Abdominal adiposity is not a mediator of the protective effect of Mediterranean diet on colorectal cancer

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Francesca Fasanelli *1*, Daniela Zugna *1*, Maria Teresa Giraudo *2*, Vittorio Krogh *3*, Sara Grioni *3*, Salvatore Panico *4*, Amalia Mattiello *4*, Giovanna Masala *5*, Saverio Caini *5*, Rosario Tumino *6*, Graziella Frasca *6*, Veronica Sciannameo *7*, 8 Fulvio Ricceri * *1, 7*, Carlotta Sacerdote* *1.*

1. Unit of Cancer Epidemiology, Department of Medical Sciences, University of Turin and Città della Salute e della Scienza University-Hospital Center for Cancer Prevention (CPO), Turin, Italy.

2 Department of Mathematics, University of Turin, Turin, Italy.

3 Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy.

4 Department of Clinical Medicine and Surgery, Federico II University, Naples, Italy.

5 Cancer Risk Factors and Lifestyle Epidemiology Unit Cancer Research and Prevention Institute - ISPO, Florence, Italy.

6 Cancer Registry, Department of Prevention, ASP, Ragusa, Italy.

7 Unit of Epidemiology, Regional Health Service, Grugliasco, Turin, Italy.

8 Department of Clinical and Biological Sciences University of Turin, Orbassano (Torino), Italy

* joint last authors.

Abstract

Adherence to the Mediterranean diet (MD) has a preventive effect on colorectal cancer (CRC). Several biological mechanisms have been hypothesized to explain this effect, but the involvement of clinical mediators has not been experimentally proven. We examined the role of abdominal adiposity (i.e. waist-to-hip ratio, WHR) as a potential mediator of the relationship between the MD and CRC in the Italian centres of the European Prospective Investigation into Cancer and Nutrition.

We evaluated the effect of the Italian Mediterranean Index (IMI) on WHR and of WHR on CRC risk. We then estimated the natural indirect effect (NIE, mediated by WHR) and the pure direct effect (PDE, unmediated) of IMI on CRC risk using mediation analyses, considering age, sex, education, physical activity, smoking, and EPIC centre as confounders.

Increased IMI was associated with significantly decreased odds of high WHR (hazard ratio [HR] for an IMI of 6-11 vs 0-1: 0.88, 95% confidence interval [CI]: 0.81-0.97). There was a positive relationship between WHR and CRC (HR for high vs low WHR: 1.34, 95%CI: 1.09-1.66). The total effect of IMI was protective on CRC risk and was mainly explained by the PDE (HR for an IMI of 6-11 vs 0-1: 0.51, 95%CI: 0.31-0.83), whereas the NIE was 1.00 (95%CI: 0.94-1.10). In this Mediterranean cohort, the protective effect of the MD on the development of CRC was not mediated by abdominal adiposity. Since this is the first study to investigate the mediating effect of abdominal obesity, other studies are needed to replicate this result.

Novelty and impact of the work

This analysis is the first attempt to generate rigorous evidence on possible biological mediators of the well-known relationship between the Mediterranean diet (MD) and colorectal cancer (CRC) risk. We concluded that abdominal adiposity is not a mediator of the association between the MD and CRC risk. In order to better explain the mechanisms of cancer development from a preventive point of view, we will undertake new research to evaluate other possible mediators of this relationship.

Introduction

Adherence to the Mediterranean diet (MD) has well-known beneficial effects on human health; studies conducted in different cohorts have shown an association between greater adherence to the MD and a reduced risk of mortality and cancer incidence (1-2). Several studies have evaluated the relationship between the MD and colorectal cancer (CRC) risk. In particular, previous studies from the European Prospective Investigation into Cancer and Nutrition (EPIC) showed that increased adherence to the MD was associated with a significantly decreased risk of CRC in men and women (3-4). Furthermore, a recent pooled analysis of three Italian case-control studies also reported that adherence to the MD played a favourable role in CRC risk (5).

Obesity is a well-known risk factor for chronic diseases, specifically CRC (6-7). The results of several studies, including EPIC, have suggested that, in addition to total body adiposity (approximated by body mass index, BMI), visceral abnormalities (approximated by waist-to-hip ratio, WHR) may play a crucial role in the onset of colon cancer (8-13). Indeed, abdominal adiposity is considered to be more closely related to insulin resistance than total body fat. In turn, insulin resistance can lead to hyperinsulinemia, which may stimulate the proliferation of colon cancer cells. As such, insulin resistance is one of the proposed mechanisms linking adiposity to cancer (14). BMI and/or WHR have also shown an inverse relationship with the MD in some (15-18), but not all, studies (19-20).

Since no specific clinical mediators or biological patterns have been experimentally proven to be involved in the relationship between the MD and CRC, and given that adiposity, particularly abdominal adiposity, has been related to both

CRC and the MD, we investigated whether the association between the MD and CRC is mediated by abdominal adiposity.

Materials and Methods

A total of 47,745 volunteers were recruited from five centres in Italy (Varese, Turin, Florence, Naples, and Ragusa) in 1993-1998 within the framework of EPIC. We excluded all participants with prevalent cancers except non-melanoma skin cancer (N=576), those with missing information on diet, anthropometry, or lifestyle (N=4059), and those with implausible energy intake (N=216). This left 42,894 participants in our study sample.

Over a mean follow-up of 11.0 years, 414 cases of CRC were diagnosed (311 colon cancers and 103 rectal cancers). Colon cancers were primary incident cases, identified as proximal (International Classification of Diseases for Oncology, 3rd Edition [ICD-O-3] codes C18.0-C18.5; N=121), distal (ICD-O-3 codes C18.6-C18.7; N=154), and over-lapping or unspecified sites (ICD-O-3 codes C18.8-C18.9; N=36). Rectal cancers were identified by ICD-O-3 codes C19.9 and C20. The end of follow-up was 31 December 2006 for Varese, Florence, and Naples; and 31 December 2008 for Turin and Ragusa.

All participants in the study sample completed a validated semi-quantitative food-frequency questionnaire at enrolment, which was designed to capture the frequency of consumption of food items and eating behaviour in the 12 months prior to enrolment. We used the Italian Mediterranean Index (IMI) (21) as a summary measure of adherence to the MD. Briefly, this index is calculated based on the intake of 11 food items: high intakes of pasta, Mediterranean vegetables (raw tomatoes, cooked leafy vegetables, raw leafy vegetables, onion or garlic, mixed salad, or mixed vegetables), fruits, legumes, olive oil, and fish (if consumption is in the 3rd tertile of the distribution, the person receives 1 point for each item, otherwise this value is set

at 0); low intakes of soft drinks, butter, red meat, and potatoes (if consumption is in the 1st tertile of the distribution, the person receives 1 point for each item, otherwise this value is set at 0); and alcohol consumption (a person receives 1 point for consumption up to 12 g/day; abstainers and people who consume more than 12 g/day receive 0). The corresponding points for these food items are summed to determine the IMI, which can range from 0-11, with increasing IMI indicating better adherence to the MD.

Upon recruitment into EPIC, self-administered questionnaires were used to collect information on lifestyle factors, and trained staff took anthropometric measurements such as weight (to the nearest 0.1 kg), height (to the nearest 0.5 or 1.0 cm), waist and hip circumferences (in cm), and blood pressure. BMI was calculated as weight in kg divided by height in m^2 (kg/m^2). Waist circumference was measured at the midpoint between the lower ribs and the iliac crest. Hip circumference was measured over the buttocks. WHR was calculated as waist circumference divided by hip circumference.

The possible confounders included in the analysis were age, sex, education level (tertiles of the relative index of inequality (22), a standardized and validated index that allows researchers to avoid residual confounding due to differences in the distribution of education levels determined by sex, birth cohort, and geographical area – the first tertile is for higher education levels), total physical activity (i.e., occupational and recreational physical activity measured according to a validated physical activity index: inactive, moderately inactive, moderately active, and active), and smoking status (never, former, and current smokers).

Statistical analysis

Descriptive statistics of CRC risk factors were reported using medians and percentages according to the IMI categories (0-1, 2-3, 4-5, and 6-11). The receiver operating characteristic curve was used to determine the optimum cut-off point of WHR for predicting CRC among men and women separately, and the best cut-off point was chosen using Youden's Index (i.e., the value that maximizes the quantity "sensitivity+specificity-1"). These cut-off values were then used to create the binary variable "high WHR" (participants below the cut-off were categorized as "no"; those above the cut-off were categorized as "yes").

A logistic regression model was used to estimate odds ratios of high WHR comparing the three highest IMI categories to the lowest one. Two different models were fitted: model A was adjusted for age, sex, and centre; and model B was adjusted for all the covariates in model A plus education level, total physical activity, and smoking status. The analysis was performed for the whole cohort and for men and women separately (first the interaction between sex and IMI score was tested by including the cross-product interaction term in the model and then using the Wald test to assess the statistical significance). Linearity of trends across IMI categories was tested by considering the categorical variable as a continuous variable in the logistic model.

A Cox regression model was fitted to estimate hazard ratios (HRs) of CRC comparing high WHR versus low WHR. Three different models were fitted: model A was adjusted for age and sex and stratified by centre; model B was adjusted for all the covariates in model A plus total physical activity and smoking status and stratified by centre and education level; and model C was adjusted for all the covariates in

model B plus IMI category and stratified by centre and education level. The Schoenfeld residuals test was used to assess the proportional hazard assumption. Sex-WHR interactions and IMI-WHR interactions were tested by including the cross-product interaction term in the model. The analysis was performed for the whole cohort and for men and women separately. Sensitivity analyses that considered only the first 2 years of follow-up were carried out to avoid reverse causality.

Mediation analysis

A mediation analysis was used to understand how much of the effect MD has CRC risk can be explained by WHR, using the weighting approach developed by Vanderweele and Vansteelandt (23). This method was chosen because it can be extended to survival outcomes without requiring the assumption of a rare outcome. We assumed that WHR measures would temporally follow overall adherence to the MD. This assumption was reasonable since the dietary questionnaire on which IMI scores were based refers to the previous 12 months, while WHR was based on measurements taken at enrolment.

We refer to the indirect effect as natural indirect effect (NIE) and to the direct effect as pure direct effect (PDE) (24). On the multiplicative scale, it has been shown that the total causal effect (TCE) is equal to the product of the NIE and the PDE (25). Identification of these effects requires several assumptions, including no unmeasured exposure-mediator, mediator-outcome, and exposure-outcome confounding; and no effect of any exposure that may confound the mediator-outcome relationship (24). We used sensitivity analysis formulae on HR scales (26) to evaluate bias in the estimates of PDE and NIE due to unmeasured

confounding of exposure-outcome and mediator-outcome associations. All analyses were performed using Stata version 13 (StataCorp, College Station, TX, USA).

Results

No crude association was evident between IMI category and WHR, and there was a suggestion of an inverse association with BMI. The percentage of women who adhered to the MD was slightly greater than that of men; and those recruited in the Naples centre fell into higher IMI categories than did participants from other centres. Subjects in different IMI categories were also different in terms of education level, physical activity, and smoking status (Table 1).

The receiver operating characteristic analysis identified the values 0.95 for men and 0.78 for women as the optimum WHR cut-offs to predict CRC (area under curve 0.59 in men and 0.55 in women). Increasing IMI category was associated with significantly decreasing odds of high WHR in the study sample (p-trend=0.003 in model B); all three higher IMI categories were associated with reduced odds of high WHR, but the association was significant only for an IMI of 6-11 vs 0-1. In men no association was found, whereas for women all three higher IMI categories were significantly associated with a decrease in odds when compared to the lowest IMI category. The p-value of the interaction between sex and IMI was nearly significant (p=0.051) (Table 2 Panel A).

Participants with high WHR seemed to be at higher risk of developing CRC (HR: 1.34, 95% CI: 1.09-1.66 in model C). The interactions between sex and WHR and between IMI and WHR were not statistically significant (p-value=0.131 and p-value=0.705, respectively). However, stratification by sex revealed stronger associations in men compared to women. In particular, in women the direction of the associations appeared to confirm the results obtained in the whole cohort, but the HRs were not significant (Table 2 Panel B).

Since IMI was strongly associated with CRC risk, we evaluated how much of this effect could be explained via WHR, which is also associated with CRC risk. Our mediation analysis showed that when an IMI of 6-11 was compared to an IMI of 0-1, the TCE on CRC risk was 0.51 (95% CI: 0.31-0.85), the PDE was 0.51 (95% CI: 0.31-0.83), and the NIE (i.e., the effect mediated by WHR) was 1.00 (95% CI: 0.94-1.10). Sensitivity analyses on the association between unmeasured confounding factors and the exposure, the mediator, and the outcome supports the conclusion that a substantial portion of the effect was direct (Table 3).

Discussion

The results of this study suggest that the MD has a beneficial effect on CRC risk in a Mediterranean population, but it has a pathway that is independent from abdominal obesity. A previous study on the Italian EPIC cohort published by our research group showed that increased IMI was associated with a significantly decreased CRC risk (4). This was consistent with several previous observational studies on cancer (2, 27), and in particular on colon cancer (3, 5, 28-29).

So far, the proposed biological mechanisms of the MD in cancer prevention have been related to the favourable effect of a balanced ratio of omega 6 and omega 3 essential fatty acids and the high amount of fibre, antioxidants, and polyphenols that can be found in fruit, vegetables, and olive oil, which can attenuate pro-inflammatory mediators (30). Another proposed mechanism is the opposition to the adipocyte-mediated chronic low-grade inflammation represented by the acute-phase C-reactive protein, which is particularly active in overweight subjects (31). In fact, adherence to the MD has been suggested to be protective against obesity (32).

In our Mediterranean population, higher adherence to the MD was associated with lower abdominal adiposity (as measured by WHR) but not with lower BMI. This is in agreement with a previous paper by Romaguera et al (17) that looked at the whole European EPIC cohort and used a modified Mediterranean Index.

There are several physiological explanations as to why adherence to the MD might protect against abdominal adiposity. Firstly, the MD is rich in dietary fibres, a nutrient group that increases the feeling of satiety (33). Furthermore, the MD has a low energy density, low glycaemic load, and high water content compared to other Western dietary patterns (32); all these characteristics could

lead to a reduction in weight gain in adulthood. In the Italian EPIC cohort, in agreement with other previous studies (19-20), the association between IMI and anthropometric measures was evident only for WHR, not for BMI or waist circumference alone (data not shown). Overweight is a major problem in several Mediterranean countries, such as Greece, Spain, and Italy, in which an important proportion of population showed good IMI scores (21). Recent studies demonstrated that the beneficial effect of the MD is not evident in general subcutaneous fat, but it is clearly associated to visceral abdominal tissue, which can be measured by WHR (15).

The association between abdominal obesity and CRC has been assessed in the present study and in previous studies (8). The hypothesized biological mechanisms involved in this association are related to insulin resistance and metabolic dysfunction (3). WHR is positively correlated with insulin resistance, hyperinsulinaemia, and chronic inflammation, which all play a role in carcinogenesis (3).

We found a clear relationship between the MD and WHR, and between WHR and CRC, both in previous studies and in the present report. This makes a strong case that WHR may be a mediator in the association between MD and CRC risk. Nevertheless, in the present study abdominal adiposity was not a mediator of the association between MD and CRC risk.

In a previous, large case-control study conducted in another Italian population, Grosso et al reported that the protective effect of the MD on CRC risk was evident even among obese patients (28). This finding addresses the possible important role of biological

mechanisms other than adiposity that could mediate the beneficial effect of the MD on CRC risk.

Intervention studies have showed that MD has a beneficial effect on several metabolic and inflammatory risk factors (such as fasting blood glucose, C-reactive protein, etc.) (34). Other observational studies have reported that the MD is associated with a reduced incidence of metabolic syndrome (35) and low inflammation levels (35). In particular, olive oil, an important food in the MD, attenuated the expression of pro-inflammatory genes cyclooxygenase-2 (34).

This analysis is the first attempt to assess the mediating role of adiposity in the well-known relationship between the MD and CRC. Further research efforts must be undertaken in order to evaluate other possible mediators of the relationship between MD and CRC, such as inflammation, fasting blood glucose, or antioxidants, either alone or in combination. Indeed, knowledge of biological mediators is fundamental to the exploration of mechanisms of cancer development, but also to the planning of prevention strategies. We found that adherence to the MD and WHR act on CRC risk through different biological pathways. This suggests that public health interventions for CRC should focus on both greater adherence to the MD and maintaining a correct body weight,

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