

To what extent does diet impact one's immune profile?

Looking for associations between 24hr food recalls and circulating chemokine levels

Materials and Methods

IRB information / Ethics Statement

In this human subjects study, all biospecimens and survey responses were obtained at the University of Georgia (Athens, GA). All research was performed following the institutional review board (IRB) guidelines. All participants were informed of the study's purpose and signed a consent prior to participation and donation of blood and questionnaire responses.

Questionnaire

At the time of sample collection, participants (n = 71) completed a questionnaire that collected information regarding sociodemographic information, medical history (including specific questions regarding reproductive history), family history, questions regarding tobacco and alcohol use, and 24-hour diet recall. Age at the time of questionnaire response and blood sample collection was calculated by subtracting the survey date from the

birth date for each participant. Body mass index (BMI) was calculated from self-reported weight and height, and placed into underweight (<18.5), normal (18.5 to <25), overweight (25.0 to <30) and obese (30+) based on CDC guidelines.¹ Age and BMI distributions are shown in **Supplemental Figure 1**.

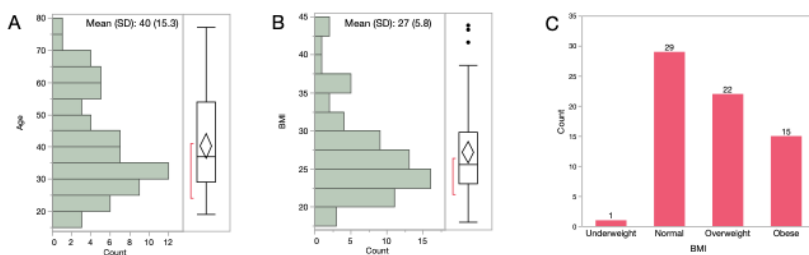


Figure 1. Distribution of Age and Body mass index (BMI).

- (A) Distribution of age in our cohort. The mean age was 40, with a standard deviation of 15.3 years.
- (B) Distribution of BMI in our cohort. The mean BMI was 27, with a standard deviation of 5.8.
- (C) Individuals with a BMI of < 18.5 were underweight, between 18.5 and <25 were normal, between 25 and <30 were overweight, and a BMI of 30 and above were considered obese. The counts of individuals in each of these BMI categories is shown in panel C.

Nutrition survey responses and analysis

From the 24-hour diet recall questionnaire responses, the various food items were organized into the following food groups: Fruits, Vegetables, Grains, Dairy, Protein, Added Sugar and No Added Sugar. The sum of all caloric servings was also calculated from the food group totals, and used as a variable in the analysis. Sum was also dichotomized for analysis (0-14 compared to 15+). For the food items, Cereal, Milk, Water and Fruits (no fruit juice) were dichotomized for analysis (Cereal: 0 compared to 1+; Milk: 0-1 compared to 2+; Water: 0-2 compared to 3+; Fruits: 0-1 compared to 2+).

¹About Adult BMI | Healthy Weight | CDC. (n.d.). Retrieved from https://www.cdc.gov/healthyweight/assessing/bmi/adult_bmi/index.html

Blood specimens and Luminex human chemokine multiplex assay

Collection, processing and Luminex multiplex assay of the samples analyzed in this study have been previously described.² Briefly, blood samples were collected at the Clinical and Translational Research Unit at the University of Georgia (CTRU, Athens, GA; n=84). The blood samples were processed within 24 hours of collected, and undiluted plasma was collected from centrifugation, and used for the Luminex human chemokine assay. The

following chemokines, cytokines and adipokines were assayed:

CCL2/MCP-1, CCL19/MIP-3b,

CCL21/6Ckine, CXCL8/IL-8,

CXCL9/MIG, IL-6, TNF- α , PAI-1,

Leptin, Adipsin, Adiponectin. The

distribution from the Luminex assay are shown in **Supplemental Figure 2.**

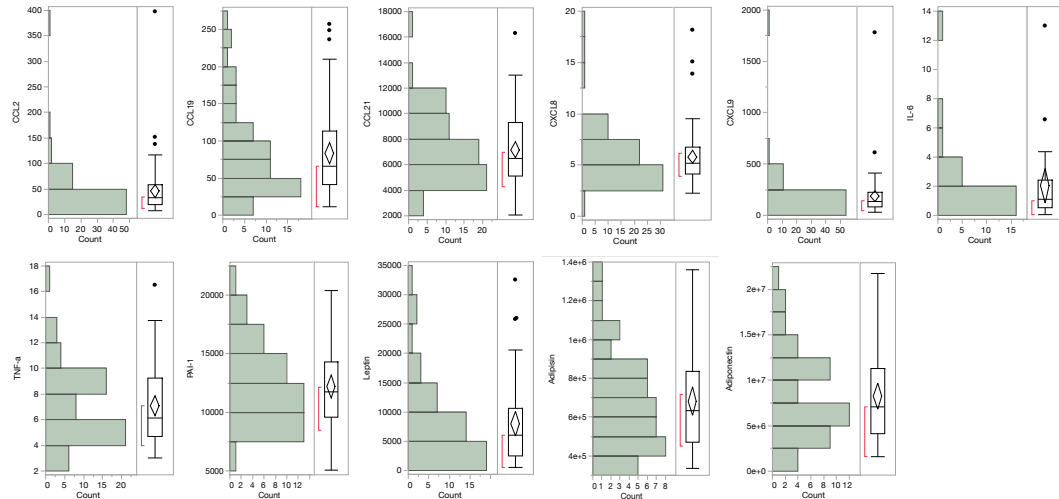


Figure 2. Distribution of chemokines.

Statistical analysis

Statistical analyses were done using JMP (SAS) Pro v. 14. All Student's t tests performed were two-tailed and the absolute P values are shown in the figure legends. F and P values are reported for ANOVA analysis and linear regression or paired t test analyses. Numbers of included data points and degrees of freedom are also shown.

²Jenkins, B. D *et al.* (2019, 04). Atypical Chemokine Receptor 1 (DARC/ACKR1) in Breast Tumors Is Associated with Survival, Circulating Chemokines, Tumor-Infiltrating Immune Cells, and African Ancestry. *Cancer Epidemiology Biomarkers & Prevention*, 28(4), 690-700. doi:10.1158/1055-9965.epi-18-0955