

# Influence of Dietary Patterns on Plasma Soluble CD14, a Surrogate Marker of Gut Barrier Dysfunction

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## Abstract

**Background:** Specific foods and nutrients, including alcohol, may contribute to gut barrier dysfunction. However, to our knowledge, the influence of whole diets is currently unknown.

**Objective:** We aimed to cross-sectionally investigate associations of dietary patterns with plasma soluble CD14 (sCD14), which is released by macrophages on stimulation with endotoxin and has been used as a marker of gut hyperpermeability.

**Methods:** We used food-frequency questionnaire data collected from 689 women in the Nurses' Health Study and 509 men in the Health Professionals Follow-Up Study. Our principal component analysis identified 2 dietary patterns: "Western" (higher intakes of red meat, processed meat, desserts, and refined grains) and "prudent" (higher intakes of fruits, vegetables, fish, and whole grains). In multivariable-adjusted logistic regression analyses, we estimated ORs and 95% CIs for high (equal to or greater than the median compared with less than the median) sCD14 concentrations in quintiles of each dietary pattern. Using logistic regression, we also investigated the joint association of the Western dietary pattern and alcohol intake or C-reactive protein (CRP) with sCD14 concentrations.

**Results:** Western dietary pattern scores were positively associated with sCD14 concentrations (OR: 1.86; 95% CI: 1.24, 2.79; *P*-trend = 0.0005; comparing extreme quintiles). Analyses of joint associations suggested that the strongest associations with higher sCD14 concentrations were for persons with both high Western pattern scores and high alcohol intake compared with participants with low scores for both (OR: 2.96; 95% CI: 1.61, 5.45) or for participants with both high Western pattern scores and high CRP values compared with those with low scores for both (OR: 4.11; 95% CI: 2.57, 6.58). The prudent pattern was not associated with sCD14 concentrations.

**Conclusions:** Higher consumption of the Western dietary pattern is associated with a marker of macrophage activation and gut hyperpermeability, especially when coupled with high alcohol intake and heightened systemic inflammation. Our findings need confirmation in studies with additional markers of gut barrier dysfunction. *Curr Dev Nutr* 2017;1:e001396.

## Introduction

The human gut epithelium consists of a single layer of epithelial cells that separates the intestinal lumen from the underlying lamina propria (1). The gut epithelium acts as a selectively permeable barrier that permits the absorption of nutrients, water, and electrolytes into the gut and defends the systemic circulation against intraluminal toxins, antigens, and enteric



**Keywords:** gut barrier dysfunction, dietary patterns, soluble CD14, alcohol, C-reactive protein, hyperpermeability

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Abbreviations used: CRP, C-reactive protein; HPFS, Health Professionals Follow-Up Study; LBP, lipopolysaccharide binding protein; MET, metabolic equivalent; NHS, Nurses' Health Study; NSAID, nonsteroidal anti-inflammatory drug; PCA, principal component analysis; sCD14, soluble CD14; sGP130, soluble glycoprotein 130; sIL-2R $\alpha$ , soluble IL-2 receptor- $\alpha$ ; sTNFR2, soluble TNF receptor 2.

microbiota (2, 3). The gut epithelium is constantly challenged as a result of interactions with external stimuli such as diet and pathogenic and commensal bacteria (2). Evidence suggests that abnormal gut barrier function contributes to several gastrointestinal disorders, such as inflammatory bowel disease and colorectal cancer (4–7), and diseases involving other systems, such as type 1 diabetes, AIDS, and rheumatoid arthritis (8).

Proposed biomarkers of gut barrier dysfunction or microbial translocation include soluble CD14 (sCD14), LPS, and lipopolysaccharide binding protein (LBP) (8–10). However, not all studies have shown consistent results for LPS as a reliable marker of gut barrier dysfunction (9), probably owing to the interference of various factors in the detection of LPS or to the short half-life of LPS (11). In addition, LPS contamination of blood collection tubes is common (12), leading to the spurious detection of LPS. Therefore, it is often not possible to reliably measure LPS directly, particularly in studies using archival serum or plasma specimens. LBP specifically binds and transfers bacterial LPS and has a longer half-life than LPS (13, 14); therefore, LBP is a more attractive marker of gut hyperpermeability than LPS. However, circulating LBP concentrations may occur not only in response to gram-negative bacteria but also in systemic infection (15); therefore, changes in LBP may not always reflect gut barrier dysfunction. sCD14 is a receptor molecule produced primarily by macrophages and hepatocytes as part of the innate immune response to LPS (16–18). It functions as a cofactor along with LBP to mediate LPS recognition and response by Toll-like receptor 4, which is found on several immune cells (19, 20). Gut barrier dysfunction resulting in microbial translocation and immune activation therefore leads to elevated sCD14 concentrations (21). Stronger evidence in support of the role of sCD14 as a marker of gut barrier dysfunction is provided by studies in HIV research (22, 23). Plasma sCD14 concentrations correlate with systemic immune activation, which drives chronic HIV infection (8, 10), and immune activation in patients with HIV has been linked to gut barrier dysfunction (8, 10). Taken together, this evidence provides support for sCD14 as a surrogate marker of gut hyperpermeability because it can easily be measured in the circulation and in archival serum or plasma specimens (24). Our group previously calculated within-person intraclass correlation coefficients for 14 plasma biomarkers, including sCD14, using archived sera collected from 200 HIV-seronegative men at 3 visits spaced over ~2 y. The age- and ethnicity-adjusted intraclass correlation coefficient for sCD14 averaged across all 3 visits was 0.56 (95% CI: 0.49, 0.64) (24).

To our knowledge, factors associated with gut barrier dysfunction have not been well studied, although limited evidence in human studies suggests that diet may play a role (25, 26). Specific dietary factors such as flavonoids and some proteins and amino acids have been shown to be associated with gut hyperpermeability (27–31). Alcohol has also been shown to interfere with the absorption of several nutrients and to lead to damage in the intestinal mucosa, which facilitates gut hyperpermeability (26, 32, 33). To our knowledge, although efforts have been made toward understanding the role of specific dietary factors in gut barrier function, the role of whole diets has not yet been characterized. Dietary pattern analysis considers the diet as a whole, compared with

individual foods or nutrients, and may provide an opportunity to comprehensively investigate relations between whole diets and health, in part by accounting for collinearity between foods and nutrients (34). Our FFQ assesses long-term dietary intake over the previous 1 y. Dietary patterns and alcohol intake are fairly constant over time, and we have found reasonable reproducibility of dietary patterns in our cohorts (35, 36). In addition, it is the long-term dietary intake that is etiologically relevant; for example, if sCD14 was extremely responsive to very specific dietary factors and these could change profoundly day-to-day in an individual, it would not have any utility as a biomarker. Therefore, we are mostly interested in potential effects of long-term dietary patterns on sCD14 concentrations. With our FFQ data, we still observe very strong associations with health outcomes, although we may potentially miss some very specific acute (day-to-day) effects of specific dietary factors. In this cross-sectional study, we investigated the association of dietary patterns with plasma levels of sCD14 and with empirical patterns of plasma markers of inflammation and immune response.

## Methods

### Study population

The Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS) are ongoing prospective cohorts in the United States that were established in 1976 and 1986, respectively. The NHS ( $n = 121,701$ ) enrolled female registered nurses aged 30–55 y (37), whereas the HPFS ( $n = 51,529$ ) enrolled male health professionals aged 40–75 y. Blood samples were collected from subpopulations of the NHS ( $n = 32,826$ ) in 1989–1990 and from the HPFS ( $n = 18,225$ ) in 1993–1994 (38). Blood collection was conducted using similar protocols for all cohorts (39). Participants who donated blood samples were free of diagnosed chronic diseases such as cancer, cardiovascular disease, diabetes, and Alzheimer disease. The procedures we used (including collection, handling, and storage) were summarized previously (40). For the current study, we used biomarker data from an ongoing matched case-control study of immune deregulation and risk of non-Hodgkin lymphoma nested within the NHS and HPFS. The median time from blood collection to lymphoma diagnosis in patients was 9.1 y (range: 1 mo to 22.1 y). Data on sCD14 and other immune biomarkers were available for a total of 1198 participants (689 women in the NHS and 509 men in the HPFS). The Institutional Review Boards at Brigham and Women's Hospital and at Harvard T.H. Chan School of Public Health approved this study.

### Biomarker assessment

Assays were performed using multiplexed (Luminex platform) assay kits (Fluorokine MAP; R&D Systems) according to the manufacturer's directions and a Bio-Plex 200 Luminex instrument and Bio-Plex analysis software (Bio-Rad). We measured concentrations of C-reactive protein (CRP) and the following 4 soluble receptors in one panel: sCD14, soluble IL-6 receptor- $\alpha$  (also known as sCD126), soluble glycoprotein 130 (sGP130; also known as sCD130, which forms part of the IL-6 receptor complex), and soluble TNF

receptor-2 (sTNFR2; also known as sCD120b). We included soluble IL-2 receptor- $\alpha$  (sIL-2R $\alpha$ ; also known as sCD25) in another soluble receptor panel. The concentrations of IL-8 (a chemokine also called CXCL8) were determined as part of a high-sensitivity human cytokine panel (Human Inflammation Multiplex Kit; Bio-Rad). We selected biomarkers for this study based on their correlations with sCD14 concentrations (Spearman  $r \geq 0.1$  and  $P < 0.05$ ; both cut-points were determined a priori). For each plate of plasma samples tested, a biomarker- and plate-specific lower limit of detection was defined as the lowest detectable concentration of each analyte obtained by extrapolation using the Bio-Plex software. The mean  $\pm$  SD coefficient of variation of sCD14 among 6 subsamples was  $11.3\% \pm 9.3\%$  (range: 4.2–25.0%) among women and  $5.8\% \pm 3.3\%$  (range: 2.5–10.3%) among men. For the other biomarkers, the mean coefficient of variation ranged from 7.5% to 12.5% in women and from 3.4% to 6.1% in men. Statistical calibration was performed to adjust for batch-related variability according to the methods of Rosner et al. (41). Briefly, a batch effect correction factor was calculated using linear regression to model the association between assay batch and natural log-transformed values of each biomarker in the controls. All values were corrected by the batch-specific factor to normalize values across the batches (41).

### Assessment of dietary data and derivation of dietary patterns

Dietary data are updated every 4 y in the NHS (since 1980) and HPFS (since 1986) with a semiquantitative FFQ that has been evaluated for validity in several studies (35, 42, 43). We used dietary data from the questionnaires closest to blood draw (i.e., the 1990 FFQ for the NHS and the 1994 FFQ for the HPFS) to align with the relevant exposure period for biomarkers. The median time from FFQ return to blood collection was 2 mo (range: 1–21 mo). Participants with an excessive number of missing items ( $\geq 70$ ) on the FFQs or implausibly low or high energy intake ( $< 600$  or  $> 3500$  kcal/d for women and  $< 800$  or  $> 4200$  kcal/d for men) were excluded (36).

We derived dietary patterns by principal component analysis (PCA) using 41 previously defined food groups shown to have good reproducibility in our cohorts (36). Briefly, foods from the FFQ were classified into 41 food groups based on nutrient profiles or culinary usage. Foods that did not fit into any of the groups were left as individual categories (e.g., pizza, French fries, and tea) (36). Vitamin and mineral supplements were not included in the definition of dietary patterns. PCA was conducted followed by a varimax orthogonal rotation procedure to produce maximally uncorrelated factors (44). We retained factors for analyses based on the largest eigenvalues (i.e., the amount of total variance explained by a principal component) and scree plots. We therefore retained 2 principal components, each representing a separate, uncorrelated dietary pattern. Next, we derived factor loadings from the correlations between food groups and the 2 retained factors. The 2 factors were described as “Western” or “prudent” based on the major foods contributing to the pattern. Each participant was then assigned 2 factor scores, determined by adding the reported frequencies of food group intakes, weighted by the factor loadings for each factor. Finally, we categorized the dietary pattern score distributions into quintiles.

### Covariate data assessment

Both cohorts collected nondietary data (e.g., medical history and health practices) and updated the data through biennial self-administered questionnaires. Similar to dietary data, we used covariate data from the questionnaires administered closest to blood draw. We calculated participants' BMI (in  $\text{kg}/\text{m}^2$ ) using height (in meters) reported at baseline for each cohort and weight (in kilograms) reported in 1990 for NHS participants and 1994 for HPFS participants. Participants reported smoking status (never, former, or current), and we calculated physical activity by summing the average metabolic equivalent (MET) hours per week of each individual activity. Regular use of medications was defined as use of  $\geq 2$  standard (325-mg) aspirin tablets/wk or  $\geq 2$  tablets of other nonsteroidal anti-inflammatory drugs (NSAIDs), acetaminophen, or cholesterol-lowering drugs/wk.

### Statistical analyses

We described participants' characteristics using means for continuous variables and frequencies (in percentages) for categorical variables across quintiles of dietary pattern scores. Biomarker concentrations were transformed using natural logs to normalize their distribution (45, 46). We adjusted dietary patterns for energy intake using the residual method (47). For the statistical modeling of each dietary pattern, we first constructed age- (at blood draw) and sex-adjusted and then multivariable-adjusted logistic regression models to calculate ORs and 95% CIs for higher plasma sCD14 concentrations (greater than or equal to the median of 14.465 on the natural log scale, which corresponds to a nontransformed value of 1915 ng/mL), compared with lower (below the median) sCD14 concentrations across quintiles of dietary patterns. sCD14 data were highly skewed and, reasonable normality could not be achieved even when we log-transformed the data; we therefore used logistic regression. All multivariable analyses were adjusted for age at blood draw (continuous, years), sex (male or female), physical activity (continuous, MET hours per week), smoking status (never, former, or current), lymphoma case-control status, regular aspirin use (yes or no), other NSAID use (yes or no), acetaminophen use (yes or no), cholesterol-lowering drug use (yes or no), BMI (continuous), and chronic disease comorbidity score. Chronic diseases or conditions included in the score (0 or 1 indicates absence or presence, respectively) were hypercholesterolemia, cancer, diabetes, high blood pressure, heart disease, rheumatoid or other arthritis, and Crohn disease or ulcerative colitis.

We conducted several sensitivity analyses. First, to assess the influence of reverse causation ( $n = 1144$ ), we excluded 54 participants who developed lymphoma within 2 y of blood donation. Second, we excluded all participants with lymphoma and conducted analyses only among controls ( $n = 601$ ). Third, we performed subgroup analyses for a priori selected potential effect modifiers, which included alcohol intake (high:  $\geq 20$  g/d for men or  $\geq 10$  g/d for women; moderate: 10 to  $< 20$  g/d for men or 5 to  $< 10$  g/d for women; or low:  $< 10$  g/d for men or  $< 5$  g/d for women), BMI (obese:  $\geq 30$ ; overweight:  $\geq 25$  to  $< 30$ ; or normal weight:  $< 25$ ), CRP (high:  $\geq 3$  mg/L; or low:  $< 3$  mg/L), regular aspirin or NSAID use (yes or no), and sex (male or female). Models were adjusted for all previously listed covariates (except when stratifying

by levels of the potential effect modifier). The *P* value for interaction was estimated using the Wald test for the interaction term. Models in women were also adjusted for menopausal status and postmenopausal hormone use.

We also conducted analyses of the joint association of the Western dietary pattern and each of alcohol, BMI, and CRP levels by first constructing variables combining tertiles of the Western dietary pattern with 3 alcohol intake levels (9 categories), 2 BMI levels (6 categories), and 2 CRP levels (6 categories). We then used multivariable-adjusted logistic regression models to test the joint association of the combined variables with higher (or lower) plasma sCD14 levels, constructing separate models for each combined variable. We calculated ORs and 95% CIs for higher categories compared with the lowest category as the reference (e.g., low Western dietary score and low alcohol intake, low Western dietary score and normal weight, or low Western diet score and low CRP).

To investigate the association of dietary patterns with biomarker pattern scores that potentially characterize the pathophysiologic milieu associated with higher sCD14 levels, we integrated sCD14 with biomarkers of inflammation and immune response that were correlated with sCD14. These included CRP, sTNFR2, IL-8, sGPI30, sIL-2R $\alpha$ , and soluble IL-6 receptor- $\alpha$  (MM Epstein, B Rosner, EC Breen, JL Batista, EL Giovannucci, L Magpantay, JC Aster, SJ Rodig, KA Bertrand, F Laden, O Martínez-Maza, BM Birmann, unpublished results, 2017; data not shown). Biomarker patterns were derived empirically using factor analysis. We used the varimax orthogonal rotation procedure to apply factor analysis on the biomarkers (48). We retained 3 factors based on eigenvalues and inspection of scree plots. Each factor was calculated as a linear combination of all biomarkers weighted by their factor loadings and summed for each participant. We constructed multivariable-adjusted logistic regression models for nonreference quintiles of the dietary patterns to calculate ORs of high (equal to or greater

**TABLE 1** Participant characteristics across the lowest, middle, and highest quintiles of energy-adjusted dietary patterns (*N* = 1198)<sup>1</sup>

Characteristic	Western pattern			Prudent pattern		
	Quintile 1 ( <i>n</i> = 239)	Quintile 3 ( <i>n</i> = 237)	Quintile 5 ( <i>n</i> = 239)	Quintile 1 ( <i>n</i> = 239)	Quintile 3 ( <i>n</i> = 240)	Quintile 5 ( <i>n</i> = 239)
Age at blood draw, y	61.6 ± 7.4	61.1 ± 8.5	60.2 ± 8.4	60.2 ± 8.5	60.8 ± 8.5	62.1 ± 7.9
BMI, kg/m <sup>2</sup>	25.0 ± 3.5	26.0 ± 3.9	26.3 ± 4.5	25.8 ± 3.8	25.3 ± 3.5	25.4 ± 3.5
Overweight or obese, BMI ≥25	45.2	57.4	60.7	56.1	47.9	55.2
Physical activity, MET h/wk	32.7 ± 31.9	24.0 ± 26.7	24.4 ± 25.9	22.3 ± 26.9	29.3 ± 32.7	33.0 ± 32.3
Dietary intake, mean servings/wk						
Processed meat	0.7 ± 0.9	1.4 ± 1.4	3.2 ± 2.8	2.3 ± 2.4	1.7 ± 2.6	1.3 ± 2.0
Red meat	2.4 ± 1.9	3.6 ± 2.5	5.8 ± 4.0	5.1 ± 4.1	3.5 ± 2.2	3.1 ± 3.1
Sweets and desserts	5.4 ± 5.9	7.3 ± 7.3	12.4 ± 11.0	11.8 ± 10.7	6.1 ± 6.2	6.2 ± 7.7
High-fat dairy	2.6 ± 2.4	4.1 ± 3.5	8.6 ± 8.4	6.1 ± 6.9	4.3 ± 3.7	4.1 ± 5.8
Refined grains	7.9 ± 5.6	8.2 ± 6.2	11.9 ± 9.0	9.6 ± 7.5	8.4 ± 6.8	9.1 ± 7.6
Poultry	3.7 ± 3.8	2.5 ± 1.7	2.7 ± 2.8	2.2 ± 1.9	2.9 ± 2.7	4.0 ± 4.5
Fish	3.6 ± 2.7	2.2 ± 1.7	2.3 ± 2.5	1.5 ± 1.4	2.3 ± 1.5	4.8 ± 4.7
Vegetables	23.4 ± 15.8	16.4 ± 9.5	17.0 ± 10.3	9.7 ± 4.7	16.1 ± 5.3	33.6 ± 15.4
Fruit	18.4 ± 12.7	12.0 ± 8.2	11.5 ± 9.1	8.6 ± 6.0	12.7 ± 7.1	20.2 ± 13.8
Whole grains	14.5 ± 11.1	10.3 ± 8.7	8.6 ± 8.5	8.4 ± 8.3	11.2 ± 8.7	15.2 ± 13.1
Alcohol, g/d	9.7 ± 15.9	7.4 ± 10.7	7.6 ± 12.4	6.7 ± 11.9	9.4 ± 14.5	10.4 ± 16.7
Current smokers	5.4	4.6	11.3	12.6	5.8	3.8
Regular medication use <sup>2</sup>						
Aspirin	47.7	48.1	49.0	48.1	50.4	46.9
Acetaminophen	25.1	24.9	27.6	22.6	25.0	23.0
Other NSAIDs	23.9	30.8	28.0	27.2	27.9	18.8
Cholesterol-lowering medications	9.2	5.9	4.2	3.8	5.8	9.2
Chronic disease comorbidity score, number of chronic diseases or conditions <sup>3</sup>						
None	37.7	43.9	46.9	46.9	44.2	36.8
1	33.5	25.7	28.0	31.0	26.3	36.0
2	15.9	21.9	17.2	15.5	21.3	16.3
≥3	13.0	8.4	8.0	6.7	8.3	10.9

<sup>1</sup> Values are means ± SDs or percentages unless stated otherwise. Dietary patterns were adjusted for energy intake using the residual method (47) prior to analyses. The food group components were as follows: processed meat (bacon or hot dog), red meats (beef, pork, or lamb), sweets and desserts (chocolate bars or pieces, candy bars, cookies, brownies, doughnuts, cakes, pies, sweet rolls, coffee cake, or pastries), high-fat dairy (whole milk, cream, ice cream, or cheese), poultry (chicken or turkey with or without skin), fish (canned tuna, shrimp, lobster, scallops, or other seafood), vegetables (cruciferous vegetables, green leafy vegetables, dark yellow vegetables, or other vegetables), refined grains (white bread, English muffins, bagels, rolls, biscuits, white rice, pasta, pancakes, or waffles), fruits (raisins, grapes, avocado, banana, cantaloupe, watermelon, apple, pear, orange, grapefruit, peach, apricot, or plum), and whole grains (cooked oatmeal, cooked breakfast cereal, dark bread, brown rice, bran added to food, or wheat germ). MET, metabolic equivalent; NSAID, nonsteroidal anti-inflammatory drug.

<sup>2</sup> Regular use was defined as ≥2 standard (325-mg) aspirin tablets or ≥2 NSAID tablets/wk.

<sup>3</sup> Chronic diseases or conditions included in the comorbidity score were hypercholesterolemia, cancer, diabetes, high blood pressure, heart disease, rheumatoid or other arthritis, or Crohn disease or ulcerative colitis.

than the median) compared with low (less than median) scores of the biomarker pattern. Finally, we analyzed the joint associations between the Western dietary pattern and alcohol and BMI with high scores of the sCD14-dominant biomarker pattern.

For analysis of the linear trend across dietary pattern quintiles, we entered each dietary pattern into multivariable-adjusted models as a continuous variable and interpreted the Wald *P* value of the continuous dietary pattern as the *P* value for linear trend. We also estimated the OR and 95% CI per 1-SD increment in dietary pattern score from the trend analyses. Analyses were conducted using SAS software (version 9.4 for UNIX; SAS Inc.). All tests were 2-sided and 95% CIs not including 1 were considered to indicate statistically significant results.

## Results

We identified 2 major dietary patterns by PCA and labeled them “Western” and “prudent” (**Supplemental Table 1**). The Western dietary pattern was characterized by high intake of red and processed meats, high-fat dairy products (e.g., whole milk and cream), refined grains, and desserts, whereas the prudent dietary pattern was characterized by high intakes of vegetables, fruits, whole grains, poultry, and fish (**Table 1**). Consistent with similar dietary patterns defined in previous studies in these cohorts (49, 50), our Western pattern scores were associated with behaviors generally considered less healthy, whereas our prudent pattern scores were associated with healthier behaviors. In particular, participants with high Western dietary pattern scores were more likely to be current smokers, overweight or obese, and less physically active. In contrast, those with high prudent dietary pattern scores tended to smoke less and report more physical activity (**Table 1**).

In multivariable-adjusted analyses, participants in higher quintiles of Western dietary pattern scores had 69% (quintile 4 compared with quintile 1; 95% CI: 1.14, 2.52) and 86% greater odds (quintile 5 compared with quintile 1; 95% CI: 1.24, 2.79) of having high sCD14 concentrations (greater than or equal to the median of

1915 ng/mL) than those with scores in the lowest quintile (**Table 2**). In contrast, the prudent dietary pattern was not significantly associated with sCD14 concentrations (OR: 0.73; 95% CI: 0.49, 1.09 comparing extreme quintiles; *P*-trend = 0.30) (**Table 2**). Results did not change materially when we excluded participants with lymphoma that developed within 2 y of blood draw or when we restricted analyses to the much smaller group of lymphoma study controls (**Supplemental Table 2**).

In subgroups based on alcohol intake, associations of the Western dietary pattern were stronger among high alcohol consumers, for whom the odds of having high sCD14 concentrations were >4-fold higher for those with Western pattern scores in the highest quintile than for those with scores in the lowest quintile. The odds of having high sCD14 were also higher among low alcohol consumers but were not as consistently higher across quintiles among participants with moderate alcohol consumption (**Table 3**). In other subgroup analyses, associations between the Western pattern and sCD14 concentrations were stronger among men and among participants who were overweight or obese, had high CRP levels, or did not use aspirin or other NSAIDs. The prudent pattern showed no significant associations in subgroups, except for an inverse association with sCD14 concentrations (i.e., reduced odds of having high sCD14 if in the highest quintile of the prudent dietary pattern) among participants with high CRP values (OR per 1-SD increase in prudent dietary score: 0.83; 95% CI: 0.70, 0.99; *P*-trend = 0.03) (**Table 3**).

In the analyses of joint associations, the odds of having high sCD14 concentrations among participants who had high Western pattern scores (highest tertile) were 3-fold higher if they were also heavy alcohol drinkers (OR: 2.96; 95% CI: 1.61, 5.45), >1.6-fold higher if they were also overweight or obese (OR: 1.64; 95% CI: 1.07, 2.50), and >4-fold higher if they also had high CRP levels (OR: 4.11; 95% CI: 2.57, 6.58) (**Figure 1**).

Using factor analysis, we identified 3 biomarker patterns and named them based on the biomarkers that loaded most highly on the pattern as follows: the sGP130 and sTNFR2 dominant biomarker pattern (GP130-TNFR2), the sTNFR2 and sIL-2R $\alpha$  dominant

**TABLE 2** Adjusted ORs from multivariable models for the association of dietary patterns with high plasma sCD14 (overall *N* = 1198)<sup>1</sup>

Statistical model	Dietary pattern quintiles					<i>P</i> -trend <sup>2</sup>	Per 1-SD increase in dietary pattern score
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5		
Western dietary pattern							
High:low sCD14	115:124	116:124	110:127	125:118	133:106		
Model 1	1	1.18 (0.80, 1.74)	1.14 (0.78, 1.69)	1.59 (1.08, 2.34)	1.69 (1.15, 2.50)	0.002	1.22 (1.07, 1.38)
Model 2	1	1.25 (0.85, 1.86)	1.28 (0.86, 1.91)	1.69 (1.14, 2.52)	1.86 (1.24, 2.79)	0.0006	1.26 (1.10, 1.44)
Prudent dietary pattern							
High:low sCD14	114:125	119:121	121:119	131:109	114:125		
Model 1	1	0.85 (0.57, 1.26)	0.95 (0.64, 1.40)	1.11 (0.75, 1.64)	0.79 (0.54, 1.17)	0.46	0.95 (0.84, 1.08)
Model 2	1	0.82 (0.55, 1.22)	0.92 (0.62, 1.38)	1.09 (0.73, 1.62)	0.73 (0.49, 1.09)	0.30	0.94 (0.82, 1.06)

<sup>1</sup>Values are *n* or ORs (95% CIs) modeling the probability of high sCD14 concentrations (i.e., greater than or equal to the median of 1915 ng/mL) unless stated otherwise. sCD14 concentrations were natural log transformed prior to analyses. Dietary patterns were adjusted for energy using the residual methods. Model 1 was adjusted for age at blood draw and sex. Model 2 was additionally adjusted for physical activity, smoking status, case-control status, BMI (in kg/m<sup>2</sup>), chronic disease comorbidity score, and regular use of aspirin, acetaminophen, other nonsteroidal anti-inflammatory drugs, or cholesterol-lowering drugs. Chronic diseases or conditions included in the score were hypercholesterolemia, cancer, diabetes, high blood pressure, heart disease, rheumatoid or other arthritis, or Crohn disease or ulcerative colitis. sCD14, soluble CD14.

<sup>2</sup>The *P* value for linear trend was the *P* value for the dietary pattern as a continuous variable adjusted for all covariates listed in footnote 1.

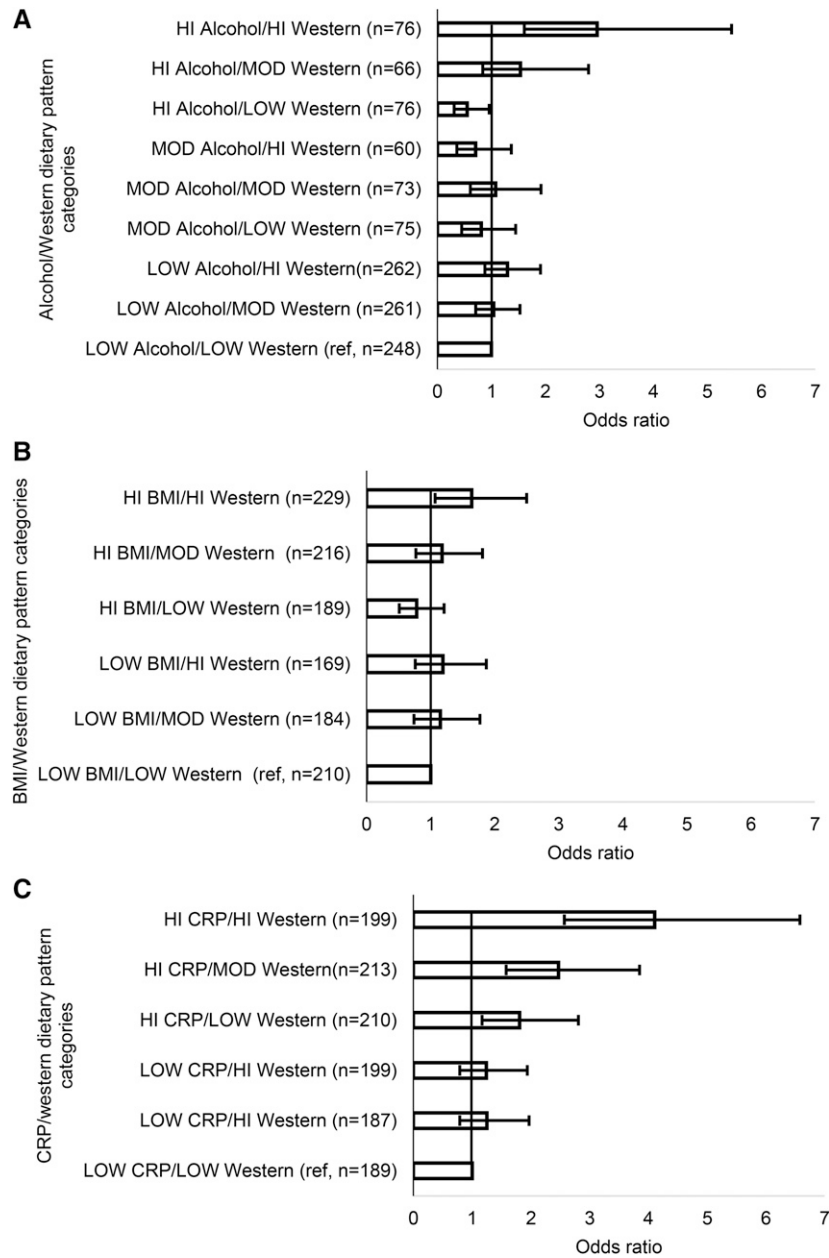
**TABLE 3** Multivariable-adjusted ORs (95% CIs) of the association of dietary patterns and high plasma sCD14 by subgroup<sup>1</sup>

Subgroup	n	Dietary pattern quintiles					P-trend <sup>2</sup>	P-interaction <sup>3</sup>	Per 1-SD increase in dietary pattern score
		Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5			
Western dietary pattern									
Alcohol intake, g/d									
High (≥20 for men and ≥10 for women)	219	1	1.75 (0.68, 4.49)	2.89 (1.10, 7.63)	3.33 (1.21, 9.15)	4.70 (1.72, 12.9)	0.004	0.01	1.67 (1.18, 2.38)
Moderate (10 to <20 for men and 5 to <10 for women)	208	1	1.74 (0.60, 5.06)	1.38 (0.47, 4.04)	3.08 (1.11, 8.53)	1.23 (0.40, 3.79)	0.49		1.12 (0.82, 1.53)
Low (10 for men and <5 for women)	771	1	1.02 (0.62, 1.68)	1.00 (0.60, 1.67)	1.27 (0.76, 2.12)	1.60 (0.96, 2.65)	0.009		1.24 (1.06, 1.46)
BMI, kg/m <sup>2</sup>									
Obese (≥30)	138	1	0.26 (0.06, 1.25)	0.39 (0.10, 1.54)	0.96 (0.25, 3.62)	2.30 (0.67, 7.86)	0.006	0.11	1.70 (1.16, 2.47)
Overweight (≥25 to <30)	497	1	2.18 (1.12, 4.23)	2.29 (1.18, 4.47)	3.90 (1.96, 7.77)	2.75 (1.41, 5.37)	0.002		1.42 (1.14, 1.76)
Normal weight (<25)	563	1	1.17 (0.67, 2.04)	1.09 (0.61, 1.96)	1.22 (0.69, 2.14)	1.41 (0.77, 2.58)	0.52	<0.0001	1.07 (0.87, 1.31)
C-reactive protein, mg/L									
High (≥3)	622	1	1.28 (0.74, 2.19)	1.55 (0.91, 2.65)	2.63 (1.47, 4.70)	2.77 (1.55, 4.94)	0.0001		1.46 (1.20, 1.78)
Normal (<3)	576	1	1.33 (0.74, 2.41)	1.06 (0.56, 2.00)	1.24 (0.69, 2.25)	1.52 (0.83, 2.75)	0.21		1.13 (0.93, 1.37)
Regular aspirin or NSAID use									
No	455	1	1.46 (0.75, 2.83)	1.80 (0.90, 3.59)	2.77 (1.42, 5.42)	2.71 (1.37, 5.35)	0.001	0.06	1.44 (1.16, 1.79)
Yes	743	1	1.27 (0.77, 2.09)	1.11 (0.67, 1.83)	1.37 (0.82, 2.29)	1.61 (0.97, 2.69)	0.07	0.20	1.17 (0.99, 1.39)
Sex									
Women	689	1	1.00 (0.61, 1.65)	1.27 (0.76, 2.13)	1.41 (0.83, 2.39)	1.62 (0.96, 2.72)	0.09		1.17 (0.98, 1.39)
Men	509	1	2.05 (1.00, 4.21)	1.70 (0.83, 3.50)	2.64 (1.32, 5.27)	2.84 (1.39, 5.81)	0.0005		1.43 (1.17, 1.76)
Prudent dietary pattern									
Alcohol intake, g/d									
High (≥20 for men or ≥10 for women)	219	1	2.34 (0.82, 6.65)	1.84 (0.66, 5.13)	4.61 (1.41, 15.1)	1.06 (0.38, 2.92)	0.87	0.72	1.03 (0.77, 1.37)
Moderate (10 to <20 for men or 5 to <10 for women)	208	1	0.52 (0.15, 1.80)	1.15 (0.34, 3.84)	1.19 (0.39, 3.58)	0.63 (0.19, 2.08)	0.52		0.91 (0.69, 1.21)
Low (<10 for men or <5 for women)	771	1	0.78 (0.48, 1.26)	0.85 (0.52, 1.39)	0.92 (0.56, 1.50)	0.79 (0.48, 1.31)	0.38		0.93 (0.78, 1.10)
BMI, kg/m <sup>2</sup>									
Obese (≥30)	138	1	1.58 (0.49, 5.11)	1.53 (0.37, 6.45)	0.95 (0.27, 3.40)	1.19 (0.30, 4.67)	0.45	0.41	0.88 (0.64, 1.22)
Overweight (≥25 to <30)	497	1	0.76 (0.40, 1.43)	0.52 (0.28, 0.97)	0.79 (0.43, 1.45)	0.66 (0.36, 1.21)	0.21		0.87 (0.70, 1.08)
Normal weight (<25)	563	1	0.91 (0.50, 1.67)	1.48 (0.82, 2.68)	1.70 (0.92, 3.14)	0.70 (0.37, 1.31)	0.75	<0.0001	0.97 (0.80, 1.18)
C-reactive protein, mg/L									
High (≥3)	622	1	0.90 (0.52, 1.53)	0.77 (0.44, 1.35)	0.80 (0.46, 1.41)	0.61 (0.35, 1.05)	0.03		0.83 (0.70, 0.99)
Normal (<3)	576	1	0.83 (0.44, 1.58)	1.35 (0.74, 2.49)	1.82 (0.99, 3.36)	0.89 (0.47, 1.71)	0.47	0.44	1.07 (0.89, 1.30)
Regular aspirin or NSAID use									
No	455	1	1.05 (0.54, 2.04)	0.56 (0.28, 1.10)	1.20 (0.62, 2.32)	0.65 (0.34, 1.25)	0.20		0.88 (0.71, 1.07)
Yes	743	1	0.78 (0.47, 1.28)	1.30 (0.79, 2.15)	1.13 (0.68, 1.86)	0.78 (0.46, 1.31)	0.70	0.73	0.97 (0.82, 1.15)
Sex									
Women	689	1	0.75 (0.45, 1.27)	0.73 (0.42, 1.25)	0.97 (0.56, 1.68)	0.71 (0.41, 1.23)	0.71		0.97 (0.82, 1.15)
Men	509	1	0.95 (0.50, 1.81)	1.35 (0.74, 2.46)	1.23 (0.67, 2.25)	0.67 (0.35, 1.28)	0.16		0.86 (0.69, 1.06)

<sup>1</sup>Values are ORs (95% CIs) modeling the probability of high sCD14 concentrations (i.e., greater than or equal to the median of 1915 ng/mL) unless stated otherwise. sCD14 concentrations were natural log transformed prior to analyses. Dietary patterns were adjusted for energy intake using the residual method prior to analyses. All analyses were adjusted for age at blood draw, sex, physical activity, smoking status, case-control status, BMI, chronic disease comorbidity score, and regular use of aspirin, acetaminophen, other NSAIDs, or cholesterol-lowering drugs. Chronic diseases or conditions included in the score were hypercholesterolemia, cancer, diabetes, high blood pressure, heart disease, rheumatoid or other arthritis, or Crohn disease or ulcerative colitis. NSAID, nonsteroidal anti-inflammatory drug; sCD14, soluble CD14.

<sup>2</sup>The P value for trend was the P value of the dietary pattern as a continuous variable adjusted for all covariates listed in footnote 1.

<sup>3</sup>The P value for interaction was estimated using the Wald test for the interaction term.



**FIGURE 1** Joint associations of the Western dietary pattern and alcohol intake categories (A), BMI (in kg/m<sup>2</sup>) categories (B), and CRP levels (C), with higher sCD14 (greater than or equal to the median of 1915 ng/mL). Variables were categorized as follows: Western pattern (first tertile: low; second tertile: moderate; or third tertile: high), alcohol intake (high:  $\geq 20$  g/d for men or  $\geq 10$  g/d for women; moderate: 10 to  $< 20$  g/d for men or 5 to  $< 10$  g/d for women; or low:  $< 10$  g/d for men or  $< 5$  g/d for women), BMI (high: overweight or obese,  $\geq 25$ ; or low: normal weight,  $< 25$ ), and CRP (high:  $\geq 3$  mg/L; or low:  $< 3$  mg/L). Bars show ORs (whiskers indicate 95% CIs) from logistic regression analyses comparing higher categories to the lowest category as the reference (OR = 1). All analyses were adjusted for age at blood draw, sex, physical activity, smoking status, lymphoma case-control status, inflammation-related chronic disease comorbidity score, or regular use of aspirin, acetaminophen, other NSAIDs, or cholesterol-lowering drugs as described in the Methods. CRP, C-reactive protein; HI, high; LOW, low; MOD, moderate; NSAID, nonsteroidal anti-inflammatory drug; ref, reference; sCD14, soluble CD14.

biomarker pattern (TNFR2-IL-2R), and the sCD14-dominant biomarker pattern (CD14) (**Supplemental Table 3**). In multivariable-adjusted models, the Western dietary pattern was associated with higher odds of only the CD14 biomarker pattern (OR comparing extreme dietary index quintiles: 2.04; 95% CI: 1.35, 3.08;  $P$ -trend = 0.0002), whereas the prudent dietary pattern was

associated with lower odds of only the TNFR2-IL-2R biomarker pattern (OR comparing extreme dietary index quintiles: 0.59; 95% CI: 0.40, 0.88;  $P$ -trend = 0.02) (**Table 4**). In analyses of joint associations, the odds of having a high CD14 biomarker pattern score among participants who had high Western pattern scores (highest tertile) were 3.7-fold higher if participants were also

**TABLE 4** Multivariable-adjusted ORs (95% CIs) of the associations between dietary patterns and high plasma biomarker pattern scores<sup>1</sup>

Biomarker pattern	Dietary pattern quintile					P-trend <sup>2</sup>	Per 1-SD increase in dietary pattern score
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5		
Western dietary pattern							
High:low GP130-TNFR2 pattern score	121:118	119:121	112:125	116:127	131:108		
Adjusted model	1	1.05 (0.72, 1.52)	0.93 (0.64, 1.36)	1.03 (0.71, 1.50)	1.30 (0.89, 1.89)	0.24	1.05 (0.97, 1.14)
High:low TNFR2-IL-2R pattern score	113:126	115:125	122:115	115:128	134:105		
Adjusted model	1	1.05 (0.72, 1.54)	1.07 (0.73, 1.57)	0.93 (0.63, 1.36)	1.35 (0.92, 1.99)	0.28	1.05 (0.96, 1.14)
High:low CD14 pattern score	111:128	113:127	116:121	123:120	136:103		
Adjusted model	1	1.27 (0.85, 1.89)	1.54 (1.02, 2.31)	1.77 (1.18, 2.66)	2.04 (1.36, 3.08)	0.0002	1.19 (1.09, 1.31)
Prudent dietary pattern							
High:low GP130-TNFR2 pattern score	125:114	112:128	112:128	129:111	121:118		
Adjusted model	1	0.72 (0.50, 1.04)	0.73 (0.50, 1.06)	0.93 (0.64, 1.36)	0.80 (0.55, 1.16)	0.67	0.98 (0.90, 1.07)
High:low TNFR2-IL-2R pattern score	141:98	115:125	107:133	122:118	114:125		
Adjusted model	1	0.65 (0.44, 0.95)	0.55 (0.37, 0.81)	0.69 (0.47, 1.01)	0.59 (0.40, 0.87)	0.03	0.91 (0.83, 0.99)
High:low CD14 pattern score	119:120	119:121	117:123	127:113	117:122		
Adjusted model	1	0.78 (0.52, 1.17)	0.84 (0.56, 1.25)	0.98 (0.65, 1.47)	0.76 (0.51, 1.14)	0.49	0.97 (0.88, 1.06)

<sup>1</sup>Values are *n* or ORs (95% CIs) unless stated otherwise. Dietary patterns were adjusted for energy intake using the residual method. Biomarker scores were classified as high (greater than or equal to the median score) compared with low (less than the median score). Median scores were  $-0.1215$  for the GP130-TNFR2 pattern score,  $-0.0875$  for the TNFR2-IL-2R pattern score, and  $-0.0170$  for the CD14 pattern score. All analyses were adjusted for age at blood draw, sex, physical activity, smoking status, case-control status, BMI (in kg/m<sup>2</sup>), chronic disease comorbidity score, and regular use of aspirin, acetaminophen, other nonsteroidal anti-inflammatory drugs, or cholesterol-lowering drugs, as described in the Methods. GP130, soluble glycoprotein 130; IL-2R, soluble interleukin-2 receptor; TNFR2, soluble tumor necrosis factor receptor 2.

<sup>2</sup>The *P* value for trend was the *P* value of the dietary pattern as a continuous variable adjusted for all covariates listed in footnote 1.

heavy alcohol drinkers (OR: 3.69; 95% CI: 1.98, 6.88) and >1.8-fold higher if they were overweight or obese (OR: 1.83; 95% CI: 1.09, 3.05) (**Supplemental Figure 1**). Among participants with low Western dietary pattern scores, higher alcohol intake was not associated with a high CD14 biomarker pattern score and, in fact, was suggestive of protection from the high CD14 biomarker pattern.

## Discussion

The gut, an important organ in the innate and adaptive immune system, is constantly being challenged as a result of interactions with external stimuli, including diet. Therefore, characterizing the role of whole diets, and not just single foods or nutrients, is important in elucidating factors that influence gut barrier dysfunction. This study conveys 2 new important findings. First, plasma concentrations of sCD14 were significantly higher in participants with higher scores on the Western dietary pattern compared with those with the lowest scores, indicating a higher likelihood for gut hyperpermeability in participants with higher Western dietary pattern scores. Second, associations between the Western dietary pattern and sCD14 concentrations were stronger among heavy alcohol consumers, overweight or obese participants, those with high CRP values, those not regularly taking aspirin or NSAIDs, and men. In contrast, the prudent dietary pattern did not show strong associations with sCD14 overall but showed a trend of inverse associations among

those with high CRP. In addition, the Western dietary pattern was associated with higher levels of an sCD14-dominated pattern of biomarkers of inflammation and immune activation, which potentially characterize the pathophysiologic milieu associated with higher sCD14 concentrations.

It has been suggested that alcohol is absorbed through the mucosa of the entire gastrointestinal tract by simple diffusion, which is determined by the alcohol concentration gradient between the intestinal lumen and the subepithelial capillaries, regional blood flow, sex, age, and fasting status, among other factors (32, 51). However, as a result of first-pass metabolism of alcohol in the stomach, the adverse effects of alcohol on the mucosa as a result of high intestinal luminal alcohol concentrations are therefore to be expected predominantly in the duodenum and upper jejunum (32). Our differential findings for alcohol intake are in line with the suggested U-shaped curve of the association of alcohol intake and some disease endpoints in previous studies (52). Gut mucosa damage as a result of heavy alcohol intake may lead to a sequence of events, including increased permeability in the gut to macromolecules, enhanced translocation of endotoxin or other bacterial toxins into the blood (32), and thus higher concentrations of circulating markers of gut hyperpermeability, including sCD14 and LBP. In addition, in *in vitro* and *in vivo* studies, consumption of moderate amounts of alcohol enhances phagocytosis and reduces inflammatory cytokine production, whereas chronic consumption of high doses inhibits phagocytosis and production of growth factors (52).



In other subgroup analyses, we observed stronger associations between the Western dietary pattern and sCD14 concentrations in overweight and obese participants, those with high CRP levels, those not regularly taking aspirin or other NSAIDs, and among men (although the *P* values for interaction for BMI and sex were not statistically significant, we observed large differences in ORs in the strata of these potential effect modifiers). These results are in line with findings in previous studies of markers of gut bacterial translocation and disease endpoints (4, 5). In a nested case-control study, Kong et al. (4) investigated the associations of serum anti-LPS and anti-flagellin IgA and IgG with colorectal cancer risk. Although they observed no significant associations with colorectal cancer risk overall, analyses by sex revealed a positive association in men for anti-LPS and anti-flagellin markers combined, whereas the associations in women were inverse. The association in men was stronger in those with higher levels of high-sensitivity CRP and with higher waist circumference, BMI, and alcohol intake (4). In an intervention study, Yang et al. (5) also found that BMI and waist circumference were positively associated with gut barrier dysfunction. This alignment of evidence, together with the findings from the joint associations of diet and these factors, suggests that alcohol intake, body size, inflammation, and sex may influence the adverse effects of poor diet quality on intestinal hyperpermeability.

Several limitations should be considered while interpreting our study findings. For example, although our cross-sectional study design is optimal for the examination of circulating biomarkers, it is not possible to determine that dietary intake preceded high plasma sCD14 concentrations. However, the consistency of our findings in strata of preselected potential effect modifiers and the adjustment for multiple potential confounding factors provide support that the high concentrations of sCD14 are likely influenced by poor diet quality. It also appears more likely that diet influences sCD14 concentrations, rather than that sCD14 levels cause individuals to adhere to major dietary patterns. Although we predefined the subgroup analyses, we cannot completely rule out that chance may account for some of the significant findings. Other factors, unrelated or indirectly related to immune function, such as drinking pattern or beverage type may be implicated in the relation between alcohol consumption and gut barrier dysfunction (53), but our sample size could not provide enough statistical power for further stratification by type of alcohol consumed. Although studies have shown sCD14 concentrations to correlate well with LBP, we did not have LBP data as a second marker of gut barrier dysfunction. To enhance statistical power, we included participants with lymphoma in the primary analyses, but blood was drawn before the diagnosis of lymphoma and results for sensitivity analyses excluding proximal lymphoma cases (within 2 y) were not materially different. We adopted data-driven approaches (factor analysis) to identify dietary and biomarker patterns. The main advantage of this technique is that it reveals hidden (unobserved), simplified structures that underlie data. However, there is subjectivity regarding the choice and interpretation of the factors (54). Nonetheless, examining the Western dietary pattern is a useful initial step in determining whether diet can influence gut barrier function, because this dietary pattern appears to encompass many adverse elements that have been consistently

associated with biomarkers (e.g., inflammation or hyperinsulinemia) and several diseases.

In our study sample, higher intake of the Western dietary pattern was associated with gut barrier dysfunction, as characterized by high plasma concentrations of sCD14 (a surrogate marker of gut hyperpermeability). We found notable differences by categories of alcohol intake, body size, generalized systemic inflammation (as characterized by CRP levels), regular use of aspirin or other NSAIDs, and sex. To our knowledge, this is the first study to examine the association of dietary patterns and a marker of gut hyperpermeability. Prospective studies are warranted in different populations with multiple biomarkers of gut barrier dysfunction to better characterize the role of whole diets, including the temporal relation between diet and gut barrier dysfunction.

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