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The Dietary Inflammatory Index is Associated with Elevated White Blood Cell Counts in the National Health and Nutrition Examination Survey

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Abstract

White blood cells (WBCs) are considered a reliable biomarker of inflammation. Elevations in both WBCs and pro-inflammatory cytokines are associated with several chronic conditions. Diet is a strong moderator of inflammation and WBCs. The purpose of this study was to examine the association between the Dietary Inflammatory Index (DII®) and WBCs using data from the United States National Health and Nutrition Examination Survey (NHANES). NHANES is a cross-sectional study that occurs in two-year cycles. Respondents from five cycles (n=26,046) with available data on diet (collected through a single 24-hour dietary recall [24HR]) and WBCs (derived using the Coulter method) were included. The DII (theoretical range is about −8 to +8) was derived from the micro and macronutrients calculated from the 24HR. Linear regression models, using survey design procedures, were used to estimate adjusted mean WBC (i.e., total, lymphocytes, monocytes, and neutrophils) counts and percentages by DII quartiles. Among all participants no statistically significant difference in WBCs were observed when comparing DII quartile 4 (most pro-inflammatory) to quartile 1 (most anti-inflammatory). However, a one-unit increase in the DII was associated with a 0.028 (1000 per μL) increase in total WBCs (p=0.01). Additionally, a 0.024 increase in neutrophils (p<0.01) was observed for a one-unit increase in the

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Declaration of Interest: Dr. Hébert owns controlling interest in Connecting Health Innovations LLC (CHI), a company planning to license the right to his invention of the dietary inflammatory index (DII) from the University of South Carolina in order to develop computer and smart phone applications for patient counseling and dietary intervention in clinical settings. Drs. Michael Wirth and Nitin Shivappa are employees of CHI.

DII. In the group of participants with normal body mass index (BMI, 18.5–24.9 kg/m^2), those in DII quartile 4 had higher levels of total WBCs compared to subjects with normal BMI in DII quartile 1 (7.12 vs. 6.88, p=0.01). Similar comparisons were observed for monocytes and neutrophils. However, these relationships were not observed for participants who were overweight or obese, which are pro-inflammatory conditions. Normal-weight individuals consuming more pro-inflammatory diets were more likely to have elevated WBCs. Because of its cross-sectional design, NHANES cannot inform directly on temporal relations, thus limiting causal inference. Future research is needed to examine the impact of anti-inflammatory diet adoption on lowering levels of WBCs, in addition to other inflammatory mediators.

Keywords

Dietary Inflammatory Index; Leukocytes; Diet; Nutrition; NHANES

1. Introduction

White blood cells (WBCs), or leukocytes, are one of three types of blood cells (other two include platelets and erythrocytes) that make up about 45% of whole blood (55% is plasma), which accounts for about 7% of an average human adult's body weight (Cameron JR, 1999; Pritchett and Reddy, 2015). Important functions of certain WBCs include, but are not limited to, destruction of virus-infected cells, directing the immune response through cytokine secretion, secretion of antibodies for phagocytosis detection (lymphocytes), destruction of pathogens by phagocytosis (neutrophils), and transformation into macrophages (monocytes) (Pritchett and Reddy, 2015). The specific properties and functions of the innate and adaptive immune systems have been described in detail elsewhere (Labrecque and Cermakian, 2015). It is important to note that elevations in WBCs can represent a normal response to infection and wound healing (Labrecque and Cermakian, 2015).

An important component of the immune system is the inflammatory response to injuries or insults. Cytokines are a type of chemoattractants synthesized by macrophages with the capacity to activate other WBCs. They can act as second messengers and induce the expression of adhesion molecules on endothelial cells that promote attachment and transmigration of leukocytes (Levy, 1996; Pober and Cotran, 1990). Again, this is a necessary mechanism for proper wound healing and for combatting infections. However, concern is warranted when insults or injuries that increase inflammatory levels or WBCs become chronic over time. Given the reciprocal relationship between inflammatory cytokines and WBCs, a chronic injury or insult to the human body could create a perpetual cycle increasing levels of inflammatory cytokines and WBCs (Libby, 2007). Chronic levels of inflammation have been associated with numerous chronic conditions including, but not limited to, type 2 diabetes mellitus (T2DM), cardiovascular diseases (CVD), gastrointestinal illnesses, and cancer (Libby, 2007). WBCs have been considered a reliable cellular biomarker of inflammation (Kounis et al., 2015). Chronic elevation of WBCs has been linked to several chronic conditions including T2DM, coronary artery disease, stroke, and leukemia (Costas et al., 2016; Danesh et al., 1998; Ford, 2002; Libby et al., 2016; Vozarova et al., 2002).

One of the strongest environmental influencers of chronic systemic inflammation is diet (Ahluwalia et al., 2013). In general, diet patterns characterized by high consumption of fruits and vegetables, whole grains, and fish (e.g., Mediterranean) are associated with lower levels of systemic inflammation; whereas diets characterized by high consumption of total and saturated fats, protein, and simple carbohydrates (e.g., Western diet) are associated with higher levels of inflammation (Ahluwalia et al., 2013). Previous research has linked dietary patterns or indices to WBC counts. For example, studies utilizing indices and patterns such as the Mediterranean Diet Score (MDS), the Italian Mediterranean Index, indices measuring adherence to the Swedish or Flemish dietary recommendations, and the Healthy Eating Index (HEI) have demonstrated that healthier scores are inversely associated with leukocyte counts (Ambring et al., 2006; Bonaccio et al., 2014; Chrysohoou et al., 2004; Dias et al., 2015; Hoebeeck et al., 2011; Loprinzi et al., 2016). However, none of these dietary indices or patterns was developed specifically with respect to dietary inflammatory potential.

The Dietary Inflammatory Index (DII®) was developed to characterize an individual's dietary inflammatory potential using a quantitative scale from pro- to anti-inflammatory (Shivappa et al., 2014a). The DII was construct validated against inflammatory biomarkers (e.g., c-reactive protein, tumor necrosis factor-alpha, interleukin-6) in numerous studies (Julia et al., 2017; Shivappa et al., 2014b; Shivappa et al., 2016b; Tabung et al., 2015; Wirth et al., 2014). Additionally, the DII has been associated with a range of other inflammatory-related conditions including obesity, various cancers, CVD, telomere length, depression, asthma, and mortality (Graffouillere et al., 2016; Harmon et al., 2017; Kesse-Guyot et al., 2016; Neufcourt et al., 2016; Ruiz-Canela et al., 2015a; Shivappa et al., 2016a; Shivappa et al., 2015a; Shivappa et al., 2017b; Shivappa et al., 2015b; Shivappa et al., 2016b; Wirth et al., 2016b; Wirth et al., 2015; Wood et al., 2015). However, the DII has yet to be examined with respect to WBC levels. Therefore, the purpose of this study was to examine the cross-sectional association between DII scores and WBCs using the National Health and Nutrition Examination Survey (NHANES). Specifically, it was hypothesized that individuals with more pro-inflammatory diets would have elevated levels of WBCs compared to those with more anti-inflammatory diets. Additionally, given that both WBC levels and DII values differ according to body mass index (BMI) (Gregor and Hotamisligil, 2011; Ruiz-Canela et al., 2015a), which is known to be associated with chronic systemic inflammation, BMI was examined as a potential effect modifier.

2. Methods

2.1 Study Population

NHANES collects cross-sectional information on American adults and children in two-year cycles. NHANES uses complex, multistage, probability cross-sectional sampling to ensure selection of participants from various geographical locations and racial/ethnic groups. This provides a representative sample of the entire United States population. NHANES participants start with an in-home interview where questionnaire information is obtained. This information includes a wide range of topics including demographics, medical histories, socioeconomic metrics, and behavioral, diet, and lifestyle habits. Participants are then invited for further examination in their mobile examination clinic (MEC) where biological

samples are run and clinical tests conducted. For a full list of data available in NHANES and precise detail on data collection, please refer to their website and documentation (http://www.cdc.gov/nchs/nhanes.htm) (Johnson et al., 2013). From five 2-year cross-sectional cycles (i.e., 2005–2014), there were a total of 30,295 individuals aged ≥18 years. Of these individuals, 2,618 were missing information on WBCs. An additional 1,624 were missing dietary information. Lastly, seven participants were removed due to a reported energy intake of less than 100 kcal. This left an analytic sample size of 26,046. Participants provided informed consent and NHANES is continually reviewed by the National Center for Health Statistics Research and Ethics Review Board.

2.2 Outcomes Assessment

To obtain leukocyte counts, blood draws were performed by trained phlebotomists during the participant visit to the MEC. Specific details on venipuncture can be found in the NHANES Laboratory Procedures Manual. Complete blood counts were conducted using the Coulter method (Coulter® HMX, Beckman Coulter, Brea, California) (CDC, 2013). The leukocytes analyzed in this study included total WBC, monocyte, lymphocyte, and neutrophil counts expressed as 1000 per microliter. Additionally, percentages of monocytes, lymphocytes, and neutrophils were examined. Although information on basophils and eosinophils was available, about 95% of participants had values below 0.4 (1000 per μL) for eosinophils and 99% had values below 0.2 (1000 per μL) for basophils. Given the limited range in values for these two leukocyte types, they were excluded from the analyses. All outcomes were treated as continuous measures.

2.3 The Dietary Inflammatory Index and Potential Covariates

NHANES used 24-hour dietary recall interviews (24HRs) to obtain dietary information. NHANES processed the dietary data to obtain micro and macronutrients by using the USDA's Food and Nutrient Database for Dietary Studies (FNDDS). The primary exposure of interest was the DII, which included the following macro and micronutrients (termed food parameters throughout): carbohydrates; fat; protein; vitamins A, B1, B2, B6, B12, C, D, E; niacin; grams of alcohol; saturated, monounsaturated, and polyunsaturated fatty acids; omega3 and omega6 polyunsaturated fatty acids; fiber; cholesterol; iron; magnesium; zinc; selenium; folic acid; beta carotene; and caffeine. Research from nearly 2,000 peer-reviewed publications formed the basis of the DII. 'Inflammatory effect scores' were created from these peer-reviewed publications for each of the DII food parameters based on their effect on inflammatory cytokines.(Shivappa et al., 2014a) At the same time, DII calculation is standardized to a regionally representative world database. This world database included dietary consumption from 11 populations around the world. The world database provided a standard mean and deviation for all DII food parameters. For each food parameter, a z-score was created by subtracting the individual's estimated intake from the standard mean. This was then divided by the world standard deviation and then converted to a distribution centered on 0 with bounds between −1 and +1. This value was then multiplied by the inflammatory effect score for each food parameter which was then summed across all food parameters to create the overall DII score. More positive scores indicate more pro-inflammatory diets and more negative values are more anti-inflammatory (Shivappa et al.,

2014a). DII scores were calculated per 1,000 calories consumed to control for the effect of total energy intake differences between participants.

Self-reported possible confounders included age; race; income; marital status; education; daily sleep duration; tobacco use; number of alcoholic drinks per week; health insurance; perceived health; family smoking status; family history of heart disease; previous diagnoses of cancer, diabetes, asthma, arthritis, or any circulatory condition; waist circumference; and moderate-to-vigorous physical activity (MVPA time in minutes). For two-year cycles between 2007 and 2012, NHANES truncated age to 80 years, but truncated to 85 years for the 2005–2006 cycle. Age was truncated to 80 years for all analyses to maintain consistency. BMI was based on clinic-measured weight and height (kg/m^2).

2.4 Statistical Analyses

All analyses were conducted using survey design procedures in SAS® (version 9.4, Cary, NC), which take into account the stratification and clustering employed by NHANES' sampling procedures. Given that 10 years of NHANES data were used, ten-year sampling weights were created by multiplying the two-year weights by one-fifth, as suggested (Johnson et al., 2013). Means and frequencies were compiled for several continuous and categorical covariates, respectively, according to DII quartile. For continuous measures, trend tests compared the change in means across DII quartiles. Chi-square tests were computed to examine the distribution of categorical covariates across quartiles. For confounder selection, potential confounders were entered into the full model if their p-value was <0.20 in bi-variable analyses (i.e., DII + confounder). The value of 0.2 for the p-value was chosen to be more inclusive for the next step to make sure no potential confounders were missed. From the full model, covariates were removed one at a time using a manual process to examine changes in the beta coefficient of the DII. The final model consisted of the DII score and any other covariate that led to a 10% change in the beta coefficient of the DII (Greenland, 1989). Statistically significant covariates also were retained.

To estimate least square means for the individual leukocytes by DII quartiles, linear regression models were employed. These analyses took into account weight, cluster, and strata variables provided by NHANES. Model residuals were examined to determine if there was departure from the assumptions of linear regression and none were noted. The primary comparison of interest was between DII quartiles 4 (most pro-inflammatory) and 1 (most anti-inflammatory). Although other groupings could have been used, quartiles are most often used with the DII. Quartile 1 represents very anti-inflammatory diets; whereas, quartile 4 represents very pro-inflammatory diets. This technique allows for comparison of the extremes. However, the DII also was used as a continuous exposure in subsequent models to examine the linear change in WBCs as the DII increased (i.e., became more pro-inflammatory). Additionally, trend tests were conducted by assigning the median value of each quartile as a continuous variable to determine if means increased across quartiles of the DII. BMI is strongly associated with leukocyte counts (Huang et al., 2001), and adiposity is known to be pro-inflammatory (Lin et al., 2015; Ouchi et al., 2011; Ramallal et al., 2017; Ruiz-Canela et al., 2015b). Thus, simply adjusting for BMI may mask effects of diet on inflammation levels. Therefore, BMI was treated as an *a priori* effect modifier and results

were stratified based on normal BMI (18.5–24.9 kg/m^2), overweight (25.0–29.9 kg/m^2), and obese (≥30 kg/m^2). Given that the decision to stratify was made *a priori*, no adjustments were made for type 1 error (e.g., Bonferroni). NHANES collects information on infections within 30 days of study examination. Specifically, additional adjustments were made for a chest or head cold; stomach or intestinal illness; and flu, pneumonia, or ear infections. Previously, Bonaccio et al., noted that the relationship between the Mediterranean diet and WBCs disappeared after adjustment for platelets (Bonaccio et al., 2014). Therefore, analyses were additionally adjusted for platelet counts by adding it as a confounder to the models. Several minor differences were noted, which are described in the Results section.

3.0 Results

Overall, the population (n=26,046) was nearly evenly split between males (48%) and females (52%). Otherwise, the population was primary European-American (69%), at least partially college educated (57%), married or living with a partner (62%), middle-aged (mean age and standard error: 46.1 ± 0.29 years), and overweight (mean BMI and standard error: 28.6 ± 0.08 kg/m^2 years, data not tabulated). Table 1 displays population characteristics by DII quartiles. Those in DII quartile 4 (i.e., most pro-inflammatory category) were more likely to be younger, male, single, and less than college educated, have lower income, smoke tobacco, and have a higher BMI and waist circumference (all p<0.01) compared to those in DII quartile 1.

The mean WBC count, expressed as 1000 per μL, by DII quartile is shown in Table 2. When comparing DII quartile 4 to quartile 1, there was no statistically significant difference in total WBC count (7.42 vs. 7.32, p=0.10). The same was true for lymphocytes, monocytes, and neutrophils. However, when the DII was analyzed as a continuous variable, there was an association with total WBC (β=0.028, standard error [SE]=0.01, p=0.01) and neutrophils (β=0.024, SE=0.01, p<0.01). It also should be noted that those in DII quartile 4 had a lower percentage of lymphocytes compared to those in DII quartile 1 (30.7% vs. 31.1%, p=0.05). Using the DII continuously indicated that as the DII increased, lymphocyte and monocyte percentages decreased, whereas neutrophil percentages increased (Table 2). Trend tests indicated a statistically significant trend for all WBCs, neutrophils, and lymphocyte percentage (data not tabulated).

The p-value for the interaction term between the DII and BMI was 0.19 which may indicate additive interaction. Regardless, the decision to stratify by BMI was made *a priori*. Interestingly, when stratified by BMI, the relationship between the DII and WBCs differed for BMI categories. Most noticeably, those in DII quartile 4 had higher values of total WBCs compared to DII quartile 1 among those with a BMI < 25.0 kg/m^2 (7.12 vs. 6.88, p=0.01). However, this was not observed among those with a BMI between 25.0 and 29.9 kg/m^2 (7.29 vs. 7.20, p=0.30) or those with a BMI ≥30.0 kg/m^2 (7.83 vs. 7.86, p=0.71). Among those with a BMI <25.0 kg/m^2, DII quartile 4 also had higher monocytes and neutrophil counts, and lower lymphocyte percentages compared to DII quartile 1 (Table 3). Among those with a BMI of 25.0 to 29.9 kg/m^2, no association between the DII and WBCs was observed. The same was true for those with a BMI ≥30.0 kg/m^2, except for a decrease in monocytes which was observed. Interestingly, waist circumference remained a significant predictor in BMI-

stratified models. To account for over-adjustment, waist circumference was removed from the BMI-stratified models and the results remained unchanged.

Results did not change after adjustment for recent infections. Likewise, additional analyses were performed after adjusting for cholesterol and blood pressure medications; no differences in results were observed. All models were additionally adjusted for platelet levels. When comparing the fourth quartile of the DII to the first, there was attenuation of several statistically significant associations among those in the normal BMI category. The difference between the first and fourth quartile for total WBCs (6.95 ± 0.10 vs. 7.11 ± 0.10, p=0.08), monocytes (0.53 ± 0.01 vs. 0.55 ± 0.01, p=0.08), and neutrophils (4.03 ± 0.08 vs. 4.18 ± 0.09, p=0.06) were no longer statistically significant. However, a one-unit increase in the DII was still associated with total WBCs (β=0.06, t-value = 3.69, p<0.01), monocytes (β=0.004, t-value = 2.84, p=0.01), and neutrophils (β=0.05, t-value = 3.71, p<0.01) after adjustment for platelets. No other difference in results was observed.

4.0 Discussion

In this cross-sectional study using data from NHANES (2005–2014), it was observed that as the DII score becomes more pro-inflammatory there was a corresponding increase in total WBCs, as well as neutrophils. However, it should be noted that these relationships were only significant when the DII was used continuously and not when comparing quartiles 1 vs. 4 among all participants. Additionally, lymphocyte and monocyte percentages decreased, whereas the percentage of neutrophils increased. Interestingly, these results were only seen among those in the normal BMI range (i.e., 18.5 – 24.9 kg/m^2). In fact, differences in total WBCs were significantly different between quartiles 1 and 4 among this group. It is possible that when all BMI groups were combined the relationship between the continuous DII and WBCs persisted because of increased power. However, among all participants, quartiles 1 vs. 4 may not have been significant because all BMIs were considered together. These relationships were nonexistent among those with a BMI of 25+ kg/m^2. Obesity is a pro-inflammatory state and this is driven primarily by adipose tissue signaling (Enos et al., 2013; Tsatsoulis et al., 2013).

Previous research corroborates the current study's findings. For example, using NHANES (2003–2006), Loprinzi and colleagues examined the relationship between healthy lifestyle habits, including the HEI, and WBCs. They found that individuals with healthy HEI scores had a −0.24 × 1000 per μL decrease in total WBCs compared to those with unhealthy HEI scores (Loprinzi et al., 2016). Using adherence to the Mediterranean diet, as measured by the MDS, those in the high-adherence group had significantly lower WBC counts compared to the low adherence group (6.17 vs. 6.31 × 1000 per μL, p<0.01) (Bonaccio et al., 2014). Based on adherence to Swedish national dietary recommendations, those with high adherence had lower WBC (5.93 vs. 6.82 × 1000 per μL, p<0.01) neutrophil (3.48 vs. 4.07 × 1000 per μL, p<0.01), and lymphocyte (1.79 vs. 1.99 × 1000 per μL, p<0.01) counts compared to low adherence (Dias et al., 2015).Others also found decreased WBC counts with increasing dietary quality (Chrysohoou et al., 2004; Hlebowicz et al., 2011; Hoebeeck et al., 2011). A case-crossover clinical trial indicated lower WBC counts after four weeks of a Mediterranean-inspired diet compared to four weeks of a normal Swedish diet (4.51 vs.

4.97 × 1000 per μL, p=0.02) (Ambring et al., 2006). However, caution is warranted when comparing these studies to the current study. Although most utilized the same study design (i.e., cross-sectional), they represent a range of populations (e.g., United States, Italy, Belgium, Greece, Sweden), dietary assessment tools (e.g., 24HRs, food frequency questionnaire, 7-day dietary diary), dietary indices (e.g., MDS, DII, adherence to national recommendations), and confounder sets for adjustment. Additionally, it should be noted that no other dietary index mentioned above was developed solely based on inflammation. It is quite possible that other dietary indices may predict WBCs because they inherently measure other aspects of diet which may be associated with WBCs as well. It should be noted that despite these differences, for the most part, the studies are in agreement. Typically, healthier diets are associated with lower WBC counts compared to less healthy diets.

Unlike other dietary indices and patterns, the DII was developed with a focus on dietary sources of inflammation and utilized data from nearly 2,000 peer-reviewed journal articles during development and is comprised of up to 45 different food parameters. Additionally, the DII is standardized to dietary intake from numerous populations around the world. Therefore, the DII is not subject to biases related to applying a dietary index based on a cultural food-way (e.g., MDS) to a different culture. A previous analysis indicated that DII scores are correlated with those of other dietary indices including the HEI, Alternative Healthy Eating Index-2010, and the Dietary Approaches to Stop Hypertension with correlation coefficients between −0.52 and −0.65, $p<0.01$). It should be noted that the DII is reverse scored compared to these other indices; hence, the negative correlations (Wirth et al., 2016a). Although the correlations are moderate, they are not perfect (i.e., the correlation coefficient was not close to 1.00). The DII most likely accounts for different sources of variability providing additional valuable information beyond other dietary indices. Presumably, the additional source of variation the DII explains is related to inflammation. Given that the DII presumably focuses on inflammation more so than other dietary indices, it is conceivable that the DII is more predictive of inflammation-related conditions than other dietary indices. However, this may not be true in all instances.

Inflammation is responsible for wound repair in the body. However, proper resolution of inflammation is necessary after tissue repair and regeneration have occurred (Kulkarni et al., 2016). Chronic exposures to pro-inflammatory stimuli may cause shifts in this homeostatic process leading to a failure to control the injury, potentially leading to additional tissue damage (Kulkarni et al., 2016; Medzhitov, 2008). As inflammation increases, the numbers of WBCs may increase or there may be a shift in WBC subtypes leading to a further increase in pro-inflammatory cytokines, as well as reactive oxygen species and other molecules that can perpetuate the inflammatory response (Libby, 2007). This is disconcerting given that WBCs can contribute to chronic disease, especially CVD (Danesh et al., 1998). For example, accumulation of excess leukocytes may lead to thickening of the arterial walls. At the same time, increases in leukocyte accumulation in plaques can increase inflammation in that affected area leading to further damage (Conti and Shaik-Dasthagirisaeb, 2015; Libby et al., 2016). Interestingly, results were strongest among those with a normal BMI. Hypertrophied adipocytes and adipose tissue-resident immune cells found in adipose tissue of those who are overweight or obese contribute to the levels of circulating pro-inflammatory cytokines (Gregor and Hotamisligil, 2011; Makki et al., 2013). Obesity

influences both the quantity and subtypes of WBCs (Grant and Dixit, 2015). The main WBC type in adipose tissue is macrophages. Specifically, M1 macrophages are particularly elevated; and this, in turn, is correlated with increased inflammation, as well as insulin resistance (Castoldi et al., 2015). It is conceivable that among those who are overweight or obese, the inflammation-contributing adipose cells overwhelm the effect of diet-associated inflammation among these individuals. Hence, positive associations were observed among the normal-weight group. A similar conclusion was noted by Shivappa and colleagues who found that the DII was associated with renal cancer among normal weight women in the Iowa Women's Health Study, but not among overweight or obese women (Shivappa et al., 2017a). In the current study, it is not surprising to observe that WBCs, expressed as 1000 per μL, increased across BMI categories (normal = 6.83±0.03, overweight = 7.08±0.04, obese = 7.70±0.04, all p-values for the test of differences <0.01).

This study had several strengths. The use of multiple two-year cycles allowed for a large sample size that is representative of the general United States population. Additionally, the range of information collected by NHANES allowed for many factors to be examined as possible confounders. However, lack of adjusting for unmeasured confounders can never be ruled out. Lastly, this was the first study to use the DII in relation to WBCs. The DII has advantages over other dietary indices and patterns, as noted above, and it can be calculated from various dietary reporting techniques (e.g., 24HR, food frequency questionnaire, 7-day dietary recall). It is important to note that the DII can span various food cultures and is not specific to a particular way of eating or set of recommendations.

Despite its strengths, this study had several weaknesses. Only one 24HR was used to calculate the DII. One 24HR may not account for the day-to-day variability in dietary intake which may lead to imprecise estimates (Basiotis et al., 1987). Also, dietary reporting biases, such as social desirability and approval, were not obtained (Hebert et al., 1997). Most, if not all WBCs, have a daily circadian rhythm, both in terms of circulating cell populations and functions (Scheiermann et al., 2012). Most circulating WBCs peak in numbers between 2000 and 0200 hours (Mazzoccoli et al., 2011; Sennels et al., 2011). The exact timing of blood draws was not available for analysis. The cross-sectional nature of NHANES cannot be ignored. It is not possible to determine whether pro-inflammatory diets led to increased WBC levels or *vice versa*. However, it is hard to conceive how high levels of WBCs would drive an individual to eat a pro-inflammatory diet. It also is important to note that the DII has been found to be relatively stable over a period of 6 years (Tabung et al., 2016). Lastly, it is possible that BMI may act a mediator between the DII and WBCs. However, research indicates that it is not advisable to explore mediation using cross-sectional designs, as it may lead to substantial bias (Maxwell et al., 2011). To fully explore mediation, a longitudinal study examining this association would be best. It is possible to at least conclude that those with higher levels of WBCs consume more pro-inflammatory diets than those with lower levels of WBCs.

5. Conclusions

This study found that consumption of more pro-inflammatory diets is associated with higher levels of WBCs, specifically total WBCs and neutrophils. Results were observed among

those with normal BMI. Elevated levels of WBCs have been linked to several chronic diseases (Libby et al., 2016; Vozarova et al., 2002). Given that pro-inflammatory diets were found to be associated with elevated WBCs, it is possible that some of the effect of pro-inflammatory diets on chronic disease functions through elevated WBC levels. However, that specific hypothesis cannot be addressed in the current study. Therefore, future longitudinal studies should consider examining mechanisms involving a pro-inflammatory diet influencing WBCs which then influence chronic disease pathways. Likewise, future studies should determine the impact of adopting an anti-inflammatory diet (e.g., one especially high in spices, herbs, green leafy vegetables, and certain fruits like berries with low consumption of fats, red meat, and sweets) on reducing elevated levels of WBCs, among those with a normal BMI. However, adoption of such a diet will have other beneficial effects among those of any BMI.

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- The Dietary Inflammatory Index measures the inflammatory potential of diet.
- A one-unit increase in DII increased white blood cells by .028 (1000 per uL).
- The DII was more strongly associated with WBCs among normal weight (BMI<0.25).

Table 1

Baseline population characteristics according to The Dietary Inflammatory Index quartiles, National Health and Nutrition Examination Survey (NHANES), 2005–2014.

Characteristic	Frequency (%) or Mean ± STE				p-value
	Quartile 1 (n=6,491)	Quartile 2 (n=6,599)	Quartile 3 (n=6,608)	Quartile 4 (n=6,348)	
Age (years)[b]					<0.01
Mean ± STE	51.0 ± 0.41	47.6 ± 0.38	44.1 ± 0.36	41.7 ± 0.34	
Sex[a]					<0.01
Female	3774 (60%)	3396 (52%)	3159 (48%)	3033 (49%)	
Male	2717 (40%)	3203 (48%)	3449 (52%)	3315 (53%)	
Marital Status[a]					<0.01
Married or living w/partner	4035 (68%)	3873 (65%)	3615 (63%)	3234 (59%)	
Widowed/Divorced/separated	1466 (18%)	1421 (19%)	1316 (18%)	1228 (18%)	
Single, never married	849 (14%)	1076 (16%)	1339 (19%)	1534 (23%)	
Education Status[a]					<0.01
Less than high school	1501 (14%)	1657 (17%)	1635 (18%)	1555 (20%)	
High school	1197 (18%)	1338 (21%)	1451 (24%)	1645 (29%)	
Some college	1637 (27%)	1807 (32%)	1862 (34%)	1757 (33%)	
College graduate or above	1988 (41%)	1479 (31%)	1144 (24%)	817 (18%)	
Race[a]					<0.01
Non-Hispanic White	2999 (71%)	2923 (69%)	2877 (68%)	2973 (69%)	
Non-Hispanic Black	1010 (7%)	1175 (9%)	1544 (12%)	1781 (15%)	
Mexican American	1037 (8%)	1272 (10%)	1164 (9%)	828 (7%)	
Other	1445 (14%)	1229 (12%)	1023 (11%)	766 (9%)	
Perceived Health[a]					<0.01
Excellent	1069 (21%)	949 (17%)	879 (15%)	743 (12%)	
Very Good	1838 (34%)	1763 (32%)	1720 (30%)	1675 (31%)	
Good	2148 (30%)	2378 (34%)	2523 (38%)	2393 (37%)	
Fair or poor	1435 (15%)	1505 (16%)	1482 (16%)	1533 (19%)	
Insurance[a]					<0.01

Characteristic	Frequency (%) or Mean ± STE				p-value
	Quartile 1 (n=6,491)	Quartile 2 (n=6,599)	Quartile 3 (n=6,608)	Quartile 4 (n=6,348)	
Yes	5328 (86%)	5084 (82%)	4900 (79%)	4566 (76%)	
No	1156 (14%)	1512 (18%)	1698 (21%)	1772 (24%)	
Income[a]					<0.01
<20,000	1435 (16%)	1640 (17%)	1680 (18%)	1782 (21%)	
20,000–34,999	1162 (14%)	1271 (16%)	1366 (17%)	1372 (18%)	
35,000–64,999	1462 (24%)	1484 (24%)	1531 (25%)	1480 (27%)	
65,000+	2137 (46%)	1900 (42%)	1783 (40%)	1516 (34%)	
Smoking Status[a]					<0.01
Current	631 (10%)	1078 (16%)	1580 (24%)	1931 (31%)	
Former	1741 (28%)	1685 (26%)	1419 (22%)	1155 (19%)	
Never	3992 (61%)	3582 (56%)	3189 (50%)	2777 (46%)	
Smoking Family Member[a]					<0.01
Yes	508 (9%)	839 (15%)	1260 (21%)	1673 (28%)	
No	4759 (91%)	4685 (85%)	4437 (79%)	3918 (72%)	
History of Diabetes[a]					<0.01
Yes	923 (10%)	819 (10%)	680 (8%)	510 (6%)	
No	5412 (90%)	5626 (90%)	5813 (92%)	5711 (94%)	
Body Mass Index (kg/m^2)[b]					<0.01
Mean ± STE	27.8 ± 0.12	28.5 ± 0.11	28.9 ± 0.13	29.3 ± 0.11	
Waist Circumference (cm)[b]					<0.01
Mean ± STE	96.0 ± 0.32	98.0 ± 0.30	98.9 ± 0.32	99.7 ± 0.32	

Column percentages may not equal 100 due to rounding. Frequencies may not equal population total due to missing data. Percentages are based on weighted frequencies. Quartile samples sizes differ slightly due to use of weighting. DII quartile ranges: Quartile 1 = −5.96 to −0.87; Quartile 2 = −0.86 to 0.704; Quartile 3 = 0.71 to 1.97; Quartile 4 = 1.98 to 4.82.

[a] p-value based on chi-square tests.

[b] p-value based on trend test across quartiles.

Abbreviations: STE = standard error.

Table 2

Mean Blood Cell Counts and Percentages by DII Quartiles, National Health and Nutrition Examination Survey (NHANES), 2005–2014.

Immune Marker	Quartile 1 (n=6,491)	Quartile 2 (n=6,599)	Quartile 3 (n=6,608)	Quartile 4 (n=6,348)	P-value 1 vs. 4	P-value Cont.
WBC Count (1000 per uL)	7.32 (7.21–7.44)	7.33 (7.21–7.45)	7.45 (7.34–7.57)	7.42 (7.30–7.54)	0.10	0.01
Lymphocyte Count (1000 per uL)	2.20 (2.16–2.24)	2.20 (2.16–2.24)	2.21 (2.17–2.25)	2.20 (2.15–2.34)	0.73	0.81
Monocyte Count (1000 per uL)	0.55 (0.53–0.58)	0.55 (0.53–0.58)	0.56 (0.53–0.58)	0.56 (0.53–0.58)	0.58	0.36
Neutrophil Count (1000 per uL)	4.33 (4.24–4.41)	4.33 (4.23–4.43)	4.43 (4.34–4.52)	4.41 (4.31–4.51)	0.06	<0.01
Lymphocyte %	31.1 (30.7–31.6)	31.0 (30.5–31.5)	30.7 (30.3–31.2)	30.7 (30.35–31.2)	0.05	<0.01
Monocyte %	7.8 (7.6–7.9)	7.7 (7.6–7.9)	7.7 (7.6–7.8)	7.7 (7.6–7.9)	0.32	0.04
Neutrophil %	57.6 (57.1–58.1)	57.6 (57.0–58.2)	58.0 (57.5–58.5)	57.9 (57.4–58.4)	0.19	0.03

Adjustment: All leukocyte models adjusted for age, sex, race, education, marital status, smoking status, perceived health, self-reported diabetes, family member smoking status, infections in the past 30 days, waist circumference, and systolic blood pressure. DII quartile ranges: Quartile 1 = −5.96 to −0.87; Quartile 2 = −0.86 to 0.704; Quartile 3 = 0.71 to 1.97; Quartile 4 = 1.98 to 4.82. Quartile determinations were based on weighted values. Therefore, displayed sample size does not indicate equal quartiles; however, the weighted values were evenly distributed among quartiles.

Table 3

Mean Blood Cell Counts and Percentages by DII Quartiles Stratified by Body Mass Index Status, National Health and Nutrition Examination Survey (NHANES), 2005–2014.

Immune Marker	Quartile 1 (n=6,491)	Quartile 2 (n=6,599)	Quartile 3 (n=6,608)	Quartile 4 (n=6,348)	P-value 1 vs. 4	P-value Cont.
BMI <25.0						
WBC Count (1000 per uL)	6.88 (6.67–7.10)	6.84 (6.64–7.05)	7.09 (6.89–7.30)	7.12 (6.90–7.34)	0.01	<0.01
Lymphocyte Count (1000 per uL)	2.12 (2.04–2.20)	2.07 (1.98–2.16)	2.11 (2.03–2.18)	2.12 (2.04–2.21)	0.97	0.68
Monocyte Count (1000 per uL)	0.53 (0.51–0.55)	0.53 (0.51–0.55)	0.55 (0.53–0.57)	0.55 (0.53–0.57)	0.02	0.01
Neutrophil Count (1000 per uL)	3.98 (3.82–4.15)	3.98 (3.82–4.14)	4.17 (3.99–4.34)	4.18 (4.00–4.36)	0.01	<0.01
Lymphocyte %	32.1 (31.3–33.0)	31.3 (30.4–32.3)	31.1 (30.2–32.1)	31.3 (30.3–32.2)	0.02	0.01
Monocyte %	7.9 (7.6–8.2)	7.9 (7.7–8.2)	7.9 (7.7–8.2)	7.9 (7.6–8.2)	0.87	0.54
Neutrophil %	56.4 (55.4–57.4)	57.0 (55.9–58.0)	57.3 (56.2–58.4)	57.1 (56.0–58.2)	0.12	0.02
BMI 25.0–29.9						
WBC Count (1000 per uL)	7.20 (700–7.39)	7.25 (7.07–7.42)	7.26 (7.07–7.45)	7.29 (7.10–7.49)	0.30	0.05
Lymphocyte Count (1000 per uL)	2.19 (2.11–2.26)	2.21 (2.13–2.28)	2.17 (2.10–2.24)	2.19 (2.12–2.26)	0.96	0.89
Monocyte Count (1000 per uL)	0.55 (0.53–0.57)	0.54 (0.52–0.56)	0.54 (0.52–0.56)	0.56 (0.54–0.58)	0.44	0.73
Neutrophil Count (1000 per uL)	4.22 (4.08–4.36)	4.25 (4.10–4.40)	4.29 (4.13–4.45)	4.32 (4.17–4.47)	0.13	0.08
Lymphocyte %	31.4 (30.6–32.1)	31.4 (30.6–32.3)	30.9 (30.1–31.7)	31.0 (30.2–31.7)	0.24	0.07
Monocyte %	7.9 (7.6–8.1)	7.7 (7.5–8.0)	7.7 (7.5–8.0)	7.8 (7.6–8.1)	0.85	0.33
Neutrophil %	57.2 (56.3–58.0)	57.2 (56.2–58.2)	57.8 (56.9–58.8)	57.6 (56.8–58.4)	0.29	0.07
BMI ≥ 30.0						
WBC Count (1000 per uL)	7.86 (7.64–8.08)	7.85 (7.65–8.05)	7.95 (7.75–8.16)	7.83 (7.64–8.02)	0.71	0.95
Lymphocyte Count (1000 per uL)	2.32 (2.24–2.41)	2.35 (2.27–2.42)	2.36 (2.28–2.44)	2.31 (2.22–2.39)	0.59	0.89
Monocyte Count (1000 per uL)	0.58 (0.56–0.60)	0.57 (0.56–0.59)	0.58 (0.55–0.60)	0.56 (0.54–0.58)	0.03	0.06
Neutrophil Count (1000 per uL)	4.71 (4.54–4.87)	4.67 (4.50–4.83)	4.75 (4.60–4.91)	4.69 (4.54–4.83)	0.64	0.77
Lymphocyte %	30.2 (29.4–31.0)	30.7 (29.9–31.5)	30.4 (29.7–31.1)	30.2 (29.4–31.1)	0.94	0.78
Monocyte %	7.6 (7.4–7.8)	7.5 (7.3–7.7)	7.4 (7.2–7.6)	7.4 (7.2–7.6)	0.02	<0.01
Neutrophil %	58.7 (57.8–59.7)	58.1 (57.2–59.1)	58.6 (57.6–59.5)	58.7 (57.6–59.7)	0.88	0.85

Adjustment: All leukocyte models adjusted for age, sex, race, education, marital status, smoking status, perceived health, self-reported diabetes, family member smoking status, infections in the past 30 days, waist circumference, and systolic blood pressure. DII quartile ranges: Quartile 1 = −5.96 to −0.87; Quartile 2 = −0.86 to 0.704; Quartile 3 = 0.71 to 1.97; Quartile 4 = 1.98 to 4.82. Quartile determinations were based on weighted values. Therefore, displayed sample size does not indicate equal quartiles; however, the weighted values were evenly distributed among quartiles.