Dietary Assessment as a Means to Modulate Inflammation in Patients with Prostate Cancer

By

Hilary Robertson
RDN, LD 2017
BS, Kansas State University, 2016
Submitted to the graduate degree program in Dietetics and Nutrition and the Graduate Faculty
of the University of Kansas in partial fulfillment of the requirements
for the degree of Master of Science.

_____

Chair: Dr. Jill Hamilton-Reeves

_____

Dr. Debra Sullivan

_____

Dr. William Parker

Date Defended: 19 April 2018

The thesis committee for Hilary Robertson certifies that this is the approved version of the following thesis:

Dietary Assessment as a Means to Modulate Inflammation in Patients with Prostate Cancer

_____

Chair: Jill Hamilton-Reeves, PhD

Date Approved: 27 April 2018

Abstract

Introduction

Inflammation is associated with increased risk of prostate cancer. Diet can modulate inflammation through changes in energy and diet quality. Diet quality scores, assessed by measuring what individuals eat, are related to inflammatory markers measured in the blood. The purpose of this project is to measure diet quality using verified diet quality indices, and then to evaluate which of these indices scores is most closely associated with certain inflammatory biomarkers in overweight men with prostate cancer.

Methods

Data was collected at three separate time points; baseline, surgery (after weight loss), and study end (after 12 weeks weight maintenance). Two 24-hour recalls were entered into the Nutrition Data System for Research software and the dietary output was averaged and used, with authors' guidance, to obtain scores for the Alternative Healthy Eating Index (AHEI), the Dietary Inflammatory Index (DII), and the energy-adjusted Dietary Inflammatory Index (e-DII). Blood was centrifuged to obtain serum, which was then used to obtain high sensitivity C-Reactive Protein (hs-CRP), and Interleukin-6 (IL-6) measurements. Inferential statistics were calculated to find associations between the dietary scores and the inflammatory biomarkers, hs-CRP and IL-6.

Results

At baseline the DII scores had the strongest correlation to IL-6 (R=0.0404), and none of the dietary scores correlated with hs-CRP in the appropriate direction. When looking at changes from baseline to the surgery time-point, during weight loss, the e-DII scores had the strongest correlation to IL-6 (R=0.0554), and the AHEI scores had the strongest correlation to hs-CRP (R=

-0.258). From the surgery time-point to the study end, during weight maintenance, the e-DII scores had the strongest correlation to IL-6 (R=0.3932), and the DII scores had the strongest correlation to hs-CRP (0.1741).

Conclusion

The associations in this study were weak, and several results were unanticipated. Out of all three dietary scores, the e-DII was most strongly associated with IL-6, and the AHE was most strongly associated with hs-CRP. It would be worthwhile to replicate these methods on a trial of a larger size and lower IL-6 detection range kit, and to see if these results would be replicated or if stronger correlations could be found.

Acknowledgements

I'd like to acknowledge and thank everyone who contributed to this project. Dr. Hamilton-Reeves conducted the pilot study of which this analysis was derived from, advised this project, acted as committee chair person, and also gave support and assistance as needed. Misty Betchel assisted with conducting the pilot study, and Misty and Emily Newbold ran the Interleukin 6 sample kits for the benefit of this project. Dr. Sullivan and Dr. Parker both served and contributed as committee members. Dr. Chalise performed the statistical analysis. Finally, Carrie Michael, Jessica Keller, and Shelby Rissler were all constant support as both co-workers and friends during the writing of this project. I am filled with immense gratitude for all of the support and guidance I have received from each individual.

Table of Contents

List of Figures

List of Tables

Chapter 1: Justification

Chronic inflammation is an important mechanism in the development and progression of cancer. Chronic inflammation causes tissue damage, releasing inflammatory cytokines, and as the tissue attempts to repair itself, DNA may become damaged, which may eventually lead to cancer development (1). Furthermore, inflammatory cytokines have been identified as potential sources for prostate cancer initiation and progression (2, 3). As prostate cancer is the second most common cancer in men and the second leading cause of cancer death in non-smoking American men (4), modulating chronic inflammation through lifestyle modifications, such as dietary choices, may affect health outcomes for men with prostate cancer.

Excess body fat contributes to chronic inflammation. Adipocytes secrete inflammatory cytokines, such as Interleukin-6 (IL-6) and C-reactive protein (CRP)(5), yet diet quality has also been shown to play a role in modulating inflammation. As such, a patient's diet may be a potential way to address and lower chronic inflammatory markers. There are several tools that can yield a score related to diet quality and inflammation. In this project we compare two of these indices, the Alternative healthy eating index (AHEI) and Dietary Inflammatory Index (DII) in order to evaluate which of these tools best correlates with inflammatory biomarkers in patients with prostate cancer.

STATEMENT OF PURPOSE: To investigate how the AHEI and DII relate to the inflammatory biomarkers IL-6 and high sensitivity C-reactive protein (hs-CRP) in overweight men with prostate cancer.

Research Questions:

1. Will the AHEI scores have a negative correlation to the IL-6 and hs-CRP values in overweight men with localized prostate cancer; will the DII scores have a positive

correlation with the IL-6 and hs-CRP values, and will the energy adjusted version of DII show a stronger positive relationship?

2. Are changes in the AHEI score and/or the DII score associated with changes in circulating inflammatory biomarkers?
3. Which diet score has a stronger correlation to the inflammatory biomarkers evaluated?

Chapter 2: Review of the Literature

Introduction and Background

Tools that measure diet quality, such as the AHEI and DII, have shown that dietary patterns are associated with inflammatory biomarkers. A study by Fung et al. compared the accuracy of various dietary indices ability to evaluate inflammation by assessing each indexes relationship to inflammatory markers, including: CRP, IL-6, E-selectin, soluble intercellular cell adhesion molecule 1, and soluble vascular cell adhesion molecule 1 (6). Fung compared the AHEI, the Healthy Eating Index (HEI), the Diet Quality Index Revised (DQI-R), the Recommended Foods Score (RFS), and the alternative Mediterranean Diet Index (aMED) scores of nurses from the Nurses' Health Study cohort (n=660). Fung reported that several dietary indices were associated with inflammatory biomarkers, but the AHEI was more strongly associated with these inflammatory biomarkers (6). Unfortunately the DII was not included in this assessment. Considering that the DII was created specifically to assess inflammation and given inflammation may be important to prevent cancer progression, a comparison between DII and AHEI is critically needed to improve our ability to assess dietary inflammation in patients with cancer.

Prostate Cancer and Chronic Inflammation

It is widely accepted that inflammation is associated with increased cancer risk (7). Chronic systemic inflammation, in particular, has been shown to be associated with increased risk of both the initiation of prostate cancer formation and with continued cancer growth (3). Circulating inflammatory biomarkers are typically used as surrogate markers for measuring chronic and systemic inflammation. For example, CRP has been shown to be a significant predictor of prostate cancer survival (8). In this study 98 men with prostate cancer were followed for 10 years with measurements on Gleason score and CRP. CRP at high circulating levels was related to poorer survival rates in the prostate cancer patients (8). Interleukin-6 is another inflammatory marker of interest, and is considered one of the best surrogate markers for chronic inflammation in regards to those with prostate cancer, according to a review by Nguyen et al. (9). Higher levels of CRP and IL-6 are related to poorer outcomes in men with prostate cancer and greater carcinogenesis in the tissues (8, 10). Given this existing evidence regarding the relationship of circulating CRP and IL-6 to prostate cancer pathogenesis and survival, these specific biomarkers will be used to assess the ability of dietary indices to evaluate inflammation in patients with prostate cancer..

Diet and Inflammation

Diet can modulate inflammation in the body. Several studies, including randomized control trials (11, 12), have shown a relationship with dietary components and certain dietary patterns and their ability to modulate inflammation (13). Other studies show relationships between diet and the risk of several chronic diseases (14, 15), including cancer (16, 17). The influence of diet on inflammation may explain the attenuation of the risk for certain chronic diseases. There are foods that exhibit anti-inflammatory effects such as fruits and vegetables, and food components such as omega-3 fatty acids, fiber and phytochemicals. Likewise, there are

foods that have been shown to exhibit a pro-inflammatory effect as well. Overall dietary patterns that increase the consumption of anti-inflammatory foods, while simultaneously decreasing the consumption of pro-inflammatory foods may be helpful for prostate cancer patients. Capturing the overall net effect of diet quality is difficult to define and quantify, which is why tools like the AHEI and DII are useful. In the intervention from where we obtained this dataset, coaching was given to the participants, which emphasized that participants consume more non-starchy vegetables, plant-based protein such as soy, fruit, and starchy vegetables, while also reducing consumption of red meat, processed meats, and sugar-sweetened beverages (SSB).

Evidence supports that increased intakes of fruits and vegetables can combat chronic inflammation (11), and non-starchy vegetable intake in particular may be most impactful (18). Increasing consumption of these beneficial foods is important but lowering consumption of refined carbohydrates and high glycemic foods may also lead to improvements in the inflammatory markers of interest (12). A study by Watzl in 2005 (11) found that increased fruit and vegetable consumption appear to lower CRP plasma concentration. In this randomized control trial (n=64), significant differences in CRP levels were observed when participants were given a low, medium, or high intake of fruits and vegetables, despite no differences in baseline measures. The authors focused on both fruits and vegetables that were rich sources of carotenoids, dietary fiber, and phytochemicals, such as lycopene, which are considered beneficial for overall health (11). A separate cross-sectional study found that women (n=486) who consumed higher amounts of fruits and vegetables had lower CRP and E-selectin serum levels, compared to those with diets higher in refined carbohydrates and saturated fat (18). Refined carbohydrates offer little fiber and fewer vitamins, minerals, and phytonutrients compared to diets that replace them with fruits and vegetables. Non-starchy vegetables, such as spinach and

cabbage, typically have a much lower glycemic index than their starchy vegetable counterparts, such as white potatoes. Low-glycemic index diets typically contain larger amounts of non-starchy vegetables and fewer starchy vegetables, refined carbohydrates, and sugar sweetened beverages have been shown to exhibit anti-inflammatory effects by lowering IL-6 and TNF-alpha (12). In one randomized control trial (n=28), participants exhibited much lower IL-6 levels after an intervention, which included a low-glycemic index diet plus exercise, and were compared to a high-glycemic index diet plus exercise (12).

Reducing red and processed meats and replacing those meat proteins with soy or plant protein is a dietary strategy that could potentially modulate chronic inflammation.
Many cancers progress through inflammation, and the relationship between increased colon cancer risk and consumption of red and processed meats is well established (19). The American Institute of Cancer Research recommends eating no more than 18 oz. of red meat per week (20).

Red meat and processed meat are known to be carcinogenic with several proposed mechanisms (21). Nitrates, nitrites, heme iron, and saturated fat are found in these food products, and have been shown to have mechanistic role in carcinogensis. Red meat contains a nonhuman sialic acid, N-glycolylneuraminic acid (Neu5Gc), which is not found in poultry or fish. It's been proposed that Neu5Gc acts as an antigen in humans, and humans then create antibodies for this antigen, and this process has been illustrated in several mice models where Neu5Gc antibodies were shown to enhance tumor growth (22, 23).

Processed meat may be from red mammalian meat or other meats, and they are defined by the method of preservation or processing, such as smoking, salting, curing, or adding a preservative such as nitrate to the meat. These methods of processing and preservation may increase the risk of cancer and may increase inflammation. The sodium content of processed

meats is significantly greater than their fresh meat counterparts, and sodium intake is associated with higher CRP levels in non-diabetic hypertensive patients (n=224) (24). Schwedhelm, et al. found that red processed meats in comparison to unprocessed red meats, were borderline associated with increases in IL-6, but not significantly associated with CRP, despite the lack of statistically significant associations there was an observed positive correlation between the inflammatory biomarkers, which included CRP, IL-6, total TNF-alpha, sTNF-R1, and STNF-R2 (25). This evidence suggests further investigation into this area is needed.

The association between both red and processed meat and inflammation, may be arbitrated by increasing body weight, despite this finding there continues to be an indication that intake of both red and processed meats is still associated with worsening health conditions (26). In contrast, increased intake of isoflavones, which are phytonutrients found in soy, are associated with lower inflammatory cytokines, including IL-6, in prostate cancer patients who were given a soy bread intervention in one clinical trial (27). Soy foods contain unsaturated fats, along with the bioactive phytonutrients, such as isoflavones, which gives them a competitive advantage over the red and processed meats, in regards to the health of prostate cancer patients.

Sugar sweetened beverage (SSB) intake is known to contribute to weight gain and obesity (28). Due to the relationship of increased adipocytes and how they increase inflammatory cytokines sugar sweetened beverages should be reduced or avoided. Sugar sweetened beverages include drinks sweetened with fructose (i.e. juice), glucose, or high fructose corn syrup, and evidence from a crossover control randomized design study shows there is no difference between the type of sugar consumed and inflammation measured by CRP and IL-6 (29).

Increasing fruit and vegetable consumption, emphasizing plant protein and simultaneously reducing red meat, processed meats, and SSB may modulate chronic inflammation, and, coincidentally, align with the cancer survivorship guidelines from the American Cancer Institute (30). The dietary indices, the AHEI and DII, which are used to measure diet quality and relate it to chronic disease risk, also align with these guidelines.

The Alternative Healthy Eating Index

The AHEI is a tool that was developed to predict chronic disease risk, including using dietary intake analysis. As previously reviewed, the AHEI has been favorably compared to other dietary indices – except the DII (6). The AHEI was developed using nutrients and foods that are consistently related to chronic disease risk in the literature. A score is yielded, and this score illuminates either a lower or increased chronic disease risk. The scores range from 0 to 110, a higher score indicates a lower risk of chronic disease, while a lower score indicates a greater risk of chronic disease.

Typically, a food frequency questionnaire (FFQ) is given to participants, but other standardized methods of diet intake recording may be applied as well. Output from the diet analysis software is used to create the AHEI score. Servings of whole fruits and vegetables, whole grains, nuts, legumes, and of long chain omega-3 fatty acids (mg/day) will raise the score. Servings of SSB, juice, red meat, processed meat, trans fat (g/day), and sodium (mg/day) will lower the score. Moderate consumption (1-2 servings) will raise the total score by 10, while no consumption of alcohol will raise the total score by 2.5, but consumption greater than what is considered moderate will lower the score (31).

The Dietary Inflammatory Index

The DII aims to quantify the inflammatory potential of the diet itself, and through this inflammatory potential the risk of chronic disease, including prostate cancer. Prostate cancer risk was found to be associated with higher DII scores in a prospective cohort study (n=2771) (32), and similar associations between DII scores and prostate cancer risk were found in a separate case control design study (n=120) (33). Evidence from a retrospective cohort study (n=726) shows that DII scores are also associated with survival in prostate cancer patients with more aggressive forms of prostate cancer (34).

The DII was developed based on published data of foods and food components and their effects on various inflammatory biomarkers, including but not limited to the two of interest in this paper, C-reactive protein and IL-6 (35). Through the literature review process, the investigators defined 45 different components of diet on which to base a score, but only 25 of which are needed to generate a score, therefore those who use this tool do not need to measure all 45 components in order to obtain a score (35).

For our analysis we used 28 components or parameters for producing our scores: alcohol, vitamin B-12, vitamin B-6, beta-carotene, caffeine, total carbohydrate, cholesterol, total calories (energy), total fat, total dietary fiber, folate, iron, magnesium, mono-unsaturated fatty acids (MUFA), niacin, total protein, omega-3 fatty acids, poly-unsaturated fatty acids, selenium, riboflavin, saturated fat, thiamin, trans fat, vitamin A activity, vitamin C, vitamin D, vitamin E, and zinc. Of these components some are pro-inflammatory such as trans-fat consumption, while others are anti-inflammatory such as poly-unsaturated fatty acid consumption. Pro inflammatory food components will result in a higher score, while anti-inflammatory food components will lower the score. Scores range from a minimum of -8.87 to a maximum of 7.98. A higher score

using the DII is more associated with chronic disease risk, including cancer. Conversely a lower score from the AHEI is more associated with disease risk.

Excess Body Fat and Inflammation

There is a well-known link between obesity and cancer, as well as obesity and chronic inflammation. Obesity is partially driven by a constant state of positive energy balance, where caloric intake exceeds energy expenditure. A review by Hursting et al. notes that obesity is associated with greater levels of inflammatory cytokines including IL-6 and CRP in both humans and rat models (36). This same review summarizes data showing the protective effect of caloric restriction for cancer prevention (36). A single arm intervention study, where 20 obese women were placed on a caloric restricted formula type diet of 800 kcals for 28 days, found that the caloric restriction appeared to lower the serum inflammatory markers significantly, further supporting the role of energy intake in inflammation (37).

A modified version of the DII has been developed to adjust for the effects of energy intake when scoring inflammatory potential of the diet (38). Scores from both the original DII and the energy-adjusted version (e-DII) will be explored to see if energy density will have a greater affect than the nutrients of the diet and which score performs better for predicting inflammation. The e-DII is calculated by dividing each component by the total energy consumed in kilocalories and then multiplying by 1000 kilocalories.

Chapter 3: Methods

Study Design

Data for this project were collected from the Prostate Cancer: Guys Resilient by Individualized Training (Energy Balance for Prostate Cancer Survivorship) (PCaGRIT). The PCaGRIT trial was a pilot study that aimed to test the feasibility of obtaining significant weight

loss in overweight men with prostate cancer using a comprehensive weight loss program prior to a prostatectomy surgery and weight maintenance post-operatively. The weight management program used weekly behavioral face-to-face coaching sessions as well as self-monitoring using HIPAA compliant technology. Weekly phone calls were also used for further coaching and accountability

Two, 24-hour dietary recalls were obtained at baseline, after weight loss and prior to a prostatectomy surgery, and after twelve weeks of weight maintenance. These visits are referred to as baseline, surgery, and final study end. Dietary recalls from a weekend and a weekday were entered into the NDSR database for analysis, and diet output data was then averaged from these two recalls. The outputs from NDSR yielded the information on dietary components necessary to calculate the AHEI and DII scores. The original indices were both developed using food frequency questionnaires (FFQ), and not 24-hour recalls. The authors of the AHEI; Stephanie E. Chiuve and Teresa T. Fung were contacted to guide our approach for adapting scores from the 24-hour recalls. For scoring the DII and the energy adjusted DII (e-DII) the dataset of the averaged recalls was scored by the authors of the DII, Nitin Shivappa and James R Hebert. Twenty study subjects have dietary data, fifteen of which were in the experimental group, and five of which were controls. From the intervention group, thirteen of the fifteen participants have data for all time points, one participant had data missing from the surgery time point, and two were missing the study end time point. Of the five control subjects, four have data for the baseline time point, three have data for the prior to surgery time point as well as the post-surgery time point.

Blood samples were drawn at the baseline, surgery, and study end time points. The blood samples were centrifuged to obtain serum samples, which were then used to measure hs-CRP

and IL-6. To measure the hs-CRP, 1 mL of serum from the blood sample was sent to Quest Diagnostics lab in Lenexa, KS and analyzed using nephelometry and a detection range of 0.2-11.0mg/L. Hs-CRP is the same as CRP, except that it is a more sensitive test and can detect lower levels of CRP than a typical CRP test. All intervention participants had hs-CRP values at the baseline time point, fourteen of the fifteen intervention participants had hs-CRP values for the surgery time point, and thirteen had hs-CRP values for the study end post-surgery time point. All of the control participants had hs-CRP for baseline, while three of the five controls had hs-CRP values for the surgery time point, and four of the five controls had hs-CRP values for the study end time point. The MILLIPLEX® MAP (Billerica, MA) human high sensitivity T cell magnetic bead panel assay (Cat. # HSTCMAG-28SK) was used to measure IL-6, and the sample plates were processed according to manufacturer's instructions and read on Luminex 200 instrument with xPONENT software. The detection range for the IL-6 assay was 0.17 to 750 pg/ml. There were six time-points of IL-6 data from the intervention, and two time-points from the control, which were below the detection range for IL-6 of the sixty total time point samples for the study. When the IL-6 value was below detection limits but above zero, a value of 0.085 pg/ml was imputed to calculate for the disparity, and to ensure that these values were not assumed zero during analysis. There were ten time-points of IL-6 data from the intervention, and six time-points of data from the control, which were not obtained due to not having a blood draw or having limited sample amounts for testing.

Statistical Methods

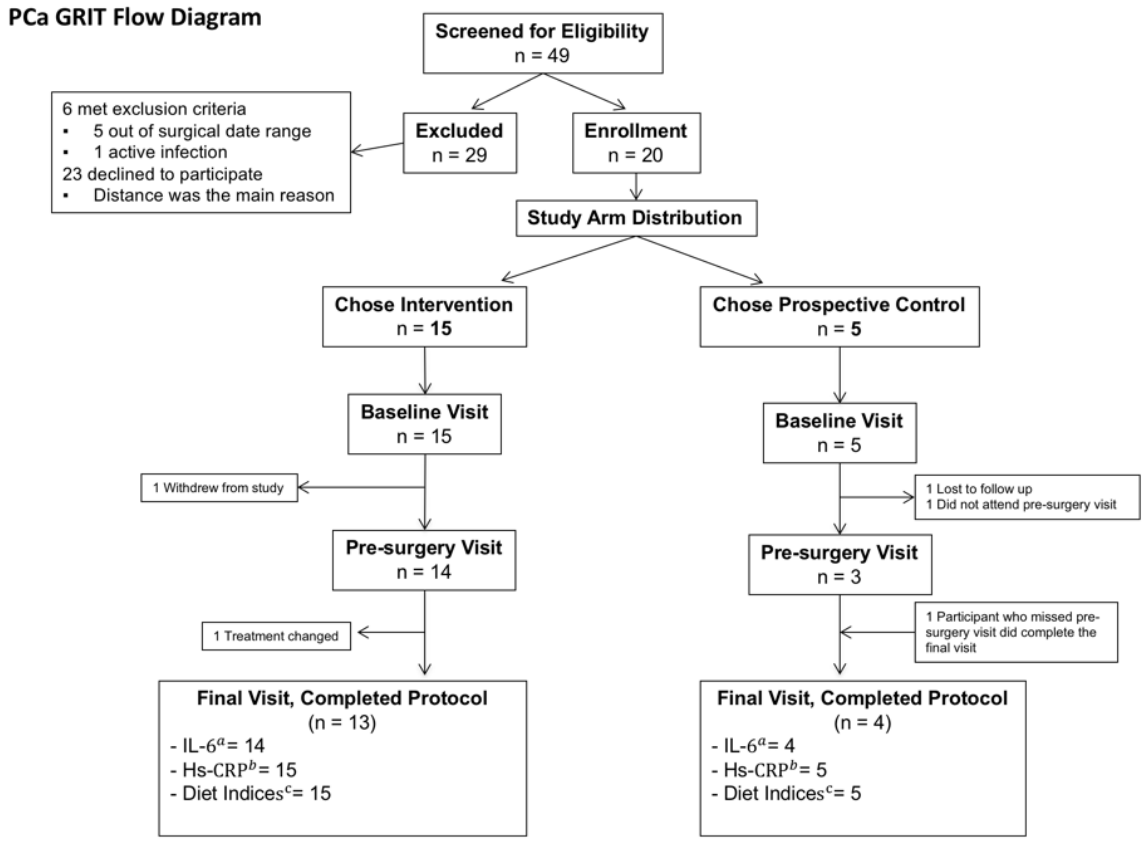
Spearman correlations were calculated by statistician, Dr. Chalise, to find associations between the inflammatory markers and diet scores. Dietary scores and inflammatory markers obtained at baseline were compared for associations. Changes in values from baseline to the

surgery time point, during weight loss, were also compared for associations between the change in dietary score to changes in inflammatory markers. From the surgery time point to the study end, the participants were in weight maintenance, and associations between changes in the dietary scores and the inflammatory markers were also compared in this time frame as well.

Chapter 4: Results

The flow diagram in figure 1 was adapted from the poster for PCaGRIT, presented at the American Institute of Cancer Research conference in 2016. Inferential statistics are described in the following tables. Line graphs depicting all comparative associations are shown in figure 2.

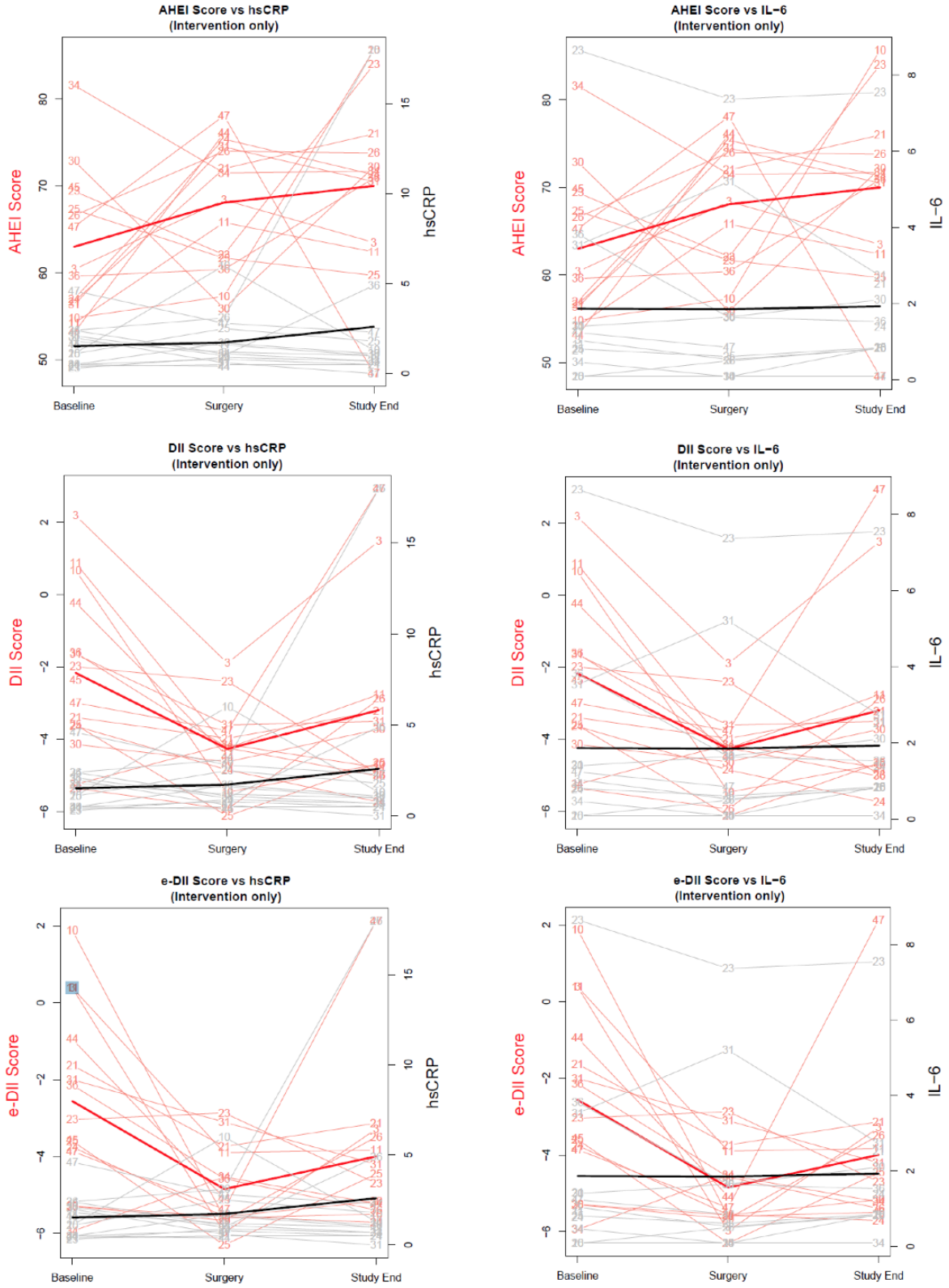
Figure 1



a.) IL-6 analyzed in intervention at baseline= 12, surgery= 12, study end= 11; in control at baseline= 3, surgery= 3, study end= 3.
b.) Hs-CRP analyzed in intervention at baseline= 15, surgery= 14, study end= 13; in control at baseline= 5, surgery= 3, study end= 4.
c.) Diet indices scores in intervention at baseline= 15, surgery= 14, study end=13; in control at baseline= 4, surgery= 3, study end= 4.

(39)

Figure 2


AHEI Score vs hsCRP
(Intervention only)
AHEI Score
hsCRP
Baseline
Surgery
Study End
AHEI Score vs IL−6
(Intervention only)
AHEI Score
IL−6
Baseline
Surgery
Study End
DII Score vs hsCRP
(Intervention only)
DII Score
hsCRP
Baseline
Surgery
Study End
DII Score vs IL−6
(Intervention only)
DII Score
IL−6
Baseline
Surgery
Study End
e-DII Score vs hsCRP
(Intervention only)
e-DII Score
hsCRP
Baseline
Surgery
Study End
e-DII Score vs IL−6
(Intervention only)
e-DII Score
IL−6
Baseline
Surgery
Study End

Refer to table 1 to see correlations between IL-6, hs-CRP and the dietary scores at baseline. Positive associations between AHEI scores and both hs-CRP and IL-6, and a positive association between the DII score and IL-6 were detected. The DII score was negatively associated with hs-CRP, and the e-DII score was negatively associated with both IL-6 and hs-CRP. The e-DII had stronger correlations with both IL-6 and hs-CRP compared to both the AHEI and the original DII.

TABLE 1 BASELINE

MARKER	Spearman correlation
IL-6	
AHEI	0.0039
DII	0.0404
E-DII	-0.0433
HS-CRP	
AHEI	0.0101
DII	-0.1288
E-DII	-0.157

During the weight loss phase of the trial, which took place between baseline and surgery time points, dietary scores changed over time. The associations between the score changes and the IL-6 and hs-CRP inflammatory marker changes are summarized in table 2. The AHEI score was positively associated with IL-6, but negatively associated with hs-CRP. The DII score and e-DII scores were both positively associated with IL-6, and also both negatively associated with hs-CRP.

TABLE 2 BASELINE TO SURGERY DURING WEIGHT LOSS

MARKER	Spearman correlation
IL-6	
AHEI	0.3507
DII	0.0133
E-DII	0.0554
HS-CRP	
AHEI	-0.258
DII	-0.4533
E-DII	-0.4709

From the surgery time point to the final study time point, the participants were to maintain their weight for twelve weeks. The associations between dietary scores and the inflammatory markers are located in table 3. The AHEI scores were positively associated with IL-6, but negatively associated with hs-CRP. The DII and e-DII scores were both positively associated with IL-6 and hs-CRP. The AHEI score had the strongest association with IL-6, and the DII had the strongest association to hs-CRP.

TABLE 3 SURGERY TO STUDY END DURING WEIGHT MAINTENANCE

MARKER	Spearman correlation
IL-6	
AHEI	0.4518
DII	0.2031
E-DII	0.3932
HS-CRP	
AHEI	-0.1396
DII	0.1741
E-DII	0.1196

Chapter 5: Discussion and Conclusion

Discussion

The present study examined associations between dietary index scores and the inflammatory biomarkers, IL-6 and hs-CRP in overweight men with prostate cancer. These associations were analyzed at baseline, from baseline to surgery (during weight loss), and from surgery to study end (during weight maintenance). Among our cohort of patients with prostate cancer, the AHEI scores at baseline had a very weak positive relationship with IL-6 and hs-CRP, while the DII scores had a very weak positive relationship to IL-6, and a very weak negative relationship to hs-CRP. Using the directionality of the associations as previously defined for each instrument, the DII was most strongly associated with IL-6 and the e-DII was most strongly associated with hs-CRP. Stronger relationships were observed in this present study; however,

these stronger associations were inverse the anticipated association. Looking at changes in dietary scores over time, during the weight loss phase of the trial, these data show that the e-DII scores were more strongly associated with IL-6 (0.0554, very weak), and the AHEI was more associated with hs-CRP (-0.258, weak). During weight maintenance the e-DII score changes were most associated with changes in IL-6 (0.3932, weak), while the DII score changes were most associated with changes in hs-CRP (0.1741, very weak). The majority of the reported associations are weak (<0.4).

There were several unanticipated results. These unanticipated findings include that the AHEI scores were positively associated to IL-6 and hs-CRP at baseline, instead of negatively associated as expected, given the higher AHEI scores relate to lower risks of chronic disease (40). At baseline the DII was negatively correlated with hs-CRP, and the e-DII was negatively correlated with both hs-CRP and IL-6. This is unexpected because higher scores in the DII are typically correlated with higher amounts of inflammation (35), yet these findings indicate the opposite. During the weight loss phase, changes in the AHEI scores were positively associated with IL-6, while the DII and e-DII scores were both negatively associated with hs-CRP. During the weight maintenance phase of the trial the AHEI scores were positively associated with IL-6. These findings counter findings by other researchers, and may be related to study limitations, which will be discussed further on.

The AHEI was created as a way to measure the impact diet choices would make on the risk of developing chronic diseases, including cancer. Several studies show that higher AHEI scores are inversely related to circulating levels of CRP and IL-6 (6, 41, 42). In this sample AHEI scores held a weak positive association to hs-CRP at baseline, while changes in the scores showed a weak negative association. The present study was a pilot trial, while the other studies

were epidemiological designs with much larger in sample sizes, and these differences in sample size may account for some of the differences observed in the findings. Furthermore, the primary aims of epidemiologic studies are different than the aims of a weight loss intervention trial. Our population in PCaGRIT included only overweight men with prostate cancer while the comparable epidemilogical studies included disparate populations of adults with a wide range of health statuses. The DII was purposefully created to measure dietary parameters which would affect inflammation (35). DII scores have been shown to predict levels of CRP (n=1,054) (43) and IL-6 (n=11,053) (44). In the present study the DII was negatively associated with hs-CRP at baseline and changes in the DII were negatively associated with changes in hs-CRP during the weight loss phase of the study as well. This association is unexpected considering a higher DII score should relate to greater inflammatory potential, and thus hypothesized to be positively associated with hs-CRP. Prior studies using the DII (43, 44) were done in an observational manner, and not a weight loss trial, and had much larger sample sizes than the present trial. The e-DII was created to correct and adjust scores for energy intake, because energy intake may become a confounding variable in creating a score. The e-DII scoring pattern is expected to behave similarly to the DII. In the present study the findings show the e-DII negatively associated with IL-6 and hs-CRP at baseline, and negatively associated with hs-CRP during the weight loss phase of the trial. These findings are surprising because the expected relationship is a positive association between the e-DII score and both of these inflammatory markers.

There were several limitations to this analysis. The trial was originally a pilot study looking at the feasibility of weight loss in overweight men with prostate cancer, and it was not designed to answer the aforementioned research questions regarding diet scores and their relationship to inflammatory markers. The sample size was very limited, and of that sample not

all of the participants had data available for dietary analysis and some participants were missing measured inflammatory markers, and these limitations reduce statistical power. Both of the AHEI and the DII were originally created to yield a score using food frequency questionnaires (FFQ), however, this analysis was done using 24-hour recalls. The authors of both dietary indices guided the approach for scoring, but there are differences between these two methods. FFQ are representative of "habitual" intake over several months to a year period of time, but FFQ are not appropriate given the nature of a trial aimed at changing habitual intake over the course of the study. 24-hour recalls were more appropriate for this trial, but these recalls rely on the respondent's memory and the skill of the interviewer to capture the most accurate information. 24-hour recalls can also give information on foods not commonly eaten, which is beneficial for a trial looking at changes over time, such as PCaGRIT, but if the participant anticipated the recall and changed their eating habits, this may have resulted in an inaccurate picture of the participants daily eating habits and thus an inaccurate dietary score. The DII has 45 components in total that can be used to conceive a score, but our analysis only used 28 of these. Despite assertions that a realizable score can be conceived with as few as 25 components, not using all 45 of the components may have impacted the realizable scores. When running the blood serum samples to obtain IL-6, the first kit ran did not have a low enough detection range, and thus a large amount of data could not be acquired. A kit with a lower detection range was found, and the samples were run again at a much later date. IL-6 is known to degrade over time, and storage methods may impact the sample profile (45). It is possible, given the storage time of the samples, that the IL-6 cytokines were affected, leading to inaccurate data.

Despite the limitations there were several strengths to this analysis. The nature of the three separate time-points in the trial made it possible to observe change over time. Two 24-hour

recalls were obtained, from a weekday and weekend day, and averaged to get a more accurate picture of habitual consumption. Trained staff took the recalls. The intervention participants in the trial also were able to lose 5-10% of their baseline body weight. The WARRIOR study, which builds upon the PCaGRIT trial, but with a larger sample size, is now in progress, and may be able to further look into dietary index score changes and their relationship to circulating inflammatory biomarkers, as well as delineate more clearly, which diet index performs the best. In this new trial it may be possible to include more components of the DII, to yield more accurate scores, and use greater stringency with storage and measuring of IL-6, now that a lower detection range IL-6 kit has been described.

Conclusion

The associations in this study were weak, and several results were unanticipated. Out of all three dietary scores, the e-DII was most strongly associated with IL-6, and the AHE was most strongly associated with hs-CRP. It would be worthwhile to replicate these methods on a trial of a larger size and lower IL-6 detection range kit, and to see if these results would be replicated or if stronger correlations could be found.

References

1. Singh R, Mishra MK, Aggarwal H. Inflammation, Immunity, and Cancer. Mediators Inflamm 2017;2017:6027305. doi: 10.1155/2017/6027305.
2. Puhr M, De Marzo A, Isaacs W, Lucia MS, Sfanos K, Yegnasubramanian S, Culig Z. Inflammation, Microbiota, and Prostate Cancer. Eur Urol Focus 2016;2(4):374-82. doi: 10.1016/j.euf.2016.08.010.
3. Nakai Y, Nonomura N. Inflammation and prostate carcinogenesis. Int J Urol 2013;20(2):150-60. doi: 10.1111/j.1442-2042.2012.03101.x.
4. Steiner JE, McCoy GN, Hess CP, Dobyns WB, Metry DW, Drolet BA, Maheshwari M, Siegel DH. Structural malformations of the brain, eye, and pituitary gland in PHACE syndrome. Am J Med Genet A 2018;176(1):48-55. doi: 10.1002/ajmg.a.38523.
5. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001;60(3):349-56.
6. Fung TT, McCullough ML, Newby PK, Manson JE, Meigs JB, Rifai N, Willett WC, Hu FB. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction. Am J Clin Nutr 2005;82(1):163-73.
7. Heikkila K, Harris R, Lowe G, Rumley A, Yarnell J, Gallacher J, Ben-Shlomo Y, Ebrahim S, Lawlor DA. Associations of circulating C-reactive protein and interleukin-6 with cancer risk: findings from two prospective cohorts and a meta-analysis. Cancer Causes Control 2009;20(1):15-26. doi: 10.1007/s10552-008-9212-z.
8. McArdle PA, Qayyum T, McMillan DC. Systemic inflammatory response and survival in patients with localised prostate cancer: 10-year follow-up. Urol Int 2010;85(4):482. doi: 10.1159/000320242.
9. Nguyen DP, Li J, Tewari AK. Inflammation and prostate cancer: the role of interleukin 6 (IL-6). BJU Int 2014;113(6):986-92. doi: 10.1111/bju.12452.
10. Culig Z, Puhr M. Interleukin-6: a multifunctional targetable cytokine in human prostate cancer. Mol Cell Endocrinol 2012;360(1-2):52-8. doi: 10.1016/j.mce.2011.05.033.
11. Watzl B, Kulling SE, Moseneder J, Barth SW, Bub A. A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. Am J Clin Nutr 2005;82(5):1052-8.
12. Kelly KR, Haus JM, Solomon TP, Patrick-Melin AJ, Cook M, Rocco M, Barkoukis H, Kirwan JP. A low-glycemic index diet and exercise intervention reduces TNF(alpha) in isolated mononuclear cells of older, obese adults. J Nutr 2011;141(6):1089-94. doi: 10.3945/jn.111.139964.
13. Casas R, Sacanella E, Urpi-Sarda M, Corella D, Castaner O, Lamuela-Raventos RM, Salas-Salvado J, Martinez-Gonzalez MA, Ros E, Estruch R. Long-Term Immunomodulatory Effects of a Mediterranean Diet in Adults at High Risk of Cardiovascular Disease in the PREvencion con DIeta MEDiterranea (PREDIMED) Randomized Controlled Trial. J Nutr 2016;146(9):1684-93. doi: 10.3945/jn.115.229476.
14. Harding AH, Wareham NJ, Bingham SA, Khaw K, Luben R, Welch A, Forouhi NG. Plasma vitamin C level, fruit and vegetable consumption, and the risk of new-onset type 2 diabetes mellitus: the European prospective investigation of cancer--Norfolk prospective study. Arch Intern Med 2008;168(14):1493-9. doi: 10.1001/archinte.168.14.1493.

15. Stamler J. The INTERSALT Study: background, methods, findings, and implications. Am J Clin Nutr 1997;65(2 Suppl):626S-42S.
16. Agudo A, Slimani N, Ocke MC, Naska A, Miller AB, Kroke A, Bamia C, Karalis D, Vineis P, Palli D, et al. Consumption of vegetables, fruit and other plant foods in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohorts from 10 European countries. Public Health Nutr 2002;5(6B):1179-96. doi: 10.1079/PHN2002398.
17. Riboli E, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. Am J Clin Nutr 2003;78(3 Suppl):559S-69S.
18. Esmaillzadeh A, Kimiagar M, Mehrabi Y, Azadbakht L, Hu FB, Willett WC. Dietary patterns and markers of systemic inflammation among Iranian women. J Nutr 2007;137(4):992-8.
19. Zhao Z, Feng Q, Yin Z, Shuang J, Bai B, Yu P, Guo M, Zhao Q. Red and processed meat consumption and colorectal cancer risk: a systematic review and meta-analysis. Oncotarget 2017;8(47):83306-14. doi: 10.18632/oncotarget.20667.
20. McMichael AJ. Food, nutrition, physical activity and cancer prevention. Authoritative report from World Cancer Research Fund provides global update. Public Health Nutr 2008;11(7):762-3. doi: 10.1017/S1368980008002358.
21. Cascella M, Bimonte S, Barbieri A, Del Vecchio V, Caliendo D, Schiavone V, Fusco R, Granata V, Arra C, Cuomo A. Dissecting the mechanisms and molecules underlying the potential carcinogenicity of red and processed meat in colorectal cancer (CRC): an overview on the current state of knowledge. Infect Agent Cancer 2018;13:3. doi: 10.1186/s13027-018-0174-9.
22. Samraj AN, Pearce OM, Laubli H, Crittenden AN, Bergfeld AK, Banda K, Gregg CJ, Bingman AE, Secrest P, Diaz SL, et al. A red meat-derived glycan promotes inflammation and cancer progression. Proc Natl Acad Sci U S A 2015;112(2):542-7. doi: 10.1073/pnas.1417508112.
23. Hedlund M, Padler-Karavani V, Varki NM, Varki A. Evidence for a human-specific mechanism for diet and antibody-mediated inflammation in carcinoma progression. Proc Natl Acad Sci U S A 2008;105(48):18936-41. doi: 10.1073/pnas.0803943105.
24. Yilmaz R, Akoglu H, Altun B, Yildirim T, Arici M, Erdem Y. Dietary salt intake is related to inflammation and albuminuria in primary hypertensive patients. Eur J Clin Nutr 2012;66(11):1214-8. doi: 10.1038/ejcn.2012.110.
25. Schwedhelm C, Pischon T, Rohrmann S, Himmerich H, Linseisen J, Nimptsch K. Plasma Inflammation Markers of the Tumor Necrosis Factor Pathway but Not C-Reactive Protein Are Associated with Processed Meat and Unprocessed Red Meat Consumption in Bavarian Adults. J Nutr 2017;147(1):78-85. doi: 10.3945/jn.116.237180.
26. Chai W, Morimoto Y, Cooney RV, Franke AA, Shvetsov YB, Le Marchand L, Haiman CA, Kolonel LN, Goodman MT, Maskarinec G. Dietary Red and Processed Meat Intake and Markers of Adiposity and Inflammation: The Multiethnic Cohort Study. J Am Coll Nutr 2017;36(5):378-85. doi: 10.1080/07315724.2017.1318317.
27. Lesinski GB, Reville PK, Mace TA, Young GS, Ahn-Jarvis J, Thomas-Ahner J, Vodovotz Y, Ameen Z, Grainger E, Riedl K, et al. Consumption of soy isoflavone enriched bread in men with prostate cancer is associated with reduced proinflammatory cytokines and immunosuppressive cells. Cancer Prev Res (Phila) 2015;8(11):1036-44. doi: 10.1158/1940-6207.CAPR-14-0464.

28. Malik VS, Pan A, Willett WC, Hu FB. Sugar-sweetened beverages and weight gain in children and adults: a systematic review and meta-analysis. Am J Clin Nutr 2013;98(4):1084-102. doi: 10.3945/ajcn.113.058362.
29. Kuzma JN, Cromer G, Hagman DK, Breymeyer KL, Roth CL, Foster-Schubert KE, Holte SE, Weigle DS, Kratz M. No differential effect of beverages sweetened with fructose, high-fructose corn syrup, or glucose on systemic or adipose tissue inflammation in normal-weight to obese adults: a randomized controlled trial. Am J Clin Nutr 2016;104(2):306-14. doi: 10.3945/ajcn.115.129650.
30. Rock CL, Doyle C, Demark-Wahnefried W, Meyerhardt J, Courneya KS, Schwartz AL, Bandera EV, Hamilton KK, Grant B, McCullough M, et al. Nutrition and physical activity guidelines for cancer survivors. CA Cancer J Clin 2012;62(4):243-74. doi: 10.3322/caac.21142.
31. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr 2012;142(6):1009-18. doi: 10.3945/jn.111.157222.
32. Graffouillere L, Deschasaux M, Mariotti F, Neufcourt L, Shivappa N, Hebert JR, Wirth MD, Latino-Martel P, Hercberg S, Galan P, et al. The Dietary Inflammatory Index Is Associated with Prostate Cancer Risk in French Middle-Aged Adults in a Prospective Study. J Nutr 2016. doi: 10.3945/jn.115.225623.
33. Shivappa N, J RH, Jalilpiran Y, Faghih S. Association between Dietary Inflammatory Index and Prostate Cancer in Shiraz Province of Iran. Asian Pac J Cancer Prev 2018;19(2):415-20. doi: 10.22034/APJCP.2018.19.2.415.
34. Zucchetto A, Gini A, Shivappa N, Hebert JR, Stocco C, Dal Maso L, Birri S, Serraino D, Polesel J. Dietary inflammatory index and prostate cancer survival. Int J Cancer 2016;139(11):2398-404. doi: 10.1002/ijc.30208.
35. Shivappa N, Steck SE, Hurley TG, Hussey JR, Hebert JR. Designing and developing a literature-derived, population-based dietary inflammatory index. Public Health Nutr 2014;17(8):1689-96. doi: 10.1017/S1368980013002115.
36. Hursting SD, Digiovanni J, Dannenberg AJ, Azrad M, Leroith D, Demark-Wahnefried W, Kakarala M, Brodie A, Berger NA. Obesity, energy balance, and cancer: new opportunities for prevention. Cancer Prev Res (Phila) 2012;5(11):1260-72. doi: 10.1158/1940-6207.CAPR-12-0140.
37. Ott B, Skurk T, Hastreiter L, Lagkouvardos I, Fischer S, Buttner J, Kellerer T, Clavel T, Rychlik M, Haller D, et al. Effect of caloric restriction on gut permeability, inflammation markers, and fecal microbiota in obese women. Sci Rep 2017;7(1):11955. doi: 10.1038/s41598-017-12109-9.
38. Shivappa N, Hebert JR, Anderson LA, Shrubsole MJ, Murray LJ, Getty LB, Coleman HG. Dietary inflammatory index and risk of reflux oesophagitis, Barrett's oesophagus and oesophageal adenocarcinoma: a population-based case-control study. Br J Nutr 2017;117(9):1323-31. doi: 10.1017/S0007114517001131.
39. Jill M. Hamilton-Reeves MDB, Cole Chana, Abigail Stanley, Lauren Hand, Tanner Isaacson, Debra K. Sullivan, Prabhakar Chalise, David Duchene, J. Brantley Thrasher. Effectiveness of a Weight Loss Program Tailored for Overweight Men with Localized Prostate Cancer: a Pilot Study. American Institute of Cancer Research Conference, 2016.

40. Chiuve SE, Sampson L, Willett WC. The association between a nutritional quality index and risk of chronic disease. Am J Prev Med 2011;40(5):505-13. doi: 10.1016/j.amepre.2010.11.022.
41. Huang T, Tobias DK, Hruby A, Rifai N, Tworoger SS, Hu FB. An Increase in Dietary Quality Is Associated with Favorable Plasma Biomarkers of the Brain-Adipose Axis in Apparently Healthy US Women. J Nutr 2016;146(5):1101-8. doi: 10.3945/jn.115.229666.
42. Akbaraly TN, Shipley MJ, Ferrie JE, Virtanen M, Lowe G, Hamer M, Kivimaki M. Long-term adherence to healthy dietary guidelines and chronic inflammation in the prospective Whitehall II study. Am J Med 2015;128(2):152-60 e4. doi: 10.1016/j.amjmed.2014.10.002.
43. Shivappa N, Steck SE, Hurley TG, Hussey JR, Ma Y, Ockene IS, Tabung F, Hebert JR. A population-based dietary inflammatory index predicts levels of C-reactive protein in the Seasonal Variation of Blood Cholesterol Study (SEASONS). Public Health Nutr 2014;17(8):1825-33. doi: 10.1017/S1368980013002565.
44. Tabung FK, Smith-Warner SA, Chavarro JE, Fung TT, Hu FB, Willett WC, Giovannucci EL. An Empirical Dietary Inflammatory Pattern Score Enhances Prediction of Circulating Inflammatory Biomarkers in Adults. J Nutr 2017;147(8):1567-77. doi: 10.3945/jn.117.248377.
45. de Jager W, Bourcier K, Rijkers GT, Prakken BJ, Seyfert-Margolis V. Prerequisites for cytokine measurements in clinical trials with multiplex immunoassays. BMC Immunol 2009;10:52. doi: 10.1186/1471-2172-10-52.