Nitrosyl-heme and Heme Iron Intake from Processed Meats and Risk of Colorectal Cancer in the EPIC-Spain Cohort

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ABSTRACT

Background: The International Agency for Research on Cancer classified processed meats (PM) as "carcinogenic" and red meat as "probably carcinogenic" for humans. The possible relationship between colorectal cancer risk and the mechanisms involved in the carcinogenesis of PMs have not been established yet. Nitrosyl-heme and heme iron have been proposed as potential-related compounds. The aim of this study was to determine the association between nitrosyl-heme and heme iron intake and colorectal cancer risk among participants from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Spain study.

Methods: This prospective study included 38,262 men and women from the EPIC-Spain study. Food consumption was assessed using diet history and food composition tables. Heme iron and nitrosyl-heme intake were determined by estimating the intake of PM items and conducting laboratory analyses. HR estimates were obtained by proportional hazard models, stratified by age at recruitment and study center and adjusted for sex, total energy intake, education, smoking, body mass index, waist size, physical activity, lifetime alcohol, fibre, calcium, and familiar colorectal cancer history.

Results: During a mean follow-up of 16.7 years, 577 participants were diagnosed with colorectal cancer. We found no overall association between nitrosyl-heme [HR_{T3vsT1}, 0.98; 95% confidence interval (CI), 0.79–1.21] or heme iron intakes (HR_{T3vsT1}, 0.88; 95% CI, 0.70–1.10) with colorectal cancer risk, nor according to tumor subtypes.

Conclusions: Our study found no evidence supporting a link between nitrosyl-heme or heme iron intake and colorectal cancer risk in Spanish subjects.

Impact: As research on nitrosyl-heme is preliminary, more heterogeneous studies are necessary to provide more convincing evidence on their role in colorectal cancer carcinogenesis.

Introduction

Colorectal cancer is the third most common type of cancer in adults worldwide with a high number of new cases and deaths annually. In 2022, Spain estimated almost 40,000 new colorectal cancer cases, becoming the most frequently diagnosed tumor, ranking as the second most common for both men and women (1). The International Agency for Research on Cancer (IARC) classified processed meats (PM) as "carcinogenic" and red meat as "probably carcinogenic" for colorectal cancer (2). Various mechanisms have been suggested to explain the link between red and PM consumption and colorectal cancer risk. Nevertheless, their specific key compounds remain unclear. Nitrosyl-heme has been suggested as a potential related compound (3). The aim of this study was to determine the association between nitrosyl-heme and heme iron intake and colorectal cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) Spain cohort.

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Materials and Methods

Between 1992 and 1996, 41,437 healthy volunteers (ages, 25–70) from five Spanish regions were enrolled in the EPIC-Spain cohort study. Questionnaire information on sociodemographic characteristics, lifestyle factors, and medical history was gathered during personal interviews. Dietary intake was assessed through a validated diet history questionnaire (EPIC-DH; ref. 4).

To estimate the exposure to nitrosyl-heme and heme iron, we used biochemical data from a previous study from our group (5) which included a total of 52 PM items from EPIC-DH questionnaires. Food and nutrient intake, including PMs consumption, were estimated using the EPIC Nutrient Database (6). Finally, nitrosyl-heme and heme iron intake was estimated by multiplying this information by PMs by each subject's daily food intake in the cohort.

We included 38,262 individuals (61.5% females) after excluding prevalent cancer cases ($n = 612$) and incomplete/improbable dietary data ($n = 695$). Among them, 577 incident colorectal cancer cases were identified after a mean follow-up of 16.7 years. Proportional hazards models were used to examine the association between sex-specific tertiles of nitrosyl-heme and heme iron intake and colorectal cancer risk. Age served as the time scale, stratified by age and center with adjustments for sex, energy intake, body mass index (BMI), waist size, education, smoking, physical activity in mets (PA), lifetime alcohol, dietary fibre, calcium, and family colorectal cancer history. Interactions with smoking, BMI, PA, and alcohol were also examined. Homogeneity of location subtype risk was assessed. Sensitivity analyses were conducted excluding the first three years of follow-up, but the results remained unchanged. R studio (4.1.3) was used for analysis, with significance set at $P < 0.05$.

The study has been performed in accordance to the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments and obtained ethical approval for the EPIC study was obtained from the ethical review board of the International Agency for Research on Cancer (IARC) ethics committee (PR098/21) and the local ethics committees in the participating centers. All participants gave written informed consent.

Data availability

EPIC data and biospecimens are accessible to researchers addressing significant inquiries regarding health and disease within projects aligning with the legal and ethical standard practices of IARC/WHO and the EPIC centers. The primary responsibility for data access rests with IARC and the EPIC centers. Requests should be sent to the corresponding author.

Results

No statistically significant association was observed between nitrosyl-heme and heme iron intake and colorectal cancer risk or subtypes (**Table 1**). In multivariate models, the HR for highest versus lowest

Table 1. Adjusted HR for full cohort and colorectal cancer risk by sex-specific tertiles of heme iron and nitrosyl-heme intake.

	Nitrosyl-heme[a,b]			Heme iron[c,d]		
	Cases	HR (95% CI)	HR (95% CI)	Cases	HR (95% CI)	HR (95% CI)
Colorectal cancer risk[e]		Basic model[f]	Multivariate model[g]		Basic model[f]	Multivariate model[g]
Full cohort = 38,262[h]	577			577		
T1	214	Reference	Reference	204	Reference	Reference
T2	169	0.84 (0.68–1.03)	0.82 (0.67–1.01)	197	1.01 (0.82–1.23)	0.98 (0.81–1.20)
T3	194	1.04 (0.84–1.28)	0.98 (0.79–1.21)	176	0.93 (0.75–1.16)	0.88 (0.70–1.10)
Proximal colon cancer risk[i]						
T1	49	Reference	Reference	50	Reference	Reference
T2	38	0.85 (0.55–1.31)	0.83 (0.54–1.28)	42	0.89 (0.58–1.34)	0.86 (0.57–1.31)
T3	44	1.11 (0.71–1.73)	1.03 (0.65–1.61)	39	0.87 (0.55–1.38)	0.80 (0.51–1.29)
Distal colon cancer risk[i]						
T1	72	Reference	Reference	65	Reference	Reference
T2	53	0.78 (0.54–1.12)	0.77 (0.53–1.10)	65	1.07 (0.75–1.52)	1.04 (0.73–1.48)
T3	64	1.05 (0.73–1.52)	0.98 (0.67–1.42)	59	1.02 (0.70–1.50)	0.95 (0.65–1.41)
Rectum cancer risk[e]						
T1	59	Reference	Reference	59	Reference	Reference
T2	61	1.14 (0.79–1.64)	1.10 (0.76–1.58)	66	1.18 (0.83–1.69)	1.16 (0.81–1.66)
T3	55	1.12 (0.75–1.66)	1.04 (0.70–1.56)	50	0.90 (0.60–1.35)	0.84 (0.55–1.26)

[a]Nitrosyl-heme (μg/day) for women: T1, 0–≤321.21; T2, >321.21–≤739.91; T3: >739.91; Nitrosyl-heme (μg/day) for men: T1, 0–≤579.72; T2, >579.72–≤1370.54; T3: >1,370.54.

[b]Person-years by nitrosyl-heme tertiles: T1, 204,237.4 person-years; T2, 203,347.7 person-years and T3, 203,068.9 person-years.

[c]Heme iron (μg/day) for women: T1, 0–≤882.0; T2, >882.0–≤2,341.41; T3: >2,341.41; Heme iron (μg/day) for men: T1, 0–≤1,652.30; T2, >1,652.30–≤2,341.41; T3: >3,950.97.

[d]Person-years by heme iron tertiles: T1, 202,420.2 person-years; T2, 203,479.1 person-years and T3, 204,754.8 person-years.

[e]$P_{homogeneity}$ colon versus rectum = 0.12 and 0.51 for nitrosyl-heme and heme iron intake, respectively.

[f]Basic model: stratified by age and center, and adjusted for sex and energy intake.

[g]Multivariate model: basic model and further adjusted by: BMI (≤25, 25–30, ≥30 kg/m^2), waist size (low:<88 cm for women and <102 cm for men; high: ≥88 cm for women and ≥102 cm for men), educational level (none, primary school, technical/professional school, secondary school, longer education, and unknown), smoking status (never, former, and current), physical activity in mets (as continuous variable), lifetime alcohol consumption (as continuous variable), fiber (as continuous variable), calcium (very low: ≤650.3 mg/day; low: >650.3 mg/day–<1,000 mg/day and normal: ≥1,000 mg/day) and family history (mother/father/siblings; no; no response).

[h]Each tertile is divided by the same number of participants ($n = 12,754$), varying in the number of cases per tumor location.

[i]$P_{homogeneity}$ proximal versus distal = 0.77 and 0.37 for nitrosyl-heme and heme iron intake, respectively.

tertile of intake was 0.98 (95% CI, 0.79–1.21) for nitrosyl-heme and 0.88 (95%CI, 0.70–1.10) for heme iron intake, respectively.

We found a nonlinear relationship between nitrosyl-heme intake and colorectal cancer risk, with weaker associations at higher intakes. Despite no heterogeneity was observed in the associations between nitrosyl-heme and heme iron intake and the risk of colorectal cancer by tumor subtype, a nonstatistically significant positive association between nitrosyl-heme intake and proximal colon and rectum cancer was observed (for proximal colon cancer, HR = 1.03; 95% CI, 0.65–1.61; and for rectum cancer, HR = 1.04; 95% CI, 0.70–1.56). There were no relevant interactions between nitrosyl-heme and heme iron and smoking status, BMI, PA, nor lifetime alcohol consumption on colorectal cancer risk.

Discussion

To the best of our knowledge, this is the first prospective study that analyzed the exposure of nitrosyl-heme in relation to the risk of CRC using analytical data on their content for the most consumed PMs.

We observed no overall association between nitrosyl-heme and colorectal cancer risk, neither by subtypes. Only one prospective study has studied the relationship between nitrosyl-heme intake and colorectal cancer risk, whose results showed a positive association with proximal colon cancer (7). However, the study assigned a constant coefficient for the nitrosyl-heme content for all PMs (0.67). In contrast, our study is the first to present nitrosyl-heme data based on direct content measurements in PMs, analyzing a wide variety of them.

Regarding heme iron, a greater number of studies investigated its associations to colorectal cancer risk. Prior analysis in EPIC-Europe (8) revealed a positive association limited to the proximal colon for heme iron intake. However, our study, possibly due to different methodology, did not find a significant link between these subtypes and heme iron intake.

Concerning limitations, diet and lifestyle variables were measured only at the beginning; changes over the nearly 17-year follow-up cannot be assessed. Regarding the strengths, it includes data on nitrosyl-heme and heme iron derived from direct laboratory measurements in PMs, its prospective design and the inclusion of potential confounders.

In summary, our findings do not support the hypothesis that nitrosyl-heme is associated with the risk of developing colorectal cancer. Given the plausibility of the biological mechanism between nitrosyl-heme, heme iron intake, and the risk of colorectal cancer, additional research with heterogeneous cohorts is warranted, especially considering the currently scarce evidence on this topic.

Authors' Disclosures

No disclosures were reported.

Authors' Contributions

L. Rizzolo-Brime: Conceptualization, data curation, formal analysis, funding acquisition, visualization, methodology, writing–original draft, writing–review and editing. **L. Lujan-Barroso:** Formal analysis, methodology, writing–review and editing. **A. Farran-Codina:** Writing–review and editing. **R. Bou:** Writing–review and editing. **C. Lasheras:** Writing–review and editing. **P. Amiano:** Writing–review and editing. **A. Aizpurua:** Writing–review and editing. **M. Sánchez:** Writing–review and editing. **E. Molina-Montes:** Writing–review and editing. **M. Guevara:** Writing–review and editing. **C. Moreno-Iribas:** Writing–review and editing. **A. Gasque:** Writing–review and editing. **M.D. Chirlaque-López:** Writing–review and editing. **S. Colorado-Yohar:** Writing–review and editing. **J. Huerta:** Writing–review and editing. **R. Zamora-Ros:** Writing–review and editing. **A. Agudo:** Writing–review and editing. **P. Jakszyn:** Conceptualization, supervision, funding acquisition, methodology, writing–review and editing.

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