REPORT
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BIOACTIVE COMPOUNDS OF FOOD AND RISK OF PROSTATE CANCER. SCIENTIFIC BASES FOR FUTURE DIETARY RECOMMENDATIONS

Dr Raúl Zamora Ros

IDIBELL Institut d'Investigació Biomèdica de Bellvitge

Dr Maria Cristina Andrés Lacueva

Facultat de Farmàcia i Ciències de l'Alimentació - UB Universitat de Barcelona

1. Abstract

Background: Prostate cancer is a heterogeneous disease, and it is important to identify associations especially with more advanced/aggressive tumors. There is evidence showing that bioactive compounds in foods, especially (poly)phenols, can have a preventive effect against prostate cancer. But epidemiological evidence regarding dietary (poly)phenols is still limited. Nutritional biomarkers are more precise measures than dietary questionnaires. Nutrimetabolomics provides quantitative and accurate data on biomarkers of food intake, microbiota activity, and cellular metabolism. To date, this is the first study evaluating the associations between biomarkers of bioactive compounds, which accurately reflect dietary patterns, and the risk of prostate cancer.

Objectives: To prospectively evaluate the associations between the intake of bioactive compounds in foods, especially (poly)phenols, the effect of microbiota activity, and prostate cancer risk, particularly in more advanced cases. Explore biomarkers of advanced prostate cancer and the metabolic pathways altered in the tumor development/progression.

Methods: The European Prospective Investigation in Cancer and Nutrition (EPIC) study is a cohort with 142,239 men from 8 countries. During the 14 years of follow-up, 7,036 cases of prostate cancer have been identified. (Poly)phenol intake is estimated using a validated dietary questionnaire and Phenol-Explorer. In an EPIC nested case-control of 891 cases and 981 matched controls, nutrimetabolomic analysis was performed using UPLC-QTRAP. This new methodology allows us the quantification of approximately 600 plasma metabolites (biomarkers of food intake and cellular metabolism). Survival models, supervised multivariate analyzes and ROC curves were used to identify dietary (poly)phenols and plasma metabolites related to prostate cancer risk.

Work plan: 1) Preparation of the EPIC study database; 2) To evaluate the associations between dietary (poly)phenol consumption and prostate cancer risk and its subtypes in the entire EPIC-Europe cohort; 3) Estimate the associations between glucosinolate consumption and the risk of prostate cancer and its subtypes in the entire EPIC-Europe cohort; 4) Analyze around 600 plasma metabolites (quantitative nutrimetabolomics) in samples from 891 prostate cancer cases and 8910 controls in a case-control study

nested in the EPIC-Europe cohort; 5) Investigate the associations between the profile of plasma metabolites and the risk of suffering from prostate cancer, especially the most advanced cases of the disease.

Impact: Epidemiological evidence is generated on the beneficial effects of the consumption of dietary bioactive compounds, especially (poly)phenols, and the microbiota activity on the prostate cancer risk. It is used to improve current dietary guidelines. Moreover, it is possible to discover biomarkers of advanced prostate cancer and altered metabolic pathways during the initiation/progression of that tumor in order to promote an improvement in the prognosis of the disease.

2. Results

The project "Bioactive compounds in foods and prostate cancer risk. Scientific bases for future dietary recommendations" is based on three sections:

1) Estimation of bioactive food compounds (polyphenols and glucosinolates) measured with dietary questionnaires and prostate cancer risk in the large epidemiological study: the EPIC cohort.

2) Analysis and quantification of around 600 metabolites, using nutrimetabolomics UPLC-QTRAP, in 891 couples in a case-control study nested in the EPIC cohort.

3) Estimation of food bioactive compounds quantified with biomarkers of dietary intake and microbiota activity (nutrimetabolomics) and prostate cancer risk in the nested case-control study within the EPIC cohort.

Dietary bioactive compounds and prostate cancer risk

Associations between intake of dietary (poly)phenol classes and subclasses and prostate cancer risk in the EPIC-Europe cohort were evaluated and no statistically significant associations were observed [1]. In total, 131,425 adult men from seven European countries, including Spain, participated in the EPIC cohort. During the 14 years of mean follow-up, 6,939 incident cases of prostate cancer were newly diagnosed, including subtypes by stage and severity of prostate cancer. Our results are

consistent with other previous results and with the WCRF/AICR report in which it is concluded that there is insufficient scientific evidence on any dietary factor (food or nutrient) that reduces the risk of developing prostate cancer.

A food composition table of glucosinolates has also been developed to evaluate their intake and their associations with prostate cancer risk. The table is finalized and is in the publication phase: "Development of an updated food composition database on glucosinolates". It includes data from 58 scientific articles with data on 27 glucosinolates in 24 foods (raw and cooked) [2]. Unfortunately, we have not yet had access to the EPIC database to calculate their intake, and therefore, we have not been able to calculate its associations with prostate cancer risk, because it is currently done remotely on a server of the International Agency for Research on Cancer (IARC/WHO) and this process is still very slow. Despite not being able to carry out dietary evaluations, they have been analyzed using nutritional biomarkers and the results are non-significant. Therefore, glucosinolates seem not to be associated with prostate cancer risk.

In addition, a perspective has been published on the importance of nutritional education, public health policies, and the availability of healthy foods, among others, to globally improve both diet and health at the population level [3]. This is very important because diet is a modifiable risk factor in many chronic diseases, including obesity, which in turn is a risk factor for many diseases, including prostate cancer prognosis.

Quantitative nutrimetabolomic analysis

Blood samples, plasma in this case, from 891 couples of prostate cancer cases and matched controls from the EPIC cohort were analyzed using a nested case-control design. The methodology used was validated by the nutrimetabolomics group of the University of Barcelona-CIBERFES. The metabolomic profile was adapted to the project, expanding the methodological coverage to include glucosinolates, bioactive compounds from food intake related to the project's objectives. Finally, 592 metabolites were included. After the application of strict quality controls, including the use of labelled standards, the preprocessing and filtering of the data which are essential stages to ensure the quality of the analytical data in experiments with metabolomic methodologies, 147 metabolites (45 from endogenous metabolism and 102 exogenous biomarkers from diet or environmental exposure) were selected. Exogenous

metabolites are those that come from the diet or environmental factors, such as bioactive compounds in the diet, and endogenous metabolites are those that are part of human metabolism. Using data from previous scientific studies, possible nutritional biomarkers of various subclasses of (poly)phenols and foods rich in (poly)phenols have been identified in the samples of this project. Firstly, it has been observed that urinary concentrations of catechin and epicatechin are moderately correlated with the intake of these bioactive compounds and slightly with their food sources, such as tea, chocolate, apple, red wine, and therefore, they can be considered nutritional biomarkers of them [4]. Moreover, the concentrations of hydroxytyrosol and tyrosol have been correlated with their intake and especially with the consumption of olive oil, wine and beer [5]. Finally, a review of nutritional biomarkers of legume intake has been published, highlighting that 478 phytochemicals, including 405 phenolic compounds, can be used to differentiate the consumption of different types of legumes [6].

Nutrimetabolomics and prostate cancer risk

This section is the main focus of the project and it is based on the investigation with the nutrimetabolomic data obtained, plasma concentrations of metabolites, in the case-control study nested in the EPIC-Europe cohort. As explained above, 592 metabolites have been analyzed, which after preprocessing and filtering resulted in 147 metabolites (45 endogenous and 102 exogenous) measured in 851 pairs of prostate cancer cases and matched controls. The cases were diagnosed during follow-up with an average time of 14 years. From these cases, we have information about the stage and severity of prostate cancer. So, blood samples were collected at baseline long before diagnosis. After statistical analysis, it was observed that 33 metabolites (9 endogenous and 24 exogenous) were associated with prostate cancer risk. Among the metabolites associated with a lower risk, the following stand out: 6-amino-5-(N-methylformylamino)-1-methyluracil; N-(2-hydroxyphenyl)acetamide sulfate; dihydroferulic acid 4-glucuronide; dihydroferulic acid 4-sulfate; gallic acid 4-sulfate. While those highlighted with associated with a greater risk are: 3,4-dihydroxybenzoic acid; trimethylamine N-oxide (TMAO); and cyclamate. Our results showed that metabolites derived from (poly)phenols, especially those coming from the microbiota, can reduce prostate cancer risk, highlighting the relevant role of the microbiota in this disease. On the other hand, the consumption of artificial sweeteners, such as cyclamate, and substances from the digestion of animal proteins such as TMAO may be associated with a greater risk and should, therefore, be considered in further studies.

These results are in the publication phase of the article entitled Prediagnostic plasma nutrimetabolomics and prostate cancer risk: A nested case-control analysis within the EPIC Study [7]. Due to the high-throughput methodology (as in any spectrometry technique, like nutrimetabolomics), results should be replicated in other observational studies before they can be generalized, and public health recommendations can be made.

In the preparation for the previous article, a complete literature search on metabolomics and prostate cancer in prospective epidemiological studies was carried out. This review includes 29 studies and highlights 42 metabolites statistically significant in at least two studies, such as citric acid, some amino acids (tryptophan, glycine, glutamine, sarcosine, among others) and some metabolism derivatives of lipids. Despite these promising results, the conclusion is that there is still no metabolite that increases the current diagnostic or prognostic value of prostate cancer. Despite that, more studies are necessary using analytical methods that measure other types of metabolites. The article is under review and is entitled "Pre-diagnostic endogenous and exogenous metabolites identified through large cohort studies conducted with prostate cancer patients. A narrative review" [8].

3. Relevance and possible future implications

Prostate cancer is the second most common cancer in men worldwide, with more than 1.4 million cases diagnosed in 2020. Despite its high incidence, it is a cancer with a high overall survival at 5 years, around 97%. However, prostate cancer is a very heterogeneous cancer, and it is still not known why some tumors are very aggressive, causing the death of 375,000 men from this disease in 2020. Therefore, prevention is still the best way to reduce the number of cases of prostate cancer.

Nowadays, there are few modifiable risk factors for prostate cancer, overweight and obesity are factors only for the advanced prostate cancer. There is also some evidence showing that the consumption of dairy products and calcium increases its risk, while low plasma concentrations of vitamin E and selenium (antioxidant and anti-inflammatory compounds) may decrease the risk of prostate cancer.

Phytochemical compounds, such as (poly)phenols and to a lesser extent glucosinolates, are bioactive compounds that have antioxidant, anti-inflammatory, anticancer effects, and in recent studies also anti-obesogenic effects. For these reasons, investigating associations between exposures to these compounds, using diet questionnaires and nutritional biomarkers (nutrimetabolomics), could have great potential in the prevention of prostate cancer.

The results using diet questionnaires were null. However, these results contradict some previous results in lower quality studies (small cases and controls) where flavonoids, one of the most abundant and relevant (poly)phenol classes, had been associated with a higher risk of prostate cancer. Therefore, the general dietary recommendations of a healthy diet rich in foods of plant origin (rich in (poly)phenols) are fully indicated for the global prevention of chronic diseases, including prostate cancer.

Our results using nutrimetabolomics are essentially positive and relevant in the prevention of prostate cancer; although, these results need to be replicated in other studies to increase the level of scientific evidence of the subjects. On one hand, it has been observed that several (poly)phenol metabolites derived from the microbiota have been associated with a lower prostate cancer risk. This gives us clues to the fundamental role played by the microbiota in the development of prostate cancer and other chronic diseases. It also highlights the importance of consuming foods rich in (poly)phenols and their possible effects on prostate cancer, but in order to appreciate these associations, the bioavailability of these compounds and possible synergisms between them would be taken into account. On the other hand, some metabolites were associated with a higher risk of prostate cancer, such as TMAO and cyclamate. TMAO is a metabolite that comes mainly from the digestion of proteins of animal origin (especially red and processed meat). These results could be incorporated into the recommendations for healthy diets in which a moderate consumption of animal foods, especially meat, is recommended, while the consumption of plant foods is encouraged. Cyclamate is an artificial sweetener. Although its acceptable daily intakes are high, it cannot be ruled out that the consumption of sweetened beverages increases the prostate cancer risk, directly or indirectly through increased obesity. This result is also already included in the healthy diet guidelines in which it is recommended to reduce the consumption of sugary beverages, and where sweetened beverages would not be the best option either.

In conclusion, we can indicate that our project has served to provide greater high-quality epidemiological evidence on dietary factors and primary prevention of prostate cancer. We highlight that the best dietary recommendations would be to follow a healthy diet, in our geographical area a Mediterranean diet, rich in foods of plant origin with high content of (poly)phenols, a moderate consumption of foods of animal origin, especially red and processed meat, and reduce the consumption of sugary and sweetened drinks. Our results increase the scientific evidence supporting the current general dietary recommendations for cancer prevention, and therefore would also be applicable for prostate cancer.

The impact of our results in primary prevention will help to reduce the number of cases of prostate cancer and other chronic diseases in the general population, if these recommendations were more actively advised from the field of public health.

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