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Intake of processed meat, but not sodium, is associated with risk of colorectal cancer: evidence from a large prospective cohort and two-sample Mendelian randomization

Qi Feng[1], Sunny H Wong[2], Jie Zheng[3], Qian Yang[3], Joseph JY Sung[2], Kelvin KF Tsoi[1, 4*]

1. Division of Epidemiology, School of Public Health and Primary Care, The Chinese University of Hong Kong
2. Department of medicine & Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong
3. MRC Integrative Epidemiology Unit, Bristol Population Health Science Institute, The University of Bristol
4. SH Big Data Decision Analytics Research Centre, The Chinese University of Hong Kong

*: corresponding author (Kelvin KF Tsoi, Division of Epidemiology, School of Public Health and Primary Care, The Chinese University of Hong Kong; Email: kelvintsoi@cuhk.edu.hk)

Abstract

Background & Aims: Processed meat and high sodium intake are common in Western diet. The objective was to examine their independent effects on the risk of colorectal cancer (CRC).

Methods: We performed both observational analysis with UK Biobank and genetic analysis with Mendelian randomization (MR). The 24-hour urinary sodium (UNa) and reported intake of processed meat were fitted on incident CRC by multivariable Cox proportional hazard model, adjusted for covariates, such as age, gender, family history, etc. Different sodium measures were used for sensitivity analyses. Two-sample MR analyses were performed using summary data from genome-wide association studies of UNa and CRC. Multivariable MR was adjusted for body mass index.

Results: We included 415 524 eligible participants from UK Biobank. During a median follow-up of 11.1 years, 2 663 participants were diagnosed with CRC. High intake of processed meat independently increased risk of CRC by 23% (HR 1.23; 95% CI: 1.03 to 1.46), but 24-hour UNa was not significantly associated with CRC (HR 0.96; 95% CI: 0.87 to 1.06). Furthermore, MR also showed little evidence for the effect of UNa on CRC (OR 1.02; 95% CI: 0.11 to 9.42). Sensitivity analyses showed consistent results across different measurements of sodium intake.

Conclusions: Intake of processed meat had an independent effect on the risk of CRC, but the risk was not associated with sodium level. Reduction of processed meat intake may be an effective strategy for CRC prevention, while sodium reduction should still be recommended to achieve other health benefits.

Keywords: Sodium intake; urinary sodium; processed meat; colorectal cancer; UK Biobank; Mendelian randomization

Introduction

Colorectal cancer (CRC) is one of the most prevalent cancers worldwide. GLOBOCAN 2018 estimated CRC contributed 10.2% of cancer incidence and 9.2% of cancer mortality among all cancer types [1]. Numerous lifestyle factors have been noted to increase CRC risk, including obesity, smoking, physical inactivity, insufficient intake of fresh vegetables and fruits, etc. [2–4]. Excessive consumption of processed meat has been regarded as a convincing cause of CRC [5]. Increased CRC risk has been observed in people with high processed meat intake consistently in large prospective cohorts [6,7] and meta-analyses [8,9].

Since high sodium intake has shown both direct and indirect carcinogenic effects on gastric cancer [10–13], studies have hypothesized similar effects on other gastrointestinal cancers, such as CRC, but current evidence remains unclear. Two case-control studies found increased CRC risk in people with high dietary sodium intake [14] and in people who usually add extra salt to food [15], while other studies failed to observe similar significant associations [16,17]. Although a recent qualitative systematic review found seven studies with inconsistent results, the majority favored a positive association [18]. However, the methodological quality of these studies was limited by small sample size, unclear temporality and residual confounding.

Processed meat generally has high sodium concentration [19]. Intake of processed meat is closely correlated with sodium intake and is a big contributor for total sodium intake in typical Western pattern diet [20,21]. This complicated the relationship between processed meat intake, sodium intake and CRC risk. It remains unclear whether high sodium intake contributes to the effect of processed meat intake on CRC. The objective of this study was to examine the independent effects of processed meat intake and sodium intake on CRC; the potential results could be used to inform primary prevention of CRC. We aimed to provide both observational

and genetic evidence (Figure 1) [22]. The observational evidence was generated from UK Biobank cohort, and the genetic evidence from Mendelian randomization (MR) analysis.

Method

This study included two independent parts of data analysis, that is an observational analysis using UK biobank and a genetic analysis using MR. The overall methodology flowchart was shown in Figure 1.

a) Observational analysis

Data source and participants

UK Biobank is a large prospective population-based cohort. Half million participants aged 40 to 69 years old were recruited during year 2006 to 2010. Baseline data were collected from each participant, using questionnaire, physical examination and biochemical analysis of blood, urine and saliva samples. The baseline questionnaire collected information on sociodemographic characteristics, family history, health status, comorbidities, medication use, reproductive and environmental exposures and lifestyle (smoking, drinking, physical activity, dietary factors, use of nutrition supplements, etc.). Physical examination measured anthropometric traits (e.g., body weight, body height, waist circumference, etc.) and blood pressure, all with standardized procedures. Clinical outcomes during follow-up were extracted from cancer and death registries. UK Biobank was approved by the North West Multicenter Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and Community Health Index Advisory Group in Scotland. All participants have provided informed consents. The UK Biobank protocol is available online (https://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf). We excluded those who were lost to follow-up (n = 1 298), pregnant at baseline (n = 370), reported

clinically-diagnosed cancer (other than non-melanoma skin cancer) at baseline (n = 41 248) and had missing data on exposure and other covariates (n = 44 054). Therefore, among all 502 494 participants, 415 524 were eligible and included (Figure 1).

Exposure

The primary exposure variables were 24-hour urinary sodium (UNa) and processed meat intake. Because dietary sodium intake was not directly measured in UK Biobank, we used 24-h UNa as its substitute, as used in previous studies [23,24]. 24-h UNa was estimated from spot UNa, urinary creatinine (UCr), body mass index (BMI) and age using the gender-stratified INTERSALT equation, which was developed and validated in Western population [25]. It has been shown to be less biased compared to other equations [26,27], and has been used in UK Biobank data previously [28]. Intake of processed meat (such as bacon, ham, sausages, meat pies, kebabs, burgers, chicken nuggets) was measured from baseline questionnaire and coded as a categorical variable (never, ≤ once a week and ≥ twice a week).

For sensitivity analyses, we used other substitute measures for dietary sodium intake, including spot UNa, urinary sodium-to-potassium (UNa/UK) ratio, urinary sodium-to-creatinine (UNa/UCr) ratio and adding salt to food. We dichotomized continuous 24-h UNa, spot UNa, UNa/UK ratio and UNa/UCr ratio using their median values as cutoff. Urinary sodium and potassium were measured with ion selective electrode analysis from participants' urine sample using Beckman Coulter AU5400, while UCr was measured with enzymatic analysis. Adding salt to food was measured with a question ("Do you add salt to your food?") in baseline questionnaire and categorized as never/rarely *vs*. usually/always.

Outcomes

The primary outcome was incident CRC (ICD-10 code: C18 - C20) during follow-up, while the secondary outcomes included incident colon cancer (ICD-10 code: C18) and incident rectal cancer (ICD-10 code: C19 - C20). Outcome data were extracted via linkage to cancer registries: the Medical Research Information Services for participants in England and Wales, and the Information Services Division for participants in Scotland. The time to outcome was calculated from the attendance date at the baseline assessment center to the date of outcome diagnosis, the date of death or the last date of follow-up (31 March 2016 for England and Wales, 31 October 2015 for Scotland), whichever was the earliest.

Covariate

We included sociodemographic, lifestyle, health status, medication use and dietary factors as covariates. Sociodemographic variables included age, gender, ethnicity (White, Black, Asian, Mixed and others), Townsend deprivation index (in quintiles), educational attainment (below college, college and above). Townsend deprivation index is a measure of material deprivation, calculated for each area based on the national census data, and each participant was assigned a Townsend deprivation index score corresponding to the area in which their postcode was located at baseline. CRC family history referred to the presence of diagnosed CRC on the participant's mother, father or siblings. Smoking and drinking status were coded as never, previous or current regular users. Physical activity was categorized to low, moderate and high levels based on their weekly metabolic equivalent values measured with the International Physical Activity Questionnaire. BMI was assessed in physical examination and calculated as body weight in kilogram divided by height square in meter. Estimated glomerular filtration rate (eGFR) was calculated based on serum cystatin C concentration with the gender-specific CKD-EPI cystatin C equations [29], as used in previous research [30]. Regular use of aspirin and/or ibuprofen was self-reported. The weekly intake frequencies of processed meat, fresh fruits,

fresh vegetables, cooked vegetables, oily fish, red meat and cereal (dietary fiber) were measured with baseline questionnaire. Intakes of unprocessed beef, lamb/mutton and pork were combined as red meat intake [31]. All answers of "prefer not to say", "do not know", "unknown" were combined as a category of "unknown".

Statistical analysis

For descriptive statistics, mean with standard deviation was used for continuous variables and frequency with proportion was for categorical variables. We compared the 24-h UNa across different levels of processed meat intake, and evaluated their association in a multivariable logistic regression with processed meat intake as independent variable and 24-h UNa as dependent variable, adjusted for age, gender, ethnicity, smoking, drinking, physical activity, BMI, eGFR, intake of fresh fruits, fresh vegetables, cooked vegetables, oily fish, red meat and cereal.

We quantified the association between the exposure and CRC risk using multivariable Cox proportional hazard model, stratified by gender, ethnicity and age (< 50, 50 - 60, ≥ 60 years old). Assumption of hazard proportionality was examined with scaled Schoenfeld residual plot, and was satisfied in this study. We fitted a first model with only processed meat intake, a second model with only 24-h UNa, and a third model with both processed meat intake and 24-h UNa. The above three models were adjusted for multiple covariates (educational attainment, Townsend deprivation index, CRC family history, physical activity, smoking, drinking, regular use of aspirin/ibuprofen, BMI, eGFR, intake of fresh fruits, fresh vegetables, cooked vegetables, oily fish, red meat and cereal), to reduce potential confounding biases. In sensitivity analysis, we replaced 24-h UNa with other sodium intake measures (spot UNa, UNa/UK ratio, UNa/UCr ratio and adding salt to food).

b) Two-sample MR

MR is a study design to make causal inference between an exposure and an outcome using genetic variants as instrumental variables [32]. We adopted two-sample MR approach [33] to examine the association between UNa and risk of CRC, colon cancer and rectal cancer. Genome wide association (GWA) studies of urine sodium, CRC, colon cancer and rectal cancer were identified to perform the MR analysis. GWA analysis is a statistical technique to investigate the association between a phenotype (e.g., a disease) and multiple genetic variants (usually millions of single nucleotide polymorphisms (SNPs)). Data on the effects of SNPs on phenotype were used for data analysis.

Data sources

We identified 50 SNPs associated with log-transformed spot UNa at a significance level of 5×10^{-8} from a GWA study of 446 237 unrelated European-ancestry individuals [34]. The 50 SNPs are located in different genes, distributed independently, and explain totally 6.4% of the trait variance (Supplementary table 1). GWA analyses of CRC, colon cancer and rectal cancer were conducted in 135 538 individuals from FinnGen cohort (1 573 CRC cases, 968 colon cancer cases and 521 rectal cancer cases) [35].

Since obesity was associated with both urinary sodium and CRC [2,36], multivariable MR adjusted for BMI was performed. A GWA study of 322 154 European-ancestry individuals from the Genetic Investigation of Anthropometric Traits (GIANT) consortium identified 98 SNPs significantly associated with BMI, which explained totally 2.5% of the trait variance [37]. For sensitivity analysis, similar to the observational analysis, we used UNa/UK ratio and UNa/UCr ratio as substitute measures for UNa. The GWA study of UNa/UK ratio and

198 UNa/UCr ratio was conducted in 327 616 unrelated European-ancestry individuals, which
199 identified 8 and 12 SNPs for UNa/UK ratio and UNa/UCr ratio, respectively [38]. All the above
200 GWA studies were adjusted for age, sex, ancestry principle components and array information.
201 Summary-level statistics from the GWA studies were extracted for analysis (Figure 1).
202 Processed meat intake was not included in multivariable MR because there is no relevant GWA
203 study on it so far.
204
205 *Statistical analysis*
206 We firstly matched the 50 UNa-associated SNPs with the summary-level statistics of the
207 outcome GWA studies in univariable MR. When a SNP could not be matched, we searched for
208 its potential proxies (with $r^2 \geq 0.80$, distance $\leq$ 500 000 bytes;
209 https://ldlink.nci.nih.gov/?tab=ldproxy); in case of no eligible proxy, we removed the SNP
210 from analysis. Summary-level statistics across different GWA studies were harmonized so that
211 their effect estimates were aligned on the same allele [39]. The strength of the SNPs as
212 instrumental variables was measured with F statistics, with a value > 10 suggesting good
213 instrument strength [40]. For multivariable MR, we matched the UNa-associated SNPs and the
214 BMI-associated SNPs with the outcome GWA studies and applied the same proxy-searching
215 strategy.
216
217 Inverse-variance weighted method with random-effects model was used as primary analysis in
218 univariable and multivariable MR. For sensitivity analysis, weighted median and MR-Egger
219 methods were used in univariable MR, while only MR-Egger method was used in multivariable
220 MR (Figure 1) [41]. Presence of pleiotropy was examined with MR-Egger intercept test, with
221 a p value < 0.05 suggesting pleiotropy. MR-PRESSO was planned to examine the effect of
222 horizontal pleiotropy, in case of any [42]. Additional sensitivity analyses was performed by (1)

using UNa/UK ratio and UNa/UCr ratio as sodium measure and (2) removing the SNPs that were associated with risk factors for the outcomes (e.g., obesity) at GWA significance level (5×10^{-8}) in manually searching PhenoScanner v2 database [43] (http://www.phenoscanner.medschl.cam.ac.uk/).

The effect measure in observational analysis was hazard ratio (HR) and its 95% confidence interval (CI), while the effect measure in MR was odds ratio (OR) and its 95% CI. A statistically significant effect was suggested by a CI not including null effect value of 1. The interpretation of HR was the excessive outcome risk for high 24-h UNa compared to that for low 24-h UNa. The interpretation of OR should be the excessive outcome risk for each 1 standard deviation increment in log-transformed spot UNa. Data analysis was performed in *R* environment, with "*rms*" (version 5.1-4), "*MendelianRandomization*" (version 0.4.2) and "*MVMR*" (version 0.2) [44] packages.

Results

The baseline characteristics of the 415 524 eligible participants were shown in Table 1. The the mean age was 56.3 years old and 53.2% were female. Almost 95% of them were White. The mean BMI was 27.4kg/m^2. The mean values for 24-h UNa, log transformed spot UNa, UNa/UK ratio and UNa/UCr ratio were 2.8, 4.2, 1.4 and 10.7, respectively. About 44.5% of participants usually/always added salt to their food. Over 30% had processed meat more than twice a week.

During a median follow-up of 11.1 (interquartile range 10.4 to 11.8) years, 2 663 participants was diagnosed with CRC (1756 colon cancer cases, 907 rectal cancer cases). Compared with the overall cohort, CRC cases were more like to be male, older, White, affluent, previous

smokers, and had lower eGFR, more frequent red meat and processed meat intake and adding salt to food, but less frequent consumption of fresh vegetables and cereal.

Processed meat intake was highly associated with 24-h UNa, spot UNa, UNa/UK ratio, UNa/UCr ratio and adding salt to food. Frequent processed meat consumer (≥ twice a week) had increased risk of high 24-h UNa (OR 1.21 (95%CI 1.17 to 1.25)), high spot UNa (1.45 (1.41 to 1.49)), high UNa/UK ratio (1.38 (1.34 to 1.42)), high UNa/UCr ratio (1.11 (1.08 to 1.14)) and were more likely to add salt to food (1.64 (1.59 to 1.68)) (Table 2).

When fitting processed meat intake and 24-h UNa in separate models, increased CRC risk was observed in high processed meat intake (HR 1.14 (0.97 to 1.34) and 1.23 (1.03 to 1.46) for ≤ once/week and ≥ twice/week, respectively), but not in high 24-h UNa (0.96 (0.87 to 1.06)). When fitting processed meat intake and the 24-h UNa in the same model, we observed an independent effect of processed meat intake (1.14 (0.97 to 1.34) and 1.23 (1.03 to 1.46) for ≤ once/week and ≥ twice/week, respectively), but still not of 24-h UNa (0.96 (0.87 to 1.05)). The effect size of processed meat intake remained similar between models (Table 3). Regarding the subtypes of CRC, neither processed meat intake nor 24-h UNa was associated with colon cancer, but processed meat intake ≥ twice/week was associated with rectal cancer (1.45 (1.06 to 1.98)) (Supplementary table 2). When using other sodium intake measures (spot UNa, UNa/UK ratio, UNa/UCr ratio and adding salt to food) in sensitivity analyses, the results remained similar, that is, no evidence of association between the sodium intake measures with CRC or its subtypes (Supplementary table 2).

In Mendelian randomization, we used 48 SNPs (F statistic = 611.25) and 125 SNPs (28 UNa-associated SNPs and 97 BMI-associated SNPs, F statistic = 14.75 and 34.70 for UNa and BMI,

respectively) in univariable and multivariable MR, respectively. We found little evidence for an association between UNa and CRC in both univariable MR (0.89 (0.21 to 3.74)) and multivariable MR (1.43 (0.30 to 6.81)) (Table 3, Figure 2). MR-Egger intercept tests showed no evidence of pleiotropy. UNa showed similarly insignificant effects on colon cancer and rectal cancer (Supplementary table 3; Supplementary figure 1). Using UNa/UK ratio and UNa/UCr ratio also demonstrated similarly null evidence for associations (Supplementary table 3). Removing the 22 SNPs associated with obesity-related traits also yielded similar results (Supplementary table 4).

Table 1: Baseline characteristics of the UK Biobank cohort

	Overall cohort (n=415 524)	Participants with colorectal cancer (n=2 663)
Female gender (n, %)	221 102 (53.2)	1 108 (41.6)
Age (mean, SD)	56.3 (8.1)	60.6 (6.6)
Ethnicity (n, %)		
White	392 008 (94.7)	2 563 (96.8)
Black	6 519 (1.6)	21 (0.8)
Asian	9 410 (2.3)	32 (1.2)
Mixed and others	6 175 (1.5)	33 (1.2)
Townsend deprivation (n, %)		
Q1 (most affluent) (mean = -4.52)	103 837 (25.0)	695 (26.1)
Q2 (mean = -2.90)	103 923 (25.0)	669 (25.1)
Q3 (mean = -0.97)	103 874 (25.0)	648 (24.3)
Q4 (most deprived) (mean = 3.19)	103 890 (25.0)	651 (24.4)
Qualification (n, %)		
College or above	135 554 (39.7)	778 (37.7)
Below college	206 228 (60.3)	1 286 (62.3)
Body mass index (mean, SD)	27.4 (4.8)	27.9 (4.6)
Family history of colorectal cancer	49 295 (11.9)	433 (16.3)

Regular user of aspirin/ibuprofen	123 406 (29.7)	831 (31.2)
eGFR (mean mL/min/1.73m^2, SD)	94.2 (9.0)	90.8 (8.4)
Smoking (n, %)		
Never smoker	228 260 (55.1)	1 236 (46.6)
Previous smoker	142 272 (34.4)	1 157 (43.6)
Current smoker	43 496 (10.5)	260 (9.8)
Drinking (n, %)		
Never drinker	17 885 (4.3)	88 (3.3)
Previous drinker	14 365 (3.5)	92 (3.5)
Current drinker	382 811 (92.2)	2 478 (93.2)
Physical activity level (n, %)		
Low	62719 (18.6)	419 (19.5)
Moderate	137295 (40.7)	886 (41.2)
High	137191 (40.7)	848 (39.4)
Sodium level		
Spot urinary sodium (mean μmol/L, SD)	77.87 (44.6)	78.73 (43.0)
Log-transformed spot urinary sodium (mean, SD)	4.18 (0.6)	4.2 (0.6)
24-h urinary sodium (mean g/d, SD)	2.76 (0.5)	2.67 (0.5)
Urinary sodium/potassium ratio (mean, SD)	1.44 (0.9)	1.41 (0.9)
Urinary sodium/creatinine ratio (mean, SD)	10.7 (7.1)	10.5 (6.2)
Adding salt to food (n, %)		
Never/rarely	230 638 (55.5)	1 424 (53.5)
Usually/always	184 826 (44.5)	1 239 (46.5)
Red meat intake frequency (mean, SD)	2.12 (1.45)	2.29 (1.53)
Processed meat (n, %)		
Never	38 194 (9.2)	174 (6.5)
≤ 1 time/week	246 588 (59.5)	1 531 (57.6)
≥ 2 times/week	129 810 (31.3)	954 (35.9)
Fresh fruit intake (n, %)		
< 1 time/week	38 692 (9.4)	270 (10.2)
1-2 times/week	225 826 (54.6)	1 417 (53.5)
3-4 times/week	117 663 (28.4)	760 (28.7)
≥ 5 times/week	31 626 (7.6)	203 (7.7)

Fresh vegetable intake (n, %)		
< 1 time/week	59 360 (14.5)	413 (15.8)
1-2 times/week	222 627 (54.3)	1 419 (54.2)
3-4 times/week	87 767 (21.4)	543 (20.7)
≥ 5 times/week	40 302 (9.8)	243 (9.3)
Cooked vegetable intake (n, %)		
< 1 time/week	20 650 (5.0)	132 (5.0)
1-2 times/week	191 569 (46.7)	1 187 (45.3)
3-4 times/week	156 241 (38.1)	1 022 (39.0)
≥5 times/week	41 792 (10.2)	277 (10.6)
Oily fish (n, %)		
< 1 time/week	183 609 (44.4)	1 103 (41.7)
1 time/week	155 718 (37.7)	1050 (39.7)
>1 times/week	73 784 (17.9)	495 (18.7)
Cereal (n, %)		
< 1 time/week	71 497 (17.3)	501 (18.8)
1-2 times/week	46 373 (11.2)	296 (11.1)
3-4 times/week	53 853 (13.0)	344 (12.9)
≥5 times/week	242 342 (58.5)	1 517 (57.1)

294 Table 2: Association between processed meat intake with sodium intake in UK Biobank

	Processed meat intake: Never	Processed meat intake: ≤ 1/week	Processed meat intake: ≥ 2/week
N	38 194	246 588	129 810
24-hour urinary sodium			
Low (n, %)	21 728 (56.9)	128 167 (52.0)	57 474 (44.3)
High (n, %)	16 466 (43.1)	118 421 (48.0)	16 466 (12.7)
Mean (SD)	2.7 (0.5)	2.7 (0.5)	2.8 (0.5)
OR (95%CI)**	1	1.01 (0.98, 1.04)	1.21 (1.17, 1.25)
Spot Urinary sodium			
Low (n, %)	23 354 (61.2)	130 657 (53.0)	53 173 (41.0)
High (n, %)	14 840 (38.8)	115 931 (47.0)	76 637 (59.0)
Mean (SD)*	4.0 (0.7)	4.1 (0.6)	4.3 (0.6)
OR (95%CI)**	1	1.18 (1.15, 1.22)	1.45 (1.41, 1.49)
Urinary sodium/potassium ratio			
Low (n, %)	21 366 (55.9)	129 233 (52.5)	56 631 (43.7)
High (n, %)	1 673 (43.8)	116 825 (47.5)	72 909 (56.3)
Mean (SD)	1.3 (0.9)	1.4 (0.9)	1.6 (0.9)
OR (95%CI)**	1	1.12 (1.09, 1.14)	1.38 (1.34, 1.42)
Urinary sodium/creatinine ratio			
Low (n, %)	18 043 (47.2)	124 822 (50.6)	64 633 (49.8)
High (n, %)	20 151 (52.8)	121 766 (49.4)	65 177 (50.2)
Mean (SD)	11.3 (6.8)	10.6 (7.4)	10.7 (6.4)
OR (95%CI)**	1	0.94 (0.92, 0.97)	1.11 (1.08, 1.14)
Adding salt to food			
Never/rarely (n, %)	24 390 (63.9)	141 426 (57.3)	64 417 (49.6)
Usually/always (n, %)	13 794 (36.1)	105 418 (42.7)	65 381 (50.4)
OR (95%CI)**	1	1.29 (1.26, 1.33)	1.64 (1.59, 1.68)

295 *: showing log-transformed spot urinary sodium.

296 **: odds ratio (95% confidence interval), estimated with processed meat intake as independent
297 variable while sodium intake measures and salt intake behaviour as dependent variable, adjusted for
298 age, sex, ethnicity, smoking, drinking, physical activity, body mass index, regular use of

aspirin/ibuprofen, estimated glomerular filtration rate, intake of fresh fruit, fresh vegetable, cooked vegetables, red meat, oily fish and cereal.

Table 3: Observational analysis and genetic analysis with Mendelian randomization (MR) for processed meat intake and urinary sodium on colorectal cancer

	Processed-meat Intake (≥ 2/week vs. never)	Urinary Sodium
Observational Analysis*:		(24-hour urinary sodium)
Model 1	1.23 (1.03, 1.46)	Not Included
Model 2	Not Included	0.96 (0.87, 1.06)
Model 3	1.23 (1.03, 1.46)	0.96 (0.87, 1.05)
Genetic Analysis[+]:		(Log-transformed spot urinary sodium)
Univariable MR[¶]	NA	0.89 (0.21, 3.74)
Multivariable MR[§]	NA	1.43 (0.30, 6.81)

*Models were stratified by age, sex, ethnicity, and adjusted for education attainment, Townsend deprivation index, colorectal cancer family history, regular use of aspirin/ibuprofen, IPAQ physical activity status, smoking, drinking, body mass index, eGFR, fresh fruit intake, fresh vegetable intake, cooked vegetable intake, cereal intake, red meat and oily fish;

[+]: Inverse-variance weighted method;

[¶]: including 48 SNPs, F statistics = 611.25, p for MR-Egger intercept test = 0.32;

[§]: including 125 SNPs (28 UNa SNPs + 97 BMI SNPs), F statistics = 14.75 and 34.70 for UNa and BMI), p for MR-Egger intercept test = 0.10;

NA: not applicable;

SNP: single nucleotide polymorphism.

Discussion

This study shows evidence that processed-meat intake, but not sodium intake, is associated with an increased risk of CRC. It provided both epidemiological and genetic evidence that the effect of sodium intake on CRC was minimal; therefore, reducing intake of processed meat is a more important strategy for CRC prevention.

There has been plentiful evidence for the association between processed meat intake and CRC risk. Vieira *et al.* found that 100 g/d increment of processed meat intake increased CRC risk by 18% in a meta-analysis of 10 studies with 10 356 participants [9]. Handel *et al.* observed high intake of processed meat increased CRC risk by 13% in a more recent meta-analysis of 15 cohorts [8]. Previous analysis with UK biobank also demonstrated similarly 20-25 g/d higher intake of processed meat increased CRC risk by 18-19% [6,7]. An early pooled analysis of UK dietary cohort consortium failed to observe a significant association, but that was probably due to small number of CRC cases during follow-up [45]. As for the CRC subtypes, inconsistency remains across studies. Händel *et al.* found significant associations with both colon cancer and rectal cancer, and the effect was stronger on colon cancer than the other (excessive risk 25% vs. 18%) [8]. Vieire *et al.* found significantly positive association with colon cancer, but not rectal cancer [9], opposite to the findings in this study. Therefore, future research is merited to delineate its effect on CRC subtypes.

Although the intakes of dietary sodium and processed meat are highly correlated as shown in this study and previous evidence [21], sodium intake did not demonstrate significant effects on CRC or its subtypes as processed meat intake did. Even if the insignificance here was caused by the limited number of events and a true association existed, the effect size should be actually minor, as the HR point estimates are very close to one. The validity of the results is enhanced

by the use of the largest sample size so far and the careful controlling for a range of confounders. The null effect was similarly observed in sensitivity analyses using other substitutes for sodium intake and MR analysis, as well as previous studies, such as a case-control study of 2 226 Western people [16] and a prospective cohort of 77 500 Japanese people [17]. Although other studies found a significant association, such as Kune *et al* [14], Alegria-Lertxundi *et al* [15] and Yakoob *et al* [18], it was likely due to uncontrolled confounding. Future studies on this topic should be evidence-based, carefully designed with larger sample size, higher statistical power and more effective measures for controlling confounding, to reduce research waste [46].

The findings indicated that sodium intake had null carcinogenic effect on CRC, while processed meat had independent carcinogenic effect, to which its high sodium concentration made little contribution. Carcinogenesis of processed meat is believed to be caused by its mutagenic chemicals, including heterocyclic amines, polycyclic aromatic hydrocarbons, haem iron, N-nitroso compounds, etc. [5,47]. Recent evidence has found that high sodium diet was associated with cancer at gastrointestinal tract, especially stomach [12,13,48]. In the case of stomach cancer, high sodium causes direct gastric epithelial cell damage and weakens mucous barrier, thus indirectly enhancing the carcinogenesis of mutagenic compounds and *Helicobacter pylori* infection [49–52]. However, colorectal epithelia are able to actively maintain intra-cellular and inter-cellular sodium homeostasis, via amiloride-sensitive epithelial sodium channel (ENaC), mineralocorticoid receptor, etc. [53,54]. *In vivo* and *in vitro* studies showed that high sodium diet down-regulates expression and activation of mineralocorticoids receptor and ENaC subunits, thus decreasing sodium absorption and consequently maintaining sodium balance [55]. These mechanisms may help prevent or mitigate the potential harmful effects of high sodium in colon and rectum as it does to gastric epithelia. Nevertheless, high

sodium may still cause colon inflammation and exacerbate colitis as shown in animal research [56].

The findings provided further evidence that reduction of processed meat intake may be a potentially effective strategy for primary prevention of CRC, but sodium reduction may not. Nevertheless, we are not encouraging high sodium intake, because no evidence shows it can decrease CRC risk. More importantly, high sodium intake is a well-known risk factor for multiple health outcomes, such as hypertension [57], metabolic syndrome [58], cardiovascular diseases [24] and mortality [59]. Randomized controlled trials have demonstrated the heath benefits of dietary sodium reduction [60,61]. Future studies are warranted to investigate how to improve general population's compliance and adherence to intake reduction of sodium and processed meat.

One of the strengths of our study is that we used the large UK Biobank cohort in observational analysis and large GWA studies in genetic analysis, which generated consistent results of null effect of sodium intake on CRC. We examined the independent effect of processed meat intake and urinary sodium, after adjusting for multiple covariates, including family history, use of aspirin, intake of red meat, fruits and vegetables, etc. In MR analysis, using SNPs as instrumental variables mainly relies on three assumptions: (1) the SNPs are associated with the exposure, (2) the SNPs should be independent of unobserved confounders after conditioning on observed confounders, and (3) associated with the outcome only through the exposure. For assumption (1) and (2), our SNPs are associated with UNa, forming strong instrumental variables, and not associated with the outcomes. For assumption (3), we performed multivariable MR adjusted for BMI, and sensitivity analysis by removing SNPs that are

potentially associated with confounders, such as various obesity measures, which yielded consistent findings.

This study has some limitations. First, we used 24-h UNa as a substitute for dietary sodium intake. Direct measurement of sodium intake is difficult and unavailable in UK Biobank, while 24-h UNa is a commonly acceptable substitute [23,24]. Using sodium intake behavior and other urinary sodium measures in sensitivity analyses showed similar results, which further enhanced the reliability of our results. Second, UK biobank only measured the frequency of food intake at baseline assessment, which may not completely represent the quantity consumed and introduce information bias. Third, we observed wide CIs under MR-Egger method in MR analyses, which may suggest underpower. However, MR-Egger method is often underpowered in studies and thus used as secondary sensitivity analysis [62], but the results were qualitatively consistent with the primary analysis of inverse-variance weighted method. Fourth, we did not specifically adjust for macronutrients (e.g., fat, protein, carbohydrates); instead, we adjusted for intakes of food (such as fruit, vegetables, red meat, fish, cereal), as they are the major sources of macronutrients as well as micronutrients. Fifth, although we have adjusted for multiple covariates in analyzing UK Biobank data, residual confounding is still possible, as in other observational studies, but its consistency with MR results increased its validity. Sixth, in multivariable MR analysis, we did not adjust for processed meat intake due to lack of relevant GWA study.

Conclusion

Although sodium intake and processed meat intake are highly correlated, processed meat intake is an independent risk factor for CRC risk, while sodium intake is not. Reducing intake of processed meat may be an effective measure for CRC prevention. Dietary sodium reduction

should still be recommended to achieve health benefits in blood pressure, metabolic syndrome, cardiovascular diseases, etc. Future research is merited to study how to improve compliance and adherence to intake reduction of processed meat and sodium.

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Declaration

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Data availability: The UK Biobank individual data are accessible under application at https://www.ukbiobank.ac.uk/. The FinnGen GWA summary data of colorectal cancer, colon cancer and rectal cancer are accessible under application at https://www.finngen.fi/en. The BMI GWA summary data is accessible at GIANT consortium https://portals.broadinstitute.org/collaboration/giant/index.php/GIANT_consortium_data_files. The GWA sunnary data of urinary sodium, urinary sodium-to-potassium ratio and urinary sodium-to-creatinine ratio can be accessed via the methods described in the original publications.

Analysis code availability: Available under reasonable request.

Author contribution: QF conceived the research idea. QF and KKFT designed the study. JZ and QY provided methodological guidance on Mendelian randomization. QF analyzed the data and draft the manuscript. QF and KKTF interpreted the results. All authors critically reviewed the manuscript.

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675 Figure legend

676 Figure 1: The flowchart of method framework and data sources

677 GWAS: Genome-wide association study. MR: Mendelian Randomization. BMI: Body Mass Index.

678 GIANT consortium: Genetic Investigation of Anthropometric Traits consortium. SNP: Single

679 Nucleotide Polymorphism.

680