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

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iv. **Abbreviations:** 24hdr, 24-hour dietary recall; BMI, body mass index; BVSII, Bavarian Food Consumption Survey II; CRP, C-reactive protein; CVD, cardiovascular disease; FFQ, food frequency questionnaire; HDL, high density lipoprotein; IL-6, interleukin-6; SES, socioeconomic status; sTNF-R1, soluble tumor necrosis factor receptor 1; sTNF-R2, soluble tumor necrosis factor receptor 2; T2D, type 2 diabetes; TNF, tumor necrosis factor; WC, waist circumference.

ABSTRACT

Background: High consumption of red and processed meat 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 and soluble TNF-receptors (sTNF-R1, sTNF-R2) in German adults.

Design: Inflammation markers were quantified in the plasma of 553 adults (233 male, 320 female, aged 18-80 years) within the cross-sectional Bavarian Food Consumption Survey. Dietary intake was estimated from three 24-hour dietary recalls (24hdr). The association between red meat consumption and inflammation markers was analyzed using 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 with sTNF-R2 (-4%; 95% CI: -7%, -2%).

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

Keywords: processed meat, unprocessed red meat, inflammation, C-reactive protein, interleukin-6, tumor necrosis factor alpha, soluble tumor necrosis factor receptors.

INTRODUCTION

High consumption of red meat, mainly processed meat, has been linked to higher risk of chronic diseases, in particular cardiovascular diseases (CVD), type 2 diabetes (T2D), and colorectal cancer (1-3). In 2015, the International Agency for Research on Cancer (IARC) concluded that there is convincing evidence that consumption of processed meat (and probably red meat) are causes of colorectal cancer (4). However, the underlying mechanisms for these associations are unclear. Higher concentrations of plasma 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 (NHS) (8) and the European Prospective Investigation into Cancer and Nutrition (EPIC) (9). However, this association was attenuated in both studies after adjusting for body mass index (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 protein CRP, while studies

investigating other major pro-inflammatory cytokines, such as interleukin-6 (IL-6), tumor necrosis factor (TNF)-α, or the soluble TNF receptors (sTNF-R1, sTNF-R2) are scarce. Soluble TNF receptors bind free TNF-α, thereby regulating its inflammatory activity (10). Although sTNF-R1 and -R2 show both anti-inflammatory and pro-inflammatory functions, they are usually interpreted as pro-inflammatory cytokines in epidemiological studies (11-13) since 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's 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, and sTNF-R1 and -R2 in the general Bavarian population.

METHODS

Study population

As part of the Bavarian Food Consumption Survey II (BVS II) in Bavaria, Germany, 1050 individuals aged 13-80 years were randomly recruited in 2002-2003. Overall response rate for this study was 71%. As has been described previously (15), all adult participants (18-80 years old) who had completed the personal interview and a minimum of one dietary recall were invited for blood sampling and anthropometric measurements. 568 individuals (65% of the eligible sample) participated in the blood sampling and anthropometric measurements, which took place up to six weeks after 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 in the study report (16).

Laboratory methods

Venous blood samples were drawn and extracted into EDTA tubes. Samples were chilled at 4-8°C and were centrifuged to separate plasma from blood cells. Samples were kept at a temperature of -80°C until analysis.

Inflammation markers (hs-CRP [high sensitivity CRP, referred to as CRP for simplicity reasons in this manuscript], IL-6, total TNF-α, and sTNF-R1 and sTNF-R2) were measured immediately after accomplishment of the study and according to manufacturer's instructions [Biosource, Brussels, Belgium] with commercial enzyme-linked immunosorbent assays (detailed methods described elsewhere (17)). Intra-assay coefficients of variation for all assays were below 7% and inter-assay coefficients of variation below 9% (17, 18).

Dietary intake (meat consumption) assessment

Dietary intake including meat consumption was assessed through three 24-hour dietary recalls (24hdr). Dietary recalls were conducted by trained interviewers by telephone and were performed unannounced both on weekdays and weekend days (2 in weekdays, 1 in weekend day). Average daily food intake was calculated by weighing recalled intake correspondingly to weekday and weekend day. Red meat was considered as any meat coming from beef, veal, pork, mutton/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

For the present analysis, a total of 553 BVS II participants with complete information on inflammation markers and at least two 24hdr (mean number of recalls 2.99 ± SD 0.07) were included (14 participants had missing data on inflammation markers of interest and one participant was excluded due to implausible IL-6 levels) (Supplemental Figure 1). Missing values for waist circumference (n=8) and smoking status (n=1) were replaced with sex-specific 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 due to a relatively high proportion of participants who did not report any intake on the recalled days (34.3% of men, 39.7% of women), with all non-reporters grouped in the first category. We compared participants' characteristics across meat consumption categories using generalized linear models for continuous non-dietary variables, Chi-square test for categorical variables, and non-parametric Kruskal Wallis test for dietary variables. The main analysis consisted of multivariable linear regression with robust variance (proc mixed) using SAS (Version 9.3; Enterprise Guide 4.3, SAS Institute Inc., Cary, NC, USA) with inflammatory markers as dependent and meat intake as independent variables. Statistical significance was defined by $\alpha<5\%$ (P values < 0.05). Adjusted geometric means and 95% confidence intervals (CI) 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 Wald's Test. We also investigated continuous estimates, which can be interpreted as relative difference (%) in markers' concentrations per 50g increment in processed meat or unprocessed red meat consumption (increment based on approximate standard deviations).

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 molar concentrations of sTNF-R1 and -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 up together. For score 2, we used Score 1 and then subtracted the z-scores of sTNF-R1 and sTNF-R2 under a physiological function rationale since they keep TNF-α inactive while bound, contributing to a lesser effect from this pro-inflammatory cytokine. Overall, larger scores would represent a higher inflammation load.

We show three different regression models: age and sex adjusted (M1); multivariable model additionally adjusted for socioeconomic status (SES), smoking status, physical activity, total energy intake (excluding energy from alcohol), and alcohol intake (M2); and a multivariable model where we additionally adjusted for BMI and BMI-adjusted waist circumference (WC) residuals (M3). As a sensitivity analysis, we additionally show M3 model excluding participants with a ratio of energy intake to estimated basal metabolic rate of less than 0.80 (n=17 men, n=27 women) ($M3^{\dagger}$), since 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 (of 9 hours or more), we tested for an association of fasting status with inflammation markers using Wilcoxon two-sample, two sided tests. Of these, all but CRP showed a significant association, reason 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 both for processed meat and unprocessed red meat consumption.

A diet rich in fruits and vegetables has been associated with lower intake of red meat and lower plasma inflammation markers (6, 8, 26). Furthermore, 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 with exclusion of participants who reported 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), leaving 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 (<25, $\geq$ 25 kg/m^2), and physical activity (< median, $\geq$ median) using cross-product terms and using Wald test to evaluate statistical significance.

RESULTS

The final study sample consisted of 553 men and women from the BVSII Study. Overall, women consumed half of the amount of processed meat and about two thirds of the amount of unprocessed red meat compared with men. 6% of the male participants and 13.1% of the women participants did not report consumption of processed meat. Unprocessed red meat consumption was not reported by 34.3% of men and 39.7% of women (Table 1). Baseline characteristics of the study participants by meat consumption quartiles were similar for processed meat and for unprocessed meat consumption quartiles (Table 2). Individuals in the highest quartiles were more likely to be of the lowest SES class, less physically active, consumed 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 each other (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), reason why we show 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 WC residuals (M2). After excluding potential underreporters as a sensitivity analysis in the fully adjusted model, the continuous analysis remained borderline significant (5%, 95% CI: -1%, 10% difference per 50g of processed meat) while the quartile analysis showed a statistically significant trend (*P*-trend= 0.046). No significant linear association was observed with plasma concentrations of CRP (2%, 95% CI: -6%, 10% difference per 50g of processed meat), although these were slightly higher in the highest processed meat consumption quartile compared to the lower quartiles. Processed meat consumption was statistically significantly inversely associated with total TNF-α, sTNF-R1, and sTNF-R2 concentrations (-3%, 95% CI: -6%, -1%; -3%, 95% CI: -4%, -1%; and -2%, 95% CI: -4%, 0% difference per 50g of processed meat, respectively). However, we observed no association with 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% difference per 50g of processed meat consumed) (Table 4). Likewise, the sensitivity analysis excluding participants with chronic diseases showed a significant association of processed meat consumption with IL-6 plasma concentrations (7%, 95% CI: 1%, 14% difference per 50g of processed meat consumed). The associations for the TNF pathway markers in the sensitivity analyses remained unchanged after additional adjustment 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 M2 and M3 noticeably modified the continuous estimates for CRP and IL-6 but not for the other markers. However, these associations were statistically non-significant. There was an inverse association with sTNF-R1 and sTNF-R2 (-3%, 95% CI: -5%, -1%; and -4%, 95% CI: -7%, -2% difference per 50g of unprocessed red meat, respectively) and a positive association with TNF molar ratio (4%, 95% CI: 0%, 7% difference per 50g of unprocessed red meat), suggesting a positive association with free circulating TNF-α. Excluding individuals who did not consume unprocessed red meat on the recalled days did not importantly 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 showed 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 exclusion of participants with chronic diseases (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

exclusion of potential underreporters in sensitivity analyses. Unexpectedly, processed meat intake was inversely association with total TNF- α. However, the TNF molar ratio, a measure for free TNF-α, was not associated with processed meat and showed a positive association with unprocessed red meat consumption. While 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 suggests heme-catalyzed lipid peroxidation products and the presence of Neu5Gc, a sialic acid found in mammalian cells that provokes an immune response in humans, as a plausible mechanism triggering chronic inflammation in response of both red and processed meat consumption (29). 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 with those of other observational studies, since 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 NHS found neither unprocessed meat nor processed meat to be associated with CRP plasma concentrations after adjustment for BMI (8). However, another cross-sectional study found total red meat consumption to be positively associated with CRP concentrations independent of BMI

(6). This study though did not differentiate unprocessed red meats from processed meats in the analysis. Other studies, where meat consumption was assessed as part of secondary analyses, have also shown mixed findings; for example, a nested case-control study within the EPIC study found only unprocessed red meat to be positively associated with CRP plasma concentrations but not processed meats (30). Another study of cross-sectional design saw no association between meat consumption and CRP or IL-6 concentrations (31) but 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 methods of analysis and small study effects where small associations may be missed.

Studies investigating dietary factors in relation to TNF-α and its soluble receptors are scarce, so confirmation of our findings in other studies is warranted. However, the here observed inverse associations of processed meat and unprocessed red meat intake with the soluble TNF receptors suggest that interpreting these markers as merely pro-inflammatory may be misleading. As stated in the introduction, these receptors have a complex regulatory role on TNF-α's pro-inflammatory function (14). A reason why these markers are often interpreted as pro-inflammatory is a positive correlation with other pro-inflammatory 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 2 diabetes populations (32, 33), as well as with disease progression of cancer, Crohn's 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.

Strengths and limitations

Having a wide variety of inflammation markers allowed us to build the TNF molar ratio (and investigate bioavailable TNF-α independent from the cancelling effect of its soluble receptors) and to build scores of overall inflammation. However, there is no standard method to build such inflammation scores and our results found for Score 2 need to be interpreted cautiously since (1) the results observed are mainly due to the TNF soluble receptors, which have a complex regulatory role over TNF-α activity, and since (2) it was built *a posteriori* by subtracting the scores of sTNF-R1 and sTNF-R2 under a physiological rationale. Another strength of this study is that we used multiple standardized 24hdr; it has been demonstrated that typically 24hdr result in smaller measurement error than food frequency questionnaires (FFQ) (34), in particular for frequently consumed foods (35). Nevertheless, an important limitation in our study is that unprocessed red meat was not frequently consumed in our study sample, underestimating the true mean consumption of this group if the dietary recall was conducted on a non-consumption day or overestimating 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 24hdr would reduce measurement error. Alternatively, a combination of dietary assessment methods such as the NCI method

combining 24hdr data with auxiliary data from food frequency questionnaires, would improve the accuracy of usual food intake estimation (35). Finally, although the 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.

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. Finally, 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 exclusion of 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 risk and progression of chronic diseases.

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TP and KN designed research; JL provided essential materials; CS analyzed data, performed statistical analysis, and wrote manuscript; KN has primary responsibility for final content. TP, JL, SR, HH edited the manuscript for important intellectual content. All authors read and approved the final manuscript.

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

Sex	Label	Median	[p25;p75]	[Min-Max]	No consumption on reported days (%)
Male (*N*=233)	Processed meat (g/d)	74.0	[40.0;121]	[0.00-415]	6.01
	Unprocessed red meat (g/d)	35.2	[0.00;84.9]	[0.00-306]	34.3
Female (*N*=320)	Processed meat (g/d)	34.3	[11.4;64.3]	[0.00-245]	13.1
	Unprocessed red meat (g/d)	22.3	[0.00;47.5]	[0.00-176]	39.7

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

	Quartiles of processed meat intake			Quartiles of unprocessed red meat intake		
	Q1 (*N*=138)	**Q4 (*N*=137)**	***P***	**Q1 (*N*=207)**	**Q4 (*N*=117)**	***P***
Range (g/day)						
Male	(0.00-39.9 g/d)	(121-415 g/d)		(0.00 g/d)	(90.0-306 g/d)	
Female	(0.00-11.4 g/d)	(64.6-245 g/d)		(0.00 g/d)	(55.0-176 g/d)	
			Frequency n(%)			
Sex (male)	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 (lowest)	14 (10.1)	26 (19.0)		21 (10.1)	16 (13.7)	
5 (highest)	12 (8.70)	13 (9.49)	0.99	34 (16.4)	4 (3.42)	
			Mean ± SD			
Age (years)	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/day)	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.

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

	Quartiles of processed meat consumption – Geometric mean (95%CI)					
	Q1 (*N*=138); (*M*=57, *F*=81)	**Q2 (*N*=138); (*M*=59, *F*=79)**	**Q3 (*N*=140); (*M*=59, *F*=81)**	**Q4 (*N*=137); (*M*=58, *F*=79)**	***P*-trend**	**Continuous β (95%CI) % difference per 50g**
Range (g/day)						
Male (*N*=233)	(0-39.9 g/d)	(40-73.9 g/d)	(74-121 g/d)	(121-415 g/d)		
Female (*N*=320)	(0-11.4 g/d)	(11.5-34.2 g/d)	(34.3-64.5 g/d)	(64.6-245 g/d)		
CRP (mg/L)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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						
M1	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)
M2	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)
M3	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)
M3†	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]						
M1	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)
M2	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)
M3	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)
M3†	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-a, sTNF-R1, and sTNF-R2; model 3 was additionally adjusted for BMI (in kg/m2) 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-a molecules per 100 molecules of soluble TNF receptors.

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 (*N*=102 excluded)**	
	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 50g					
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 50g					
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/m2), BMI-adjusted waist circumference residuals, and fasting status in the case of IL-6, total TNF-a, 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-a 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]

	Quartiles of unprocessed red meat consumption – Geometric mean (95%CI)					
	Q1 (*N*=207); (*M*=80, *F*=127)	**Q2 (*N*=114); (*M*=50, *F*=64)**	**Q3 (*N*=115); (*M*=51, *F*=64)**	**Q4 (*N*=117); (*M*=52, *F*=65)**	***P*-trend**	**Continuous β (95%CI) % difference per 50g**
Range (g/day)						
Male (*N*=233)	(0 g/d)	(0.1-39.9 g/d)	(40-89.9 g/d)	(90-306 g/d)		
Female (*N*=320)	(0 g/d)	(0.1-27.9 g/d)	(28-54.9 g/d)	(55-176 g/d)		
CRP (mg/L)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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)						
M1	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)
M2	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)
M3	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)
M3†	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]						
M1	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)
M2	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)
M3	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)
M3†	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-a, sTNF-R1, and sTNF-R2; model 3 was additionally adjusted for BMI (in kg/m2) 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-a molecules per 100 molecules of soluble TNF receptors.

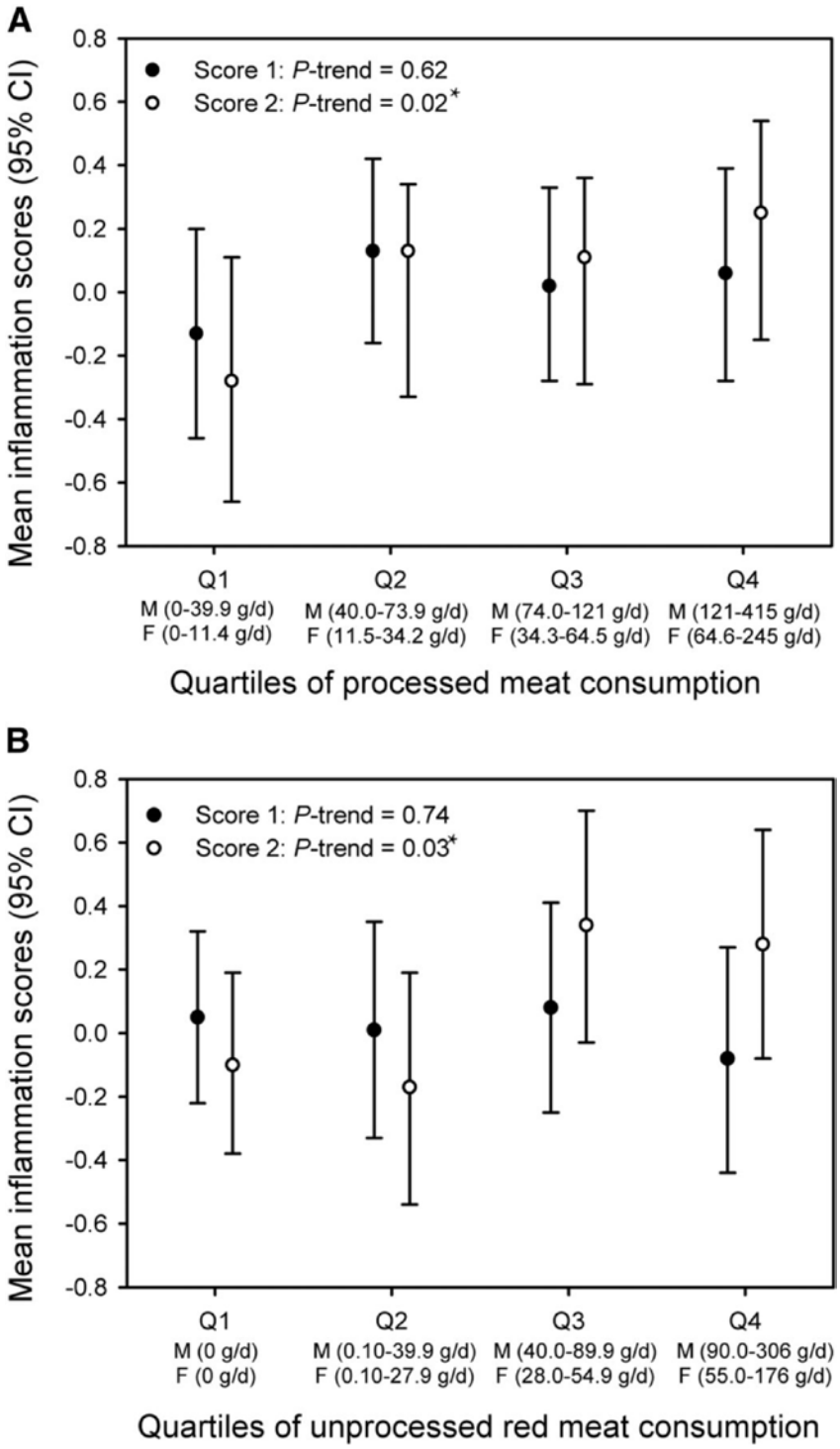
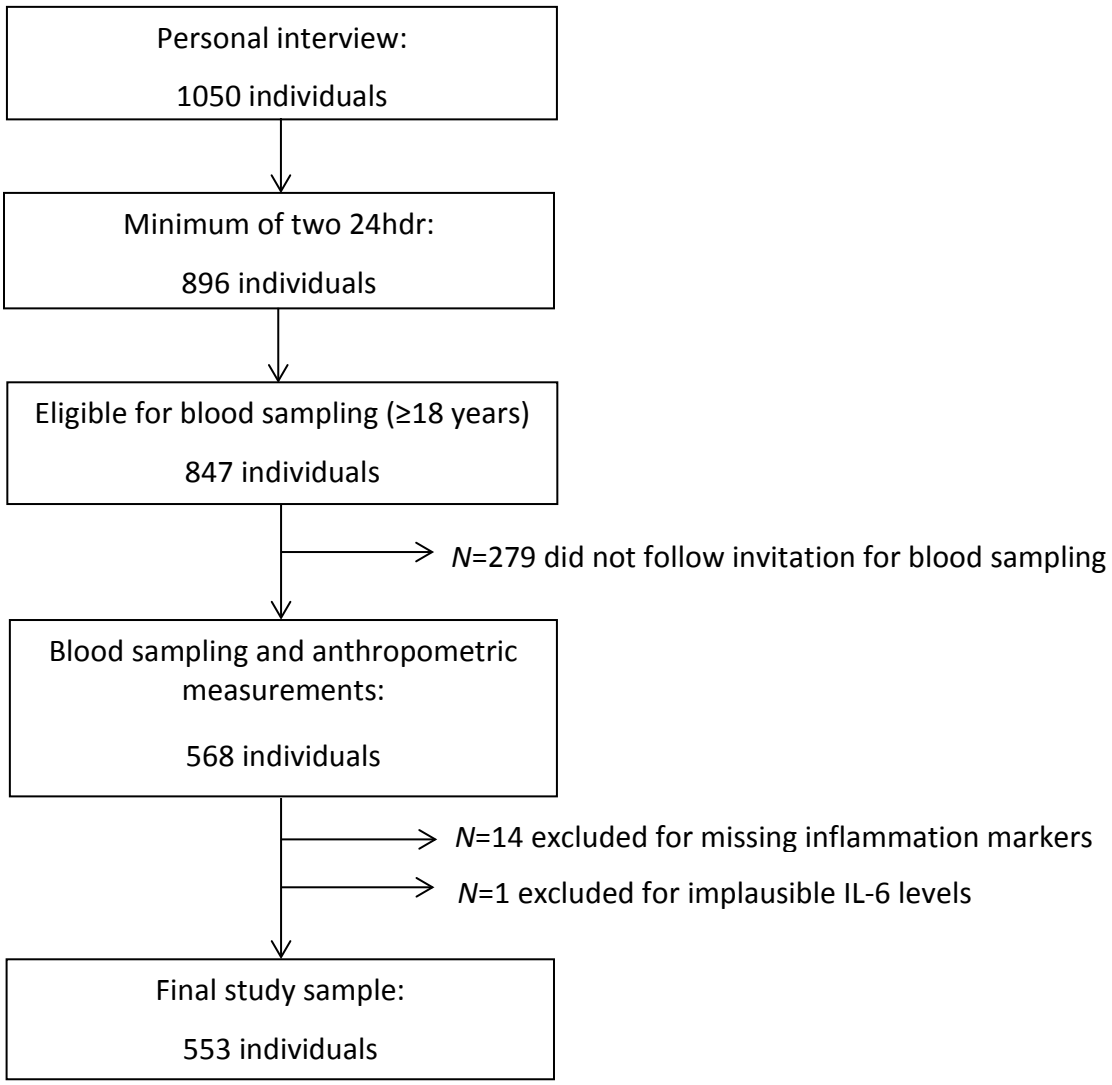


Figure 1: Overall inflammation scores_ least squares means (95% CIs) according to processed meat (A) and unprocessed red meat (B) quartiles in Bavarian adults (n = 553). The multivariable model plus body fatness (model 3) adjusted for sex, age, socioeconomic status, smoking status, physical activity, total nonalcohol energy intake,

alcohol intake, BMI (in kg/m2), 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-a. 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.



Supplemental Figure 1: Flow-chart of participants within the Bavarian Food Consumption Survey II included in this study

Supplemental Table 1: Correlation among inflammation markers in adults in the Bavarian Food Consumption Survey II (Spearman correlation coefficients, (*P* value))

	CRP	IL-6	Total TNF-α	sTNF-R1	sTNF-R2	TNF molar ratio
CRP	-					
IL-6	0.51 (<0.0001)	-				
Total TNF-α	0.21 (<0.0001)	0.27 (<0.0001)	-			
sTNF-R1	0.29 (<0.0001)	0.38 (<0.0001)	0.47 (<0.0001)	-		
sTNF-R2	0.26 (<0.0001)	0.36 (<.0001)	0.43 (<0.0001)	0.71 (<0.0001)	-	
TNF molar ratio[1]	0.02 (0.57)	0.05 (0.27)	0.75 (<0.0001)	-0.07 (0.09)	-0.18 (<0.0001)	-

[1] TNF molar ratio represents free TNF-α molecules per 100 molecules of soluble TNF receptors.

Supplemental Table 2: Associations of unprocessed red meat consumption with plasma inflammation markers among adults in the Bavarian Food Consumption Survey II, excluding participants with no consumption on reported days (Q1)[1]

Inflammation marker Male (*N*=153) Female (*N*=193)	**Continuous β (95%CI)** **% difference per 50g**
CRP (mg/L)	-9 (-23; 4)
IL-6 (pg/mL)	3 (-5; 12)
Total TNF-α (pg/mL)	-1 (-6; 4)
sTNFRI (ng/mL)	-5 (-8; -3)
sTNFRII (ng/mL)	-5 (-8; -2)
TNF molar ratio[2]	4 (0; 9)

[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). Using the fully adjusted model M3: adjusted for sex, age, socioeconomic status (SES), smoking status, physical activity, total non-alcohol energy intake, alcohol intake, BMI, BMI-adjusted waist circumference residuals, 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-a molecules per 100 molecules of soluble TNF receptors.

Supplemental Table 3: Sensitivity analyses on the association between unprocessed red 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 (N=102 excluded)**	
	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 50g					
CRP (mg/L)	-5 (-15; 5)	0.40	-5 (-15; 5)	0.39	-5 (-15; 5)	0.42	-6 (-016; 5)	0.35	-4 (-15; 7)	0.48
IL-6 (pg/mL)	-1 (-9; 7)	0.41	-1 (-9; 7)	0.47	-1 (-9; 7)	0.41	-2 (-10; 6)	0.36	-1 (-10; 8)	0.52
Total TNF-α (pg/mL)	0 (-4; 3)	0.72	0 (-3; 3)	0.60	0 (-4; 3)	0.71	0 (-4; 3)	0.73	-1 (-5; 2)	0.91
sTNF-R1 (ng/mL)	-3 (-5; -1)	0.02*	-3 (-5; -1)	0.03*	-3 (-5; -1)	0.03*	-3 (-5; -1)	0.02*	-4 (-6; -2)	0.01*
sTNF-R2 (ng/mL)	-4 (-7; -2)	0.001*	-4 (-6; -2)	0.002*	-4 (-7; -2)	0.001*	-4 (-7; -2)	0.001*	-5 (-7; -2)	0.001*
TNF molar ratio[2]	4 (0; 7)	0.02*	4 (0; 7)	0.02*	4 (0; 7)	0.02*	4 (0; 7)	0.03*	3 (-1; 7)	0.06
					Absolute units per 50g					
Score 1[3]	-0.04 (-0.22; 0.13)	0.74	-0.04 (-0.22; 0.14)	0.78	-0.04 (-0.22; 0.14)	0.76	-0.05; (-0.23; 0.12)	0.68	-0.05 (-0.23; 0.14)	0.78
Score 2[4]	0.25 (0.04; 0.45)	0.03*	0.25 (0.04; 0.45)	0.03*	0.25 (0.04; 0.45)	0.03*	0.24 (0.04; 0.44)	0.03*	0.29 (0.07; 0.52)	0.01*

[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/m2), BMI-adjusted waist circumference residuals, and fasting status in the case of IL-6, total TNF-a, 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-a 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.