levels have skewed distributions, with an interquartile spacing of CRP values that increases across the CRP range. The third quartile range of CRP levels tends to be twice as wide as lower quartiles; the fourth quartile concentration range is larger (Campbell et al., 2003; Ockene et al., 2001). Second, to the extent that body mass index (BMI) is responsive to cannabinoids, and BMI might fall on a causal pathway that leads from CS toward immunomodulation, in the current study we study BMI as a stratification variable to detect subgroup variations. Formal mediational analysis is not possible, given uncertainty about temporal sequencing and feedback loops in the cross-sectional NHANES data. We return to both of these issues in this paper's discussion section.

2. METHODS

This study's estimates are based on cross-sectional survey data gathered in the 2005–2010 NHANES cycles. By design, NHANES seeks nationally representative sample survey estimates for the United States non-institutionalized civilian population, with multistage area probability sampling prior to recruitment of designated respondents. NHANES sampling works downward from its primary sampling units through US counties, blocks, households, and individuals within households, with an oversampling of certain subgroups to increase statistical precision of estimates for these subgroups (United States Centers for Disease Control and Prevention, 2010). In analysis steps, the use of analysis weights takes into account this over-sampling, with post-stratification adjustments that bring study estimates into balance with known US Census Bureau population distributions.

For this study, we specified a sample that includes all NHANES designated respondents aged 20–59 years on the assessment date. Some did not consent to participate; others had missing or invalid responses to key variables under study. For this reason, the effective unweighted sample size for the present study is 9156. Supplemental Figure 1 provides flow chart that leads from all NHANES participants to those who contribute information for this study's estimates¹.

The key response variable in this study is the level of serum CRP (mg/L). In NHANES, CRP has been quantified by latex-enhanced nephelometry, with no apparent changes in

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equipment, lab methods, or lab sites across the years from 2005–2010 (United States Center for Disease Control and Prevention, 2006).

The explanatory covariate of central interest in this study is recently active cannabis smoking, assessed via a confidential Audio Computer Assisted Self-Interviews (ACASI) approach on the day of a physical examination at the NHANES mobile examination center. The ACASI approach is intended to promote accuracy and completeness of reporting on sensitive topics, including age of first cannabis smoking, and how many days cannabis was used in the 30 days just prior to assessment. On this basis, NHANES respondents can be classified as never smokers; past CS (smoked cannabis at least once in lifetime but not in the past 30 days); and recently active CS (smoked cannabis at least once in the past 30 days).

The initial guiding conceptual model was one in which the serum CRP levels are expressed as a function of recent and past cannabis smoking, with ‘never CS’ as a reference subgroup, and with statistical adjustment for covariates: age (years), sex (male/female), ethnic self-identification (ESI: coded for non-Hispanic White/non-Hispanic Black/Hispanics/all others), educational attainment (coded for less than high school/high school/above high school), income-poverty ratio (coded for less than 1 versus 1 or more), tobacco cigarette smoking (coded for never/past/current), and past-year alcohol consumption (coded for yes/no).

The plan for data analysis was organized in relation to standard “explore, analyze, explore” cycles. First, in the “explore” step, we examine univariate distributions and the first five moments of each variable’s distributions; there is no exploration of the CS-CRP relationships under study. In the subsequent main “analysis/estimation” step and due to the skewed nature of the CRP levels, we turned to the quantile regressions, estimating the degree to which serum CRP level might depend upon presence of recently active cannabis smoking (with never smokers as the referent group and a covariate term for former CS). Final post-estimation “exploratory” steps include stratified QR analyses that addressed sex, ESI, tobacco smoking, alcohol consumption and BMI, one by one.

As mentioned before, quantile regression can be used to model any percentile. Here, for illustrative purposes, the manuscript reports quantile estimates based on the 10th, 25th (Q1), 50th (Median), 75th (Q3) and the 90th percentiles. In the QR context, the best estimation approach for variances, standard errors, and 95% confidence intervals (CI) is based on bootstrap re-sampling, according to statistical theory worked out and published by Efron in 1979 and subsequently refined (Efron, 1979). In brief, for this study, via SAS SURVEYSELECT software, the observed sample was re-sampled with replacement 1000 times, and the QR model was fit iteratively to these samples (Lohr, 2012; Suhr, 2009). The resulting distribution of 1000 QR estimates was produced, from which lower and upper CI bounds are the 2.5th and 97.5th percentiles, respectively (Efron, 1979; Feng et al., 2011; Rust and Rao, 1996). An online appendix provides additional details, including citations to general approaches that might be useful to other readers of this article (Koenker, 2005; Koenker and Hallock, 2001). Implementing software has been described by Chen and colleagues (Chen et al., 2010). All analyses were performed in SAS 9.3 (SAS Institute, Cary NC).

3. RESULTS

Table 1 describes the study sample, showing 50:50 male-female ratio for NHANES study participants. In the sample, about one in eight (12.2%) qualified as recently active cannabis users; a majority had tried cannabis; a minority qualified as tobacco smokers. Normal weight, overweight, and obese status values were equally distributed among study participants. The arithmetic mean of serum CRP levels was 3.9 mg/L with median of 1.7 mg/L. The weighted distribution of serum CRP levels is displayed in Supplemental Figure 2.

Figure 1 is set up as a plot of the QR-estimated difference of serum CRP levels in the contrast of recently active CS values minus never CS values, derived as covariate-adjusted regression slope estimates from analysis-weighted quantile regressions modeling, with inclusion of a covariate term for past CS (with no product-terms included). The solid line depicts point estimates for the estimated difference between recent CS and never CS, all uniformly below the null difference value of 0.0, with a shaded 95% confidence interval that fails to entrap the null value of 0.0 at lower serum CRP values until just below the CRP median. From the median CRP value to the highest CRP value, the association remains inverse. Nevertheless, because the numbers of participants at these higher serum CRP levels become smaller and smaller, the width of bootstrapped confidence intervals becomes larger and larger. It should be noted that there is no appreciable variation in the size of the QR regressions slope estimate above the median. Instead, the width of the confidence interval depends strictly upon the NHANES effective sample size across these higher serum CRP levels. Studied one by one, product-terms to index subgroup variation in the CS-CRP slope estimates did not qualify for inclusion in the model, as gauged by a deliberately inclusive alpha set at 0.20. It should be noted that the difference between past CS and never CS was uniformly null in this study ($p > 0.05$), which can be seen clearly in appendix materials as Supplemental Table 1². The supplement also displays results from a standard linear regression model as well as slope estimates for selected serum CRP quantiles³.

Some readers might wish to know the results of a cannabis-CRP investigation that applies the standard cutpoint approach (0.5 mg/dL or 5 mg/L) to these newer NHANES data. For post-estimation exploratory analyses, we specified a cutpoint analysis as described by Rajavashisth and colleagues, and applied it to the newer NHANES data. The resulting multivariable odds ratios based on a logistic regression indicated that both past and recently active cannabis smokers are more likely to be in the serum CRP category < 0.5 mg/dL when compared to never users. However, the 95% CI for these point estimates entrapped the null value ($p > 0.05$). We also tested whether the slope estimate for former cannabis smokers differed from the slope estimate for currently active cannabis smokers (i.e., test of the differences of the two slopes from these independent subgroups), and found no difference ($p > 0.05$).

Because the newer NHANES data are based on better CRP detection limits, we then coarsened the newer NHANES data to simulate an imputation approach used in the prior

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study. Namely, whenever the observed CRP value was below 0.3 mg/dL, we substituted a constant value of 0.21. This analysis of the coarsened data also produced a null result.

Final post-estimation exploratory analyses disclosed no noteworthy variation in the CS-CRP relationship across subgroup strata defined by sex, ESI, tobacco smoking, and alcohol drinking analyses (Supplemental Figure 2). Similarly, BMI stratifications disclosed no appreciable variation in CS-CRP relationships. As a check on the CS-BMI relationship in the NHANES 2005–2010, we regressed BMI on CS with covariate terms for age, race, sex, education, income-poverty ratio, tobacco smoking, and alcohol drinking. This analysis disclosed an inverse association (slope = -1.2 ; 95% C.I. = $-1.7, -0.6$; $p < 0.05$).

4. DISCUSSION

The study's main finding is one of generally lower serum CRP levels for recently active cannabis smokers among adults age 20–59 years old, as compared with US community residents of the same age who had never smoked cannabis, with no appreciable variation in relation to covariates or subgroups studied here. As such, our quantile regressions approach did not replicate what Rajavashisth and colleagues found as lower CRP values among former cannabis smokers. The most prominent finding in these newest NHANES data with the QR approach is an inverse association that links recently active cannabis smoking with lower CRP levels.

Our study has some limitations as mentioned in our introduction, including uncertainty about temporal sequencing and the cross-sectional research design. These uncertainties place clear constraints on study inferences. Nonetheless, we note that lower CRP levels most likely do not prompt onset or persistence of cannabis smoking, and no study has nominated background variables that might produce a spurious CS-CRP relationships.

Measurement issues also are important. We acknowledge that a single point-in-time measurement of serum CRP might raise the possibility of measurement errors of the same type that are faced when a clinician examines the CRP lab value in routine clinical practice. Even so, the prognostic value of a single CRP marker level is widely accepted (Wald et al., 2009), and we are not aware of evidence to support the idea that cannabis exposure alters the sensitivity or specificity of CRP assays.

We also acknowledge that cannabis smoking was self-reported, in the context of an ACASI assessment intended to promote accuracy and completeness of disclosure. Nonetheless, there are possibilities of measurement errors even in this context, particularly in the domain of non-reporting of actual cannabis use (Harrison and Hughes, 1997; Harrison et al., 2007). Some of those classified as non-recent users actually might have been active users of cannabis. If present, the effect of this misclassification might drive the observed study estimates toward the null value or widen the 95% CI.

Notwithstanding limitations of this type, these findings are of interest due to biologically plausible immunomodulatory effects of cannabinoids, plus the large nationally representative samples of NHANES and strong CRP assays in the context of a rigorous assessment protocols. This study's QR modeling helped account for skewness of serum CRP

levels and created a view of potential CS effects on CRP that other regression models might not disclose. In this context, we note that serum CRP level is so highly skewed that the mean CRP level fails to serve well as a central tendency indicator. Seeking to address this skew, epidemiologists and clinical researchers generally have adapted their approaches with one or another CRP cutpoint. Even though the cutoff point approach clearly yields loss of information, one goal of a cutpoint is to differentiate chronic inflammatory processes from the response to acute infection or tissue injury, which might yield CRP values as large as 1000-fold from basal concentrations (Altman and Royston, 2006; Gabay and Kushner, 1999; Kushner et al., 2010). This differentiation was not at issue in the hypothesized CS-IM relationship under study here.

Some readers might wonder why BMI was not modeled with a covariate term as a potentially confounding variable in this study. The answer involves the possibility of BMI in a mediational pathway from CS to IM. We acknowledge that reduced body adipose tissue might prompt reduced secretion of inflammatory adipokines, such as interleukin-6 and tumor necrosis factor- α , with resulting attenuation in CRP levels (Calabro et al., 2005; Moshage et al., 1988). We also note that active cannabis users have been found to be less likely to be overweight or obese when compared to never users according to recent studies with various community samples (Le Strat and Le Foll, 2011; Penner et al., 2013). With CRP and BMI concurrently measured as potential medical outcomes of cannabis smoking (CS), we cannot fit any plausible model of BMI as a mediator of a CS→CRP effect. Moreover, any regression model would be mis-specified when the BMI term is thrown in the mix as if it were an exogenous covariate (e.g., completely non-dependent on CS). In an observational study like NHANES, BMI cannot be assigned at random or treated with a randomized intervention. The only alternatives available when the goal is to ‘hold constant’ a variable such as BMI are ones that involve stratification to produce the CS-CRP estimates specific for BMI strata. The stratification approach does not provide a test of mediation; it simply holds constant BMI level in a check on whether the CS-CRP estimates vary appreciably or non-appreciably across strata. Fine-grained stratification in the form of exact or caliper matching of BMI levels had to be ruled out due to the nature of the NHANES complex sample survey data and the QR approach. For this reason, we turned to the more common form of stratification and formed the three BMI levels as just described, with examination of the CS-CRP slope estimates and their variability. As shown in our online supplement, there was no appreciable variability of the CS-CRP relationship with BMI level held constant in this fashion below [Supplement Table 2]. Again, we should stress that this inspection holds BMI constant, but it was not intended as a test of either mediation or effect-modification.

In conclusion, this study’s estimates from nationally representative samples shed new light and add useful new epidemiological facts about suspected immunomodulatory effects of cannabis smoking in relation to CRP pathways, drawing attention to a possible anti-inflammatory cannabis effect among recently active cannabis smokers. What lower CRP levels might mean for cannabis smokers and their health status is still unknown. On the positive side, our results are consistent with pre-clinical studies on the potential anti-inflammatory role of cannabinoids, especially cannabidiol, which seems to have negligible psychoactive effects, as well as clinical studies on cannabidiol efficacy across several

inflammatory disease clinical endpoints (Mecha et al., 2013; Ribeiro et al., 2012). However immunomodulation and reduced cytokine production can also mean an altered immune response, more susceptibility to infection, and hence a less favorable outcome among cannabis smokers (Friedman et al., 2003). The most fruitful lines of future research to work up this observed association most likely will include clinical translational research, with pre-clinical lines of investigation that are designed to complement patient outcomes research and epidemiological studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NHANES	National Health and Nutrition Examination Surveys
CRP	C-reactive protein
BMI	body mass index

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Highlights

- We present estimates on cannabis smoking-attributable immunomodulation as manifest in serum C-reactive protein (CRP) levels, a non-specific inflammatory marker with interpretable clinical values.
- Evidence suggesting possible cannabis-attributable anti-inflammation emerges at CRP levels below the median ($p < 0.05$).
- Stratification by BMI disclosed no appreciable variation of the cannabis smoking-CRP relationship across body mass index subgroups.

Figure 1.

Estimated Effect of Recently Active Cannabis Smoking on Serum CRP (mg/L) Levels Across the Quantile Range of Serum CRP Levels, With Those who Never Smoked Cannabis as a Reference Subgroup. Data for the US Based on the National Health and Nutrition Examination Survey 2005–2010.

- By definition, the x-axis median is at 0.5 -- i.e., 50% of the population above and below that point
- Estimates adjusted for age (years), sex (male/female), ethnic self-identification (ESI: coded for non-Hispanic White/non-Hispanic Black/Hispanics/all others), educational attainment (coded for less than high school/high school/above high school), income-poverty ratio (coded for less than 1 versus 1 or more), tobacco cigarette smoking (coded for never/past/current), and past-year alcohol consumption (coded for yes/no)

Table 1

Baseline characteristics of the study population. Data for the United States based on the National Health and Nutrition Examination Survey, 2005–2010.

Characteristics	Mean values $\pm$ standard error (SE) or Sample size (weighted percentages)
Total sample size	9156
Age	39.7 $\pm$ 0.2
Female (%)	4708 (50.0)
Ethnic self-identification (%)	
Non-Hispanic Whites	4200 (68.6)
Non-Hispanic Blacks	1786 (11.2)
Hispanics	2753 (14.4)
All others	417 (5.8)
Education categories (%)	
Below high school	2278 (16.4)
High school	2133 (23.0)
Above high school	4745 (60.6)
Income poverty ratio (%)	
Less than 1	1840 (13.0)
1 or more	6717 (82.1)
Missing	599 (4.9)
Cigarette smoking (%)	
Never	5036 (54.2)
Former	1650 (20.0)
Recently active	2470 (25.8)
Past-year alcohol drinking	
No	3054 (28.9)
Yes	6102 (71.1)
Cannabis smoking (%)	
Never	4150 (39.8)
Former	3891 (48.0)
Recently active	1115 (12.2)
Body mass index (%)	
Normal weight (<25.0 kg/m ²)	2715 (32.1)
Overweight (25.0–29.9 kg/m ²)	3025 (32.7)
Obese (≥ 30.0 kg/m ²)	3372 (34.6)
Missing	44 (0.5)
Serum CRP (mg/l)	3.9 $\pm$ 0.1
Serum CRP <0.5 mg/dL (%)	7015 (79.4)