

# The association between obesity, cardiometabolic disease biomarkers, and innate immunity-related inflammation in Canadian adults

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**Introduction:** Obesity is associated with a state of chronic inflammation, and increased cardiometabolic disease risk. The present study examined the relationship between body mass index (BMI) and cardiometabolic and inflammatory biomarkers among normal weight, overweight, and obese Canadian adults.

**Methods:** Subjects (n = 1805, aged 18 to 79 years) from the Canadian Health Measures Survey (CHMS) were examined for associations between BMI, cardiometabolic markers (apolipoprotein [Apo] A1, ApoB, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], total cholesterol, total cholesterol/HDL ratio [total:HDL-C ratio], triglycerides, and glycosylated hemoglobin [HbA<sub>1c</sub>]), inflammatory factors (C-reactive protein [CRP], fibrinogen, and homocysteine), and 25-hydroxyvitamin D [25(OH)D]. Bootstrap weights for variance and sampling weights for point estimates were applied to account for the complex survey design. Linear regression models adjusted for age, sex, physical activity, smoking status, and ethnicity (in addition to season of clinic visit, for vitamin D analyses only) were used to examine the association between cardiometabolic markers, inflammatory factors, and BMI in Canadian adults.

**Results:** All biomarkers were significantly associated with BMI ( $P \leq 0.001$ ). ApoA1 ( $\beta = -0.31$ ,  $P < 0.0001$ ), HDL-C ( $\beta = -0.61$ ,  $P < 0.0001$ ), and 25(OH)D ( $\beta = -0.25$ ,  $P < 0.0001$ ) were inversely associated with BMI, while all other biomarkers showed positive linear associations. Distinct patterns of association were noted among normal weight, overweight, and obese groups, excluding CRP which showed a significant positive association with BMI in the overall population ( $\beta = 2.80$ ,  $P < 0.0001$ ) and in the normal weight ( $\beta = 3.20$ ,  $P = 0.02$ ), overweight ( $\beta = 3.53$ ,  $P = 0.002$ ), and obese ( $\beta = 2.22$ ,  $P = 0.0002$ ) groups.

**Conclusions:** There is an apparent profile of cardiometabolic and inflammatory biomarkers that emerges as BMI increases from normal weight to obesity. Understanding these profiles may permit developing an effective approach for early risk prediction for cardiometabolic disease.

**Keywords:** obesity, inflammation, biomarkers, cardiometabolic disease

## Background

Approximately 25% of Canadians are obese according to measured height and weight data from 2007–2009.<sup>1</sup> Obesity reduces length and quality of life and is associated with a number of chronic conditions including type 2 diabetes mellitus (T2DM), hypertension, cardiovascular disease (CVD), and some forms of cancer.<sup>2</sup> In addition, obesity has been shown to adversely impact psychosocial and psychological well-being.<sup>3</sup> As levels of obesity continue to rise globally, it is critical to understand the metabolic consequences of this condition and its etiological role in the development of chronic diseases.

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Once thought only to be an energy storehouse, it is now well recognized that adipose tissue has endocrine functions including the secretion of proinflammatory cytokines. Indeed, a common link between obesity and several comorbid conditions, including CVD and T2DM, is a state of chronic low-grade inflammation that accompanies these chronic conditions.<sup>4,5</sup> Obesity is associated with an increase in adipose tissue that results in higher circulating levels of free fatty acids, which in turn, inhibit insulin-stimulated glucose uptake.<sup>6</sup> This ultimately leads to increased plasma glucose levels, and elevated insulin synthesis and production.<sup>6</sup> Adipose tissue also secretes a variety of cytokines, such as the proinflammatory Tumor necrosis factor (TNF- $\alpha$ ) and Interleukin-6 (IL-6),<sup>5</sup> which can activate signal transduction cascades that inhibit insulin action.<sup>7</sup> IL-6 also stimulates production of acute phase proteins, such as C-reactive protein (CRP) and fibrinogen in the liver, which are markers of increased CVD risk.<sup>8,9</sup>

Currently, there is great interest in understanding the potential role of vitamin D, a micronutrient with anti-inflammatory properties, in modulating low-grade inflammation.<sup>10</sup> Low circulating levels of 25(OH)D, the vitamin D metabolite used to determine vitamin D status, have been associated with an increased number of metabolic syndrome components and insulin resistance in the Canadian population,<sup>11</sup> suggesting that low concentrations of this micronutrient may be a biomarker of cardiometabolic risk. However, the relationship between vitamin D and obesity among Canadians remains poorly explored. Furthermore, despite evidence suggesting a role for cardiometabolic and inflammatory markers in chronic disorders,<sup>12</sup> the change in their profile as body weight increases from normal to obese remains poorly explored. Elucidating these relationships may enable us to further understand the metabolic changes in obesity that can be involved in the early stages of T2DM and CVD. Furthermore, it may provide the basis for developing effective population-based preventive strategies for the range of chronic diseases that are linked to obesity. The objective of the present study was to examine the association between the different body mass index (BMI) states and a number of cardiometabolic and inflammatory biomarkers, and circulating 25(OH)D, in a representative sample of the adult Canadian population.

## Methods

### Study design and population

Data come from the Canadian Health Measures Survey (CHMS) cycle 3.1, which is a population-based survey

designed to collect information on the health and wellness of Canadians aged 6 to 79 years, from households in the ten provinces and three territories of Canada. Complete details of the study design and data collection have been published elsewhere.<sup>13–16</sup> Briefly, a multi-stage sampling strategy was used to identify 15 collection sites from which data was collected between March 2007 and February 2009. Those living on Aboriginal Reserves or Crown Lands, in institutions and certain remote regions, and full-time members of the Canadian Forces were not captured by this survey. The CHMS represents 96.3% of the Canadian population. A total of 8772 dwellings were selected for survey with 6106 agreeing to participate, for a household response rate of 69.6%. From these households, 6604 subjects of the 7483 selected agreed to respond to the study questionnaire, for a response rate of 88.3%. Of those who agreed, 5604 also visited the mobile examination centre for collection of physical measurements, for a response rate of 84.9%. This resulted in an overall response rate of 51.7% at the national level.

The study was reviewed and approved by the Health Canada Research Ethics Board.<sup>15</sup> All subjects signed a consent form prior to participating in the study. Participation was voluntary and included a household interview, and visit to a mobile examination centre for physical measurements, and collection of blood and urine samples. Each dwelling was randomly selected to receive a morning or afternoon clinic appointment. Only those who received a morning appointment were required to be fasted for blood measures. In the present study, only fasting responders were included in the analysis ( $n = 2634$ ). As Health Canada recommends the use of BMI classifications for adults aged 18 years and older only,<sup>17</sup> all subjects under the age of 18 were also excluded ( $n = 829$ ). Therefore, the present analysis includes 1805 subjects, representing, when weighted, 24,624,702 Canadians.

### Metabolic markers, BMI classification, and other covariates

Assessment of biomarkers from blood samples were analyzed at the Health Canada Laboratory, Bureau of Nutritional Sciences, Nutrition Research Division using standard operating procedures. Blind replicates and quality control samples including known analyte concentrations, and field blanks were periodically sent with collected samples to monitor precision and accuracy.<sup>14</sup> A number of metabolic indices and disease markers were measured in the CHMS,<sup>14</sup> and were available for the present study. These included cardiometabolic disease markers (apolipoprotein [Apo] A1 [g/L], ApoB [g/L], low-density lipoprotein cholesterol [LDL-C]

[mmol/L], high-density lipoprotein cholesterol [HDL-C] [mmol/L], total cholesterol [mmol/L], total cholesterol/HDL ratio [total:HDL-C ratio], triglycerides [mmol/L], and glycosylated hemoglobin [HbA<sub>1c</sub>] [%]); inflammatory biomarkers (CRP [mg/L], fibrinogen [g/L], and homocysteine [ $\mu$ mol/L]); systolic and diastolic blood pressure; and plasma 25-hydroxyvitamin D (25(OH)D) (nmol/L).

Subject's BMI (weight in kg/height in m<sup>2</sup>) derived from measured height and weight was used to categorize subjects into underweight (BMI < 18.5, n = 26 – this group was excluded from the stratified analyses), normal weight (18.5  $\leq$  BMI < 25, n = 660), overweight (25  $\leq$  BMI < 30, n = 680), and obese (BMI  $\geq$  30, n = 429) groups (n = 10 missing BMI data). Waist circumference was measured at the mid-point between the top of the iliac crest and the last floating rib in the midaxillary line using a Gulick measuring tape (Fitness Mart®, Grays Mills, WI).<sup>18</sup> Smoking status was self-reported and was further subdivided into ever (daily, occasional, former daily, and former occasional) or never smoked. Ethnicity was categorized into three main subgroups from the twelve ethnic groups reported in the CHMS, to allow for adequate sample size within each group, and to minimize the associated degrees of freedom in adjusted models. The three groups included Caucasians, Asians (Koreans, Filipinos, Japanese, Chinese, South Asians, Southeast Asians, Arabs, and West Asians), and Others (African Canadians, Latin Americans, and mixed). Subjects were dichotomized based on the past-month use of cardiac medications (lipid lowering medications, blood pressure medication, or other medications with direct effects on the circulatory system). Self-reported use of medication was collected, and coded using the American Hospital Formulary Service drug code. Physical activity was assessed based on average daily energy expenditure in leisure time physical activities (kcal/kg/day) in the past 3 months, and were categorized as inactive (0–1.5), moderate (1.5–3), and active ( $\geq$ 3).

## Statistical analysis

Data analysis was conducted using survey procedures in SAS version 9.2 (SAS Institute, Inc, Cary, NC). In all analyses, bootstrap weights for variance estimates and sampling weights for point estimates were applied to account for the complex survey design. All tests were conducted with eleven degrees of freedom. The distribution of continuous variables was examined by plotting histograms. If skewed, variables were natural log transformed to improve linearity of relationships and normality of residuals. Scatter plots of the untransformed, unadjusted, and unweighted data for

BMI and biomarkers were generated using linear regression lines to fit within each BMI group. The top 1% of biomarker values for HbA<sub>1c</sub>, and 25(OH)D were excluded from the plots as potential outliers.

Linear regression was used to examine the association between BMI and biomarkers in the entire population with adjustments for age, sex, smoking status (ever, never), physical activity (inactive, moderate, active), and ethnicity (Caucasian, Asian, Other). Regressions examining associations with 25(OH)D were additionally adjusted for season of clinic visit. Linear regression was also run within each BMI group. To assess potential confounding by medications which impact on CVD markers, analyses were also run with the addition of a dichotomized variable for cardiac drug use. To further analyze potential confounding by diabetes diagnosis, analyses were also run after excluding subjects self-reporting having been diagnosed with type 1 or type 2 diabetes by a health professional, or who had a fasting plasma glucose > 7.0 mmol/L. All *P*-values < 0.05 were considered significant.

To generate adjusted mean levels of the biomarkers within each of the BMI groups, least square means were produced in regression models which examined biomarkers and BMI categories adjusted for age, sex, smoking status, physical activity, and ethnicity. Least square means for vitamin D by BMI group were adjusted for age, sex, physical activity, ethnicity, and season of clinic visit. Untransformed means are presented for interpretability.

## Results

Subject characteristics including mean levels of cardiometabolic biomarkers are presented in Table 1. Approximately half of the study population was female, with a mean age of 44 years. The majority of the sample was made up of inactive subjects (55%) and those who never smoked (53%). The population was predominantly Caucasian (84%), free of diabetes (96%), and not taking any cardiac medications (82%). Mean levels of cardiometabolic biomarkers, including blood pressure and waist circumference, fell within normal clinical ranges; however, the average BMI of the population fell within the overweight range (26.9).

The associations between cardiometabolic markers of disease and BMI are shown in the overall population in Table 2. The number of subjects, adjusted mean level, and standard error for each biomarker are also shown stratified by BMI group. All biomarkers were significantly associated with BMI in the overall study population. The association between BMI and homocysteine was no longer significant

**Table 1** Population characteristics

Characteristic	n <sup>1</sup>	% or mean (lclm, uclm) <sup>2</sup>
Sex <sup>3</sup>		
Male	861	49.2 (48.8, 49.5)
Female	944	50.8 (50.5, 51.2)
Age (years) <sup>3</sup>	1805	44.3 (44.0, 44.6)
Age group <sup>3</sup>		
18–35	506	32.5 (30.5, 34.4)
>35–65	980	56.3 (54.1, 58.5)
>65	319	11.3 (10.0, 12.6)
Ethnicity <sup>3</sup>		
Caucasian	1535	84.3 (71.8, 91.9)
Asian <sup>4</sup>	144	11.4 (6.1, 20.1)
Other <sup>5</sup>	83	
Physical activity index <sup>3</sup>		
Active	392	19.9 (16.1, 24.3)
Moderate	464	24.7 (21.1, 28.8)
Inactive	949	55.4 (48.5, 62.2)
Smoking activity <sup>3</sup>		
Ever	892	46.9 (43.2, 50.5)
Never	908	53.1 (49.5, 56.8)
Waist circumference (cm)		
Males	861	94.6 (93.2, 95.9)
Females	930	86.7 (84.7, 88.8)
BMI (kg/m <sup>2</sup> )	1795	26.9 (26.4, 27.4)
Systolic blood pressure (mmHg)	1804	111.5 (110.2, 112.7)
Diastolic blood pressure (mmHg)	1804	70.9 (70.1, 71.8)
Taking cardiac medication <sup>3</sup>	420	18.3 (15.9, 21.0)
Has diabetes <sup>3</sup>	86	4.0 (3.3, 4.9)
Metabolic markers		
Apolipoprotein A1 (g/L)	1805	1.4 (1.4, 1.5)
Apolipoprotein B (g/L)	1801	0.9 (0.9, 0.9)
LDL-C (mmol/L)	1798	3.0 (3.0, 3.1)
HDL-C (mmol/L)	1805	1.3 (1.3, 1.4)
Total cholesterol (mmol/L)	1805	4.9 (4.8, 5.0)
Total:HDL-C ratio	1805	3.9 (3.8, 4.0)
Triglycerides (mmol/L)	1803	1.3 (1.3, 1.4)
HbA <sub>1c</sub> (%)	1760	5.6 (5.5, 5.7)
Inflammatory markers		
C-reactive protein (mg/L)	1669	2.3 (2.1, 2.5)
Fibrinogen (g/L)	1752	0.0297 (0.0283–0.0309)
Homocysteine (μmol/L)	1793	7.8 (7.5, 8.2)
Other		
Vitamin D (nmol/L)	1784	67.4 (65.1, 69.8)

**Notes:** <sup>1</sup>Unweighted frequency; <sup>2</sup>weighted measure; <sup>3</sup>self-reported data; <sup>4</sup>coefficient of variation = 27% denoting a marginal estimate with a high sampling variability; <sup>5</sup>coefficient of variation = 34% denoting an unacceptable estimate. Ethnicity-specific estimates for the Other group are not presented as per Statistics Canada recommendations.

**Abbreviations:** lclm, lower confidence limit; uclm, upper confidence limit; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Total:HDL-C ratio, total cholesterol/HDL ratio; HbA<sub>1c</sub>, glycosylated hemoglobin.

after adjustment for use of cardiac medications ( $P = 0.1$ ), or when individuals with diabetes were excluded from the analysis ( $P = 0.6$ ). Most biomarkers were positively associated with BMI. Only ApoA1 ( $\beta = -0.31$ ,  $P < 0.0001$ ), HDL-C ( $\beta = -0.61$ ,  $P < 0.0001$ ), and 25(OH)D ( $\beta = -0.25$ ,  $P < 0.0001$ ) were inversely associated with BMI. Some

metabolic markers remained significantly associated with BMI within the normal weight and overweight BMI groups individually (Figure 1). Total:HDL-C ratio and triglycerides remained positively associated, while HDL-C remained inversely associated with BMI, in both the normal weight and overweight groups. ApoB, LDL-C, and total cholesterol remained positively associated with BMI in the normal weight group, while ApoA1 remained inversely associated with BMI in the overweight group. HbA<sub>1c</sub> was not significantly associated with BMI in the normal weight, overweight, or obese groups separately. Among the inflammatory markers, homocysteine was not associated with BMI in the normal weight, overweight, or obese groups, but fibrinogen was positively associated with BMI in the obese group ( $\beta = 0.50$ ,  $P = 0.01$ , Figure 2). CRP remained strongly and positively associated with BMI in normal weight ( $\beta = 3.20$ ,  $P = 0.02$ ), overweight ( $\beta = 3.53$ ,  $P = 0.002$ ), and obese groups ( $\beta = 2.22$ ,  $P = 0.0002$ ). Vitamin D was not significantly associated with BMI in any one BMI group.

## Discussion

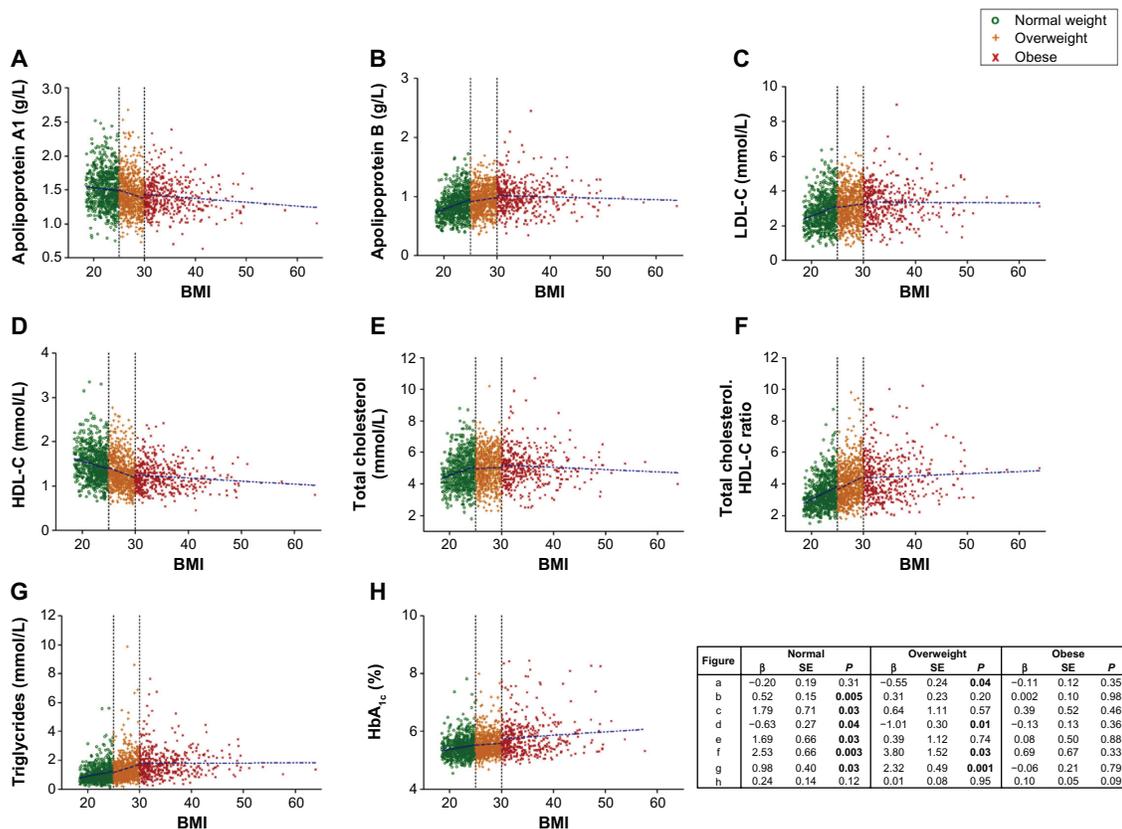
Obesity and its associated cardiometabolic and inflammatory changes contribute to increased risk of chronic conditions including T2DM and CVD.<sup>4,5</sup> Dyslipidemia associated with obesity is an example of such a change, and is characterized by increased small, dense LDL, ApoB, and triglycerides, and low HDL.<sup>19</sup> In obesity, an increase in lipolysis and fatty acid release, paired with impaired fatty acid uptake in adipocytes, results in increased hepatic uptake of fatty acids. The fatty acids are re-esterified into triglycerides, which are incorporated into VLDL along with ApoB.<sup>20</sup> One molecule of ApoB is found in atherogenic lipoprotein particles including VLDL, intermediate density lipoprotein, (IDL) and LDL, making it an important marker of CVD risk<sup>21</sup> that has been shown to be positively associated with obesity.<sup>22</sup> Levels of ApoB, LDL-C, total cholesterol, total:HDL-C ratio, and triglycerides were all positively associated with BMI in the overall population (Table 2). While these positive associations remained significant in the normal weight group, only total:HDL-C ratio and triglycerides remained significant in the overweight group, and none of these markers were significantly associated with BMI in the obese group. In contrast, HDL-C levels tend to be lower in obesity. This may be attributed to increased cholesteryl ester transfer protein and hepatic lipase activity, which results in increased HDL-C catabolism and clearance.<sup>20</sup> HDL-C has antioxidant, anti-inflammatory, and antithrombotic roles which may be attributable to the presence of ApoA1,<sup>23,24</sup> another important biomarker of CVD

**Table 2** Adjusted mean levels of biomarkers by BMI groups

Biomarker	Normal weight			Overweight			Obese			Overall population	
	n <sup>2</sup>	Mean <sup>3</sup>	SE <sup>3</sup>	n <sup>2</sup>	Mean <sup>3</sup>	SE <sup>3</sup>	n <sup>2</sup>	Mean <sup>3</sup>	SE <sup>3</sup>	$\beta \pm SE$	P <sub>Trend</sub> <sup>4</sup>
Apolipoprotein A1 (g/L)	646	1.49	0.02	663	1.40	0.02	415	1.34	0.02	-0.31 ± 0.05	<0.0001
Apolipoprotein B (g/L)	643	0.87	0.02	663	0.92	0.03	414	1.00	0.03	0.29 ± 0.04	<0.0001
LDL-C (mmol/L)	643	2.82	0.10	663	3.01	0.10	411	3.33	0.13	1.18 ± 0.17	<0.0001
HDL-C (mmol/L)	646	1.48	0.04	663	1.30	0.02	415	1.19	0.03	-0.61 ± 0.06	<0.0001
Total cholesterol (mmol/L)	646	4.86	0.10	663	4.92	0.09	415	5.10	0.11	0.64 ± 0.14	0.0009
Total:HDL-C ratio	646	3.47	0.10	663	4.01	0.09	415	4.51	0.14	2.29 ± 0.21	<0.0001
Triglycerides (mmol/L) <sup>1</sup>	645	0.96	1.04	662	1.19	1.05	415	1.56	1.05	1.02 ± 0.06	<0.0001
HbA <sub>1c</sub> (%) <sup>1</sup>	634	5.66	1.01	644	5.65	1.02	403	5.95	1.02	0.11 ± 0.01	<0.0001
C-reactive protein (mg/L) <sup>1</sup>	567	0.65	1.15	640	1.26	1.16	392	2.28	1.16	2.80 ± 0.19	<0.0001
Fibrinogen (g/L) <sup>1</sup>	623	0.03	1.02	650	0.03	1.02	404	0.03	1.02	0.32 ± 0.03	<0.0001
Homocysteine (μmol/L) <sup>1</sup>	645	7.41	1.05	659	7.39	1.05	410	7.99	1.05	0.12 ± 0.05	0.04 <sup>5</sup>
25(OH)D (nmol/L) <sup>1</sup>	644	55.36 <sup>6</sup>	1.04	657	56.42 <sup>6</sup>	1.03	410	47.65 <sup>6</sup>	1.04	-0.25 ± 0.06	<0.0001

**Notes:** <sup>1</sup>Biomarker was log-transformed for linear regression analyses producing P-value for trend results; <sup>2</sup>unweighted frequency for the number of subjects used in each analysis to produce mean, SE, and P-values; <sup>3</sup>adjusted (age, sex, smoking status, physical activity, ethnicity and season of clinic visit, for vitamin D only), untransformed, weighted means and SE; <sup>4</sup>P-value for the relationship between log-transformed BMI and biomarker adjusted for age, sex, smoking status, physical activity, and ethnicity (and season of clinic visit, for vitamin D only); <sup>5</sup>P-value for linear regression coefficient no longer significant after adjustment for cardiac drug use (P = 0.1) or when excluding subjects with diabetes (P = 0.6); <sup>6</sup>vitamin D means only adjusted for age, sex, physical activity, ethnicity, and season of clinic visit in order to maintain 11 degrees of freedom in the model.

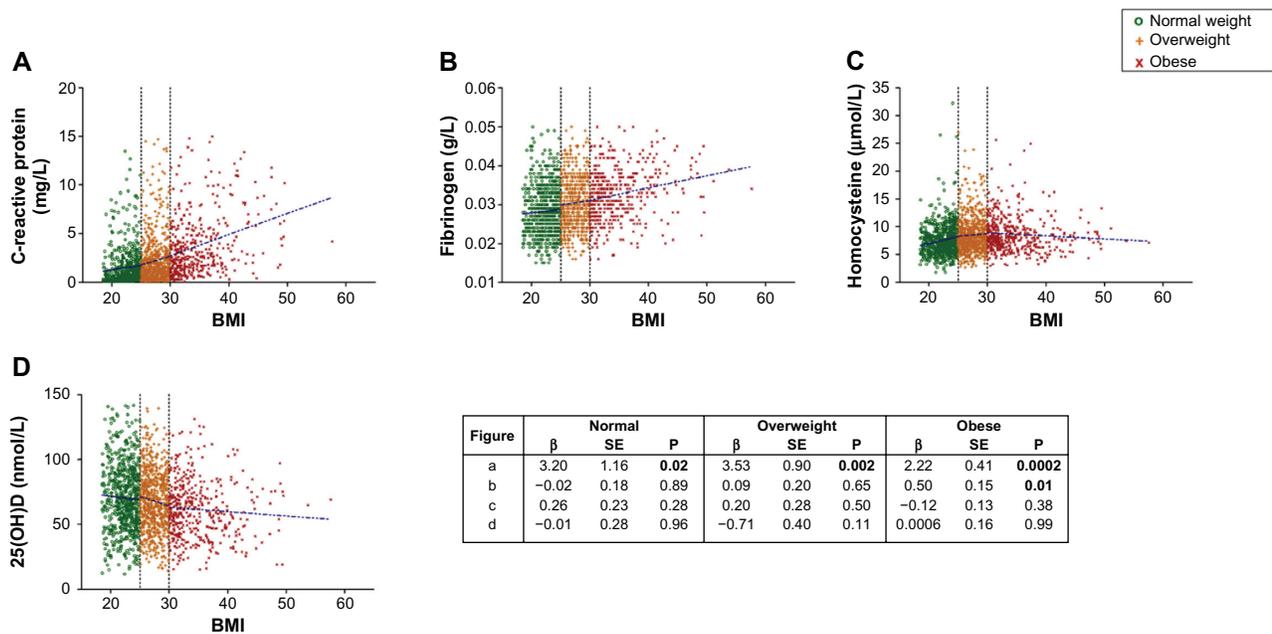
**Abbreviations:** BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Total:HDL-C ratio, total cholesterol/HDL ratio; HbA<sub>1c</sub>, glycosylated hemoglobin; 25(OH)D, 25-hydroxyvitamin D; SE, standard error.



**Figure 1** Relationship between metabolic markers and BMI by BMI group.

**Notes:** Unweighted and untransformed values for biomarkers and BMI were plotted on a single graph with green open circles (o) representing subjects who fall in the normal weight range, orange plus signs (+) representing those that fall in the overweight range, and red exes (x) representing those who fall in the obese range. The top 1% of biomarker values for HbA<sub>1c</sub> were removed from the graph to exclude potential outliers. Unadjusted linear regression lines within each BMI group were plotted to highlight trends in the data (shown in blue). Gray dotted lines are shown vertically on the graph to represent the cut-points between normal weight, overweight, and obese. Regression coefficients ( $\beta$ ), standard errors, and P-values were calculated using weighted linear regression adjusted for age, sex, smoking status, physical activity, and ethnicity. BMI, triglycerides, and HbA<sub>1c</sub> were log-transformed to improve normality in linear regression models.

**Abbreviations:** BMI, body mass index; HbA<sub>1c</sub>, glycosylated hemoglobin; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; Total:HDL-C ratio, total cholesterol/HDL ratio; SE, standard error.



**Figure 2** Relationship between BMI and biomarkers of inflammation and plasma vitamin D by BMI groups.

**Notes:** Unweighted and untransformed values for biomarkers and BMI were plotted on a single graph with green open circles (o) representing subjects who fall in the normal weight range, orange plus signs (+) representing those that fall in the overweight range, and red exes (x) representing those who fall in the obese range. The top 1% of biomarker values for 25(OH)D were removed from the graphs to exclude potential outliers. Unadjusted linear regression lines within each BMI group were plotted to highlight trends in the data (shown in blue). Gray dotted lines are shown vertically on the graph to represent the cut-points between normal weight, overweight, and obese. Regression coefficients ( $\beta$ ), standard errors, and P-values were calculated using weighted linear regression adjusted for age, sex, smoking status, physical activity, and ethnicity. BMI, CRP, fibrinogen, homocysteine, and 25(OH)D were log-transformed to improve normality in linear regression models.

**Abbreviations:** BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; CRP, C-reactive protein; SE, standard error.

risk.<sup>25</sup> In the present study, levels of both ApoA1 and HDL-C were inversely associated with BMI in the overall population and among overweight subjects.

Several of the associations identified between metabolic markers and BMI in the population as a whole were only significant in the normal weight and/or overweight groups when examined separately. This plateau effect seen in Figure 1 for the obese group would seem to suggest no further change in metabolic markers with increasing BMI in the obese group; however, these findings should be interpreted cautiously as the obese group had the smallest sample size of the BMI groups, and a strong relationship between obesity and several of these markers is well established.<sup>19,20,22</sup>

CRP and fibrinogen are acute phase proteins whose concentrations increase in response to inflammation and injury, and both biomarkers are positively associated with CVD risk.<sup>8</sup> Homocysteine, an amino acid and byproduct of methionine catabolism, is also a biomarker of inflammation<sup>26</sup> and CVD risk, although its utility with regard to the latter has been questioned.<sup>27,28</sup> All three of these inflammatory biomarkers have been positively associated with obesity.<sup>29,30</sup> In the present study, homocysteine was not associated with BMI among normal weight, overweight, or obese subjects, nor in the whole population after adjusting for cardiac

medication use, or when looking exclusively at those without diabetes. Conflicting results between BMI and circulating homocysteine levels have been reported, and a recent study of men and women in Western Japan also showed no association between BMI and plasma homocysteine.<sup>31</sup> In contrast, CRP and fibrinogen were positively associated with BMI in the overall population and among obese subjects, with strong positive associations additionally noted among overweight and normal weight subjects for CRP. A wealth of evidence suggests a mechanistic role for increased adiposity in the development of chronic systemic inflammation (for review, see Badawi et al, 2010)<sup>4</sup>. Adipose tissue secretes numerous cytokines, such as the proinflammatory TNF- $\alpha$  and IL-6, which in turn trigger the hepatic production of acute phase proteins such as CRP and fibrinogen.<sup>4,8,9</sup> In particular, visceral lipid accumulations may contribute to cytokine secretion, and exacerbate cardiometabolic risk.<sup>32-34</sup> Overall, our results support an association between chronic inflammation and increased BMI, and identify the innate immunity-related inflammatory cascade as a potential target pathway for intervention strategies aimed at reducing the risk of obesity-related chronic diseases in the general population.

Obesity is a key risk factor for T2DM, and is associated with an increased production of inflammatory cytokines and

release of free fatty acids, which disrupt insulin signaling and action, and contribute to insulin resistance.<sup>4,6</sup> HbA<sub>1c</sub>, a measure of long-term blood glucose regulation, is a diagnostic measure of T2DM,<sup>35</sup> and has been shown to be positively associated with BMI in some<sup>36,37</sup> but not all studies.<sup>38</sup> In the present study, HbA<sub>1c</sub> was positively associated with BMI in the entire study population. This significant, albeit weaker ( $\beta = 0.11$ ,  $P < 0.0001$ ) association, however, was not significant within the normal weight, overweight, or obese groups when examined separately. The absence of a significant association with BMI among the BMI subgroups may be due to lower power in these smaller groups, or may be due to the influence of other factors influencing HbA<sub>1c</sub> not accounted for here, including diet.<sup>37</sup>

Circulating levels of vitamin D have been inversely associated with obesity.<sup>39-43</sup> A potential explanation for such associations may relate to vitamin D sequestration in adipose tissue.<sup>44</sup> It has also been suggested that vitamin D may play an active role in obesity, with low levels stimulating synthesis and release of parathyroid hormone, increasing calcium in adipocytes, and promoting weight gain.<sup>45</sup> In the present study, BMI was inversely associated with plasma 25(OH)D in the entire study population (Table 2), but not within the normal weight, overweight, or obese groups (Figure 2). This lack of association within BMI subgroups may be a consequence of small sample sizes. Previous research from our group, using the same study population, showed an inverse association between circulating 25(OH)D and number of metabolic syndrome components.<sup>11</sup> Vitamin D is thought to modulate cardiometabolic disease risk through its anti-inflammatory and immunomodulatory properties.<sup>10,46</sup> When considering the population as a whole, our results lend support to previous research suggesting that 25(OH)D is inversely associated with BMI, but we are unable to determine, based on the available data, whether a low vitamin D status contributes to the development of obesity.

The present study has several limitations. Although BMI is a commonly used indicator of obesity and health risks in large epidemiological studies, BMI provides no information on the distribution and type of body fat, or amount of lean tissue. Therefore, we cannot rule out the possibility of different patterns of cardiometabolic risk being associated with different distributions of body fat (ie, android or gynecoid). However, this study aimed to characterize novel relationships between specific biomarkers and increasing obesity in the Canadian population, and therefore we employed the Health Canada weight classification scheme, which is based on BMI.<sup>17</sup> Future research efforts should be directed towards

examining whether the observed associations differ across specific obesity types. In addition, detailed information on diet was not available for the present study and associations could not be adjusted for differences in total energy or macronutrient intake. Diet can influence many of the biomarkers examined and may confound some of the associations presented. The self-reported nature of some of the data in this study is also a limitation, including the measure of physical activity, which only captures leisure-time physical activities. We cannot rule out the possibility of residual confounding from measured and unmeasured sources, including diet, work- or transportation-related physical activity, sun exposure, or other unmeasured biomarkers, such as leptin. Furthermore, metabolic dysregulation of physiological processes including for eg, glycemic control, might contribute to some of the observed associations, but this study did not consider measures of glucose metabolism such as homeostatic model assessment of insulin resistance (HOMA-IR). This study was cross-sectional in nature, and therefore causality cannot be determined from the presented associations. It is also possible that a single measure of these biomarkers may not be reflective of long-term status and disease risk. In addition, the small size of the obese subgroup may have resulted in insufficient power to detect associations. The present study consisted mainly of Caucasian subjects, and associations may differ in different ethnocultural groups. Future research should examine the association between cardiometabolic biomarkers and obesity in different ethnocultural groups, given known differences in cardiometabolic disease rates in different ethnicities. However, strength of this study includes its measurement of a range of cardiometabolic and inflammatory biomarkers, as well as anthropometrics in a nationally representative sample of the adult Canadian population.

In conclusion, we observed a distinctive profile of metabolic phenotypes – including cardiometabolic disease markers and inflammatory biomarkers – that emerges as BMI increases from normal weight to obesity. These findings may have important implications in developing public health intervention and prevention strategies to reduce the burden of chronic diseases associated with obesity in the general population.

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## Disclosure

The authors report no conflicts of interest in this work.

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