

## Weight loss via a low-carbohydrate diet improved the intestinal permeability marker, zonulin, in prostate cancer patients

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### ABSTRACT

**Background:** Accumulating evidence suggest that gut microbiota may impact urologic health including prostate cancer (PC), potentially *via* affecting intestinal permeability (IP). Studies have indicated that disrupted IP may be improved by healthy diets and weight loss. In the Carbohydrate and Prostate Study 2 (CAPS2) clinical trial, which showed that a low-carbohydrate diet (LCD) reduced weight significantly in men with PC and suggestively slowed PC disease progression, we explored the impact of LCD on an IP marker, zonulin and an inflammation marker, high sensitivity C-reactive protein (hsCRP).

**Methods:** CAPS2 was a 6-month randomized controlled trial testing a LCD intervention vs. control on PC progression using prostate-specific antigen doubling time (PSADT) as the marker. All 45 participants had prior primary PC treatment, PSADT >3 and <36 months, and body mass index (BMI)  $\geq 24$  kg/m<sup>2</sup>.

**Results:** At 6-month, zonulin decreased in the LCD arm (median  $-8.3\%$ , IQR  $-16.6, 0.3\%$ ) while the control increased slightly (median  $1.4\%$ , IQR  $-3.0, 13.3\%$ ;  $p = .014$ ). No changes were observed in hsCRP. Linear regression models showed that weight change was significantly associated with log(PSADT) such that the greater the weight loss, the longer the PSADT ( $p = .003$ ). There was a similar inverse trend between change in zonulin and log(PSADT) ( $p = .050$ ). Nevertheless, the mediation analysis showed that zonulin was not a significant intermediary mechanism of the effect of weight change on PSADT ( $p = .3$ ).

**Conclusion:** Future studies are merited to examine further the potential association of IP with inflammation and to clarify if improvement in IP is associated with decreased PC progression.

**Trial registration:** NCT01763944.

### KEY MESSAGES

- Gut microbiota may impact urologic health including prostate cancer, potentially *via* affecting intestinal permeability.
- Weight loss significantly improved intestinal permeability in prostate cancer patients.
- Improvement in intestinal permeability was associated with slowed prostate cancer progression as indicated by the PSA doubling time.

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## Introduction

Accumulating evidence suggest a role of gut microbiota in urologic health including prostate cancer (PC), potentially *via* its impact on intestinal permeability (IP) [1–3]. When the gut microbiota community is disrupted or imbalanced, i.e. gut dysbiosis, intestinal barrier function may be impaired and thus increase permeability. Greater IP allows increased leakage of

intestinal lumen fluid, macromolecules, leukocytes, toxins and compounds into circulation that may contribute to inflammation [4]. IP, as a marker of gut microbiota health, may be involved in the development of PC and/or responses to treatments, and inflammation may be a potential mediating mechanism, however, existing data are highly inadequate to draw conclusions [5]. A recent study suggests that certain gut microbes may produce androgens which

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contributes to castration resistance in PC [6]. This finding further supports the need to continue examining the association of diet and lifestyle, microbial composition, IP, PC development, progression and responses to treatment.

Studies have indicated that disrupted IP is not irreversible and may be repaired or restored [3]. Lifestyle, such as dietary intake, is an important modifiable factor of the gut microbiota community and IP [7]. Both high fiber intake and weight loss have been shown to improve IP, while high fat and Western diets disrupt microbiota composition and IP in mice and humans [3,8–10]. Zonulin is a pre-haptoglobin (Hp)-2 protein with regulating function on intestinal barriers and has been commonly used as a marker for IP [11]. Studies have shown that serum zonulin correlated with inflammation markers including C-reactive protein (CRP) [10]. Since inflammation has been suggested to be associated with PC, we examined the association between IP, inflammation marker high sensitivity CRP (hsCRP), and PC progression in the Carbohydrate and Prostate Study 2 (CAPS2) clinical trial, which showed that a low-carbohydrate diet (LCD) reduced weight significantly in men with PC and suggestively slowed PC disease progression [12].

## Methods

Details of the CAPS2 trial and its main result has been reported previously [12]. The CAPS2 study was designed to test the effect of a LCD intervention on PC progression using prostate-specific antigen doubling time (PSADT) as the marker. A total of 45 men with biochemical recurrence after primary PC treatment were enrolled and randomly assigned to either the LCD intervention or a no-dietary intervention control for 6 months. The study protocol was approved by the Duke University Medical Center Institutional Review Board. Each participant signed an informed consent prior to beginning of the study. Data and blood specimen were collected at baseline, 3- and 6-month post randomization. Participants in the LCD arm were coached to restrict carbohydrate intake to  $\leq 20$ g/day. No other PC treatments during the study were allowed.

Key eligibility criteria of the main trial included (1) prior primary PC treatment (radical prostatectomy or definitive local radiation), (2) PSA between 3 and 20 ng/ml if prior local radiation or between 0.4 and 20 ng/ml if prior radical prostatectomy within the past 2 months, (3) PSADT  $>3$  and  $<36$  months, (4) BMI  $\geq 24$  kg/m<sup>2</sup>, (5) willingness to be randomized to either

the LCD or the control arm, and (6) phone access for intervention coaching for the LCD arm. Key exclusion criteria included (1) symptomatic metastatic disease, (2) anticipation of needing secondary PC therapy within the next 6 months, (3) currently using weight loss medications or enrolled in any diet/weight loss program, (4) current therapy aimed at lowering testosterone, (5) already following a LCD, (6) being vegetarian/vegan, (7) unwilling to be randomized to either the LCD or control arm, (8) lost  $>5\%$  of body weight in the last 6 months, or (9) had comorbidities that may limit the patient's ability to complete the study.

Serum specimens were analyzed for zonulin by ELISA (Alpco, Salem, NH) and hsCRP was measured by the Duke Immunoassay Laboratory using high sensitivity immunoturbidimetric assay by Beckman analyzer. We chose these two markers for this secondary analysis because the prior research showing a correlation between zonulin and hsCRP [10], and because that both assays are commonly used as a marker of IP and inflammation, respectively. The primary outcome of the main CAPS2 trial was PSADT and was previously reported [12].

Pearson correlations between weight change and zonulin change, between weight change and PSADT, and between zonulin change and PSADT, were plotted to illustrate their respective relationships before adjusting for any covariate. The percent of changes in zonulin and hsCRP from baseline to 3-month and 6-month were compared between arms using Wilcoxon rank-sum tests. Linear regression models were used to test the association between the exposures of percent change in zonulin from baseline to 6-month and absolute weight change from baseline to 6-month (in separate models and together) with PSADT (log-transformed) as the outcome. Models were adjusted for baseline PSADT, baseline PSA, and primary treatment (surgery vs. radiation). Additional adjustments included total energy intake, fiber intake, baseline BMI and comorbidities. Linear regression was also used to test the association between percent change in zonulin and weight change. Furthermore, we formally tested the role of zonulin as a mediator between weight loss and PSADT [13]. Analyses were conducted using SAS 9.4 (SAS Institute; Cary, NC, USA). A  $p$  value of  $\leq 0.05$  is considered statistically significant.

## Results

Among the 45 patients who were randomized ( $N=26$  in LCD,  $N=19$  in control) and completed the intervention, they were older in age (median 72 years, IQR

**Table 1.** Zonulin (ng/mL) and hsCRP (mg/L) values in the CAPS2 study.

	Low-carbohydrate diet (N = 26)	Control (N = 19)	p value <sup>a</sup>
Zonulin, baseline			.346
Median	44.3	42.1	
Q1, Q3	38.8, 48.3	36.5, 47.3	
Zonulin, 3 months			.775
Median	41.5	39.4	
Q1, Q3	36.6, 44.5	34.1, 47.8	
Zonulin, 6 months			.316
Median	40.6	43.8	
Q1, Q3	35.8, 44.8	36.9, 50.4	
Percent change in zonulin, 0 to 6 months			.014
Median	-8.3	1.4	
Q1, Q3	-16.6, 0.3	-3.0, 13.3	
HsCRP, baseline			.889
Median	2.2	2.3	
Q1, Q3	1.1, 3.4	1.2, 4.0	
HsCRP, 3 months			.502
Median	2.5	1.6	
Q1, Q3	1.3, 4.9	1.1, 3.6	
HsCRP, 6 months			.434
Median	2.0	2.5	
Q1, Q3	1.3, 4.1	1.0, 3.0	
Percent change in HsCRP, 0 to 6 months			.434
Median	-0.5	-6.7	
Q1, Q3	-30.0, 43.4	-26.7, 9.3	

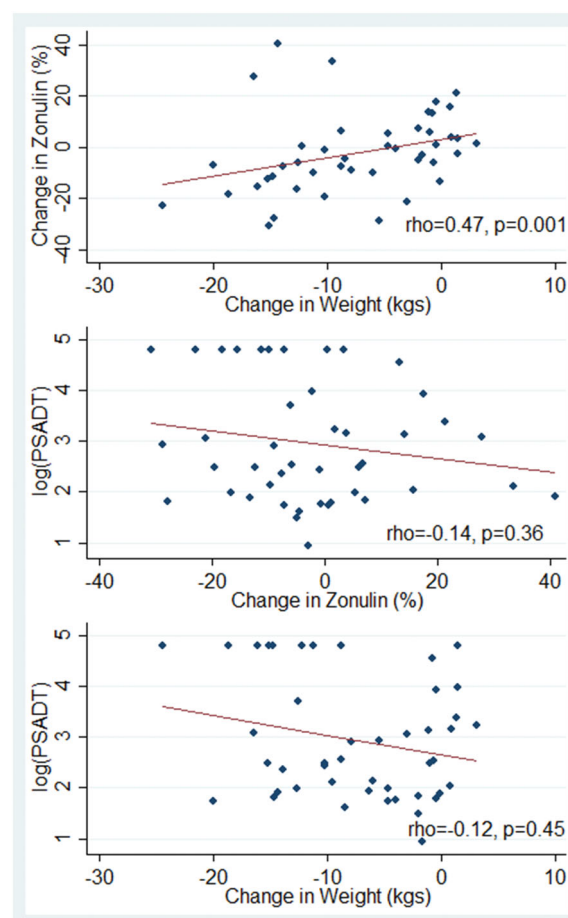
<sup>a</sup>Wilcoxon

Note: One subject in the control arm was missing zonulin measurements, one subject in the LCD arm and four subjects in the control arm were missing hsCRP measurements.

66, 74), overweight (BMI median 29.3, IQR 27.3, 32.5 kg/m<sup>2</sup>), had baseline PSADT of 11 month and 80% had prior surgery [12]. As reported previously, after the 6-month LCD intervention, LCD patients lost weight significantly more than controls (-12.1 vs. -0.5 kg,  $p < .001$ ). Though primary analysis showed no difference between arms in PSADT, LCD significantly lowered PSADT (28 vs. 13 months,  $p = .021$ ) after adjusting for key baseline covariates including PSA, PSADT, treatment received and hemoconcentration.

For the current secondary analyses, baseline serum zonulin levels were balanced between the arms which remained similar at 3-month. Between baseline and 6 months, zonulin decreased in the LCD arm (median -8.3%, IQR -16.6, 0.3%) while the control increased slightly (median 1.4%, IQR -3.0, 13.3%;  $p = .014$ ). No changes were observed in the hsCRP values at either time point (Table 1). We also examined the associations between zonulin and hsCRP but found no significant associations at baseline ( $\rho = 0$ ,  $p = .29$ ) and over the 6-month intervention as percent change of both measures ( $\rho = 0.05$ ,  $p = .76$ ).

Pearson correlation plots in Figure 1 showed that, without adjustment for any covariate, weight change was significantly associated with the change in zonulin ( $p = .001$ ), while the associations between zonulin change and PSADT ( $p = .36$ ), and between weight

**Figure 1.** Person correlation plots of weight change and zonulin change, zonulin change and PSADT, and weight change and PSADT, respectively.

change and PSADT ( $p = .45$ ), were not significant. In linear regression models, weight change was significantly associated with log(PSADT) such that the greater the weight loss, the longer the PSADT ( $\beta = -0.031$ , 95%CI: -0.050, -0.011,  $p = .003$ ). There was a similar inverse trend between change in zonulin and log(PSADT) ( $\beta = -0.020$ , 95%CI: -0.039, -0.001  $p = .050$ ). When both weight change and change in zonulin were included in the same model, weight change was significantly associated with PSADT ( $\beta = -0.027$ , 95%CI: -0.047, -0.006,  $p = .012$ ), but the relationship between zonulin and PSADT was attenuated ( $\beta = -0.012$ , 95%CI: -0.031, 0.007,  $p = .21$ ). In addition, we found that percent change in zonulin was significantly associated with weight change ( $\beta = 0.32$ , 95%CI: 0.03, 0.60,  $p = .033$ ). Nevertheless, the mediation analysis showed that zonulin was not a significant intermediary mechanism of the effect of weight change on PSADT ( $p = .3$ ). Results were consistent after further adjustment of comorbidities as metabolic syndrome, energy intake, and fiber intake or adjustment of baseline BMI.

## Discussion

Our findings showed that weight loss improved IP in PC patients while consuming a LCD ( $N=26$ ). This is consistent with a previous study showing that weight loss improved IP in a non-cancer population [10]. In addition, our data showed that weight loss directly benefits PSADT and improvement in IP, as shown by reduction in zonulin, may also benefit PSADT. Even though our findings do not confirm that the benefit of weight loss was manifested through the improvement in IP, given the positive associations and small sample sizes, future research should examine this possibility further.

Many studies have explored IP using zonulin as a marker but few in cancer patients. Our zonulin data were comparable to those with morbid obesity ( $63 \pm 32$  ng/mL) [10], normal weight adults (median 54.5 ng/mL, IQR: 45.2–64.4) [14], obese patients with colorectal cancer ( $26.57 \pm 14.95$  ng/mL), and older adults without active cancer ( $42.2 \pm 11.8$  ng/mL) [15], but higher than patients who underwent colonoscopies and those with hepatocellular carcinoma, and another group of healthy controls ( $14.72 \pm 9.57$  ng/mL) [10,16–18]. The differences may be attributed to variation in the characteristics of study populations and/or analytical kits made by different manufacturers. Thus, we focused on the percent of change when evaluating the zonulin data. A previous study showed that 7.3% weight loss reduced serum zonulin by 30% (reduction of 19 ng/mL) and with associated improvement in markers of glucose intolerance and liver disease [10]. Though our LCD intervention achieved a lower reduction in zonulin (8.3%) with 6.3% weight loss, the reduction in zonulin was associated with lengthening of PSADT with borderline significance ( $p=.05$ ). The patient characteristics of our study as compared to that of the participants in the above-mentioned weight loss study (with non-cancer patients) may also contribute to the different findings.

It is unclear why our data did not show any association of weight change, zonulin and PSADT with hsCRP. Previous research showed that lower IP was associated with reduced inflammation [19,20]. Our assessment of inflammation was very limited with only hsCRP which may limit the detection of any change in inflammation. In addition, the changes in IP may be too small to lead to any detectable changes in hsCRP.

Almost no research has examined the role of IP in PC. Previous studies showed that gut microbial composition differed significantly in men with PC compared to benign controls and a unique microbiome signature was identified for higher Gleason score

cancers [21,22]. A recent study showed that certain microbial species (e.g. *Ruminococcus* sp.) can convert androgen precursors to active androgens [6] which provides the potential strategy to minimize castration resistance with intervention in microbial community.

Due to the limitation of our design, we cannot distinguish the potential separate influence of weight loss from carbohydrate restriction. Our study is also limited in its small sample size and multiple testing was performed without corrections as this was already a post-hoc exploratory analysis. As such, these results should be viewed as hypothesis generating. Nonetheless, our findings add to the existing evidence of the impact of diet and weight loss on IP and suggests for further research in its role in PC. Although the two study arms had unequal sample sizes ( $N=26$  in LCD,  $N=19$  in control), this was unlikely to impact the analyses; if anything, a more balanced study design might have had higher power and stronger results. Future studies should measure a full panel of inflammation markers to understand more fully how improved IP may have impacted the inflammation pathway and if this impact is associated with PC progression.

## Author contributions

PHL contributed to the design, data collection and interpretation of the data and drafted the paper; LH performed all the data analyses and reviewed the paper critically, SJF reviewed the paper critically. All authors gave final approval of the version submitted; and all authors agreed to be accountable for all aspects of the work.

## Disclosure statement

The authors have no conflict of interest to report.

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## Data availability statement

The data that support the findings of this study are available upon reasonable request.

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