Dietary Pattern Low in Fruits Explains Variations in Inflammation and in Biomarkers of Cardiovascular Disease in Latinos Diagnosed with Type-2 Diabetes

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**Authors’ contributions**

This work was carried out in collaboration between all authors. Author MCC participated in the design of the research, interpreted the data and wrote the manuscript; Authors SVL, SSP participated in subject recruitment, read the manuscript and provided comments; Author RPE participated in study design, data interpretation and provided comments; Author MLF designed the experiment, interpreted data and had primary responsibility for the final content. All authors read and approved the final manuscript.

**ABSTRACT**

**Aims:** 1) To identify a dietary pattern associated with plasma interleukin-6 (IL-6), tumor necrosis factor alpha (TNF\(\alpha\)) and monocyte chemotactic protein-1 (MCP-1) in Latinos diagnosed with T2D. 2) To examine whether the “pro-inflammation” dietary pattern obtained is associated with additional cardiovascular disease risk in this Latino population.

**Methodology:** Reduced rank regression was used to determine the cross-sectional relationship between food patterns and plasma inflammatory biomarkers in Latinos (26 men/77 women, 32-76 y) diagnosed with type-2 diabetes (T2D). Reduced rank

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regression was used to create 3 dietary patterns from 33 food groups. We included IL-6, TNFα, and MCP-1 as response variables to derive 3 dietary patterns.

**Study Design:** Cross-sectional.

**Place and Duration of Study:** University of Connecticut, Hartford Hospital, and the Hispanic Health Council, between January 2010 and May 2011.

**Results:** The first dietary pattern characterized by low intake of fruits and yellow vegetables and high intake of refined grains and sugar-free beverages explained the largest proportion of variance in inflammation markers. After adjusting for confounding factors including energy intake, statin treatment, waist circumference, glycosylated hemoglobin and blood pressure, IL-6 (P< 0.0001) and TNFα (P =0.0004) were positively associated with the first dietary pattern. Food groups that were negatively associated with inflammation markers were fruits and dark yellow vegetables, explaining 10.2% and 4.6% of the variance, respectively. This dietary pattern was also significantly associated with higher number of large VLDL particles (P < 0.05) after adjusting for WC, statins use and systolic blood pressure as well as higher concentrations of apolipoprotein A-2 after adjusting for WC and energy (P< 0.05).

**Conclusion:** This dietary pattern may increase heart disease risk in this already challenged population.

**Keywords:** Dietary pattern; latinos; type-2 diabetes; cardiovascular disease risk; TNFα; inflammation.

### 1. INTRODUCTION

Type-2 diabetes (T2D) is highly prevalent in Hispanics (11.6%) according to the National Health and Nutrition Examination Survey (NHANES) 2003-2004 data [1]. T2D is linked with almost double increased risk of cardiovascular diseases (CVD) [2] and, considering the mounting T2D-associated health care costs, studying this specific population is warranted.

Diet is a modifiable lifestyle factor that contributes to the progression of chronic inflammatory diseases such as CVD [3,4]. Dietary patterns allow for the effects of the overall diet and food synergies to be evaluated instead of a single nutrient or food item [5]. A dietary pattern determined by the use of reduced rank regression (RRR), reflects a combination of foods that are associated with specific biomarkers [6]. Among the inflammatory markers associated with CVD risk, C reactive protein (CRP) is one of the most studied biomarkers establishing a relationship between inflammation and diet components [7,8] or dietary patterns [9-11]. There are scarce data on the relationship of plasma IL-6, TNFα and monocyte chemoattractant protein-1(MCP-1) and diet. Thus, these cytokines which are associated with atherosclerosis development [12] were chosen as the response biomarkers in the RRR to determine dietary patterns in Latinos. Furthermore, because RRR is relatively a new method [6], more research is needed in testing the reproducibility of the RRR approach in ethnic minorities such as Latinos [13].

The RRR method creates an “artificial” dietary pattern that can be used as a hypothesis generator for studying mechanisms linking diet and disease risk [6]. Most of the research on dietary patterns and inflammatory markers or CVD risk has been conducted in individuals at risk [4,14,15] or free of T2D [8,10,16,17]. Little is known about these relationships in diabetic individuals [7] and even less in Latino populations [15]. Our research question sought to determine if a dietary pattern derived to explain variation in inflammatory markers is associated with risk factors for cardiovascular disease in Latinos diagnosed with T2D.
Hence, the aims of this study were two-fold: 1) To identify a dietary pattern associated with plasma IL-6, TNFα and MCP-1 in Latinos diagnosed with T2D by using RRR analyses; and 2) To examine whether the "pro-inflammation" dietary pattern obtained is associated with additional cardiovascular disease risk in this Latino population.

2. MATERIALS AND METHODS

This study was approved by the Institutional Review Boards of the University of Connecticut, Hartford Hospital, and the Hispanic Health Council. Dietary patterns were determined in 103 Latinos (77 women and 26 men), 90% of which were Puerto Rican with an average age of 56.2 ±11.1 diagnosed with T2D by their physicians at the time of enrollment in the Diabetes among Latinos Best Practices Trial (DIALBEST). This subgroup of 103 subjects was selected based on the order of enrollment and the data were used for the determination of the dietary pattern associated with inflammatory markers and CVD risk factors analyses. DIALBEST, described elsewhere [18], is a parallel randomized longitudinal study with a peer counseling intervention to improve the management of T2D in this Latino population. The inclusion criteria consisted of: Latinos older than 21 years, diagnosed with T2D and with glycosylated hemoglobin (HbA1c) > 7%. All participants signed an informed consent before enrollment. The study was conducted in the Hartford area and all survey data collection and fasting blood draws took place at the participants’ household.

2.1 Diet Assessment

Bilingual trained personnel interviewed the participants to complete one 24-h dietary recall using the 4-pass methodology recommended by USDA. The diet data were entered by personnel with knowledge both in Spanish and nutrition. The dietary intake was analyzed using the Nutritional Data System for Research (NDSR) 5.0 (Minneapolis, MN). In agreement with our methodology, one 24-h dietary recall has been used previously to determine dietary patterns indexes from NHANES III data [19].

2.2 Adiponectin, Leptin, Insulin, and Inflammatory Markers

IL-6, TNFα, CRP, adiponectin, leptin, insulin, soluble intercellular cell adhesion molecule 1 (sICAM-1) and MCP-1 were measured in duplicate by using xMAP® technology on the Luminex® IS 200 system with antibodies to each biomarker as previously reported [20].

2.3 Plasma Lipids

Aprotinin (0.5mL/100), sodium azide (0.1mL/100mL), and phenylmethylsulfonyl fluoride (0.1mL/100mL) were added to preserve plasma. Plasma total cholesterol, HDL cholesterol (HDL-C) and triglycerides (TG) were measured by enzymatic methods [21]. LDL cholesterol (LDL-C) was calculated by the Friedewald equation [22].

2.4 Apolipoproteins

Apolipoprotein (Apo) Al, Apo B, Apo CII, Apo CIII and Apo E were measured in duplicate using xMAP® technology on the Luminex® IS 200 system. As previously reported, the technique uses fluorescently labeled microsphere beads with antibodies to each individual apolipoprotein [20].
2.5 Lipoprotein Particle Size and Number

Nuclear magnetic resonance (NMR) analysis was performed on a 400-MHz NMR analyzer (Bruker BioSpin, USA) as previously described to measure size and number of VLDL, LDL and HDL particles [23].

2.6 Assessment of Covariates

Anthropometrics and blood pressure: Waist circumference (WC) was measured to the nearest 0.1 cm at the midpoint between the lowest rib and the iliac crest using a flexible tape over one layer of light clothes. Body weight was measured to the closest 0.1 kg on a calibrated digital scale with participants wearing light clothes. Height was measured to the closest 0.1 cm on a portable stadiometer/scale and body mass index was calculated. Blood pressure (BP) was measured on the left arm using a manual sphygmomanometer, with the study participant in a sitting position following 5 min rest. All anthropometric and BP measurements were collected in triplicate.

Medications: DIALBEST participants completed an extensive survey. Data for hypoglycemic drugs and insulin were obtained from two closed ended questions: Are you currently taking diabetic pills? Or are you currently using insulin? The data for lipid-lowering medications were collected during the interview. Bilingual interviewers obtained medication lists by copying names and doses of drugs directly from the bottles of medications in the home of participants.

2.7 Plasma Glucose and HbA1c

Fasting plasma glucose was measured on an automated lactate/glucose analyzer (2300 STAT, YSI, Yellow Springs, OH). HbA1C was assessed at the participants’ household from capillary blood using a disposable point-of-care Metrika A1cNow device (Metrika Inc, Sunnyvale, CA). This kit is certified by the National Glycohemoglobin Standardization Program [24].

2.8 Selection of Response Variables

Plasma IL-6, TNFα and MCP-1 were chosen as the response biomarkers in the RRR to determine dietary patterns within this population based on their relationship with insulin resistance [25-29]. Food components have been directly associated with increases in inflammatory markers, such as amount of carbohydrates and saturated or TRANS dietary fat [30]. These are examples of reductionist approaches, although associations between dietary patterns and markers of inflammation have also been reported [10,16,31].

In summary, TNFα, IL-6 and MCP-1 are markers of low grade systemic inflammation and endothelial dysfunction that can be affected by food components, foods as a whole, or perhaps indirectly by improving glucose metabolism.

2.9 Statistical Analysis

Dietary patterns were derived from 33 food categories as predictors and logarithmically transformed TNFα, IL-6 and MCP-1 data (to achieve normality) as the response variables to derive the dietary pattern scores with the use of RRR. We used SAS software (version 9.2
SAS Institute) for data analysis, implementing the PLS procedure with the method=RRR option. The 33 food categories were generated by consolidating 141 food variables obtained from the NDSR output of individual dietary intake records. Each “food category” is also composed of several food variables or food groups (Supplementary Table 1). For instance, “refined grains” refers to: refined loaf bread, refined crackers, refined pasta, refined cereal, etc. These 33 food categories were created with careful consideration and determined a priori, with the main inclusion criteria based on nutrient composition and the culinary habits of Latinos and supported by previous studies [3,4,10,15].

The RRR method determined three scores that are not correlated, and each score represented a different dietary pattern. Score 1 was the dietary pattern considered for further analyses because it explained the highest variance in the combination of responses variables. Retaining the first score for statistical analyses is consistent with previous studies using the RRR method [4,32,33].

We applied a commonly used simplification approach [4,32,34] to determine which of the 33 food categories were included in dietary pattern 1: only those food categories with an absolute factor loading ≥ |0.2| were considered part of the first dietary pattern score. The RRR procedure also provided the percent of variation explained per food group for dietary pattern 1.

Multiple regression analyses were used with the GLM procedure with dietary pattern 1 as the independent variable and the different biomarkers (leptin, IL-6, adiponectin, TNFα) or the CVD risk factors (plasma lipids, apolipoproteins or particle sizes) as the dependent variables. Results were considered significant with a P-value < .05.

Three multivariate models were applied: Model 1A was adjusted for WC and lipid lowering medications, specifically statins, and Model 1B was adjusted for WC, statin use and systolic BP. Similarly, other covariates were tested alone or in combination but were not included because their addition did not improve the fit of the models (age, gender, antihypertensive drugs, HbA1C, plasma glucose, BMI, body weight). Finally, a different Model, called model 2 was adjusted for WC and energy intake to test the effect of energy without the medications.

3. RESULTS AND DISCUSSION

We used RRR analysis to identify a dietary pattern associated with plasma IL-6, TNFα and MCP-1 in Latinos diagnosed with T2D. The first dietary pattern alone or score 1, explained 24.5%, 17.3% and 4.9% of the variance in plasma IL-6, TNFα and MCP-1 respectively. Furthermore, dietary pattern 1 explained the highest percentage of the average variance in plasma IL-6, TNFα and MCP-1 (15.6%). Thus, only score 1, subsequently referred to as dietary pattern 1 was considered for further analyses. The food categories and the percentage of variance explained corresponding to dietary pattern 1 are described in Table 1 (results unadjusted). The food categories in dietary pattern 1 that were directly associated with plasma IL-6, TNFα and MCP-1 combined were: green stewed vegetables (15.2%), refined grains (11.6%), sugar-free beverages (6.5%), vegetable oil (6.2%), soup (4.1%) and high fat dairy (4.1%). The ones inversely associated with plasma IL-6, TNF-α and MCP-1 combined were fruits (10.2%), dark yellow vegetables (4.6%) and added sugars (5%). In addition, citrus fruits were positively correlated with sugar (r=0.23, P =0.02) and syrup (r=0.20, P =0.048) (data not shown).
We identified a dietary pattern associated with plasma IL-6, TNFα and MCP-1 in Latinos diagnosed with T2D by using RRR analysis. Latinos with T2D who ate more green stewed vegetables, refined grains, vegetable oil, sugar free beverages, soups and high fat dairy and concurrently consumed less fruits, dark yellow vegetables and added sugars were more likely to have higher plasma IL-6, TNFα and MCP-1. The associations between this dietary pattern with plasma IL-6 and TNFα remained significant after adjusting for WC, energy intake, statin use and systolic BP.

Table 1. Variation explained by each food category in dietary pattern 1 (score 1).

| Direct assoc.                      | Inverse assoc. | Model effect loading | % of variance |
|-----------------------------------|----------------|----------------------|---------------|
| Green stewed vegetables           | 0.39           | 15.1                 |
| Refined grains                    | 0.34           | 11.6                 |
| Sugar-free beverages              | -0.32          | 10.2                 |
| Vegetable oil                     | 0.26           | 6.5                  |
| Added sugars                      | -0.22          | 6.2                  |
| Dark-yellow vegetables             | -0.22          | 5.0                  |
| Soup broth                        | 0.21           | 4.5                  |
| High fat dairy                    | 0.2            | 4.1                  |

There are certain food groups that accounted for a larger variance in dietary pattern 1. Green stewed vegetables and refined grains accounted for 15.1% and 11.6%, respectively. These food categories were directly associated with IL-6, TNFα and MCP-1 combined. Meanwhile fruits accounted for the largest variance (10%) inversely associated with IL-6, TNFα and MCP-1. Some of the food groups in dietary pattern 1 were consistent with the food groups reported by dietary patterns from The European Prospective Investigation into Cancer and Nutrition (EPIC) [32] or the Insulin Resistance Atherosclerosis Study (IRAS) [4]. Specifically, refined grains and vegetable oils were positively, and fruits were negatively associated with inflammatory biomarkers in these studies. However, the EPIC and the IRAS studies used different response biomarkers in the RRR to determine the dietary pattern. In addition these studies used a Caucasian population who were either healthy or at risk of developing T2D, which might weaken the comparison with our study. Many of the food categories in dietary pattern 1 associated with inflammatory markers, such as low intake of fruits or high intake of refined cereals, have been also identified in other studies focusing on Latinos at risk of T2D [14,15]. This could imply that eating certain combination of foods, such as those emphasized in dietary pattern 1, could over time become a factor in the development of chronic inflammatory diseases, such as T2D and CVD.

Study participants’ scores representing dietary pattern 1 were categorized into quartiles. Participants most closely following dietary pattern 1 fell in the upper quartile. We examined anthropometric characteristics of study participants and nutrient distribution across quartile categories of the first dietary pattern (Table 2). There were no differences in body weight, BMI or WC between the quartiles. In contrast, Latinos who most closely followed dietary pattern 1 tended to have higher energy intake (1465±661 Kcal for the 1st quartile vs. 1938±992 Kcal for the 4th quartile, \( P=.002 \)). Consequently they also had higher carbohydrate (CHO), protein and fat intake than the Latinos in the other quartiles (\( P=.027, P=.007 \) and \( P=.011 \) respectively). In addition there was a higher intake of animal protein, MUFA, SFA, soluble fiber, and starch for individuals in the 4th quartile (\( P=.032, P=.008, \) respectively).
Furthermore, Latinos in the 4th quartile were more likely to have a higher glycemic load (153.8±126), than those in the 1st quartile (122.2±52, P=.024).

Latinos with T2D closely following dietary pattern 1, which was associated with inflammatory markers, tended to have higher caloric intake. Conversely there were no differences in body weight, BMI or WC between the quartiles categories of dietary pattern 1. The higher trend in animal protein, starch, soluble fiber and MUFA consumption in the 4th quartile might be related to the higher energy intake since the significance was lost when energy intake was added to the regression model (data not shown). The percentages of CHO, protein and fat consumed were 60, 14 and 26% for those in the 1st quartile and 52, 18 and 30% for those in the 4th quartile of dietary pattern 1 intake (data not shown). Regardless that energy intake from CHO appeared lower for Latinos following closely dietary pattern 1, these individuals were consuming high glycemic index foods. Data from the Nurses’ Health Study (n=902) in women with diabetes showed positive associations between CRP and TNF receptor-2 (TNF-R2) and glycemic index, [7]. TNF-R2 reflects TNF system activation and was associated with CVD in women with diabetes [7]. The glycemic index median from the Nurses’ Health Study (55.7 for the 5th quintile) was lower when compared to our study (66.5±7.2 for the 4th quartile), where 75% of the participants were women. Thus our data suggest that Latinos following dietary pattern 1 independent of quantity might be eating high glycemic foods, which may contribute to their higher inflammatory state.

Table 2. Anthropometrics characteristics of study participants and nutrients distribution across quartile categories of dietary pattern 1 (p for trend)

| Anthropometrics | 1st Quartile (n=25) Mean ±SD | 2nd Quartile (n=26) Mean ±SD | 3rd Quartile (n=26) Mean ±SD | 4th Quartile (n=26) Mean ±SD | p for trend |
|-----------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|------------|
| Weight (Kg)     | 79.5±17                    | 87.1±28                    | 93.4±26                    | 82.4±15                    | .138       |
| BMI (Kg/m²)     | 32.2±7                     | 36.1±9                     | 36.1±9                     | 32.8±6                     | .276       |
| WC (cm)         | 103.5±15                   | 109.3±19                   | 112.8±19                   | 106.9±12                   | .229       |
| **Dietary intake** |                            |                            |                            |                            |            |
| Total Energy (Kcal/d) | 1465.4±661                | 1118.2±612                 | 1663.8±762                 | 1938.0±992                 | .002       |
| CHO (g/d)       | 221.8±104                  | 152.2±87                   | 226.2±115                  | 254.9±173                  | .027       |
| Protein (g/d)   | 52.9±40                    | 48.7±34                    | 76.5±43                    | 86.9±59                    | .007       |
| Fat (g/d)       | 42.9±28                    | 35.4±25                    | 50.7±33                    | 64.8±41                    | .011       |
| Animal protein (g/d) | 33.2±37                    | 32.1±28                    | 48.3±31                    | 60.5±55                    | .032       |
| Saturated fat (g/d) | 13.6±8.9                   | 12.8±10.8                  | 17.4±11                    | 22.0±16                    | .025       |
| Cholesterol (mg/d) | 144.8±212                  | 171.5±175                  | 186.0±117                  | 252.6±230                  | .219       |
| MUFA (g/d)      | 15.9±10                    | 7.7±7                      | 18.1±12                    | 23.4±17                    | .008       |
| PUFA (g/d)      | 9.5±10                     | 11.6±7                     | 10.8±10                    | 13.6±11                    | .167       |
| Total Fiber (g/d) | 15.6±11                    | 9.9±7                      | 15.4±15                    | 18.0±17                    | .146       |
| Soluble fiber(g/d) | 2.4±2                      | 3.2±2                      | 5.0±4                      | 5.0±4                      | .012       |
| Starch (g/d)    | 129±63                     | 79.5±53                    | 135.7±74                   | 150.3±134                  | .026       |
| Glycemic index (glucose) | 60.8±7.6                   | 60.4±7.3                   | 63.2±10                    | 63.7±7.2                   | .37        |
| Glycemic Load (glucose) | 122.2±52                   | 85.3±50                    | 132.3±68                   | 153.8±126                  | .024       |

\(^7\)Body Mass Index \(^8\)Waist circumference
3.1 Dietary Pattern 1 and Inflammatory Markers

We tested the association between dietary pattern 1 and plasma insulin, leptin, adiponectin and several inflammatory markers after adjusting for WC and statin use in model 1A and for the same covariates plus systolic BP in model 1B (Table 3). Dietary pattern 1 explained 24% 16% and 9% of variance in plasma IL-6 \( (P<.0001) \), TNFα \( (P=.0004) \) and MCP-1 \( (P=.038) \), respectively (model 1A). Meanwhile in model 1B, dietary pattern 1 explained 28% and 31% of the variance in IL-6 \( (P=.002) \) and TNFα \( (P=.0017) \), respectively. When systolic BP was added to model 1B, MCP-1 was no longer associated with dietary pattern 1 \( (P=0.1) \). IL-6 \( (P<.0001) \) and TNFα \( (P=.0007) \) remained significantly associated with dietary pattern 1 after adjusting for WC and energy intake in model 2, but MCP-1 \( (P=.11) \) did not. CRP, sILCAM-1, leptin, adiponectin and insulin were not significantly associated with dietary pattern 1 in any of the tested models.

Table 3. Regression analysis to predict inflammatory markers, leptin, adiponectin and insulin from dietary pattern 1

| Biomarkers  | Model 1A Adjusted for: WC and statins use | Model 1B Adjusted for: WC, statins use and systolic BP | Model 2 Adjusted for: WC and Energy intake |
|-------------|------------------------------------------|------------------------------------------------------|-------------------------------------------|
|             | \( \beta \) coefficient (SE) \( r^2 \)    | \( \beta \) coefficient (SE) \( r^2 \)              | \( \beta \) coefficient (SE) \( r^2 \)       |
| IL-6 \( ^1 \) | 1.82 \( ^a \) (1.14) 0.24                | 1.77 \( ^b \) (1.15) 0.28                           | 1.95 \( ^a \) (1.15) 0.22                   |
| TNF-α \( ^2 \) | 1.40 \( ^b \) (1.09) 0.16                | 1.34 \( ^b \) (1.09) 0.31                           | 1.39 \( ^b \) (1.10) 0.16                   |
| MCP-1 \( ^3 \) | 1.25 \( ^b \) (1.11) 0.09                | 1.20 \( ^b \) (1.10) 0.16                           | 1.19 \( ^b \) (1.10) 0.09                   |
| l-CAM-1 \( ^4 \) | 0.99 (1.07) 0.06                         | 1.00 \( ^b \) (1.07) 0.06                           | 0.95 (1.07) 0.06                           |
| CRP \( ^5 \) | 0.92 (1.19) 0.24                         | 0.96 \( ^b \) (1.21) 0.27                           | 0.96 \( ^b \) (1.19) 0.22                   |
| Leptin      | 1.02 (1.18) 0.16                         | 1.03 \( ^b \) (1.18) 0.23                           | 0.98 (1.18) 0.17                           |
| Adiponectin | 0.88(1.12) 0.08                          | 0.90 \( ^b \) (1.13) 0.09                           | 0.86 (1.13) 0.03                           |
| Insulin     | 1.19 (1.15) 0.13                         | 1.16(1.15) 0.11                                      | 1.19(1.16) 0.12                           |

(SE)Standard errors are reported in parentheses
\( \ast r^2 \): The \( r^2 \) from Model 2 cannot be directly compared to the \( r^2 \) of the first two models (Models 1A and 1B).
\( ^a P<.0001 \)
\( ^b P<.005 \)

Latinos with T2D following dietary pattern 1 were more likely to have higher plasma IL-6 and TNFα, even after adjusting for confounding factors. These data imply that after controlling for all these variables, dietary pattern 1 can still predict a modest variation on plasma IL-6 and TNFα in Latinos with diabetes. On the other hand, the association between dietary pattern 1 and MCP-1 was no longer significant when systolic BP was added to the model. These results suggest a possible role of systolic BP in mediating the relationship between MCP-1 and dietary pattern 1. This could be expected because MCP-1 is considered a marker of endothelial dysfunction [35], where vasodilation is inappropriate and accordingly BP increases. In a study by De La Sierra et al., a more impaired endothelial dysfunction correlated with higher MCP-1 levels in never treated- newly diagnosed hypertensive adults was observed [36]. Thus it appears that systolic BP may mediate the influence of dietary pattern 1 on MCP-1.
CRP, sICAM-1, leptin, adiponectin and insulin were not significantly associated with dietary pattern 1 in any of the tested models. IL-6 and TNFα can stimulate CRP release from the liver [28]. Contrary to expectations, CRP was not associated with dietary pattern 1, neither was it higher in Latinos following dietary pattern 1. Similarly a dietary pattern called “Western dietary pattern” was not associated with CRP after adjusting for WC and BMI in middle age healthy Iranian women (n=486) [16]. The current CRP data is also in agreement with results from a study in Japanese older adults (n=7802) [11]. Researchers determined 4 dietary patterns by Principal Component Analysis (PCA) and failed to find any positive association between any dietary pattern and CRP [11]. In contrast, studies in healthy middle age men [9], women [10], and both genders [31] showed positive association between CRP and the “Western dietary pattern”. These studies [9,10,31] were conducted in people free of T2D which might weaken the comparisons with our study.

An unexpected finding was that added sugars, in the context of dietary pattern 1, were inversely associated with response biomarkers variance. Approximately 85% of the Latinos enrolled in our study were of Puerto Rican origin. Eating canned fruit is a common culinary habit for this ethnic group. Thus, we speculate that sugar intake could be associated with the fruit eating habit in this population. Another possible explanation could be that participants use the tropical fruit tamarind, high in citric acid, to prepare a homemade juice sweetened with sugar. In this study, citrus fruits were positively correlated with sugar. It was also unforeseen that green stewed vegetables were among the food categories positively associated with IL-6, TNFα and MCP-1. Nonetheless, the mean intake of green stewed vegetables for those following closely dietary pattern 1 was very low (0.12±0.4 serving/day, which is the equivalent of ¼ cup). In addition, RRR findings indicate that the food items that characterize dietary pattern 1 should be eaten together in order to explain the biomarkers variability.

3.2 Dietary Pattern 1 and CVD Risk Factors

We tested the associations between dietary pattern 1 derived to explain plasma IL-6, TNFα and MCP-1 and risk factors for CVD in Latinos with T2D (Table 4). High number of large VLDL particles was significantly associated with dietary pattern 1 after adjusting for WC and statin use in model 1A ($P=0.032$) and after adjusting for WC, statin use and systolic BP in model 1B ($P=0.0031$). After adjusting for energy intake and WC in model 2, the association between dietary pattern 1 and number of large VLDL particles was no longer significant ($P=0.089$). Dietary pattern 1 explained 7 and 8% of the variance in the number of large VLDL particles in Model 1A and 1B, respectively. Additionally, there was a trend for an association between dietary pattern 1 and apo C3 ($P=0.07$) after adjusting for WC, statin use and systolic BP in model 1B.

Dietary pattern 1 was significantly associated with Apo A2 ($P=0.038$) after adjusting for WC and energy intake in model 2. Dietary pattern 1 explained 22%, 14% and 8% of the variance in Apo A2 ($P=0.038$), Apo C3 ($P=0.071$) and large VLDL particles ($P=0.089$), respectively after adjusting for WC and energy intake in model 2. Plasma LDL-C, HDL-C, TG, large HDL particles, small LDL particles and Apo B were not significantly associated with dietary pattern 1 in any of the tested models.

Dietary pattern 1 explained a modest variance of 15% in plasma IL-6, TNFα and MCP-1. We also investigated the association between dietary pattern 1 and CVD risk in Latinos with T2D by assessing traditional risk factors such as plasma lipids, and other biomarkers associated with deranged lipoprotein metabolism such as apolipoproteins, lipoprotein particle size and...
number. Participants following more closely dietary pattern 1 were more likely to have higher proportion of large VLDL after adjusting for WC, statin use and systolic BP. Large VLDL particles develop into small LDL particles [37], which are more prone to oxidation. Oxidized LDL particles are easier to be removed by the arterial wall contributing to the progression of atherosclerosis [38]. Consistently, there was a trend for an association between dietary pattern 1 and Apo C3 after adjusting for WC, statin use and systolic BP. Apo C3 can impair VLDL lipolysis, through the inhibition of Apo C2 and as a result prevent lipoprotein lipase action [37]. Further, data from a study using kinetic modeling techniques in humans showed that Apo C3 strongly inhibits hepatic uptake of VLDL [39]. Apo C3 also increases sICAM-1 expression and monocytes adhesion in human endothelial cells via NF-κB activation [40]. Our findings on Apo C3 association with the “pro-inflammatory” dietary pattern are in agreement with reports that Apo C3 may activate extracellular signal-regulated kinase and NF-κB through toll like receptor 2 pathways (TLR2) [41]. Consequently Apo C3 induces pro-inflammatory adipokine expression in in vitro animal and human embryonic cells plus in vivo in animals [41].

### Table 4. Regression analysis to predict CVD risk factors from dietary pattern 1

| CVD biomarkers | Model 1A β coefficient | Model 1B β coefficient | Model 2* β coefficient |
|---------------|------------------------|------------------------|------------------------|
|               | r²                     | P                      | r²                     | P                      |
| LDL-C¹        | -2.20                  | .11 .559               | -0.70                  | .13 .856               | -4.48                  | .07 .253               |
| HDL-C²        | -0.40                  | .04 .88                | 0.26                   | .14 .879               | 0.31                   | .03 .913               |
| TG³           | 23.56                  | .04 .146               | 27.65                  | .05 .97               | 20.2                   | .04 .218               |
| Large VLDL⁴   | 2.48                   | .07 .032               | 2.66                   | .08 .031               | 2.03                   | .08 .089               |
| Large HDL     | -0.52                  | .03 .478               | -0.31                  | .076 .683             | -0.41                  | .02 .591               |
| Small LDL     | -42.45                 | .053 .60               | -18.99                 | .057 .83              | 84.63                  | .01 .675               |
| Apo A2 (mg/L) | 16.68                  | .14 .25                | 19.60                  | .15 .212              | 30.66                  | .22 .038               |
| Apo B (mg/L)  | 46.22                  | .001 .81               | 97.6                   | .006 .638             | 84.6                   | .008 .647               |
| Apo C3 (mg/L) | 50.01                  | .13 .139               | 61.94                  | .16 .076              | 63.7                   | .14 .071               |

Adjusted for: WC and statins use
Adjusted for: WC, statins use and systolic BP
Adjusted for: WC and energy intake

*²: The r² from Model 2 cannot be directly compared to the r² of the first two models (Models 1A and 1B).

1Low density lipoprotein, 2High density lipoprotein, 3Triglycerides, 4Very Low density lipoprotein

Dietary pattern 1 was significantly associated with Apo A2 only when energy intake was included in the model. There is genetic evidence [42] linking a specific Apo A2 polymorphism (ApoA2-265T>C SNP) with energy intake in a study conducted in 2 independent populations including Latinos from the Boston Puerto Rican Studies (n=930). Individuals carrying the CC allele had higher energy intake than the T-allele carriers [42]. Apo A2 is the second most abundant protein of HDL particles [43]. Apo A2 can activate hepatic lipase, which hydrolyses TG and phospholipids and generates more atherogenic particles [37]. Data from human female Apo A2 transgenic mice showed that Apo A2 also plays a role in TG catabolism by regulating lipoprotein lipase activity [43]. Similarly there was a trend in the association in model 3 between dietary pattern 1 and Apo C3 with higher number of large VLDL particles as well. Adding energy intake to the model weakens the associations between large VLDL particles and dietary pattern 1, which suggests a role of energy intake mediating this relationship. A VLDL kinetic study was conducted to determine factors affecting VLDL-TG production in 13 men and 12 women [44]. Resting energy expenditure (REE) and plasma insulin were independent significant predictors of VLDL-TG production [44]. A higher energy
or food intake results in REE increment. Thus, energy intake influence on VLDL particles could supersede the influence of specific food combinations.

Overall, large VLDL particles are atherogenic and in addition, they tend to form small LDL particles consistent with a more atherogenic lipid profile or Pattern B. Our results imply that dietary pattern 1 is associated with deranged VLDL lipoprotein metabolism in Latinos with T2D.

Studying dietary patterns has the advantage of evaluating food synergism. It is noteworthy that combination of all these foods groups and its distribution eaten collectively generated these associations with plasma biomarkers in diabetic Latinos. Even though we mentioned some food groups that agreed with previous reports, it is clear that one food group cannot be consider alone, unless it is in the context of the whole dietary pattern.

There are some limitations to this study including the small sample size, the type of dietary assessment, a single 24 hour dietary recall and the fact that association does not imply causation. Also we did not adjust for multiple comparisons. Some sort of bias is inherent to any type of factor analysis; nevertheless RRR appeared to be less subjective than PCA when determining the number of dietary patterns. In addition, these results apply only to this specific Latino population diagnosed with T2D.

5. CONCLUSIONS

We focused in biomarkers that, to the best of our knowledge, have not been previously studied in the context of an RRR identified dietary pattern associations in Latinos with T2D. A dietary pattern characterized by low intake of fruits, yellow vegetables and added sugars; and high intake of dark green stewed vegetables, refined grains, vegetable oil, sugar free beverages, soups and high fat dairy can predict a modest IL-6, TNFα and MCP-1 variation in Latinos diagnosed with T2D. Furthermore this dietary pattern was also associated with higher number of large VLDL particles and Apo A2; increasing CVD risk in this already challenged population. Lastly, interventional studies are needed to test the effect of diet at improving inflammatory status among Latinos with T2D.

CONSENT

All authors declare that written informed consent was obtained from all the participants in this study.

ETHICAL APPROVAL

All authors hereby declare that all human studies have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

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**SUPPLEMENTARY MATERIALS:**

**Supplemental Table 1. Food categories details**

| Category number | Food grouping / category name | Foods or food groups                                                                 |
|-----------------|--------------------------------|---------------------------------------------------------------------------------------|
| 1               | Processed meats                | cured pork, lean cured pork, cold cuts, lean cold cuts, meat savory snack              |
| 2               | Red meats                      | beef, lean beef, lamb, fresh pork, lean fresh pork, lean lamb                          |
| 3               | Fish and other seafood         | fish, lean fish, fried fish, shellfish, fried shellfish                                 |
| 4               | Poultry                        | poultry, lean poultry, fried chicken                                                   |
| 5               | Eggs                           | eggs, eggs substitutes                                                                  |
| 6               | Butter                         | butter regular, low fat butter, gravy regular, gravy low fat                           |
| 7               | Margarine                      | margarine regular, margarine low fat, shortening                                         |
| 8               | Fat free dairy products        | fat free milk, yogurt sweetened fat free, yogurt artificially sweetened cream fat free, non fat sweet milk powder, |
| 9               | High-fat dairy products        | whole milk, flavored milk whole, artificially sweetened milk regular, powder milk regular, cheese full fat, yogurt sweetened whole milk, yogurt artificially sweetened whole milk, cream regular |
| 10              | Tea                            | unsweetened tea, sweet tea                                                             |
| 11              | Coffee                         | unsweetened coffee, sweet coffee                                                       |
| 12              | Fruit                          | citrus fruit, fruit, fried fruits                                                      |
| 13              | Fruit juices                   | citrus juice, fruit juice                                                              |
| 14              | Dark-yellow vegetables         | winter squash, sweet potatoes, carrots                                                 |
| 15              | Tomatoes                       | tomato                                                                                 |
| 16              | Green stewed vegetables        | dark green vegetables (e.g: broccoli, green collard, green beans...)                   |
| 17              | Legumes                        | e.g. Beans, mature lima beans, any mature cooked dried bean                           |
| 18              | Other vegetables               | avocado, other vegetables (e.g: cabbage, beets, summer squash...), vegetable juice, vegetable snacks, pickled foods |
| 19              | Potatoes                       | white potatoes, other starchy vegetables (e.g: corn, peas, yucca, yam...)              |
| 20              | Fried vegetables               | fried potatoes, fried vegetables (e.g. onion rings)                                   |
| 21              | Whole grains                   | grains whole grain, grains some whole, loaf whole grain, loaf some whole, other bread whole, other bread some, crackers whole, crackers some whole, pasta whole, pasta some, cereal whole grain, cereal some, cereal sweet whole, cereal sweet some |
| 22              | Refined grains                 | grains refined, loaf bread refined, other bread refined, crackers refined, pasta refined, cereal sweet refined, cereal refined, non grain flour |
|   | Category                        | Description                                                                                                                                 |
|---|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------|
| 23 | Snacks                          | snack chips whole, snack chips some, snack chips refined, popcorn                                                                         |
| 24 | Nuts                            | nuts seed, nuts seed butters                                                                                                               |
| 25 | Sugar-containing beverages      | sweet water, sweet coffee subs, sweet fruit drinks, soft drinks                                                                           |
| 26 | Sugar-free beverages            | diet soda, artificially sweetened water, unsweetened water, sugar substitutes, artificially sweetened tea, unsweetened coffee substitute, artificially sweetened coffee substitute, artificially sweetened fruit drinks, unsweetened soft drinks |
| 27 | Oil                             | vegetables oils (e.g. canola, corn, sunflower...) cooking sprays                                                                             |
| 28 | Mayonnaise and other creamy salad dressings | salad dressing regular, salad dressing low fat, regular sweet sauces, low fat sweet sauces, regular sauces, low fat sauces |
| 29 | Soups                           | soups (e.g. Soup broth, consommé, bouillon...)                                                                                               |
| 30 | Sweets and desserts             | cakes whole grain, cakes refined, cakes some, snack bars whole, snack bars some, snack bars refined, frozen dairy dessert, frozen nondairy, pudding, artificially sweetened pudding, sweet meal, non chocolate candy, chocolate candy, miscellaneous dessert, |
| 31 | Sugars                          | syrup, sugar, frosting                                                                                                                     |
| 32 | Non dairy products              | nondairy yogurt, nondairy cream, nondairy milk, cheese nondairy                                                                            |
| 33 | Reduced fat dairy               | reduced fat milk, flavored milk reduced fat, cheese reduced fat, yogurt sweetened low fat, yogurt artificially sweetened low fat, cream low fat, flavored milk low fat, cheese low fat |

*Alcohol intake was not included because it was not reported*