DOI: 10.53469/jcmp.2024.06(10).02

Mendelian Randomization Analyses Identified Bioavailable Testosterone Mediates the Effect of Fat Intake on Prostate Cancer

Qiao Du[1], Xia Wang[1], Hao Yang[2], Zheng Luo[3], Youqi Xu[1,*]

[1]Department of Oncology, The Second Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing 210017, Jiangsu, China
[2]Liaocheng People's Hospital, Liaocheng 252000, Shandong, China
[3]Nanjing Hospital of Chinese Medicine, Nanjing 210000, Jiangsu, China
**Correspondence Author*

Abstract: ***Background: Dietary factors are considered significant in the risk of prostate cancer (PCa). However, observational studies concerning the influence of macronutrients and micronutrients on PCa risk have yielded inconsistent findings. Method: We employed a two-sample Mendelian randomization (MR) approach to assess the impacts of four principal macronutrients and 17 micronutrients on PCa risk. Utilizing MR, we examined the relationship between fat digestion products (glycerol, fatty acids) and PCa, and conducted a two-step MR to determine if serum testosterone mediates the impact of fat intake on PCa risk. Results: Our study revealed a strong association between genetically predicted fat intake and PCa risk [OR=1.818, 95% CI (1.136, 2.909), P=0.013], with evidence suggesting that vitamin B5, vitamin B12, carotenoids, and zinc may influence PCa risk. No genetic evidence linked glycerol and various fatty acids to PCa risk (all P>0.05). Notably, the mediator bioavailable testosterone explained of the total effect of fat intake on prostate cancer risk [mediated proportion=8.8 %, 95% CI (-4.4% , 21.9%)]. Conclusion: In conclusion, our research demonstrates that fat intake increases the risk of prostate cancer. We also provide genetic evidence that bioavailable serum testosterone mediates the effect of fat consumption on prostate cancer risk. However, we found no significant benefits from micronutrients in preventing prostate cancer, with the exception of carotenoids.***

Keywords: Prostate cancer, Macronutrients, Micronutrients, Testosterone, Mendelian randomization**.**

1. Introduction

Prostate cancer (PCa) represents a prevalent malignancy within the male genitourinary system, comprising approximately 29% of cancer cases in men and standing as the second leading cause of cancer-related mortality among men, following lung cancer [1]. The incidence of PCa is strongly associated with age [2]. Irregular androgen stimulation has been linked to the onset of prostate malignancies [3]. Presently, androgen deprivation therapy, via surgery or medication, serves as the primary treatment for PCa [4,5]. However, the development of androgen resistance and the inevitable recurrence of the disease underscore the importance of investigating non-androgenic factors influencing PCa's etiology and progression [3].

Dietary factors have been acknowledged for their critical role in the development and progression of PCa, with numerous studies documenting the relationship between PCa and various nutrients (such as fats and proteins) and dietary patterns (such as a Western diet) [6]. While several cohort and case-control studies have identified an association between increased dietary fat intake and PCa risk [7,8], others have found no such correlation [9,10]. Similarly, no significant link has been established between PCa risk and the consumption of animal or plant proteins [11], although an animal study utilizing a mouse PCa model suggested that high milk consumption could mitigate PCa progression by diminishing the expression of Ki-67 and G protein-coupled receptor family C group 6 member A [12]. Obesity, a burgeoning global health concern, is increasingly recognized as a pivotal factor in cancer development [13]. Numerous studies have demonstrated a clear positive relationship between obesity-related metrics (such as body mass index) and cancer incidence [14,15], yet observational studies reporting on the correlation between these metrics and PCa risk have produced inconsistent results [16,17].

The primary cause of variability in study outcomes is attributed to the predominant reliance on observational research, wherein findings are susceptible to being skewed by residual confounding factors and reverse causation. Mendelian randomization (MR), a method employing genetic variants as instrumental variables in place of direct risk factors, offers a robust approach to explore the associations between risk factors and diseases [18]. The random assortment of alleles at conception ensures that genetic variations remain unaffected by confounding variables such as measurement bias or reverse causation [19].

This study aims to utilize MR to examine the relationship between genetically predicted relative intake of macronutrients (fats, proteins, sugars, and carbohydrates) and circulating concentrations of 17 genetically predicted micronutrients (minerals and vitamins) with prostate cancer (PCa) risk. Furthermore, through a two-step MR analysis, it investigates whether serum testosterone acts as a mediator in this relationship.

2. Materials and Methods

2.1 Data Sources

Genetic data for PCa were sourced from a genome-wide association analysis (GWAS) conducted by the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) [20]. This analysis comprised a total sample size of 140,254, which included

79,148 cases and 61,106 controls, and analyzed 20,346,368 single nucleotide polymorphisms (SNPs).

The GWAS data pertaining to the four macronutrients were disseminated by the Social Science Genetic Association Consortium. This dataset predominantly included participants of European ancestry, aged between 27 and 71 years. Instrumental variables for the relative intake of four principal dietary nutrients (carbohydrates, sugars, proteins, and fats) were extracted from the study by Meddens et al. [21]. To estimate the relative composition of these macronutrients, a self-reported questionnaire covering more than 70 food items was utilized. The evaluation of relative intake for fats, proteins, and carbohydrates involved 268,922 individuals of European descent, whereas the sugar intake analysis encompassed 235,391 individuals.

We located 17 GWAS datasets for circulating concentrations of micronutrients within European populations, drawing from the GWAS catalog and PubMed. These datasets covered 7 essential minerals and 10 vitamins, specifically calcium [22], copper [23], iron [24], magnesium [25], phosphorus [26], selenium [27], zinc [23], vitamin A (retinol) [28], vitamin B5 (pantothenic acid) [28], vitamin B6 [28], vitamin B9 (folate) and vitamin B12 [29,30], vitamin C [31], 25-hydroxyvitamin D [32], vitamin E [33], β-carotene [34], and carotenoids (cryptoxanthin) [28]. With the exception of selenium, which was measured in toenails and blood, all other micronutrients were quantified in serum levels.

The GWAS genetic data for glycerol were obtained from the summary data on human disease-related plasma metabolites [28]. Conversely, the genetic data for fatty acids were sourced from the largest-scale genetic association dataset available, comprising over 114,000 participants from the UK Biobank. This dataset includes 15 different types and ratios of fatty acids: total fatty acids, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, omega-3 fatty acids, and omega-6 fatty acids, with specific study details provided by Borges MC et al. [35].

Within the GWAS catalog, two extensive studies on serum testosterone (encompassing total testosterone, bioavailable testosterone, and sex hormone-binding globulin (SHBG)) genetic data in European populations were identified. Given the gender-specific nature of the cancer under investigation, we opted for the study that performed GWAS analyses separately for male and female hormones [36]. All data utilized in this study were reported in European populations.

2.2 Study Design

This study adhered to the STROBE-MR guidelines (Strengthening the Reporting of Observational Studies in Epidemiology using Mendelian Randomization) [37]. Mendelian Randomization (MR) studies are predicated on three fundamental assumptions: the instrumental variable (genotype) must be strongly associated with the exposure (phenotype) (Assumption 1, Relevance assumption, with a correlation coefficient > 0.8); the instrumental variable should not be linked to any confounders (Assumption 2, Independence assumption); and the instrumental variable should influence the outcome solely via the exposure (Assumption 3, Exclusion restriction assumption) [38], as depicted in Figure 1.

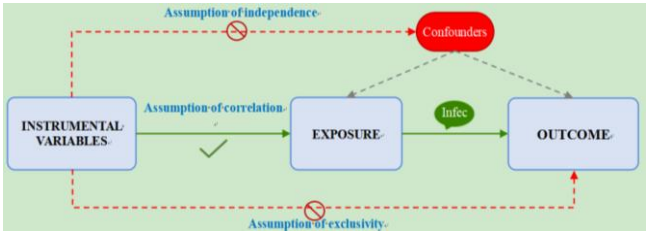

Figure 1: Flowchart of Two-Sample Mendelian Randomization

The research proceeded through multiple stages of two-sample MR analysis. Initially, MR analysis was utilized to explore the causal impacts of macronutrients and micronutrients on PCa. The subsequent phase evaluated the effects of serum testosterone levels on PCa. In the following steps, only factors established as having a causal relationship with PCa were considered. The third phase applied MR analysis to ascertain the directionality of the association between the selected nutrients and serum testosterone levels. The fourth phase probed into potential mediating effects via a two-step MR analysis.

First, appropriate instrumental variables (IVs) were identified through rigorous quality control measures. (1) The chosen IVs' genetic variants must exhibit a strong association with the exposure factor. To identify single nucleotide polymorphisms (SNPs) related to the exposure factor and ensure the validity and reliability of the causal inference between exposure and outcome, several steps were undertaken to select the most appropriate SNPs. Initially, SNPs achieving genome-wide significance ($P < 5X10\text{-}8$) were selected for their reliability and comprehensiveness. Subsequently, to mitigate bias from residual linkage disequilibrium among genetic variants, criteria of an LD $r^2 < 0.001$ and a minimum distance of 10,000 kb were applied. Furthermore, the F-statistic was employed as a selection criterion, with SNPs demonstrating an F-value > 10 considered strongly related to the exposure factor. SNPs with palindromic structures were excluded during this process. The F-statistic formula is $\left(F = \frac{R^2}{1-R^2} \cdot \frac{N-K-1}{K}\right)$, where R^2 represents the percentage of variance in the exposure explained by SNPs, calculated as $R^2 = 2 \cdot (1 - EAF) \cdot EAF \cdot \beta^2$ (EAF is the effect allele frequency for each SNP, is the effect size of the allele), K represents the number of SNPs, and N is the sample size of the exposure data [39,40]. (2) The chosen IVs' genetic variants must not be linked to any confounders. (3) The chosen IVs' genetic variants should not be associated with the outcome data. SNPs strongly associated with the exposure, identified through the aforementioned steps, were utilized in the MR analysis after excluding SNPs related to confounding factors (such as age, genetics) and SNPs associated with the outcome using Phenoscanner [39].

The primary analytical approach employed in this study was the inverse variance weighted (IVW) method, complemented by the weighted median (WM) and MR-Egger regression as secondary analytical methods. The IVW method presupposes that the instrument influences the outcome exclusively through the exposure and not via alternative pathways. In contrast, WM and MR-Egger regression yield more

conservative effect estimates but provide more robust results across a wider array of conditions [41,42]. The relationship between exposure and outcome risk was quantified using the odds ratio (OR) and its 95% confidence interval (CI), where a P-value ≤ 0.05 signifies a causal relationship between the exposure and the outcome. The analysis included tests for heterogeneity, sensitivity, and pleiotropy. Heterogeneity was assessed using Cochran's Q [43], with a significant Q statistic ($P \leq 0.05$) indicating variability in the analysis outcomes. The reliability of the results was also evaluated by examining the symmetry of the funnel plot. Sensitivity analysis employed the leave-one-out approach to determine the influence of individual SNPs as potential outliers. Horizontal pleiotropy was assessed using MR-Egger and MR-PRESSO [42]; a P-value < 0.05 for the MR-Egger intercept suggested horizontal pleiotropy, breaching the fundamental assumptions of MR, thus rendering the results invalid. Additionally, the intercept from MR-Egger's slope plot was utilized to ascertain whether genetic variations impacted the outcome through mechanisms other than the exposure.

Analytical tools utilized in this research included R (version 4.3.2), the Two-Sample MR package, and MR-PRESSO. This research relied on publicly available data, obviating the need for additional ethical approval or harmonization.

3. Results

3.1 Determining the Impact of Dietary Factors on Prostate Cancer Risk

In our analysis of macronutrients, fat intake was notably associated with a increased risk of PCa [OR=1.818, 95% CI (1.136, 2.909), P=0.013]. Conversely, there was no significant genetic evidence to indicate that the consumption of sugars (P=0.3089), carbohydrates (P=0.6825), or proteins (P=0.8758) affected PCa risk. Among the 17 examined micronutrients, we identified associations between PCa risk and vitamin B5 [OR=1.063, 95% CI (1.00, 1.129), P=0.0475], vitamin B12 [OR=1.090, 95% CI (1.021, 1.164), P=0.0103], carotenoid levels (cryptoxanthin) [OR=0.955, 95% CI (0.914, 0.998), P=0.0388], and zinc [OR=1.057, 95% CI (1.002, 1.116), P=0.0425], with no other significant causal links identified, as depicted in Figure 2.

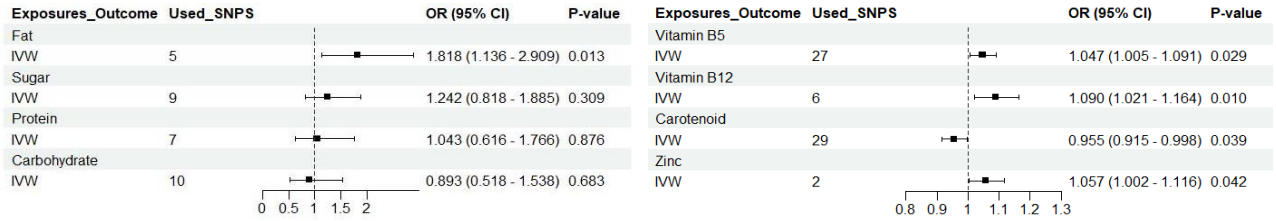

Figure 2: Forest Plot of MR analysis results of macronutrients and micronutrients in relation to PCa

Notes: PCa, prostate cancer; IVW: inverse variance weighting; CI, confidence interval; OR, odds ratio; SNPs, single nucleotide polymorphisms..

Given the pathogenic role of fat intake against PCa, subsequent MR analysis was conducted to determine if the metabolic products of fat, including glycerol and 15 types of fatty acids along with their ratios (monounsaturated fatty acids, polyunsaturated fatty acids, omega-3 fatty acids, and omega-6 fatty acids), were linked to PCa risk. However, this analysis did not yield genetic evidence supporting an association between glycerol, the different types and ratios of fatty acids, and PCa risk. The findings from the MR analysis are illustrated in supplementary material.

3.2 Identifying Potential Mediating Effects

Initially, we evaluated the influence of serum testosterone on PCa risk. After removing potential linkage disequilibrium (LD) variants and outliers, we identified 66 bioavailable testosterone (BT) SNPs, 140 total testosterone (TT) SNPs, and 206 sex hormone-binding globulin (SHBG) SNPs with strong associations with their respective exposures but not the outcomes. The MR findings revealed that an increase in serum BT levels by one unit was associated with a higher risk of PCa [OR=1.208, 95% CI (1.084, 1.346), P=0.0006], whereas no genetic evidence indicated an effect of SHBG or TT on PCa risk. Subsequently, two-sample MR analysis was utilized to examine the effect of fat intake on serum BT levels, demonstrating a significant association [OR=1.320, 95% CI (1.004, 1.340), P=0.047]. No evidence of reverse causality between serum BT levels and fat intake was found. The outcomes of the two-step MR analysis are documented in Figure 3.

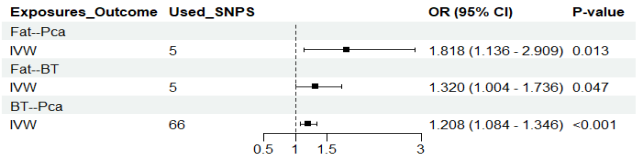

Figure 3: Three-Line Table of Two-step MR analysis results

Notes: Intake of Fat – BT - PCa) (PCa, prostate cancer; BT, bioavailable testosterone; IVW: inverse variance weighting; CI, confidence interval; OR, odds ratio.

In the next phase, leveraging the insights from the two-step MR analysis, we investigated the mediating role of serum bioavailable testosterone in the causal pathway between fat intake and PCa risk. This analysis indicated that serum bioavailable testosterone mediated 8.8% of the causal effect of fat intake on PCa risk, as illustrated in Table.1. The analyses consistently showed an absence of heterogeneity and horizontal pleiotropy.

Table 1: Serum Bioavailable Testosterone mediates the effect of Intake of Fat on the risk of PCa

Exposure	Mediation	outcome	Total effect (β)	Direct effect A(β)	Direct effect B(β)	Mediation effect	Mediated Proportion (%)
Fat	Bioavailable Testosterone	PCa	0.5978	0.2777	0.1885	0.0524	8.8%

4. Discussion

In this MR analysis leveraging large-scale GWAS summary statistics, we identified a causal relationship between fat intake from macronutrients and PCa risk. There is statistically significant evidence suggesting that genetically predicted relative fat intake may increase PCa risk. Regarding micronutrients, genetic evidence indicates that vitamin B5, vitamin B12, and zinc might elevate PCa risk, whereas carotenoids exert a protective effect. Further investigation into the effect of fat intake on prostate cancer risk revealed no significant association between the metabolic products of fat (glycerol and various fatty acids) and PCa risk. Nonetheless, two-step MR analysis suggested that bioavailable serum testosterone acts as a mediator in the relationship between fat intake and prostate cancer risk.

Our findings indicate no correlation between the intake of proteins, carbohydrates, and sugars and PCa risk, aligning with prior research [11,44,45]. Our study found that fat intake increases the risk of prostate cancer, a finding consistent with several cohort and case-control studies [7,8]. However, some studies have reported no significant association between fat intake and prostate cancer risk [9,10]. These discrepancies may stem from various sources, including confounding factors from self-reported dietary questionnaires, recall bias, and reverse causation inherent in case-control studies.

Our findings on the relationship between micronutrients and prostate cancer largely corroborate those of a previous MR analysis [46]. With the exception of carotenoids, we identified no micronutrients that offer protective benefits against PCa, a conclusion that is in agreement with the outcomes of a systematic review [47]. Specifically, our analysis suggests that vitamin B5, vitamin B12, and zinc may elevate PCa risk. Conversely, we propose that carotenoids could diminish PCa risk, potentially by modulating cell growth patterns to thwart tumor cell proliferation and promote apoptosis [48]. These observations imply that not all micronutrients should be supplemented in populations at elevated risk for PCa, and the consumption of certain micronutrients might even need to be curtailed. Additional research is required to formulate clinical guidelines and elucidate potential underlying mechanisms.

In terms of glycerol and fatty acids, including saturated, unsaturated, omega-3, and omega-6 fatty acids, we found no link to PCa risk, aligning with previous MR analysis findings [49]. Our more conservative results may stem from the use of different instrumental variables and a rigorous SNP filtering process. Epidemiological studies have indicated that a high intake of saturated fatty acids (present in meat and butter) could decrease the survival rates of PCa patients, whereas unsaturated fatty acids (found in fish and plant oils) might lower PCa risk [50,51]. The disparities between our findings and those from observational studies may arise from the latter's vulnerability to residual confounding factors and reverse causality, or the challenges in accurately measuring fatty acid intake.

Our two-step MR analysis reveals that bioavailable serum testosterone (BT) acts as a mediator in the link between fat intake and prostate cancer (PCa), a novel insight not previously documented. The findings from this analysis indicate that elevated genetically predicted BT levels constitute a risk factor for PCa, whereas the influence of total testosterone (TT) and sex hormone-binding globulin (SHBG) on PCa appears to be limited. Prior studies have established that BT is more closely associated with specific androgen-dependent outcomes than TT [52]. The association between testosterone levels and PCa in observational studies has been contentious, often attributed to short follow-up durations, small sample sizes, and the varied ages of participants [53,54]. Our research addresses these limitations by bypassing the unmeasurable confounders typical in observational studies, thus providing compelling evidence that lifelong elevated genetically predicted BT levels (but not TT or SHBG) could indeed heighten PCa risk.

However, this study is not without limitations. Firstly, the PCa data derived from European populations may not fully extend to other ethnic groups, necessitating further analysis across diverse populations to validate the universality of our findings. Secondly, since our conclusions are predicated on genetic variations, they may be particularly relevant to cases of PCa with an inherent susceptibility. Thirdly, despite utilizing the most comprehensive GWAS dataset available for extracting genetic variations, the explained variance by the instrumental variables was modest, thereby constraining our capacity to discern weak to moderate associations for many dietary factors. Future research should revisit these analyses with larger GWAS datasets.

5. Conclusion

Our research confirms a significant association between fat intake and prostate cancer risk, presenting genetic evidence that bioavailable serum testosterone may mediate the impact of fat intake on PCa risk. Apart from carotenoids, which were shown to have a protective effect, no other micronutrient was found to confer benefits in preventing PCa.

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