Plasma Inflammation Markers of the Tumor Necrosis Factor Pathway but Not C-Reactive Protein Are Associated with Processed Meat and Unprocessed Red Meat Consumption in Bavarian Adults[1–3]

Carolina Schwedhelm,[4] Tobias Pischon,[4] Sabine Rohrmann,[5] Hubertus Himmerich,[6] Jakob Linseisen,[7] and Katharina Nimptsch[4]*

[4]Molecular Epidemiology Research Group, Max-Delbrück Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany; [5]Division of Chronic Diseases Epidemiology, Epidemiology, Biostatistics and Prevention Institute, Zurich, Switzerland; [6]Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, United Kingdom; and [7]Institute of Epidemiology II, Helmholtz Centre Munich, Neuherberg, Germany

Abstract

Background: High consumption of red and processed meats has been linked to higher chronic disease risk. It has been hypothesized that inflammation markers may mediate part of this association. Most previous studies on the association of red meat intake with circulating inflammation markers used C-reactive protein (CRP) but rarely other markers, and not all differentiated between processed meat and unprocessed red meat.

Objective: We investigated the cross-sectional association of processed meat and unprocessed red meat consumption with plasma concentrations of CRP, interleukin 6 (IL-6), tumor necrosis factor (TNF)-α, soluble TNF receptor (sTNF-R) 1, and sTNF-R2 in German adults.

Methods: Inflammation markers were quantified in the plasma of 553 adults (233 men and 320 women) aged 18–80 y within the cross-sectional Bavarian Food Consumption Survey II. Dietary intake was estimated from three 24-h dietary recalls. The association between red meat consumption and inflammation markers was analyzed with the use of multivariable-adjusted linear regression.

Results: Processed meat consumption was borderline significantly associated with higher IL-6 [relative difference per 50-g increment: 5% (95% CI: −1%, 10%)] but not with CRP (2%; 95% CI: −6%, 10%), and it was inversely associated with total TNF-α (−3%; 95% CI: −6%, −1%), sTNF-R1 (−3%; 95% CI: −4%, −1%), and sTNF-R2 (−2%; 95% CI: −4%, 0%) concentrations. Unprocessed red meat consumption was not associated with CRP (−5%; 95% CI: −15%, 5%) or IL-6 (−1%; 95% CI: −9%, 7%) but was inversely associated with sTNF-R1 (−3%; 95% CI: −5%, −1%) and sTNF-R2 (−4%; 95% CI: −7%, −2%).

Conclusions: Our results suggest an inverse association between both processed meat and unprocessed red meat with inflammation markers of the TNF pathway in Bavarian adults but no association with CRP. Further research on the role of TNF pathway markers in chronic inflammation is warranted.

Introduction

High consumption of red meat, mainly processed meat, has been linked to a higher risk of chronic diseases, particularly cardiovascular diseases (CVDs)[8], type 2 diabetes (T2D), and colorectal cancer (1–3). In 2015, the International Agency for Research on Cancer concluded that there is convincing evidence that the consumption of processed meat (and probably red meat) causes colorectal cancer (4). However, the underlying mechanisms for these associations are unclear. Higher concentrations of plasma

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[3] Supplemental Figure 1 and Supplemental Tables 1–3 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

*To whom correspondence should be addressed. E-mail: katharina.nimptsch@mdc-berlin.de.

[8] Abbreviations used: BVSII, Bavarian Food Consumption Survey II; CRP, C-reactive protein; CVD, cardiovascular disease; sTNF-R, soluble TNF receptor; T2D, type 2 diabetes.

TABLE 1 Unprocessed red meat and processed meat intake in the Bavarian Food Consumption Survey II

Meat intake	Median, g/d	IQR, g/d	Range, g/d	No consumption on reported days, %
Men (n = 233)				
Processed meat	74.0	40.0–121	0.00–415	6.01
Unprocessed red meat	35.2	0.00–84.9	0.00–306	34.3
Women (n = 320)				
Processed meat	34.3	11.4–64.3	0.00–245	13.1
Unprocessed red meat	22.3	0.00–47.5	0.00–176	39.7

biomarkers of chronic low-grade inflammation have been associated with a higher risk of the chronic diseases for which an association with red and processed meat intake has been observed (5). Therefore, it has been speculated that habitual red and processed meat intake may be related to chronic subclinical inflammation and that this association may partly explain the link to chronic disease risk (6, 7). However, previous studies on the association of red and processed meat intake with blood concentrations of inflammation markers have shown mixed results. A positive association between red and processed meat consumption and circulating C-reactive protein (CRP) concentrations has been observed in cross-sectional analyses of the Nurses' Health Study (8) and the European Prospective Investigation into Cancer and Nutrition study (9). However, this association was attenuated in both studies after adjusting for BMI, which is independently associated with higher chronic subclinical inflammation and may therefore mediate part of this association (8). However, in a cross-sectional analysis in Iranian women, a persistent association of red meat intake with CRP concentrations was observed after introducing BMI in the model (6). Most previous studies investigated only the acute-phase CRP, whereas studies that have investigated other major proinflammatory cytokines such as IL-6, TNF-α, soluble TNF receptor (sTNF-R) 1, or sTNF-R2 are scarce. sTNF-Rs bind free TNF-α and thereby regulate its inflammatory activity (10). Although sTNF-R1 and sTNF-R2 show both anti-inflammatory and proinflammatory functions, they are usually interpreted as proinflammatory cytokines in epidemiologic studies (11–13) because they are secreted in response to inflammation and have been proposed as markers for disease progression, with higher concentrations in later stages of diseases such as cancer, Crohn disease, and CVD (14). Nevertheless, whether these markers are associated with disease incidence in healthy populations is less clear.

The aim of this study was to investigate the association of processed meat and unprocessed red meat intake with plasma concentrations of the inflammation markers CRP, IL-6, TNF-α, sTNF-R1, and sTNF-R2 in the general Bavarian population.

Methods

Study population. As part of the Bavarian Food Consumption Survey II (BVSII) in Bavaria, Germany, 1050 individuals aged 13–80 y were randomly recruited between 2002 and 2003. The overall response rate for this study was 71%. As has been described previously (15), all adults aged 18–80 y who had completed the personal interview and ≥1 dietary

TABLE 2 Characteristics of the study population by processed meat and unprocessed red meat consumption quartiles[1]

	Processed meat intake			Unprocessed red meat intake		
	Quartile 1 (n = 138)	Quartile 4 (n = 137)	P	Quartile 1 (n = 207)	Quartile 4 (n = 117)	P
Range, g/d						
Men	0.00–39.9	121–415		0.00	90.0–306	
Women	0.00–11.4	64.6–245		0.00	55.0–176	
Frequency, n (%)						
Men	57 (41.3)	58 (42.3)	0.99	80 (38.6)	52 (44.4)	0.65
Smoking status			0.55			0.01
Never	79 (57.2)	71 (51.8)		124 (59.9)	55 (47.0)	
Current	28 (20.3)	31 (22.6)		31 (15.0)	35 (29.9)	
SES			0.02			0.002
1	14 (10.1)	26 (19.0)		21 (10.1)	16 (13.7)	
5	12 (8.70)	13 (9.49)	0.99	34 (16.4)	4 (3.42)	
Mean ± SD						
Age, y	48.5 ± 16.6	47.9 ± 14.0	0.72	47.9 ± 15.3	46.7 ± 14.8	0.39
BMI, kg/m^2	26.2 ± 4.94	27.1 ± 4.92	0.22	26.1 ± 4.88	27.2 ± 5.56	0.08
Physical activity, MET-h/d	2.76 ± 4.12	1.57 ± 2.63	0.003	2.29 ± 3.65	2.25 ± 3.32	0.90
HDL cholesterol, mg/dL	46.5 ± 7.71	46.2 ± 7.70	0.80	47.3 ± 7.47	46.7 ± 8.23	0.28
Median [IQR]						
Energy intake, kcal/d	1724 [1361–2199]	2081 [1720–2547]	<0.0001	1884 [1477–2299]	2126 [1709–2487]	0.003
Ethanol, g/d	4.56 [0.04–14.4]	8.14 [0.54–22.0]	0.01	5.09 [0.14–17.4]	13.2 [2.30–25.5]	0.01
Vegetables, g/d	128 [70.6–193]	114 [65.0–174]	0.10	114 [60.8–178]	132 [79.8–197]	0.22
Fruits, g/d	136 [33.8–263]	79.3 [0.94–167]	0.01	100 [21.6–223]	78.8 [8.57–180]	0.04
Dairy products, g/d	175 [90.5–310]	120 [49.7–205]	0.001	160 [78.1–277]	120 [49.7–264]	0.01
Cereal and cereal products, g/d	170 [112–231]	195 [143–261]	0.09	184 [140–248]	205 [146–255]	0.11
Sugar and confectionery, g/d	28.5 [10.0–57.7]	26.9 [13.2–55.3]	0.96	31.3 [13.7–56.4]	27.8 [11.1–55.8]	0.31
Cakes, g/d	35.7 [5.71–80.6]	34.3 [8.57–85.3]	0.07	40.7 [8.57–81.1]	49.2 [10.7–91.4]	0.80

[1] P values for frequency, mean ± SD, and median IQR were obtained with the use of the chi-square test, generalized linear models, and the Kruskal-Wallis test, respectively. MET, metabolic equivalent; SES, socioeconomic status.

recall were invited for blood sampling and anthropometric measurements. In total, 568 individuals (65% of the eligible sample) participated in the blood sampling and anthropometric measurements, which took place up to 6 wk after the dietary assessment (**Supplemental Figure 1**). All participants expressed their informed consent, and the study was ethically approved by the local ethics committee (16). Study design and methods are described in detail elsewhere (16).

Laboratory methods. Venous blood samples were drawn and extracted into EDTA tubes. Samples were chilled between 4 and 8°C and centrifuged at room temperature at 1467 × *g* for 15 min to separate plasma from blood cells. Samples were kept at −80°C until analysis.

Inflammation markers (high-sensitivity CRP; referred to herein simply as CRP), IL-6, total TNF-α, sTNF-R1, and sTNF-R2 were measured immediately after completion of the study and according to the manufacturer's instructions (Biosource) with commercial ELISAs [detailed methods described elsewhere (17)]. Intra-assay CVs for all assays were <7%, and interassay CVs were <9% (17, 18).

Dietary intake (meat consumption) assessment. Dietary intake, including meat consumption, was assessed through three 24-h dietary recalls. Dietary recalls were conducted by trained interviewers by telephone and were unannounced both on weekdays and weekends (2 on weekdays and 1 during the weekend). Mean daily food intake was calculated by weighing recalled intake correspondingly to weekdays and weekends. Red meat was considered as any meat coming from beef, veal, pork, mutton or lamb, domestic rabbit, and game. Processed meat was meat bought as a ready-to-eat product or meats processed for preservation by salting, smoking, curing, marinating, or cooking (19).

Statistical analysis. A total of 553 BVSII participants with complete information on inflammation markers and at least two 24-h dietary recalls (mean ± SD number of recalls: 2.99 ± 0.07) were included in this analysis (14 participants had missing data on inflammation markers of interest, and 1 participant was excluded because of implausible IL-6 concentrations) (Supplemental Figure 1). Missing values for waist circumference (n = 8) and smoking status (n = 1) were replaced with sex-specific

TABLE 3 Associations of processed meat consumption with plasma inflammation markers among adults in the Bavarian Food Consumption Survey II[1]

	Quartile 1 (n = 138)	Quartile 2 (n = 138)	Quartile 3 (n = 140)	Quartile 4 (n = 137)	P-trend	Continuous β % difference per 50 g
Range, g/d						
Men (n = 233)	0–39.9	40–73.9	74–121	121–415		
Women (n = 320)	0–11.4	11.5–34.2	34.3–64.5	64.6–245		
CRP, mg/L						
Model 1	1.68 (1.39, 2.03)	1.89 (1.60, 2.24)	1.72 (1.45, 2.05)	2.04 (1.69, 2.47)	0.15	5 (−4, 13)
Model 2	1.69 (1.39, 2.06)	1.95 (1.64, 2.31)	1.74 (1.46, 2.07)	2.09 (1.72, 2.54)	0.13	5 (−3, 14)
Model 3	1.72 (1.44, 2.05)	1.85 (1.58, 2.16)	1.72 (1.47, 2.02)	1.94 (1.60, 2.35)	0.40	2 (−6, 10)
Model 3†	1.61 (1.34, 1.94)	1.74 (1.47, 2.06)	1.62 (1.37, 1.91)	1.88 (1.55, 2.29)	0.24	3 (−6, 11)
IL-6, pg/mL						
Model 1	1.42 (1.25, 1.63)	1.53 (1.36, 1.73)	1.62 (1.44, 1.82)	1.75 (1.56, 1.98)	0.02*	6 (1, 12)
Model 2	1.42 (1.25, 1.61)	1.54 (1.34, 1.76)	1.62 (1.44, 1.82)	1.74 (1.53, 1.97)	0.03*	6 (1, 12)
Model 3	1.44 (1.28, 1.62)	1.50 (1.32, 1.71)	1.62 (1.45, 1.80)	1.69 (1.49, 1.92)	0.07	5 (−1, 10)
Model 3†	1.40 (1.24, 1.60)	1.46 (1.28, 1.68)	1.58 (1.41, 1.76)	1.67 (1.47, 1.91)	0.046*	5 (−1, 10)
Total TNF-α, pg/mL						
Model 1	11.8 (11.0, 12.6)	11.9 (11.1, 12.7)	11.8 (10.9, 12.7)	10.8 (10.2, 11.5)	0.05	−3 (−5, 0)
Model 2	11.8 (11.0, 12.7)	12.0 (11.2, 12.8)	11.8 (10.9, 12.7)	10.8 (10.1, 11.5)	0.03*	−3 (−6, −1)
Model 3	11.8 (11.0, 12.8)	11.9 (11.1, 12.7)	11.8 (10.9, 12.7)	10.8 (10.1, 11.5)	0.02*	−3 (−6, −1)
Model 3†	11.7 (10.8, 12.7)	11.8 (11.0, 12.7)	11.7 (10.8, 12.7)	10.6 (9.96, 11.4)	0.02*	−4 (−6, −1)
sTNF-R1, ng/mL						
Model 1	1.86 (1.79, 1.94)	1.81 (1.74, 1.89)	1.77 (1.70, 1.84)	1.76 (1.70, 1.82)	0.01*	−2 (−4, −1)
Model 2	1.85 (1.77, 1.92)	1.80 (1.72, 1.87)	1.76 (1.69, 1.83)	1.74 (1.67, 1.81)	0.01*	−2 (−4, −1)
Model 3	1.85 (1.78, 1.93)	1.79 (1.72, 1.86)	1.76 (1.69, 1.83)	1.73 (1.67, 1.80)	0.003*	−3 (−4, −1)
Model 3†	1.83 (1.75, 1.91)	1.79 (1.71, 1.87)	1.75 (1.68, 1.82)	1.72 (1.65, 1.79)	0.01*	−3 (−4, −1)
sTNF-R2, ng/mL						
Model 1	4.61 (4.38, 4.85)	4.57 (4.38, 4.77)	4.50 (4.30, 4.70)	4.33 (4.16, 4.50)	0.02*	−2 (−4, 0)
Model 2	4.63 (4.38, 4.88)	4.58 (4.38, 4.79)	4.53 (4.32, 4.75)	4.34 (4.16, 4.54)	0.02*	−2 (−4, 0)
Model 3	4.63 (4.39, 4.89)	4.56 (4.37, 4.77)	4.53 (4.32, 4.75)	4.33 (4.14, 4.51)	0.01*	−2 (−4, 0)
Model 3†	4.60 (4.33, 4.88)	5.48 (4.38, 4.79)	4.51 (4.30, 4.74)	4.30 (4.12, 4.49)	0.01*	−2 (−4, 0)
TNF molar ratio[2]						
Model 1	0.72 (0.68, 0.77)	0.74 (0.70, 0.79)	0.75 (0.70, 0.80)	0.71 (0.67, 0.74)	0.71	−1 (−3, 2)
Model 2	0.73 (0.68, 0.77)	0.75 (0.70, 0.80)	0.75 (0.70, 0.80)	0.71 (0.67, 0.75)	0.56	−1 (−3, 1)
Model 3	0.73 (0.68, 0.77)	0.75 (0.70, 0.80)	0.75 (0.70, 0.80)	0.71 (0.67, 0.75)	0.61	−1 (−3, 1)
Model 3†	0.73 (0.68, 0.78)	0.74 (0.69, 0.79)	0.75 (0.69, 0.80)	0.70 (0.67, 0.74)	0.49	−1 (−4, 1)

[1] All values are geometric means (95% CIs) unless otherwise indicated. Mixed linear regression models were used. Dependent variables were log-transformed (geometric means and their respective 95% CIs were back-transformed to concentrations for an easier interpretation). Model 1 was adjusted for sex and age; model 2 was additionally adjusted for socioeconomic status, smoking status, physical activity, total nonalcohol energy intake, alcohol intake, and fasting status in the case of IL-6, total TNF-α, sTNF-R1, and sTNF-R2; model 3 was additionally adjusted for BMI (in kg/m^2) and BMI-adjusted waist circumference residuals; and model 3† additionally excludes potential underreporters (n = 44) as a sensitivity analysis. The P-trend was calculated by treating the median values of each meat consumption quartile as a continuous variable. *$P < 0.05$. CRP, C-reactive protein; sTNF-R, soluble TNF receptor.

[2] Free TNF-α molecules per 100 molecules of soluble TNF receptors.

median values. We log-transformed all plasma analytes (CRP, IL-6, total TNF-α, sTNF-R1, and sTNF-R2) for analysis.

Because of a substantial sex-related difference in meat intake, we categorized participants in sex-specific quartiles of processed meat and unprocessed red meat intake. Approximate quartiles were used for unprocessed red meat because of a relatively high proportion of participants who did not report any intake on the recalled days (34.3% and 39.7% of men and women, respectively), with all nonreporters grouped in the first category. We compared participants' characteristics across meat consumption categories with the use of generalized linear models for continuous nondietary variables, the chi-square test for categorical variables, and the nonparametric Kruskal-Wallis test for dietary variables. The main analysis consisted of multivariable linear regression with robust variance (mixed procedure) with the use of SAS version 9.3 (SAS Institute Inc.), with inflammatory markers as dependent variables and meat intake as independent variables. Statistical significance was defined as $\alpha < 0.05$ ($P < 0.05$). Adjusted geometric means and 95% CIs of inflammatory marker concentrations are presented by quartiles of processed or unprocessed red meat intake. Trends across meat consumption quartiles were calculated by treating the median values for each category as a continuous variable and examined for significance with the Wald test. We also investigated continuous estimates, which can be interpreted as relative difference (percentage) in markers' concentrations per 50-g increment in processed meat or unprocessed red meat consumption (increment based on approximate SDs).

We also calculated the so-called TNF molar ratio (estimated bioavailable TNF-α fraction) by dividing the molar concentration (concentration/molecular weight) of total TNF-α by the sum of the molar concentrations of sTNF-R1 and sTNF-R2 (20). The ratio was multiplied by 100 and can be interpreted as bioavailable TNF-α molecules per 100 soluble receptor molecules. The molar ratio was log-transformed for analysis. In addition, we created inflammation scores to estimate overall inflammation (21, 22). For these variables, individual observations of each marker were ranked in percentiles and standardized as *z* scores. For score 1, the *z* scores for CRP, IL-6, and total TNF-α were summed together. For score 2, we used score 1 and then subtracted the *z* scores of sTNF-R1 and sTNF-R2 under a physiologic function rationale because they keep TNF-α inactive while bound, contributing to a lesser effect from this proinflammatory cytokine. Overall, larger scores would represent a higher inflammation load.

We show 3 different regression models: age- and sex-adjusted (model 1); multivariable model additionally adjusted for socioeconomic status, smoking status, physical activity, total energy intake (excluding energy from alcohol), and alcohol intake (model 2); and a multivariable model in which we additionally adjusted for BMI (in kg/m^2) and BMI-adjusted waist circumference residuals (model 3). As a sensitivity analysis, we also show a model that excluded participants with a ratio of energy intake to estimated basal metabolic rate of <0.80 (n = 17 men; n = 27 women) because such low values may reflect potential underreporting of diet (23). Covariables were chosen based on clinical relevance and on adjustments in comparable investigations (8, 9, 17, 24, 25). Because 372 study participants had the blood samples taken in fasting status (≥9 h), we tested for an association of fasting status with inflammation markers with the use of Wilcoxon 2-sample and 2-sided tests. Of these, all but CRP showed a significant association, which was why we incorporated fasting status into the multivariable model in the case of IL-6, total TNF-α, sTNF-R1, and sTNF-R2. Analyses are shown for both processed meat and unprocessed red meat consumption.

A diet rich in fruits and vegetables has been associated with a lower intake of red meat and lower plasma inflammation markers (6, 8, 26). Furthermore, a higher consumption of dairy products has been associated with higher inflammation (27). To examine whether confounding by fruit, vegetable, and dairy consumption may have affected our results, we adjusted for these food groups in further sensitivity analyses. As an additional sensitivity analysis, we ran models that excluded participants who reported a previous diagnosis of the following diseases associated with chronic inflammation (5): T2D (n = 37), CVD (n = 18), cancer (n = 16), and inflammatory bowel disease (n = 48). This left a total number of 451 individuals for analysis. Potential effect modification was evaluated by testing for interaction in the association between meat consumption (processed meat, unprocessed red meat) and all markers by sex, BMI, and physical activity with the use of cross-product terms and the Wald test to evaluate statistical significance.

Results

The final study sample consisted of 553 men and women from BVSII. Overall, women consumed half the amount of processed meat and approximately two-thirds the amount of unprocessed red meat compared with men. In total, 6% of the men and 13.1% of the women did not report consumption of processed meat. Unprocessed red meat consumption was not reported by 34.3% of the men and 39.7% of the women (**Table 1**). Baseline characteristics of the study participants by meat consumption quartiles were similar for processed meat and unprocessed meat

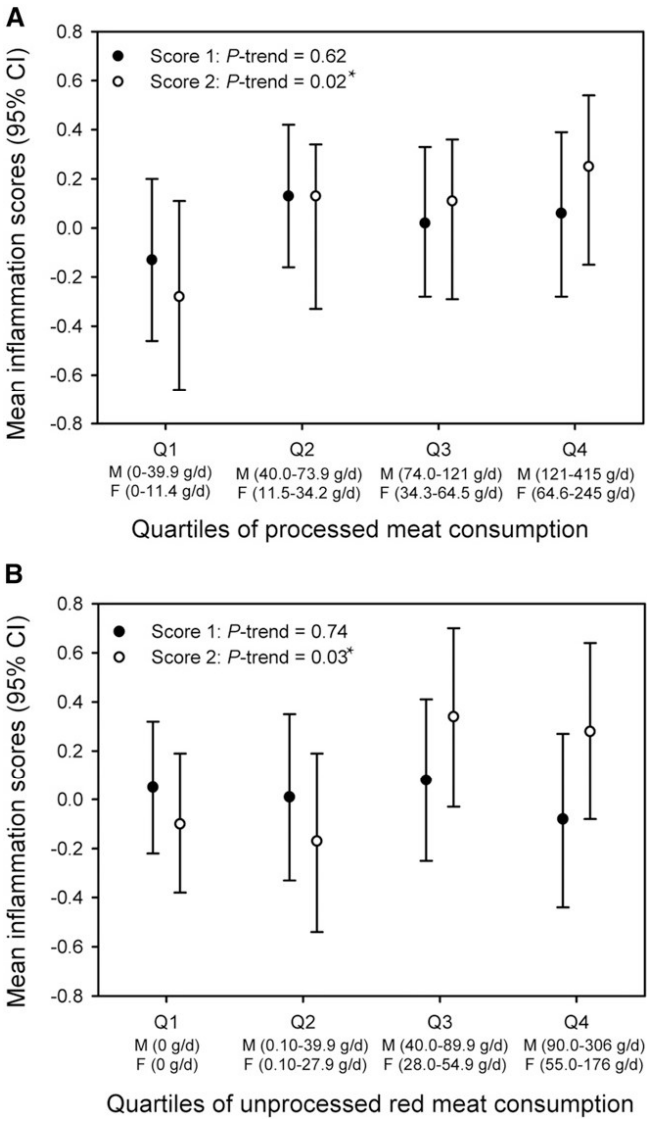

FIGURE 1 Overall inflammation scores' least squares means (95% CIs) according to processed meat (A) and unprocessed red meat (B) quartiles in Bavarian adults (n = 553). The multivariable model plus body fatness (model 3) adjusted for sex, age, socioeconomic status, smoking status, physical activity, total nonalcohol energy intake, alcohol intake, BMI (in kg/m^2), and BMI-adjusted waist circumference residuals is shown. Score 1 is the sum of the ranked and *z*-standardized scores for C-reactive protein, IL-6, and total TNF-α. Score 2 additionally subtracts the scores of soluble TNF receptors 1 and 2 from score 1. *$P < 0.05$. F, female; M, male; Q, quartile.

consumption quartiles (**Table 2**). Individuals in the highest quartiles were more likely to have the lowest socioeconomic status, to be less physically active, and to consume more calories and grams of ethanol and less fruits and dairy products than individuals in the lower meat quartiles. Smoking status, age, BMI, and HDL cholesterol did not differ across meat consumption quartiles.

The inflammation biomarkers CRP, IL-6, total TNF-α, sTNF-R1, and sTNF-R2 were positively correlated with one another (**Supplemental Table 1**). We did not observe any significant interactions in the association between processed meat or unprocessed red meat consumption and inflammatory markers by sex, BMI, or physical activity (results not shown). Sex-stratified analyses were similar for men and women (results not shown), which is why we show the main results aggregated for men and women.

For processed meat consumption, linear regression results did not differ substantially between the different adjustment models (**Table 3**). A positive association between processed meat consumption and IL-6 plasma concentrations was significant only before adjusting for BMI and BMI-adjusted waste circumference residuals (model 2). After excluding potential underreporters as a sensitivity analysis in the fully adjusted model, the continuous analysis remained borderline significant (5%; 95% CI: −1%, 10%), whereas the quartile analysis showed a statistically significant trend (*P*-trend = 0.046). A significant linear association was not observed with plasma concentrations of CRP (2%; 95% CI: −6%, 10%), although these were slightly higher in the highest processed meat consumption quartile than in the lower quartiles. Processed meat consumption was statistically significantly inversely associated with total TNF-α, sTNF-R1, and sTNF-R2 concentrations (TNF-α: −3%, 95% CI: −6%, −1%; sTNF-R1: −3%, 95% CI: −4%, −1%; and sTNF-R2: −2%, 95% CI: −4%, 0%). However, we observed no association with the TNF molar ratio. Processed meat consumption was not associated with the overall inflammation score 1 but was statistically significantly positively associated with score 2 across quartile categories (*P*-trend = 0.02) (**Figure 1**).

In further sensitivity analyses, processed meat consumption was positively associated with IL-6 after adjusting for fruit and vegetable consumption (but not dairy consumption) (5%; 95% CI: 0%, 10%) (**Table 4**). Likewise, the sensitivity analysis that excluded participants with chronic diseases showed a significant association of processed meat consumption with IL-6 plasma concentrations (7%; 95% CI: 1%, 14%). The associations for the TNF pathway markers in the sensitivity analyses remained unchanged after additionally adjusting for fruit, vegetable, and dairy intake but were attenuated and no longer statistically significant after excluding participants with chronic diseases.

Unprocessed red meat consumption was not associated with plasma concentrations of CRP, IL-6, or total TNF-α (**Table 5**). Additionally adjusting for covariables in multivariable models 2 and 3 noticeably modified the continuous estimates for CRP and IL-6 but not the other markers. However, these associations were statistically nonsignificant. There was an inverse association with sTNF-R1 and sTNF-R2 (sTNF-R1: −3%, 95% CI: −5%, −1%; sTNF-R2: −4%, 95% CI: −7%, −2%) and a positive association with the TNF molar ratio (4%; 95% CI: 0%, 7%), suggesting a positive association with free circulating TNF-α. Excluding individuals who did not consume unprocessed red meat on the recalled days did not substantially change the results (**Supplemental Table 2**).

Similar to the observations on processed meat, unprocessed red meat consumption showed no association with overall inflammation score 1 but a positive association with score 2, with a statistically significant trend across quartiles (*P*-trend = 0.03) (Figure 1). No substantially different results were observed in the sensitivity analyses adjusting for fruit, vegetable, or dairy consumption or when participants with chronic diseases were excluded (**Supplemental Table 3**).

Discussion

In this study, we did not observe any association between processed meat or unprocessed red meat consumption and CRP concentrations. Processed meat was borderline-significantly positively associated with plasma concentrations of IL-6 after excluding potential underreporters in sensitivity analyses. Processed meat intake was unexpectedly inversely associated with total TNF-α. However,

TABLE 4 Sensitivity analyses on the association between processed meat consumption and plasma concentrations of inflammation markers among adults in the Bavarian Food Consumption Survey II[1]

	Main analysis		Plus fruit consumption		Plus vegetable consumption		Plus dairy consumption		Excluding chronic diseases	
	β (95% CI)	*P*-trend	β (95% CI)	*P*-trend	β (95% CI)	*P*-trend	β (95% CI)	*P*-trend	β (95% CI)	*P*-trend
% Difference per 50 g										
CRP, mg/L	2 (−6, 10)	0.40	2 (−7, 10)	0.41	1 (−7, 9)	0.47	1 (−7, 9)	0.54	3 (−6, 12)	0.25
IL-6, pg/mL	5 (−1, 10)	0.07	5 (0, 10)	0.03*	5 (0, 10)	0.049*	4 (−2, 10)	0.12	7 (1, 14)	0.02*
Total TNF-α, pg/mL	−3 (−6, −1)	0.02*	−3 (−6, 0)	0.049*	−4 (−6, −1)	0.02*	−4 (−6, −1)	0.02*	−2 (−5, 1)	0.14
sTNF-R1, ng/mL	−3 (−4, −1)	0.003*	−3 (−4, −1)	0.01*	−3 (−5, −1)	0.002*	−3 (−5, −1)	0.003*	−1 (−3, 0)	0.18
sTNF-R2, ng/mL	−2 (−4, 0)	0.01*	−2 (−4, 0)	0.02*	−2 (−4, 0)	0.01*	−2 (−4, 0)	0.01*	−2 (−4, 0)	0.10
TNF molar ratio[2]	−1 (−3, 1)	0.61	−1 (−3, 2)	0.75	−1 (−3, 1)	0.58	−1 (−3, 1)	0.62	−1 (−4, 2)	0.63
Absolute units per 50 g										
Score 1[3]	0.00 (−0.14, 0.14)	0.62	0.01 (−0.14, 0.15)	0.56	−0.01 (−0.15, 0.13)	0.68	−0.02 (−0.16, 0.13)	0.77	0.05 (−0.11, 0.21)	0.27
Score 2[4]	0.15 (0.01, 0.29)	0.02*	0.15 (0.00, 0.29)	0.03*	0.14 (0.00, 0.29)	0.03*	0.14 (−0.01, 0.28)	0.04*	0.14 (−0.04, 0.32)	0.06

[1] Mixed linear regression models were used. Dependent variables were log-transformed (geometric means and their respective 95% CIs were back-transformed to concentrations for an easier interpretation). The fully adjusted model 3 was used to adjust for sex, age, socioeconomic status, smoking status, physical activity, total nonalcohol energy intake, alcohol intake, BMI (in kg/m^2), BMI-adjusted waist circumference residuals, and fasting status in the case of IL-6, total TNF-α, sTNF-R1, and sTNF-R2. The *P*-trend was calculated by treating the median values of each meat consumption quartile as a continuous variable. *$P < 0.05$. CRP, C-reactive protein; sTNF-R, soluble TNF receptor.

[2] Free TNF-α molecules per 100 molecules of soluble TNF receptors.

[3] Score 1 is the sum of the ranked and *z*-standardized scores for CRP, IL-6, and total TNF-α.

[4] Score 2 additionally subtracts the scores of sTNF-R1 and sTNF-R2 from score 1.

TABLE 5 Associations of unprocessed red meat consumption with plasma inflammation markers among adults in the Bavarian Food Consumption Survey II[1]

	Quartile 1 (n = 207)	Quartile 2 (n = 114)	Quartile 3 (n = 115)	Quartile 4 (n = 117)	P-trend	Continuous β % difference per 50 g
Range, g/d						
Men (n = 233)	0	0.1–39.9	40–89.9	90–306		
Women (n = 320)	0	0.1–27.9	28–54.9	55–176		
CRP, mg/L						
Model 1	1.67 (1.43, 1.96)	2.11 (1.74, 2.55)	1.89 (1.58, 2.27)	1.80 (1.48, 2.21)	0.45	3 (−8, 14)
Model 2	1.78 (1.52, 2.09)	2.09 (1.72, 2.54)	1.88 (1.57, 2.26)	1.79 (1.45, 2.22)	0.81	0 (−11, 11)
Model 3	1.79 (1.54, 2.09)	1.99 (1.66, 2.38)	1.87 (1.58, 2.21)	1.60 (1.32, 1.93)	0.40	−5 (−15, 5)
Model 3†	1.70 (1.45, 1.99)	1.96 (1.61, 2.40)	1.73 (1.45, 2.06)	1.53 (1.26, 1.85)	0.42	−5 (−15, 5)
IL-6, pg/mL						
Model 1	1.58 (1.40, 1.78)	1.57 (1.42, 1.74)	1.56 (1.41, 1.74)	1.59 (1.39, 1.83)	0.76	3 (−5, 11)
Model 2	1.62 (1.43, 1.83)	1.54 (1.38, 1.72)	1.52 (1.36, 1.70)	1.57 (1.36, 1.82)	0.93	1 (−7, 10)
Model 3	1.64 (1.45, 1.85)	1.52 (1.38, 1.67)	1.53 (1.38, 1.70)	1.48 (1.29, 1.71)	0.41	−1 (−9, 7)
Model 3†	1.61 (1.42, 1.82)	1.51 (1.36, 1.68)	1.47 (1.32, 1.64)	1.46 (1.27, 1.68)	0.41	−2 (−10, 7)
Total TNF-α, pg/mL						
Model 1	11.6 (11.0, 12.2)	11.3 (10.4, 12.2)	11.3 (10.6, 12.1)	12.0 (11.2, 12.9)	0.43	0 (−3, 4)
Model 2	11.7 (11.0, 12.4)	11.3 (10.4, 12.3)	11.4 (10.6, 12.2)	12.0 (11.1, 12.9)	0.59	0 (−3, 3)
Model 3	11.7 (11.0, 12.4)	11.3 (10.3, 12.3)	11.4 (10.6, 12.2)	11.9 (11.0, 12.9)	0.72	0 (−4, 3)
Model 3†	11.5 (10.8, 12.2)	11.2 (10.2, 12.4)	11.3 (10.5, 12.2)	11.7 (10.8, 12.7)	0.65	0 (−4, 3)
sTNF-R1, ng/mL						
Model 1	1.79 (1.74, 1.84)	1.90 (1.79, 2.00)	1.78 (1.72, 1.85)	1.74 (1.67, 1.81)	0.18	−2 (−4, 0)
Model 2	1.79 (1.73, 1.85)	1.87 (1.77, 1.97)	1.77 (1.70, 1.84)	1.72 (1.65, 1.80)	0.12	−3 (−5, −1)
Model 3	1.79 (1.74, 1.85)	1.86 (1.76, 1.96)	1.77 (1.70, 1.84)	1.70 (1.63, 1.77)	0.02*	−3 (−5, −1)
Model 3†	1.79 (1.73, 1.85)	1.86 (1.76, 1.98)	1.74 (1.68, 1.81)	1.69 (1.62, 1.76)	0.01*	−4 (−6, −2)
sTNF-R2, ng/mL						
Model 1	4.58 (4.43, 4.73)	4.76 (4.49, 5.05)	4.26 (4.06, 4.48)	4.36 (4.18, 4.55)	0.01*	−3 (−6, −1)
Model 2	4.63 (4.45, 4.81)	4.76 (4.48, 5.05)	4.28 (4.08, 4.50)	4.36 (4.16, 4.57)	0.01*	−4 (−6, −2)
Model 3	4.64 (4.46, 4.82)	4.74 (4.47, 5.03)	4.28 (4.08, 4.50)	4.32 (4.12, 4.53)	0.001*	−4 (−7, −2)
Model 3†	4.64 (4.46, 4.83)	4.75 (4.45, 5.07)	4.26 (4.05, 4.47)	4.29 (4.09, 4.50)	0.001*	−5 (−7, −2)
TNF molar ratio[2]						
Model 1	0.72 (0.69, 0.76)	0.67 (0.63, 0.73)	0.74 (0.70, 0.79)	0.78 (0.73, 0.83)	0.03*	3 (0, 7)
Model 2	0.73 (0.69, 0.77)	0.68 (0.63, 0.73)	0.75 (0.70, 0.79)	0.78 (0.73, 0.84)	0.03*	3 (0, 7)
Model 3	0.73 (0.69, 0.76)	0.68 (0.63, 0.73)	0.75 (0.70, 0.79)	0.79 (0.73, 0.84)	0.02*	4 (0, 7)
Model 3†	0.71 (0.68, 0.75)	0.67 (0.62, 0.73)	0.75 (0.70, 0.80)	0.78 (0.73, 0.84)	0.01*	4 (1, 8)

[1] All values are geometric means (95% CIs) unless otherwise indicated. Mixed linear regression models were used. Dependent variables were log-transformed (geometric means and their respective 95% CIs were back-transformed to concentrations for an easier interpretation). Model 1 was adjusted for sex and age; model 2 was additionally adjusted for socioeconomic status, smoking status, physical activity, total nonalcohol energy intake, alcohol intake, and fasting status in the case of IL-6, total TNF-α, sTNF-R1, and sTNF-R2; model 3 was additionally adjusted for BMI (in kg/m^2) and BMI-adjusted waist circumference residuals; and model 3† additionally excludes potential underreporters (n = 44) as a sensitivity analysis. The P-trend was calculated by treating the median values of each meat consumption quartile as a continuous variable. *$P < 0.05$. CRP, C-reactive protein; sTNF-R, soluble TNF receptor.

[2] Free TNF-α molecules per 100 molecules of soluble TNF receptors.

the TNF molar ratio, a measure of free TNF-α, was not associated with processed meat and showed a positive association with unprocessed red meat consumption. Although both processed meat and unprocessed red meat consumption were inversely associated with sTNF-R1 and sTNF-R2, we observed a statistically significant positive association when considering TNF-α and its receptors in the inflammation score 2.

Various components of red and processed meat have been proposed to contribute to chronic inflammation and disease risk, such as advanced glycation end products, heme iron, and nitrosamines (28). A recent review (29) suggests heme-catalyzed lipid peroxidation products and the presence of *N*-glycolylneuraminic acid, a sialic acid found in mammalian cells that provokes an immune response in humans, as a plausible mechanism that triggers chronic inflammation in response to both red and processed meat consumption. In the case of processed meats, other compounds such as nitrosamines can contribute to higher oxidation and inflammation (28).

Our results are difficult to compare to those of other observational studies because most studies on the association between meat consumption and markers of inflammation only examined CRP, whereas other inflammatory markers were less often measured. Furthermore, findings from previous studies have been inconsistent. For instance, similar to our results, a cross-sectional study within the Nurses' Health Study found neither unprocessed meat nor processed meat to be associated with CRP plasma concentrations after adjusting for BMI (8). However, another cross-sectional study found total red meat consumption to be positively associated with CRP concentrations independent of BMI (6), but this study did not differentiate unprocessed red meats from processed meats. Other studies in which meat consumption was assessed as part of secondary analyses have also shown mixed findings. For example, a nested case-control study within the European Prospective Investigation into Cancer and Nutrition study found only unprocessed red meat to be

positively associated with CRP plasma concentrations but not processed meats (30). Another cross-sectional study (31) reported no association between meat consumption and CRP or IL-6 concentrations, but this study did not differentiate between unprocessed red meats and processed meats. Inconsistencies between these findings may be attributable to different populations (different eating habits and potentially different susceptibility to inflammation), differences in the measurement of exposure, and the methods of analysis and small study effects for which small associations may be missed.

Studies that have investigated dietary factors in relation to TNF-α and its soluble receptors are scarce, so confirming our findings in other studies is warranted. However, the inverse associations of processed meat and unprocessed red meat intake with the soluble TNF receptors observed herein suggest that interpreting these markers as merely proinflammatory may be misleading. As stated previously, these receptors have a complex regulatory role on the proinflammatory function of TNF-α (14). One reason why these markers are often interpreted as proinflammatory is because they are positively correlated with other proinflammatory cytokines, which was also observed in our study. Positive associations of sTNF-R1 and sTNF-R2 have been observed with renal function loss in type 1 and T2D populations (32, 33), as well as with the progression of cancer, Crohn disease, and CVD (14). After we excluded participants with T2D, cancer, CVD, and inflammatory bowel disease in our sensitivity analyses, the previously observed associations between processed meat consumption and the markers of the TNF pathway as well as the inflammation score 2 were no longer observed, suggesting a different response of sTNF-R1 and sTNF-R2 to red meat consumption in healthy participants compared with participants with chronic diseases.

Having a wide variety of inflammation markers allowed us to build the TNF molar ratio (and investigate bioavailable TNF-α independently from the cancelling effect of its soluble receptors) and to build scores of overall inflammation. However, there is no standard method to our knowledge for building such inflammation scores, and our results for score 2 need to be interpreted cautiously because *1*) they were observed mainly because of the soluble TNF receptors, which have a complex regulatory role over TNF-α activity, and *2*) this score was formulated a posteriori by subtracting the scores of sTNF-R1 and sTNF-R2 under a physiologic rationale. Another strength of this study is that we used multiple standardized 24-h dietary recalls. It has been demonstrated that 24-h dietary recalls typically result in smaller measurement errors than FFQs (34), particularly for frequently consumed foods (35). Nevertheless, an important limitation of our study is that unprocessed red meat was not frequently consumed in our study sample. This could have possibly resulted in an underestimation of the true mean consumption of this group if the dietary recall was conducted on a nonconsumption day or an overestimation of the true mean consumption if the recall was on a consumption day. Therefore, the findings on unprocessed red meat should be interpreted cautiously. For measuring the consumption of foods that are not frequently consumed, more repeated 24-h dietary recalls would reduce measurement errors. Alternatively, a combination of dietary assessment methods such as the National Cancer Institute method combining 24-h dietary recall data with auxiliary data from FFQs would improve the accuracy of usual food intake estimation (35). Finally, although BVSII was designed to be representative of the Bavarian population, the response rate for the blood sampling was 67% (after an initial 71% response rate in BVSII), limiting generalizability to the adult Bavarian population.

In conclusion, both processed meat and unprocessed red meat consumption showed inverse associations with sTNF-R1 and sTNF-R2, and unprocessed red meat consumption showed a positive association with bioavailable TNF-α. Both processed meat and unprocessed red meat were positively associated with an overall inflammation score (score 2) considering sTNF-R1 and sTNF-R2. In the case of processed meat, the inverse associations with markers of the TNF pathway were attenuated after excluding chronic diseases. Our findings suggest that taking the inflammation markers of the TNF pathway into account may contribute to understanding the link between red meat consumption, chronic low-grade inflammation, and the risk and progression of chronic diseases.

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