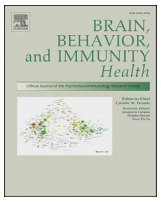
Brain, Behavior, & Immunity - Health 14 (2021) 100240

Contents lists available at ScienceDirect

Brain, Behavior, & Immunity - Health

journal homepage: www.editorialmanager.com/bbih/default.aspx



Full Length Article

Methodological and conceptual complexities of assessing relationships between single-occasion CRP inflammation and daily affect

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ARTICLE INFO

Keywords:
Bayesian statistics
Momentary data
Inflammation
Mood
CRP

ABSTRACT

Inflammation is commonly implicated in sustained levels of depressed mood, chiefly with concurrent measures. There is a dearth of research on understanding how mood-inflammation relationships change on a day-to-day timescale. Determining how inflammation and mood may fluctuate and interact with each other is imperative to determining which pathways may lead to a depressed mood due to inflammation, and, more broadly, which factors induce inflammation in the first place. Therefore, we explored a means of elucidating the nature of mood-inflammation relationships using daily measures of mood and a single time-point measure of inflammation, C-Reactive Protein (CRP). We predicted that the relationship between affect and this measure of inflammation would be time-invariant because of evidence suggesting factors contributing to inflammation are persistent over time, such as obesity or poor gut-microbiome health. Our sample consisted of 1397 young adult participants who completed daily surveys for thirteen days and provided a blood sample for CRP measurement once at the conclusion of the study. A Bayesian multivariate regression model was performed to determine how daily levels of positive and negative mood could be predicted by this single time-point measure of inflammation. As part of our analysis, we sought to control for two key moderators, BMI and physical activity. Results indicated that moderate levels of inflammation were not associated with poor mood when the individual exercised. We also determined that high BMI participants exhibited a greater impact of inflammation on their mood relative to low BMI participants. However, contrary to our primary prediction that this mood-inflammation relationship would be time-invariant, we did indeed find that the relationship was time-variant. This result indicated that research examining associations involving inflammation daily will be required to understand which causative factors may contribute to fluctuations of a mood-inflammation relationship on a daily basis.

Introduction

Recent studies implicate inflammatory processes as having a continuous negative effect on mood, contributing to psychological impairment. Most current research has tested for inflammation using acute inflammatory markers such as C-reactive protein (CRP) or IL-6, which are then used to predict current or recently experienced affect or depressive symptoms. In this vein, a recent study found empirical evidence that the relationship between inflammation and affect is a dynamic process and can vary daily (Graham-Engeland et al., 2018). The Graham-Engeland et al. study poses important methodological questions about how fluctuations in inflammation covary with changes in affect as research methods need to capture the nature of this key association because they have implications for identifying upstream causative factors such as gut microbiome health.

A key point of investigation of the gut-brain axis interaction is the health of the gut flora (i.e., the gut microbiome). An unhealthy microbiome, characterised by low diversity and imbalance of microorganisms, has been shown to contribute to inflammation within the body (Bischoff, 2011; Boulangé et al., 2016). Inflammation, in turn, is implicated in causing or maintaining maladaptive moods and behaviours, such as greater negative affect and depression (Clemente et al., 2018; Lamers et al., 2013; Qin et al., 2017). Further clouding the issue of inflammation measurement and associated outcomes are a plethora of confounding (or moderating) factors such as obesity, heart disease risk, rheumatoid arthritis, and physical activity (Choy and Panayi, 2001; Danesh et al.,

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https://doi.org/10.1016/j.bbih.2021.100240
Received 28 November 2020; Received in revised form 28 February 2021; Accepted 28 February 2021
Available online 10 March 2021

2004; Fantuzzi, 2005; Suzuki et al., 2002). In the present study, we sought to determine whether the strength of the relationship between inflammation and affect varies based on the timing of the measurement of momentary affect (i.e. a time-variant relationship between inflammation and affect). We also sought to address methodological and statistical considerations raised from previous work (e.g. Graham-Engeland et al., 2018; Shrout et al., 2018; Rooijen et al., 2006), and finally, we discuss what implications these findings may have for future work in inflammation-related outcomes such as microbiome research.

At present, we are aware of only one study, namely by Graham-Engeland et al. (2018), that has assessed time-variance in the inflammation-affect relationship. The study aimed to determine if affect measures taken closer to the time of a blood test exhibited stronger associations with CRP. To test this hypothesis, they conducted an Ecological Momentary Assessment (EMA) study on positive and negative affect assessed five times a day for 14 days in a sample of 220 middle aged community adults, and they conducted a blood test at the end of 14 days (to measure CRP). Although affect reports closer to the time of blood draw yielded stronger associations with CRP, low statistical power meant no firm conclusions could be drawn. Nevertheless, improving our understanding of the nature of a potential time-variant relationship between CRP and affect may have key implications for determining how readily CRP and/or mood can be modified through factors such as gut microbiome ill-health or potential interventions such as probiotics. Thus, in the present study, we sought to replicate Graham-Engeland et al.'s finding on a new, larger sample with an entirely different demographic makeup. We then extend their study by accounting for methodological and sample limitations that are important in work linking inflammatory markers to affect. In particular, we realised that controlling for confounds such as oral contraceptives is essential in this type of research, as well as considering moderators such as body fat, and accounting for skewed distributions for variables such as negative affect (Qin et al., 2017; Rooijen et al., 2006).

Methodological considerations of linking CRP to outcome variables

Often studies involving CRP require large samples in the thousands of participants to overcome error caused by noisy biological measurements (Valkanova et al., 2013). As indicated above, CRP and similar inflammatory markers are also influenced by a range of 'third variable' factors. Capturing all of these main effects and their interactions accurately can be a daunting undertaking; an important realisation is that researchers need a large sample size to achieve appropriate statistical power to detect real and significant relationships. Moreover, adding many control variables to statistical models necessitates a large sample size to maintain adequate statistical power. Thus, large samples are required both to overcome the inherent noise in inflammatory measurements and to sensitively capture the effects of the many covariates in statistical models investigating inflammation. A pragmatic approach is to find a balance between the breadth of investigated confounds and the sample size.

In particular, the oral contraceptive pill (OCP) has been demonstrated to have a large impact on CRP and poses a major confound that is seldom accounted for in studies of CRP. For example, it is not uncommon to see increases to levels above 2 mgL^{-1} in individuals taking OCP (Rooijen et al., 2006). The effect of OCP should be considered in CRP research, given that a CRP above 2 mgL^{-1} is considered indicative of moderate heart disease (Ridker, 2007). Controlling for an OCP effect can also be difficult, given it only impacts a subsample of females and the effect varies widely depending on formula of the pill (Rooijen et al., 2006). Leaving the effect of OCP unaccounted for can either diminish the effect of CRP on affect generally or give rise to erroneous estimates of relationships between gender, CRP, and affect. One key limitation of the only time-variant study of affect and CRP was that it did not control for OCP (Graham-Engeland et al., 2018), despite its reasonably high use in the United States according to the CDC (23% of woman aged 30–39 and 12% of women aged 40–49 years old; Daniels and Abma, 2019). In our replication and extension of this research, we sought to replicate the analysis, but removed females taking OCP to address this confounding effect on CRP.

Adipose tissue is also a key driver of CRP, often measured via Body Mass Index (BMI). Some evidence suggests BMI can also exacerbate the effect of CRP on depressive symptomology (Qin et al., 2017). That is, as BMI increases, higher CRP is more likely to be associated with higher levels of depression. Over time, obesity can lead to metabolic syndrome, a disruption to homeostasis in the body that coincides with a marked increase in systemic inflammation as measured by CRP (Aronson et al., 2004). The process by which adipose tissue contributes to inflammation is multi-faceted. Adipose tissue can directly release proinflammatory cytokines, and this tissue can contribute to atherosclerosis, that is, the build-up of plaques in artery walls. However, atherosclerosis would be unlikely in adolescent or young adults samples (Fantuzzi, 2005). Obese individuals are also more likely to report a poor diet and a relatedly poor microbiome, both of which contribute to inflammation (Turnbaugh et al., 2009). Clearly BMI is associated with a raft of proinflammatory mechanisms which can contribute to changes in mood. Thus, it is important to not only control for BMI but also investigate the moderating effect of BMI on associations between CRP and affective outcomes. The target study by Graham-Engeland et al. (2018) used CRP as an outcome in their models, and BMI was covaried out as a main effect rather than investigated as a moderator (as we sought to do herein).

A recent meta-analysis sheds more light on the complexities of examining these relationships. Marsland et al. (2017) investigated an association between CRP and stress (among a selection of other inflammatory markers), and despite disambiguating stress from affect, Marsland et al. could not confirm that CRP influenced stress. This null result was possibly attributable to the small study samples, i.e., the aggregate total consisted of only 266 participants spread across nine studies. Similarly the breadth of covariates included varied across the literature and in some cases, CRP was tested without using a high sensitivity assay, which is a problem because measures of CRP below 3 mgL^{-1} are unreliable (Windgassen et al., 2011; the lower bound on CRP estimates can also vary between assays).

Once a sample and a range of covariates are determined, further thought is required for study design effects and model selection when conducting ecological momentary assessment (EMA) studies. EMA studies are considered the gold standard approach for measuring affect as the assessments are obtained in real time (or shortly after the event in question). In EMA studies, people are signalled periodically throughout the day using smartphones or other technology to report how they are feeling at that moment; these assessments occur over a period of time, usually from one to three weeks, to obtain a reliable sample of each participant's emotional experience. There is evidence that EMA-reported affect can be more sensitive for detecting links with physiological processes than retrospectively-reported affect (Conner and Barrett, 2012; Finan et al., 2012). The researchers should also consider EMA design effects, and one such consideration is that participants typically evidence elevated reports of psychometric measures such as affect and anxiety early in an EMA study due to novelty and acquiescence bias. This initial elevation should be accounted for by trimming leading data points until data reach a stable plateau or baseline or by adding time as a covariate when modelling affect as an outcome (Shrout et al., 2018). Improper handling of the elevation bias leads to increased variance and estimation of erroneous time-variant effects. Other potential problems are that the variables of CRP and negative affect are heavily positively skewed and the data need to be handled by log transformation or with a model that accounts for skew. In the present paper, we address these distributional and psychometric concerns to yield results which hopefully contribute to a clearer understanding of inflammation–affect relationships.

Mechanisms of affect-inflammation relationships

Notwithstanding the sheer methodological complexity of assessing inflammation in an accurate and unbiased fashion, the biological model illuminating why inflammation is associated with affect is similarly complex. In our own work we base our investigations of the gut-brain-axis on the assumption that gut microbiome ill health can precipitate inflammation that is exhibited by an increase in the level of CRP. Inflammation can then lead to innervation of the vagus nerve, which bidirectionally connects the gut and brain, resulting in an inflammatory reflex to regain homeostasis (Cryan & O'Mahony, 2011; Forsythe et al., 2014; Lyte, 2013). The vagus nerve terminates on multiple sub-cortical nuclei, including the amygdala, which may explain why an inflammatory reflex attributed to the vagus nerve is also associated with affective change (Berthoud and Neuhuber, 2000).

However, the gut-brain axis does not account for all the variance in the inflammation-affect feedback loop in the human body (Forsythe et al., 2014). Rheumatoid arthritis is characterised by inflammation in the joints, and there is good support for the idea that arthritis-related inflammation can have a negative impact on mood (Margaretten et al., 2011). Arthritis, BMI, and other factors may cloud the inflammation-mood mechanism as hypothesised by the gut-brain axis. If adipose tissue in high BMI individuals releases inflammatory cytokines, it is not clear if this process would operate similarly or differently to the gut-brain axis. A first step to understanding whether there are multiple pathways being utilised by different drivers of inflammation is to understand whether affect and inflammation relationships are time-variant and if so, on what time scale variance can be detected. In the case outlined above, it would stand to reason that an inflammation-microbiome connection is unlikely if inflammation fluctuates over a matter of days whereas the same subject's gut-microbiome and diet underwent no extreme change over the same time period (a fair assumption in a random sample).

Purposes of the present study

In our study we attempted to replicate the study by Graham-Engeland et al. (2018) using a larger sample and a more sensitive approach to measuring the presumed association between CRP and daily measurements of affect. To extend the research, we also controlled for a number of potential confounds, namely we removed participants reporting use of OCP, we accounted for the initial elevation in responses expected in EMA studies, and accounted for BMI. Lastly, we adopted a Bayesian analytic framework which explicitly accounts for skewness in affective responses and included prior information on the directionality and magnitude of effects. Our overall aim was to determine if, with this more sensitive statistical approach, the association between affect and inflammation would change as a function of time lag between respective measurements. In other words, we sought to investigate whether the relationship between affect and inflammation fluctuates over time and whether this varying association can be deduced by studying the lag in time between when affect is measured and when blood is drawn to assess inflammation.

In our study, we expected similar trends to the original Graham-Engeland et al. (2018) study. The authors identified large variance in daily lagged estimates and they used an exploratory analysis to discern a general trend for of the association between inflammation and momentary negative affect to increase as the time between blood test and momentary affect measurement decreased (Fig. 1). Pertaining to their primary hypothesis, they noted that weekly recall of affect had a much stronger relationship with inflammation in the week of the blood test relative to the first week of the study. The conclusions that Graham-Engeland et al. (2018) reached are worth pursuing, given their theoretical import. Their findings can be viewed to be exploratory given that they were not considered to be statistically significant, which was possibly due to the small sample size used (N = 220). We seek to determine whether we would find similar results using our larger sample size (N = 903) with differing demographic characteristics.

We also proposed refinements in the assessment of CRP-mood relationships. After controlling for an initial elevation bias, we predicted time-invariance because our optimised approach controlled for the main effect of time on affective responses which should account for the spurious initial elevation in affective responses typical of these types of studies. We also sought to determine whether we would find a significant interaction between BMI and CRP in predicting momentary affect. Specifically, we expected that inflammation, as measured by CRP, would more likely be associated with lower positive affect and higher negative affect when body fat (BMI) is elevated. That is, we expected to find a

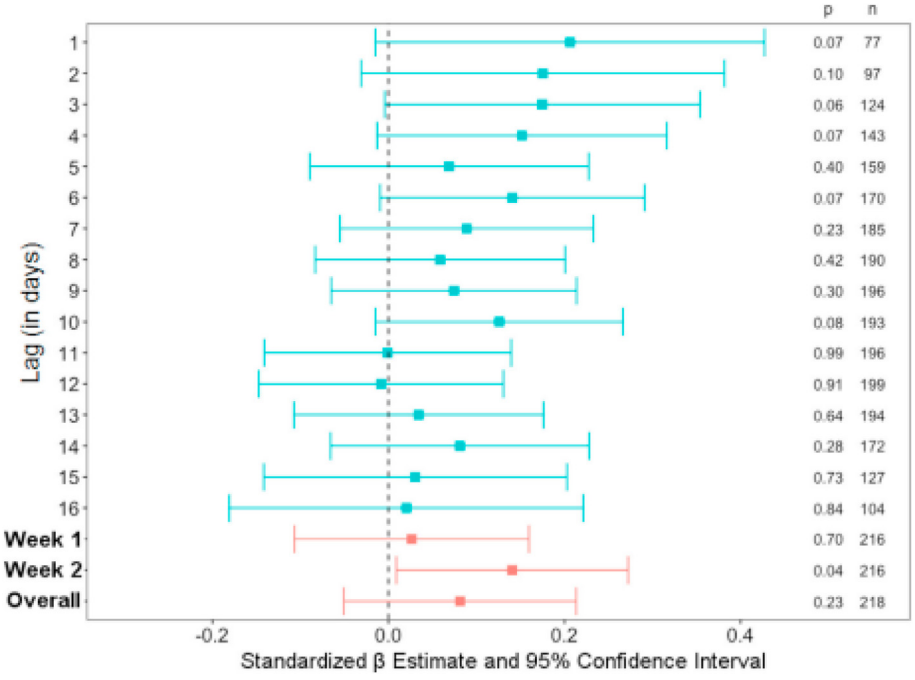

Fig. 1. Association between negative affect and a composite of inflammatory cytokines. The lag is the number of days between measuring momentary affect and the blood draw at the conclusion of the study. Separate affect measurements were captured at the end of each week and an overall average was also estimated and compared to a composite inflammation measure. Error bars are 95% confidence intervals and p-values and number of participants are presented in the right margin. Reprinted from Graham-Engeland et al. (2018).

moderation such that BMI exacerbated the influence of CRP on negative affect (Aronson et al., 2004; Qin et al., 2017). Given this is a moderation, any additive effects of stress or other factors due to BMI would be controlled within the main effect. If other factors pertaining exclusively to BMI influence the moderation, then it can only be because they are having a multiplicative effect dependent on levels of CRP.

Methods

Participants

Participants came from the Daily Life Study, a study of the health and well-being of young adult university students in Dunedin New Zealand. We blood tested 1397 participants, of which 49 were removed because they had a CRP over 10, which is indicative of clinically relevant infection. Three were removed for having a BMI of more than 50. Six were removed because they were older than a young adult upper age limit of 30 years old. And 436 females were removed because they took some form of OCP, which elicits spurious levels of systemic inflammation. Of the remaining 903, 471 (52%) were female, the mean age was 19.8 years old and the sample consisted predominantly of New Zealand Europeans (566; 63%) with minorities consisting of Asian (124; 14%), Maori/Pacific Islander (46; 5%), or other ethnicity (167; 19%). We also used the Centre for Epidemiological Disease – Depression (CES-D) scale to assess participants displaying depressive symptomology (Radloff, 1977). Using a generally accepted cut-off of 20 or more out of 60 (Vilagut et al., 2016), 207 (23%) of participants reported depressive symptoms higher than considered normal. A further 33 (4%) of respondents also took some form of pharmaceutical intervention for mental illness.

Procedure

Participants were recruited from the student volunteer pool in a New Zealand Psychology Department and from the wider university through both Facebook and noticeboard advertisement. After participants provided consent, we administered a survey on demographics and behavioural traits. Each day thereafter for 13 days, participants conducted a daily survey each evening. After 13 days of momentary assessments, participants attended a follow-up appointment where a non-fasting blood sample was drawn and anthropomorphic data (i.e., height and weight) were collected by a nurse.

Measures

Momentary affect. At the end of each day, participants reported their mood using Likert scale responses to nine positive prompts (happy, excited, cheerful, pleased, calm, energetic, enthusiastic, good, relaxed) and nine negative word prompts (nervous, dejected, irritable, hostile, sad, angry, unhappy, anxious, tense). Confirmatory Factor Analysis (CFA) was used to generate a single measure of positive affect and a single measure of negative affect for each day.

Inflammation. We used high sensitivity C-reactive protein (hs-CRP) to determine the level of acute inflammation. We measured hs-CRP using blood samples collected at the end of the study period (14th day), they were separated and refrigerated within 4 h of collection. Serum was then used to measure hs-CRP using an immunoturbidimetric assay with a Roche Cobas C502 analyzer (Roche Diagnostics, Mannheim, Germany). In order to account for most people having very low levels of inflammation (i.e., a positive skew), we used the log of hs-CRP to transform the level of acute inflammation.

Anthropomorphic measures. Height and weight was measured three times by a nurse and averaged. Height and weight measures were then used to calculate BMI using the standard method.

Comparability of the target Study's methodology to the present methodology

In comparison to the target study, i.e., Graham-Engeland et al. (2018), the present study had many similarities in collection. The measure of the hs-CRP was the same between both studies as well as anthropomorphic measures, although the present study did not consider alternative cytokines. In the target study, a range of inflammatory cytokines were used to form a composite measure and tested alongside CRP (the exploratory trend over time reported above in Fig. 1 was a cytokine composite and the CRP relationship was weaker in the target study). In the target study, affect was collected five times a day and averaged across the day, whereas we used a daily diary once per day. In the target study, for each momentary response, participants answered four questions for positive affect and five questions for negative affect. The mean responses across affective states were then averaged across momentary responses to get daily, weekly, or total levels of affect in the target study. In contrast, we used 18 questions, split evenly across positive and negative affect and used Confirmatory Factor Analysis (CFA) to generate daily levels of positive and negative affect. Lastly, and perhaps most significant, we recruited a young university student population from average to high socioeconomic backgrounds in Dunedin, New Zealand. Graham-Engeland et al. (2018), by contrast, used a small adult population from low socio-economic government housing complexes in New York, United States.

Analytical approach

We incorporated several covariates and used a Bayesian statistical method to more sensitively identify relationships in the data through the use of weakly informative priors. We used two multi-level Bayesian regression models using the *brms* package (Bürkner, 2017) implemented in R (R Core Team, 2019): one model for daily positive affect and one model for daily negative affect as outcome variables. We would argue that positioning affect as the outcome variable makes better conceptual sense than CRP scores because of our working model that diet and/or the microbiome can lead to inflammation and psychological illbeing through innervation of the vagus nerve. Including positive and negative affect as predictors of CRP may also lead to problems in multicollinearity due to their inherent inverse correlation. Predictors in these regressions included CRP, BMI, physical activity, sex, and day of study. The effect for timing of the blood test from the day of affect measure was measured through a CRP*day interaction (this term assesses the key prediction based on findings from the original study). We also included interactions of CRP with BMI and with physical activity due to their prior identification as moderators in the literature. Random intercepts were included for each participant to account for repeated measures of mood and a skew normal link function was used to account for the negative skew in positive affect and the positive skew in negative affect.

Formulation of priors

An advantage of Bayesian methods is the use of prior information. Given that momentary analysis of affect–inflammation relationships are still relatively unexplored, we sought to use weakly informative priors. Using weakly informative priors means we can slightly reduce variance when an effect is likely, but if the data suggest there is no effect, the priors will not prevent a null finding from being expressed in the posterior distribution. A summary of the priors for standardised effects can be seen in Table 1. We expected small decreases in positive affect and corresponding increases in negative affect as BMI (Gibson-Smith et al., 2018) and the log of CRP increased (De Berardis et al., 2006; Fiedorowicz et al., 2015). We also expected an effect in the opposite direction as amount of physical activity increased, since positive affect would likely be higher and negative affect would likely be lower (Kanning and Schlicht, 2010). Finally, we formed a prior for the moderation of a CRP-affect relationship based on time between blood draw and time of affect measurement (day). We expected that as the day variable

Table 1
Means (M) and standard deviations (SD) for weakly informative priors used in Bayesian analyses for positive and negative affect models.

	Positive Affect		Negative Affect	
Effect	M	SD	M	SD
BMI	−0.1	0.05	0.1	0.05
Exercise	0.1	0.05	−0.1	0.05
log (CRP)	−0.1	0.05	0.1	0.05
log (CRP)*Day	0.1	0.05	−0.1	0.05

decreased, i.e., getting closer to time of blood draw, the effect of CRP would increase and be more strongly associated with lower positive affect and higher negative affect.

All priors were normally distributed with a mean of either −0.1 or 0.1 and standard deviation of 0.05. Our choice of priors was constrained by the fact we expected a good probability of an effect in a given direction (represented by a bulk of probability on either side of a coefficient of zero), but wanted to allow a small probability of a null effect (represented by having the tails of prior distributions slightly overlapping a coefficient of zero). We determined if priors were appropriate by plotting the posterior distributions against the priors to assure priors did not have too great an influence on the data. We also compared informed analyses with the presented priors to a non-informative analysis with flat priors to determine that no effects were being classed as meaningful due to the influence of priors.

Results

Control variables

The main effect of day controls for the initial elevation bias typically observed in EMA studies. In both models, we supported a main effect of day on levels of reported affect with over 99% probability (Table 2). Positive affect started high and decreased, and in the opposite direction negative affect started low and increased throughout the study. We then sought to control for BMI which can impact on mood independently of one's level of inflammation. We obtained a high probability of negative affect increasing and positive affect decreasing as BMI increases (Over 99% probability), consistent with reports in the literature. Females typically report higher levels of both positive and negative affect relative to males, hence we controlled for gender as well. In our study, females were more likely to report higher negative affect but reported similar levels of positive affect relative to males. Increased levels of exercise, which can increase inflammation despite being associated with more positive moods, were associated with higher levels of positive affect but showed no association with levels of negative affect.

For both positive and negative affect, physical activity (exercise) had over 99% probability of moderating an association between CRP and levels of affect. It appeared that when CRP was high, physical activity had little influence on levels of affect, but when CRP was low, physical activity was associated with a large increase in positive affect and a small decrease in negative affect.

Table 2
Coefficients and 95% credible intervals for positive and negative affect. Posterior probabilities represent the percentage of posterior samples above a coefficient of zero for positive coefficients and vice versa for negative coefficients. The informed posterior probabilities result from models using priors in Table 1, whereas non-informed priors use flat priors.

	Effect	Estimate	Lower	Upper	Posterior Probability	
					Informed	Non-Informed
Positive Affect	Intercept	−0.06	−0.17	0.05	91.5%	88.2%
	BMI	−0.06	−0.12	−0.01	100.0%	97.9%
	log (CRP)	−0.06	−0.19	0.06	88.1%	71.3%
	Exercise	0.07	0.01	0.13	100.0%	90.4%
	Gender	−0.19	−0.32	−0.05	100.0%	100.0%
	Day	0.02	0.02	0.03	100.0%	100.0%
	log (CRP) * BMI	−0.06	−0.20	0.09	77.1%	82.2%
	log (CRP) * Day	0.01	0.00	0.02	95.5%	86.1%
	log (CRP) * Exercise	−0.22	−0.41	−0.05	100.0%	100.0%
Negative Affect	Effect	Estimate	Lower	Upper	Posterior Probability	
					Informed	Non-Informed
	Intercept	0.08	0.00	0.14	100.0%	100.0%
	BMI	0.03	0.00	0.07	100.0%	95.1%
	log (CRP)	0.08	−0.01	0.17	100.0%	100.0%
	Exercise	−0.01	−0.05	0.04	64.3%	75.1%
	Gender	0.00	−0.08	0.08	54.4%	50.4%
	Day	−0.01	−0.01	0.00	100.0%	100.0%
	log (CRP) * BMI	0.14	0.05	0.23	100.0%	100.0%
	log (CRP) * Day	−0.01	−0.02	0.00	100.0%	100.0%
	log (CRP) * Exercise	0.04	−0.07	0.16	85.5%	90.2%

Variables of interest to our hypotheses

Graham-Engeland et al. suggested that affect measurements closer to the time of CRP measurement were more highly associated, i.e., day of affect measurement moderated the relationship between CRP and levels of affect (captured through a day * CRP interaction). We found such an effect unlikely for positive affect (86.1% probability) but highly probable for negative affect (more than 99% probability; see Table 1). Specifically, CRP appeared to manifest a strong association with negative affect towards the end of the study period (i.e., close to the time of CRP testing), but yielded almost no association with negative affect at the start of the study period (Fig. 2). In particular, it should be noted that any third variables, such as stress, are assumed to vary randomly and consequently yield no significant associations with the time of the blood draw.

We also predicted that BMI would moderate the effect of CRP on level of momentary affect. Indeed, participants with a high BMI reported a higher level of negative affect if they exhibited higher levels of CRP relative to high BMI individuals with lower level of CRP (see Fig. 2). In contrast, participants with a low BMI reported lower levels of negative affect as CRP increased (over a 99% probability). However, BMI was considerably less likely to moderate the effects of CRP on positive affect, which yielded a 77.1% probability.

Discussion

In the present study, we sought to replicate a hypothesis put forward by Graham-Engeland et al. (2018) that suggested a time-variant relationship between levels of inflammation and affect based on a single measurement of CRP and multiple measurements of daily affect. Our approach included important moderators of an inflammation and affect relationship, accounted for an initial elevation bias, and used a Bayesian multilevel regression (as opposed to a series of OLS regression models). Out data supported a time-variant relationship between inflammation and negative affect, whereby inflammation was better able to estimate levels of affect when the two measurements had close temporal proximity.

A further point of difference between Graham-Engeland et al. (2018) study, besides methodology, was our substantially different sample. Our sample was large with vastly different demographic characteristics (i.e., age, education, and socio-economic status). Despite these differences between the two studies, we supported a time-variant relationship between inflammation and affect. This replication with a different sample adds strong support to a presumed association between inflammation and affect, but also raises questions about the source of inflammation and

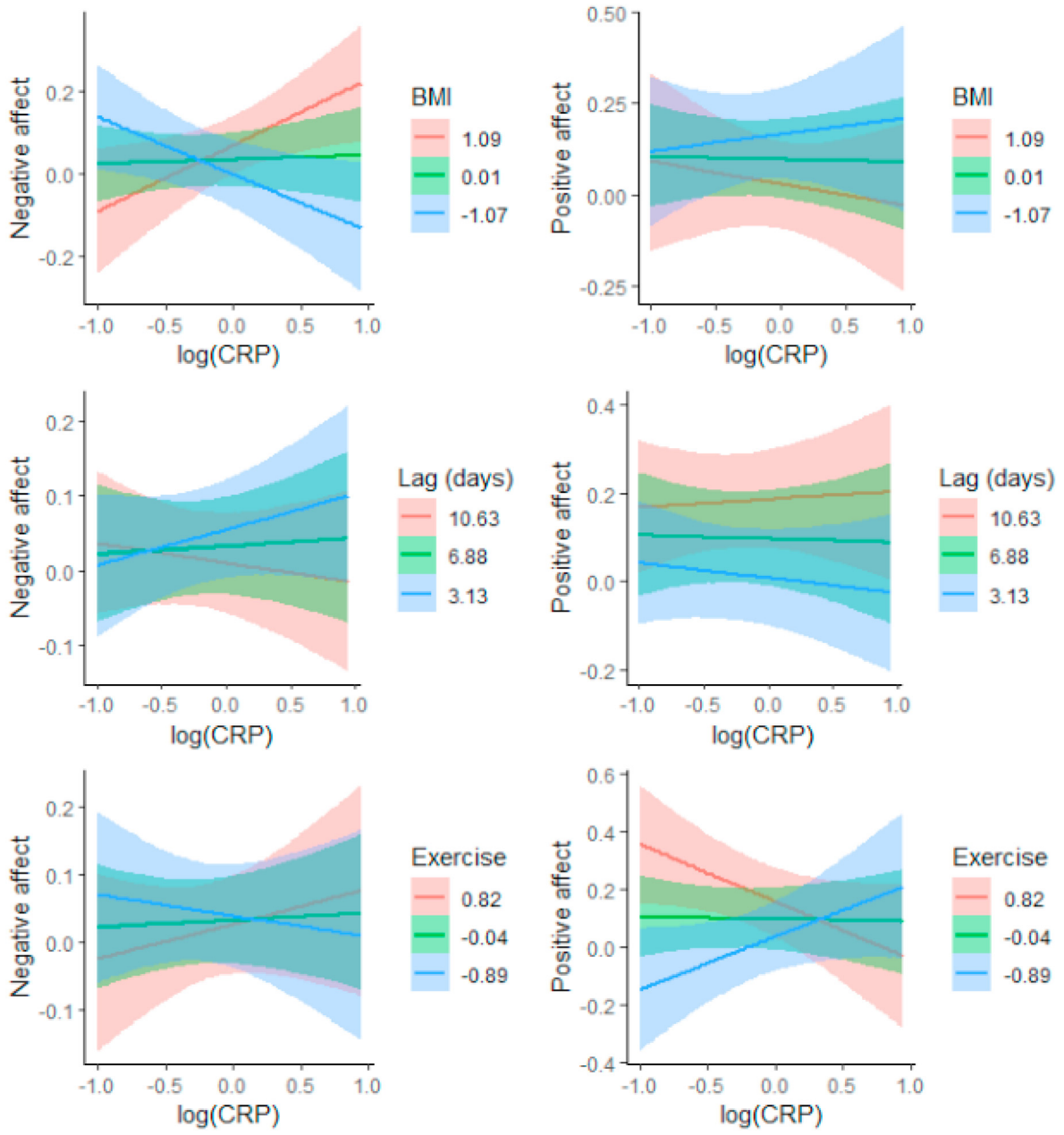

Fig. 2. Marginal effects of BMI (top panel), lag in days from blood draw (middle panel), and exercise (bottom panel) as moderators of log (CRP) on positive and negative affect. BMI and exercise are standardised whereas lag in days is the unstandardized number of days prior that affect measurement was taken relative to blood draw. Error bands represent 95% credible intervals.

how it might relate to causative factors of inflammation that are thought to be stable and persistent, such as the gut-microbiome (David et al., 2014).

On this note of mechanism implicated in inflammation processes, it should be noted that despite our more thorough investigation of potential moderators, the possibility of third variable confounding is far from exhausted given the complexity of the immune system and modes of brain-behaviour interaction. Another such possibility could be daily stress, which some researchers believe can have a top-down influence on an individual's inflammation, and it would also evoke higher negative and lower positive affect.

Of the moderators that were investigated, however, we saw that higher BMI predicted increases in the association between inflammation with negative affect, consistent with previous research that has supported a similar effect in depression (Qin et al., 2017). In contrast to previous research, however, we used momentary affect rather than depression as an outcome, and we used a much younger sample (Qin et al. used an elderly Chinese sample). Thus, the mechanism by which BMI moderates affect is likely attributable to adipose tissue, rather than a comorbidity such as atherosclerosis which is likely to develop in later life.

Our findings also supported the view that physical activity would function as a moderator of inflammation and mood. Specifically, we found that people who engaged in physical activity manifested a smaller increase in negative affect associated with inflammation relative to people who did not engage in physical activity. However, physical activity did not moderate the relationship between inflammation and affect when levels of inflammation were high. Literature suggests that physical activity causes acute inflammation (Suzuki et al., 2002), so perhaps the moderation we observed obscured or controlled for small amounts of inflammation associated with physical activity, whereas higher levels of inflammation are more likely to be associated with adverse factors such as illness, injury, diet, or a dysfunctional gut microbiome.

Conclusions

Our study identified temporal variation in the strength of the relationship between a single measure of inflammation (CRP) and negative affect as assessed over a series of consecutive days: the association between negative affect and CRP, positive in direction, was stronger for daily assessments nearer to the time of CRP assessment. Our findings replicated results obtained by Graham-Engeland et al. (2018) with a dissimilar sample (different demographic characteristics). Collectively our findings suggest that when investigating relationships of other variables with CRP inflammation, it is important to collect measurements on mood and behaviour near the time of blood collection. Our initial motivation to replicate and enhance Graham-Engeland et al.'s study was due to our interest in the association between inflammation and the gut microbiome. However, our findings would suggest a more thorough investigation of inflammation on a momentary basis is required before any assertions can be made about the use of inflammatory markers as proxies of chronic health factors.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was supported by the Health Research Council of New Zealand (#12/709) with a grant for 149,976NZD awarded to Tamlin S. Conner (PI) and Tony R. Merriman (AI).

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