

# BMJ Open Examining chronic inflammatory markers on blood pressure measures in the presence of vitamin D insufficiency among indigenous cree adults: results from the cross-sectional Multi-Community Environment-and-Health Study in *Eeyou Istchee*, Quebec, Canada

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## ABSTRACT

**Objective** High blood pressure (BP) is a risk factor for cardiovascular disease. Examining the role of inflammatory mediators on BP is important since vitamin D (VD) is a modifiable risk factor, which possibly modulates inflammatory cytokines. This study simulated what are known as average ‘controlled direct effects (CDE)’ of inflammatory markers, C reactive protein (CRP), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-6 (IL-6) on continuous BP measures, while fixing VD, an intermediate variable to specific level.

**Design** Cross-sectional study.

**Setting** We analysed data from the Multi-Community Environment-and-Health Study, 2005–2009, conducted in *Eeyou Istchee*, Quebec, Canada.

**Participants** This study recruited 1425 study Indigenous Cree participants from seven Cree communities. Only adults with serum VD levels, inflammatory markers and BP measures were included in this data analysis.

## Primary and secondary outcomes

**measures** Inflammatory markers examined the top 25th exposure percentiles. VD ‘insufficiency’ (ie, 25-hydroxyvitamin-D levels <50 nmol/L) defined by the Institute of Medicine. CDE for each inflammatory marker in the presence and absence of population VD insufficiency simulated the average direct effect change for systolic and diastolic BP (SBP and DBP) measures. All models were adjusted for exposure-and-mediator outcome relationship.

**Results** Among 161 participants, 97 (60 %) were female. The prevalence of VD insufficiency was 32%. CDE estimates show in the presence and absence of population vitamin D insufficiency, inflammatory markers have a slightly different association on BP. TNF- $\alpha$  significantly and inversely associated with SBP in the presence of vitamin D insufficiency, fully adjusted model  $\beta = -13.61$  (95% CI  $-24.42$  to  $-2.80$ ); however, TNF- $\alpha$  was not associated with SBP in the absence of vitamin D insufficiency. CRP, IL-6 were also not significantly associated with BP measures, although the magnitude of association was

## Strengths and limitations of this study

- This was a unique sample population with measurements of both inflammatory markers and vitamin D.
- This is the first study to examine simulated ‘controlled directed effects’ between inflammation and serum vitamin D on blood pressure, a cardiovascular risk factor.
- This study is limited in sample size, and due to the nature of the study design the temporal relationship between inflammatory markers, vitamin D and blood pressure cannot be established. The study design precludes any causal interpretation of our results.

greater for those with elevated inflammation and VD insufficiency.

**Conclusion** This novel analysis shows in the presence of VD insufficiency, inflammation (particularly TNF- $\alpha$ ) may affect SBP. Additional research is needed to elucidate these findings, and the temporal relationship between these variables.

## INTRODUCTION

Hypertension or high blood pressure (BP) is a risk factor for cardiovascular disease (CVD),<sup>1</sup> which is the leading cause of death and disability worldwide.<sup>2</sup> In Canada, 25% of Canadians are reported to have hypertension.<sup>2</sup> Among Indigenous peoples in Canada, the prevalence of hypertension is also progressively increasing, and CVD is reported to be the leading cause of death among Indigenous Peoples in Canada.<sup>3</sup> Inflammation has been associated with hypertension in both experimental animal models and human studies<sup>4–9</sup>; however, due to the complexity of this relationship, the mechanisms between inflammation



and vascular involvement is still unclear. Proinflammatory markers are shown to be important in the development of atherosclerotic CVD complications.<sup>10</sup> Acute C reactive protein (CRP) is a systemic marker inflammation, and has been shown to contribute to hypertension.<sup>11 12</sup> Previous studies have shown that elevated CRP concentrations contribute to CVD events, such as myocardial infarctions<sup>13–15</sup> and stroke.<sup>16–19</sup> CRP concentrations have also been associated with both incident and prevalent hypertension.<sup>12 20–23</sup> Tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ), a proinflammatory cytokine primarily produced by macrophages, is necessary for normal immune function, and inflammatory response; however, inflammatory imbalances can contribute to chronic inflammatory diseases, such as arthritic-related conditions and inflammatory bowel diseases.<sup>24</sup> TNF- $\alpha$  concentrations are found to be elevated in various cardiovascular conditions (eg, advanced health failure, cardiomyopathy),<sup>25</sup> and may also induce vascular inflammation, which may contribute to the pathogenesis of atherosclerosis.<sup>24</sup> Similarly, interleukin-6 (IL-6), another important proinflammatory cytokine has been shown to play a critical role in the development of atherosclerosis and atherosclerotic disease.<sup>26</sup> Cross-sectional and prospective studies have shown that IL-6 is associated with elevated BP measures.<sup>4–7 27–29</sup> Thus, the role of inflammatory mediators on cardiovascular risk factors such as BP is important, particularly since modifiable risk factors such as vitamin D may potentially modulate inflammation, and possible inflammatory related diseases.<sup>30 31</sup>

Vitamin D is obtained through synthesis in the skin following exposure to ultraviolet-B light, as well as from dietary sources. Ultraviolet-B light is too weak to permit synthesis during the winter, and at latitudes above and below 37° North and South, respectively.<sup>32 33</sup> Vitamin D is essential for human health, as it promotes bone mineralisation and maintains calcium homeostasis.<sup>34</sup> Vitamin D has also been reported to have anti-inflammatory effects by attenuating inflammatory cytokines<sup>35 36</sup>; however, opposing views have been reported in that inflammation may potentially lower vitamin D concentrations.<sup>37</sup> Furthermore, vitamin D receptors (VDR) located in cells throughout the body act as biological mediators capable of immuno-modulating regulating effects through the interaction between the active form of vitamin D and VDR expression.<sup>38</sup> A recent systematic review and meta-analysis of randomised controlled trials evaluated the effect of vitamin D<sub>3</sub> supplements on BP among persons with vitamin D deficiency (ie, 25-hydroxyvitamin D 25(OH) D < 50 nmol/L or 20 ng/mL). The results from the subgroup analysis report that participants over 50 years of age and those with body mass index (BMI) greater than 30 kg/m<sup>2</sup> showed a significant reduction in BP measures (ie, systolic and diastolic BP (SBP/DBP)).<sup>39</sup> Other reviews of the literature have come to similar conclusions, although there is still no consensus on the relationship between vitamin D and regulation of BP.<sup>40</sup> Thus, investigating the effect of inflammatory markers (ie, CRP, TNF- $\alpha$  and IL-6) in the presence of vitamin D insufficiency is warranted

given the public health importance, particularly among Indigenous People who are greater risk of lower serum vitamin D levels due to living in northern latitudes. To date, no study has been specially designed to examine multiple longitudinal venous measures of serum vitamin D and inflammatory markers, which could possibly elucidate temporal variations in high-risk populations. Thus, importantly, these hypothesis-generating study results preclude any causal interpretation or conclusion. Therefore, we simulated what are known as average ‘controlled direct effects’ (CDE) of inflammatory markers on continuous BP measures fixing the intermediate variable vitamin D to a specific level in the population among Cree communities residing in Eeyou Istchee, northern Quebec, Canada.

## METHODS

### Data sources

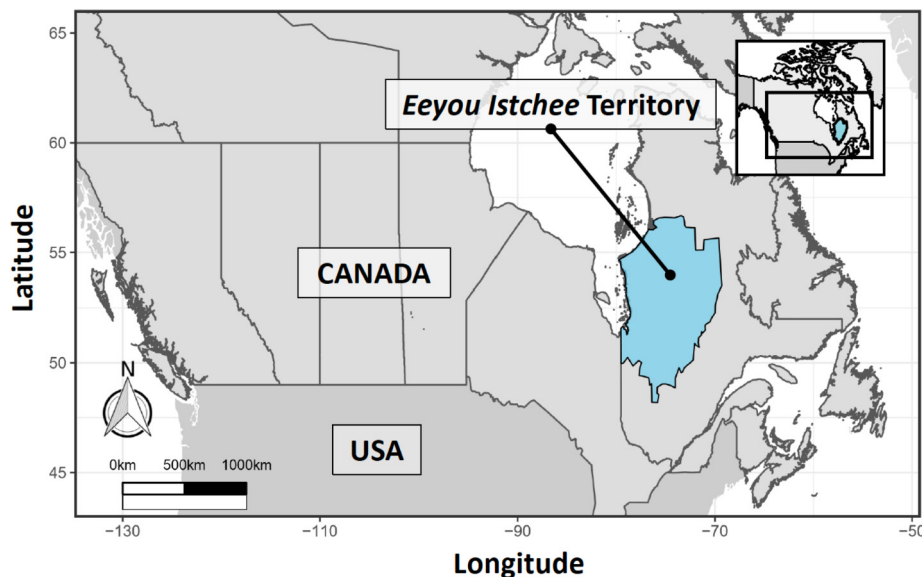
The *Nituuchiscaayihitaa Aschii* (‘Learn about ourselves and our earth’), Multi-Community Environment-and-Health Study, 2005–2009, was conducted in the Eeyou Istchee territory, located in the James Bay Region of northern Quebec, Canada (figure 1). Complete study details about the Multi-Community Environment-and-Health Study are provided elsewhere.<sup>41</sup> In brief, seven Cree communities in this region were included in this cross-sectional study. Both children and adults were enrolled; specifically, participants were stratified according to age ranges: children (0–7 years, and 8–14 years), and adults (15–39 years, and 40 years and older), which included adolescent participants. The primary aim of the study was to assess the link between diet, environmental contaminants exposures, lifestyle factors and overall health status. Written and informed consent was obtained from all participants or their guardians in Cree, English or French.

### Study population

The Environment-and-Health Study, 2005–2009, recruited 1425 study participants. Full details regarding the study recruitment and sampling are described elsewhere.<sup>41</sup> Our analysis included adults between 20 and 80 years of age who had undergone a physical examination, completed interviewer-administered health questionnaires, and completed a phlebotomy blood draw. Only adults with the following measures were included in the analysis: (1) valid BP measures; (2) complete inflammatory marker exposure profiles (ie, CRP, TNF- $\alpha$  and IL-6); and had (3) valid measure of 25-hydroxyvitamin D, 25(OH)D. The final analysis included 161 cases from seven of the nine communities from the Eeyou Istchee territory. A flow chart of the sample is presented in the online supplemental figure S1.

### Exposures and intermediate variable assessment

Whole blood samples were collected among participants and temporarily stored and frozen at –20 °C or –80 °C,



**Figure 1** Eeyou Istchee Territory, Quebec, Canada.

until aliquoted samples were sent to the Centre Hospitalier Universitaire de Québec, in Québec City. High-sensitivity CRP (hs-CRP) was measured by nephelometry using a BN ProSpec station (Dade Behring, Mississauga, Ontario). Expected values for healthy individuals are typically  $<3$  mg/L (Eastmain-Wemind 2011). TNF- $\alpha$  and IL-6 were measured in EDTA-plasma using respective human ELISA kits (Quantikine HS, R&D System, Minneapolis, Minnesota, USA). Quantitative measure of serum 25-hydroxyvitamin D, [25(OH)D], which is a marker of total vitamin D status was carried out by a procedure including protein extraction and quantitation by competitive radioimmunoassay using the IDS RIA kit (Medicorp, Montréal, Québec). Total vitamin D [25(OH)D] has a half-life range of  $\approx 2$ –3 weeks.<sup>42–45</sup> The Institute of Medicine (IOM) report 25-hydroxyvitamin D [25(OH)D] levels greater than 50 nmol/L (20 ng/mL) as ‘sufficient’ for the majority of the population.<sup>46</sup> For this analysis, we report vitamin D levels according to the IOM definitions. However, the Endocrine Society Task Force on vitamin D report different definitions and cut-off levels for vitamin D (ie, sufficient [25(OH)D] levels are greater than 75 nmol/L or 30 ng/mL).<sup>47</sup>

### Outcome assessment

For participants who completed the physical examination, BP measurements were taken in accordance with WHO clinical guidelines for the management of hypertension.<sup>41</sup> Specifically, participants were instructed to refrain from eating and smoking at least 30 min before BP assessment, and just prior to BP measurements participants rested for 5 min. SBP and DBP were measured in units of millimetres of mercury (mm Hg). Three BP readings were taken in a sitting position, and the mean measurement of SBP and DBP was calculated using the last two measurements. Classification of BP was according to the new 2017 clinical BP guidelines.<sup>48</sup>

### Risk factors

Interviewer-administered health questionnaires collected sociodemographic and health-related behaviour information. Information was gathered on the following covariates: age (years continuous) and sex. Data related to smoking habits were collected, two broad group categories are available for smoking habits, current and occasional smokers, and never and former smokers. The clinical physical exam measured weight in kilograms (kg), and height in metres. BMI was calculated by dividing weight by height in metres squared (BMI, kg/m<sup>2</sup>).

### Statistical analysis

Descriptive statistics were calculated for all covariates, and stratified by an intermediate variable ( $m$ ), serum vitamin D status (ie, vitamin D ‘insufficiency’  $<50$  nmol/L, and vitamin D ‘sufficiency’  $\geq 50$  nmol/L). Means (or geometric means)  $\pm$ SD are presented where appropriate. Categorical data are reported as frequencies and percentages.

Using multivariable regression models under potential outcomes framework, CDE were estimated.<sup>49,50</sup> Inflammatory marker levels (ie, hs-CRP, TNF- $\alpha$ , IL-6) at or above the 75th percentile were compared with values below the 75th percentile among participants, classifying inflammatory markers into two exposure levels,  $a=1$  and  $a^*=0$ . CDE for each inflammatory marker exposure level indicate the average direct effect for a population, that is, change of SBP and DBP outcomes if the exposure is contrasted (ie,  $a=1$ , and  $a^*=0$ ), while uniformly fixing the intermediate variable ( $m$ ) to either designated level, vitamin D insufficiency or vitamin D sufficiency (ie,  $m(1)$  and  $m(0)$ , respectively) in the population.<sup>51</sup> CDE estimates can be written in the following form:

$$E[Y(a, m) - Y(a^*, m)] \text{ or equivalently}$$

$$E[Y(a, m(1) - Y(a^*, m(1)))] \text{ and } [Y(a, m(0) - Y(a^*, m(0))]$$

Marginal structural models (MSM) with stabilised inverse probability weighting techniques were used to estimate CDE.<sup>49 50 52</sup> Using SAS software PROC GENMOD procedures, a final generalized estimating equation (model) was fit with an independent working correlation structure (type=ind), created stabilised weights and robust sandwich estimator to produce 95% CIs. All models were adjusted for the following a priori covariates of the exposure-outcome and mediator-outcome relationship: age (continuous), sex, smoking status (categorical) and BMI (continuous). Estimates of CDE assume no further unmeasured confounding of the exposure-outcome relationship and the mediator-outcome relationship. Statistical analyses were carried out using SAS V.9.4 (SAS Institute), and geographical map was generated using R (V.3.5.3; Vienna, Austria).

### Patient and public involvement

There was no patient or public involvement in the design, conduct or results interpretation of this study. Peer-reviewed publications using data from the Multi-Community Environment-and-Health Study are disseminated to the Cree Board of Health (CBHSSJB).

## RESULTS

### Descriptive results

Population study characteristics are presented in table 1 (and online supplemental table 1S). In total, there were 161 participants, and 97 (60%) were female. The overall mean age ( $\pm$ SD) was 42.7 $\pm$ 14.3 years. Among participants, 43% self-reported being 'current and occasional' smokers compared with former or never. At the time of examination, the mean BMI among all participants was (34 kg/m<sup>2</sup>), and this was similar among adults with and without vitamin D insufficiency. Prevalence of vitamin D insufficiency <50 nmol/L was 32% among Indigenous Cree adults. The geometric means for serum CRP, TNF- $\alpha$  and IL-6 were 3.7 mg/L, 1.9 pg/mL and 2.9 pg/mL, respectively. According to the new 2017 clinical BP guidelines, 39% of Indigenous adults were classified as having hypertension (ie, stage 1 or higher), 16.1% had elevated BP and 44.7% has normotensive BP readings.

### Controlled direct effects

CDE estimates in the presence of vitamin D insufficiency, and vitamin D sufficiency are presented in tables 2 and 3. Average CDE for a population represent the association of high inflammatory makers (ie,  $\geq$ 75%) on continuous SBP or DBP measures if everyone in the population had either vitamin D insufficiency, or intervening to eliminate vitamin D insufficiency. Across all weighted models, high TNF- $\alpha$  levels and vitamin D insufficiency significantly decreased SBP  $\beta = -13.61$  (95% CI -24.42 to -2.80) among adults (table 2, model 3); however, high TNF- $\alpha$  levels and vitamin D sufficiency was not significantly associated with SBP measures. DBP results were not significantly associated with high TNF- $\alpha$  levels and vitamin D

insufficiency, or with vitamin D sufficiency (table 3). In the fully weighted model (table 2, model 3), high levels of CRP non-significantly increased SBP (and DBP) by  $\beta = 1.76$  (95% CI -14.75 to 18.29) among vitamin D insufficiency, but with vitamin D sufficiency, SBP and DBP differences were negligible  $\beta = 0.41$  (95% CI -6.93 to 7.65). Lastly, though not statistically significant, high IL-6 concentrations and vitamin D insufficiency show a larger increase in both SBP and DBP, when compared with only high IL-6 levels with sufficient vitamin D levels.

## DISCUSSION

The effect of inflammatory markers and vitamin D status on cardiometabolic risk factors like SBP and DBP has not been studied. In this cross-sectional analysis using data from the Environment-and-Health Study, we show that inflammatory markers have a slightly different association on BPs among adults, particularly in the presence and absence of population vitamin D insufficiency. Specifically, high levels of TNF- $\alpha$  were inversely associated with SBP measures in the presence of vitamin D insufficiency, whereas at sufficient vitamin D levels, (ie, if vitamin D insufficiency was blocked for the population), TNF- $\alpha$  appears not to have the same relationship. The average CDE of BP for the population under the potential outcomes framework compares the presence and absence of high levels of inflammation with fixed vitamin D levels either insufficiency or vitamin D sufficiency.<sup>53</sup>

To date, this is a novel association. Very few studies have examined the effect between serum vitamin D and inflammatory markers among adults without chronic inflammatory diseases.<sup>54</sup> TNF- $\alpha$  is a proinflammatory cytokine that has immunomodulatory effects. However, not many studies have aimed to examine the association between inflammation and BP measures. TNF- $\alpha$  has been associated with both an increase and decrease in BP, but the cause remains unclear.<sup>55</sup> Particularly, studies have shown mixed results between TNF- $\alpha$  and BP measures. For example, TNF- $\alpha$  was shown to have a significant positive correlation with SBP and DBP measures among healthy Japanese women.<sup>56</sup> Among Japanese adults, plasma levels of TNF- $\alpha$  were higher among adults with essential hypertension, even among the controlled hypertensive group compared with normotensive adults.<sup>57</sup> Similarly, among healthy Columbian adults, TNF- $\alpha$  levels in the fourth quartile were 1.45 times more likely to have high BP than those in the lowest quartile.<sup>4</sup> However, in matched analysis, Sheu *et al*<sup>58</sup> report that TNF- $\alpha$  concentrations were not significantly different between adults with and without hypertension. Furthermore, in a study among older Caucasian adults aged 50–69 years, TNF- $\alpha$  from the 25th to the 75th centile found a non-significant negative association with SBP, -1.26 (95% CI -4.64 to 2.12).<sup>59</sup> The negative association found in our analysis and others is reported to be due to TNF- $\alpha$  concentrations, that is, higher concentrations or more severe inflammation are associated with an inverse relationship with BP, whereas

**Table 1** Descriptive statistics stratified by vitamin D status among Indigenous Cree adults from the *Nituuchischaayihitaaau Aschii*—Multi-Community Environment-and-Health Study, 2005–2009

Participant characteristics	Total population (n=161)	Range (IQR)	Serum vitamin D(25(OH)D)*	
			Insufficiency N (%); or mean±SD	Sufficiency N (%); or mean±SD
Total population			52 (32.3%)	109 (67.7%)
Serum vitamin D, 25(OH)D	63.8±25.9		40.4±5.8	71.5±24.6
Demographic information				
Sex (n, %)				
Females	97 (60.2%)		35 (36.1%)	62 (63.9%)
Males	64 (39.8%)		17 (26.6%)	47 (73.4%)
Age (years)	42.7±14.3		35.4±11.8	46.1±14.1
Risk factors				
Smoking status				
Current or occasional smokers	69 (42.9%)		32 (46.4%)	37 (53.6%)
Former or never (R)	92 (57.1%)		20 (21.7%)	72 (78.3%)
Anthropometry				
Body mass index, BMI (kg/m <sup>2</sup> )	34.2±6.1		35±6.6	33.8±5.8
Inflammatory markers (GM and GSD)†				
hs-CRP (mg/L)	3.71±2.52	0.40–71.40 (IQR: 1.70–6.84)	3.98±2.47	3.59±2.54
TNF-α (pg/mL)	1.93±1.92	0.55–9.53 (IQR: 1.12–3.06)	1.87±1.93	1.96±1.93
IL-6 (pg/mL)	2.89±1.86	0.83–26.61 (IQR: 1.80–3.97)	3.22±1.75	1.17±0.56
Chronic conditions				
Blood pressure (BP) measures‡				
Systolic BP	122.4±16.6		119.5±16.9	123.7±16.3
Diastolic BP	73.0±11.2		73.2±10.8	72.9±11.9
Hypertensive (stage 1 and 2)	63 (39%)		21 (33.3%)	42 (66.7%)
Elevated BP	26 (16.1%)		8 (30.8%)	18 (69.2%)
Normotensive	72 (44.7%)		23 (31.4%)	49 (68.1%)
MCR type 2 diabetes§				
Present	35 (23.8%)		10 (28.6%)	25 (71.4%)
Absent	112 (76.2%)		34 (30.4%)	78 (69.6%)

Missing values: body mass index, BMI, n=2 missing.

\*Baseline serum vitamin D [25(OH)D] was defined as vitamin D insufficiency, that is, [25(OH)D]<50 nmol/L or <20 ng/mL, or vitamin D sufficiency [25(OH)D]≥50 nmol/L or ≥20 ng/mL according to the Institute of Medicine [25(OH)D] definitions, Ross *et al*, 2011. In our analysis, only n=5 and n=2 participants had values above 125 nmol/L, and 150 nmol/L, respectively.

† Presented as geometric means (GM)±SD (GSD).

‡ Blood pressure definitions according to new 2017 Clinical Practice Guidelines from the American College of Cardiology/American Heart Association (ACC/AHA), Whelton *et al*, 2017.

§ Medical chart reviewed (MCR) diagnosis of type 2 diabetes among adults over 20 years, verified individual health-related information ascertained from health questionnaires. In total, (n=14) adults were missing MCR type 2 diabetes data.

%, percentage; hs-CRP, high-sensitivity C reactive protein; IL-6, Interleukin 6; N, frequency value; R, reference group; TNF-α, tumour necrosis factor.

moderate levels are reported to increase hypertension.<sup>55</sup> Though not entirely elucidated, possible proposed mechanisms include the overall health status of the individual, the source of the inflammatory stimulus, or different varying renal TNF-α concentrations, which may induce renal haemodynamic disruption (eg, vasoconstriction and hypofiltration) and change the excretory function of the kidneys causing diuresis and natriuresis.<sup>55 60</sup> Interestingly, a recent study discusses a new concept that TNF

plays a role in regulating skeletal muscle resistance and thereby able to adapt haemodynamic parameters such as BP, which was found among several species including humans.<sup>61</sup> Kroetsch *et al*<sup>61</sup> also report the possibility that TNF acts as a mechanosensor; this novel TNF function would impact pathogenesis of certain underlying disease processes.

The link between elevated C reactive protein (CRP), a marker of systemic inflammation and cardiovascular

**Table 2** Adjusted marginal structural models estimating controlled direct effects (CDE) of inflammatory markers (i.e., high-sensitivity C reactive protein (CRP), tumour necrosis factor (TNF), and Interleukin 6 (IL-6)) on systolic blood pressure (SBP) measures in the presence and absence of vitamin D (VD) insufficiency [25(OH)D<50 nmol/L] among Indigenous Cree adults from the Multi-Community Environment-and-Health Study, 2005–2009

	CDE for SBP measures					
	VD insufficiency present ( $m=1$ )			VD insufficiency absent ( $m=0$ )		
	$\dagger E(Y(a,m(1)) - Y(a^*,m(1)))$			$\dagger E(Y(a,m(0)) - Y(a^*,m(0)))$		
	Estimate $\beta$	95% CI	P value*	Estimate $\beta$	95% CI	P value*
<b>CRP</b>						
Model 1	-2.32	-15.19 to 10.54	0.724	0.78	-5.30 to 6.85	0.803
Model 2	-1.06	-14.50 to 12.37	0.877	0.90	-5.72 to 7.56	0.789
Model 3	1.76	-14.75 to 18.29	0.839	0.41	-6.93 to 7.75	0.913
<b>TNF-<math>\alpha</math></b>						
Model 1	-13.67	-23.78 to -3.56	<b>0.008*</b>	-5.70	-11.47 to 0.08	0.053
Model 2	-13.61	-23.54 to -3.07	<b>0.011*</b>	-5.92	-11.65 to -0.18	0.043
Model 3	-13.61	-24.42 to -2.80	<b>0.014*</b>	-5.73	-11.59 to 0.13	0.052
<b>IL-6</b>						
Model 1	5.79	-15.26 to 26.85	0.589	1.85	-7.94 to 11.64	0.711
Model 2	7.81	-13.59 to 29.22	0.474	1.54	-7.70 to 10.80	0.743
Model 3	13.9	-13.32 to 41.15	0.317	2.62	-10.75 to 15.99	0.701

\*Statistical significance ( $p<0.05$ ).

$\dagger$ CDE for the each inflammatory marker exposure level indicating the average direct effect change on SBP and diastolic blood pressure outcomes if the exposure is contrasted (ie,  $a=1$ , and  $a^*=0$ ), while uniformly fixing the intermediate variable ( $m$ ) to either designated level, VD insufficiency or VD sufficiency (ie,  $m(1)$  and  $m(0)$ , respectively) in the population. Model 1: age, sex; Model 2: model 1, plus smoking status; Model 3: model 2, plus body mass index.

risk, has been extensively studied; however, a causal link is yet to be determined.<sup>62–64</sup> In a recent meta-analysis of prospective and retrospective studies, higher circulating inflammatory markers (eg, CRP and IL-6) were found to be associated with hypertension risk.<sup>65</sup> Likewise, in a review of CRP and hypertension, antihypertensive medication has been shown to lower circulating CRP levels independently of BP effects, which is possibly why in our study results CRP was found not to be associated with continuous measurement of BP.<sup>11</sup> Additionally, in a meta-analysis of cohort studies and randomised controlled trials examining the effect of vitamin D on BP, Zhang *et al*<sup>66</sup> report a L-shaped relationship between circulating 25(OH)D levels and hypertension risk; specifically, risk of hypertension was shown to be greater with 25(OH)D levels less than 75 nmol/L. However, when examining pooled results of the randomised control trials, vitamin D supplementation was not associated with a significant decrease in the weighted mean differences for either SBP or DBP reduction.<sup>66</sup> Among Eastern James Bay Cree, mean vitamin D concentrations are reported to be lower when compared with data from the Canadian Health Measures Survey.<sup>67</sup>

Changes in diet (ie, limited traditional food consumption) and changes to the subsistence way of life influence vitamin D status and bone mineral density levels.<sup>67 68</sup> Racial and ethnic differences have been reported to affect vitamin D status. Data from the National Health and

Nutrition Examination Survey report that African and Mexican Americans have significantly lower 25(OH)D levels when compared with white Americans.<sup>69</sup> However, African Americans paradoxically have higher bone mineral density scores despite lower reported vitamin D concentrations.<sup>70–72</sup> Similarly, Indigenous people living at northern latitudes are susceptible to lower vitamin D levels, which may have led to biological adaptations that result in lower but still ‘optimal’ vitamin D levels.<sup>73 74</sup> VDRs are widely distributed in tissues throughout the human body, being important in mediating the biological actions of the active form of vitamin D.<sup>75</sup> A few studies (with mixed results) have examined VDR polymorphisms and risk of hypertension. Interestingly, a gene-nutrient interaction study among a Canadian northern First Nations (Dené) cohort found that vitamin D binding protein and VDR gene polymorphisms affect the bioavailability and regulation of serum vitamin D.<sup>76</sup> Specifically, in this cohort, VDR *FokI*-f allele was found in 82% of participants, and is reported to be associated with downregulating T helper type 1 (Th1) immune response (ie, cells that secrete TNF- $\alpha$  and cytokine interferon  $\gamma$ ).<sup>76 77</sup> Possibly, VDR gene polymorphisms are linked to chronic inflammation,<sup>78</sup> which may play a role in high BP. In a matched case-control study of 280 adults with hypertension, compared with normotensive controls, genotypes of VDR *FokI* polymorphisms (ie, ‘FF’ and ‘Ff’ vs ‘ff’ genotypes) show that the odds of hypertension were significantly higher for the

**Table 3** Adjusted marginal structural models estimating controlled direct effects (CDE) of inflammatory markers (i.e., high-sensitivity C reactive protein (CRP), tumour necrosis factor (TNF), and Interleukin 6 (IL-6)) on diastolic blood pressure (DBP) measures in the presence and absence of vitamin D (VD) insufficiency [25(OH)D<50 nmol/L] among Indigenous Cree adults from the Multi-Community Environment-and-Health Study, 2005–2009

	CDE DBP					
	VD insufficiency present (m=1)			VD insufficiency absent (m=0)		
	†E(Y(a,m(1)) – Y(a*,m(1)))			†E(Y(a,m(0)) – Y(a*,m(0)))		
	Estimate β	95% CI	P value*	Estimate β	95% CI	P value*
<b>CRP</b>						
Model 1	2.04	–4.28 to 8.35	0.526	3.29	–0.71 to 7.31	0.107
Model 2	1.54	–4.73 to 7.82	0.630	3.24	–1.10 to 7.94	0.137
Model 3	0.69	–5.70 to 7.07	0.834	1.59	–2.69 to 5.89	0.466
<b>TNF-α</b>						
Model 1	–5.08	–13.31 to 3.15	0.226	–2.29	–6.62 to 2.02	0.298
Model 2	–5.05	–13.24 to 3.15	0.228	–2.69	–6.92 to 1.52	0.210
Model 3	–5.25	–13.46 to 2.95	0.209	–2.53	–6.67 to 1.61	0.231
<b>IL-6</b>						
Model 1	6.28	–3.32 to 15.88	0.199	3.33	–1.53 to 8.20	0.179
Model 2	6.40	–3.00 to 15.81	0.182	3.43	–1.44 to 8.30	0.167
Model 3	7.88	–3.74 to 19.52	0.184	2.51	–3.83 to 8.87	0.437

\*Statistical significance (p<0.05)

†CDE for the each inflammatory marker exposure level indicating the average direct effect change on systolic blood pressure and DBP outcomes if the exposure is contrasted (ie, a=1, and a\*=0), while uniformly fixing the intermediate variable (m) to either designated level, VD insufficiency or VD sufficiency (ie, m(1) and m(0), respectively) in the population. Model 1: age, sex; Model 2: model 1, plus smoking status; Model 3: model 2, plus body mass index.

FF genotype and allele F.<sup>79</sup> In contrast, a prospective study of men without hypertension shows the risk of hypertension was higher for ff genotype carriers of *FokI* polymorphisms compared with FF and Ff genotypes combined.<sup>80</sup> Although further research is needed, genetic, environmental and lifestyle factors are shown to contribute to vitamin D status and the innate immune response, which could have effects on risk factors such as BP measures.

This is the first study to contrast counterfactual outcomes between specific inflammatory markers and vitamin D status, which is reported to possibly modulate the inflammatory response on cardiometabolic risk factors such as BP measures among Indigenous Cree adults. MSM that estimate CDE offer the capability of setting different set of confounders for the exposure-outcome, and mediator-outcome relationship, which are especially important in the presence of confounders between vitamin D and BP measures that are affected by inflammatory markers. Standard regression models in such cases would not be sufficient and lead to biased estimates.<sup>81 82</sup> Furthermore, the intermediate variable (ie, vitamin D status) is also amenable to human intervention, and CDE are often noted as having greater public health interest.<sup>82 83</sup> However, this study had several limitations. First, this is a cross-sectional analysis and temporality between inflammatory markers, vitamin D and measures of BP is not established as all measures are taken at the same point in time. Second, only a single measure of vitamin D was obtained,

although this may be an adequate reflection of yearly average of vitamin D concentrations.<sup>84</sup> Third, smoking status is a broadly defined variable, and therefore, may result in residual confounding by smoking. Smoking is a known risk factor for cardiovascular outcomes; however, the connection between smoking and BP measures are reported to be small, and the exact mechanism remains to be determined.<sup>85</sup> Furthermore, research has shown that vitamin D levels tend to be significantly lower among current smokers compared with non-smokers.<sup>86</sup>

In conclusion, this novel analysis shows that TNF-α in the presence of population vitamin D insufficiency was significantly associated with lower SBP. However, further research is needed to elucidate this CDE association given the important modulatory effects of vitamin D on inflammatory markers and their contribution to cardiometabolic risk factors.

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