



## Conference on ‘The future of animal products in the human diet: health and environmental concerns’

### Symposium 1: Meat, health and sustainability

#### Processed meat: the real villain?

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Meat is a food rich in protein, minerals such as iron and zinc as well as a variety of vitamins, in particular B vitamins. However, the content of cholesterol and saturated fat is higher than in some other food groups. Processed meat is defined as products usually made of red meat that are cured, salted or smoked (e.g. ham or bacon) in order to improve the durability of the food and/or to improve colour and taste, and often contain a high amount of minced fatty tissue (e.g. sausages). Hence, high consumption of processed foods may lead to an increased intake of saturated fats, cholesterol, salt, nitrite, haem iron, polycyclic aromatic hydrocarbons, and, depending upon the chosen food preparation method, also heterocyclic amines. Several large cohort studies have shown that a high consumption of processed (red) meat is related to increased overall and cause-specific mortality. A meta-analysis of nine cohort studies observed a higher mortality among high consumers of processed red meat (relative risk (RR) = 1.23; 95 % CI 1.17, 1.28, top v. bottom consumption category), but not unprocessed red meat (RR = 1.10; 95 % CI 0.98, 1.22). Similar associations were reported in a second meta-analysis. All studies argue that plausible mechanisms are available linking processed meat consumption and risk of chronic diseases such as CVD, diabetes mellitus or some types of cancer. However, the results of meta-analyses do show some degree of heterogeneity between studies, and it has to be taken into account that individuals with low red or processed meat consumption tend to have a healthier lifestyle in general. Hence, substantial residual confounding cannot be excluded. Information from other types of studies in man is needed to support a causal role of processed meat in the aetiology of chronic diseases, e.g. studies using the Mendelian randomisation approach.

#### Processed meat: Red meat: Mortality: Cancer: CVD

Meat is an integral part of human diet in many cultures and in recent years, meat consumption has increased considerably in most parts of the world. According to the Food and Agriculture Organization, world *per capita* meat consumption was just over 30 kg per person per year in 1980; in 2005, it was 41 kg per person per year. As incomes rise, more meat is consumed<sup>(1)</sup>. Meat consumption contributes to intake of a number of vitamins and minerals such as vitamin B, vitamin A, zinc and

iron. It is also an important source of protein providing essential amino acids; however, meat is also rich in saturated fat and cholesterol. The total fat content of meat varies considerably with average values (median, interquartile range) of 9.3 (5.1–15) g/100 g in beef and 12.4 (4–16.2) g/100 g in pork; the same is true for SFA with values of 3.8 (2.5–6.1) g/100 g in beef and 3.5 (1.4–5.5) g/100 g in pork<sup>(2)</sup>. Total fat and saturated fat concentrations are distinctly higher in many types of processed meat, with

**Abbreviations:** EPIC, European Prospective Investigation into Cancer and Nutrition; NOC, N-nitroso compounds; PAH, polycyclic aromatic hydrocarbons; HCA, heterocyclic aromatic amines; TMAO, trimethylamine-N-oxide; HR, hazard ratio; RR, relative risk.

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**Table 1.** Association between red and processed meat consumption and all-cause and cause-specific mortality: results of meta-analyses

Effect	Larsson <sup>(24)</sup>	Abete <sup>(23)</sup>	Wang <sup>(25)</sup>
	RR top v. bottom intake category	RR top v. bottom intake category	RR increase of one serving per d
Unprocessed red meat			
All-cause mortality	1.10 (95 % CI 0.98, 1.22; six studies)	1.09 (95 % CI 0.94, 1.28; seven studies)	1.05 (95 % CI 0.93, 1.19; five studies) 1.23 (95 % CI 1.17, 1.30, USA) 0.90 (95 % CI 0.59, 1.38, Europe)
CVD mortality	–	1.16 (95 % CI 1.03, 1.32; seven studies)	1.06 (95 % CI 0.88, 1.28; six studies)
Cancer mortality	–	–	1.03 (95 % CI 0.89, 1.18; five studies)
Processed meat			
All-cause mortality	1.23 (95 % CI 1.17, 1.28; six studies)	1.22 (95 % CI 1.16, 1.29; five studies)	1.15 (95 % CI 1.11, 1.19; five studies)
CVD mortality	–	1.18 (95 % CI 1.05, 1.32; six studies)	1.15 (95 % CI 1.07, 1.24; six studies)
Cancer mortality	–	–	1.08 (95 % CI 1.06, 1.11; five studies)

extreme values of up to 90 g/100 g total fat and 25 g/100 g saturated fat in fatty bacon. Calculations of the contribution of meat and processed meat to the total daily energy intake or the total daily fat intake underpin the important role of meats in present dietary practice<sup>(3)</sup>.

Meat has already been consumed for thousands of years. Prior to the availability of adequate storage such as refrigerators or deep-freezers that can preserve fresh meat for a longer period, preservation by drying, salting, curing or smoking were the only means to be able to provide meat in times when no fresh meat was available. For example, salting and sun-drying was used in ancient Egypt<sup>(4)</sup>. In addition to improving the meat's durability, meat processing is also used to preserve the food's colour and taste.

Salting, i.e. adding NaCl to meat, increases its durability by decreasing the water content of meat and inhibiting micro-organisms<sup>(5)</sup>. Curing, that is adding salt enriched with nitrates and nitrites to meat products for preservation purposes, leads to the formation of N-nitroso compounds (NOC) and increases the originally low salt (NaCl) content of fresh meat. Similar to the developments in meat smoking, developments in manufacturing practice, e.g. addition of ascorbic acid, decreased the amount of nitrate/nitrite added to processed meat products in most European countries during past decades<sup>(6)</sup>.

In addition to this exogenous exposure, there is endogenous nitrate and nitrite generation from inducible and endogenous NO synthases, resulting in NOC production. NOC arise from the reaction of nitrite and secondary or tertiary amines in the intestine from N-nitrosation of amines, which are produced in the colon by bacterial decarboxylation of amino acids<sup>(7)</sup>. Additionally, haem iron from red but not white meat substantially increases the endogenous production of NOC<sup>(7)</sup>.

Smoking of meat inactivates enzymes and micro-organisms and influences its taste. As a downside, however, smoke contains polycyclic aromatic hydrocarbons (PAH), which are formed by pyrolytic processes at high smoking temperatures (400–1000°C). The type of wood used, the temperature, the use of smoke flavour additives and whether direct or indirect, hot or cold smoking methods determine the amount of PAH that is produced

during this process<sup>(8)</sup>. In the past couple of years, even decades, with improvements in managing the smoking process, the amount of PAH that is produced has decreased considerably<sup>(9)</sup>. In addition to PAH production, smoking also increases the concentration of NOC in food<sup>(10,11)</sup>.

In this review, we evaluate the current evidence on the association of processed meat consumption with mortality and the incidence of cancer, CVD and diabetes. In addition, we discuss effects of different components in red and processed meat and their possible role in the aetiology of these chronic diseases.

### Total mortality

Several prospective studies evaluated the association between meat intake and mortality<sup>(12–24)</sup>, some of which compared meat consumers with vegetarians<sup>(14,16,18,20)</sup>. The results of these studies mostly pointed in the direction of a positive association in particular of processed meat, consumption and all-cause mortality. Three meta-analyses have been published in the past 2 years using basically the same studies and coming up with similar results<sup>(25–27)</sup>. In all of them, processed meat consumption was significantly associated with a moderately increased all-cause mortality (Table 1), but the consumption of unprocessed red meat was not.

Several of the earlier mentioned cohorts estimated the contribution of high processed meat consumption to total mortality in terms of attributable or preventable fractions. The results of these estimates were quite heterogeneous: In the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, we estimated that a reduction of processed meat consumption to <20 g/d could reduce the total mortality by 3.3 % (95 % CI 1.5, 5.0 %). In contrast, estimates from US cohorts were much higher. In the American Association of Retired Persons cohort, the preventable fraction was estimated to be 20 % if women decreased their processed meat consumption to less than 1.6 g per 4184 kJ/d (1.6 g per 1000 kcal/d)<sup>(19)</sup> and in two other US cohorts, the preventable fraction was estimated to be 9.3 % in the Health Professionals Follow-up Study and 7.6 % in the Nurses' Health Study if the participants lowered

their red meat (processed and unprocessed) consumption to less than 0.5 servings daily. The difference between the US studies and the EPIC result is likely due to the stronger risk estimates observed in the US cohorts compared with EPIC, but may also be explained by higher meat consumption in the US than in Europe as well as differences in meat preparation and cooking.

An analysis of EPIC-Oxford participants observed that vegetarians as well as non-vegetarians with a health-conscious lifestyle had a statistically significantly lower mortality compared with the British general population<sup>(20)</sup>. Similarly, in a study in Germany, both vegetarians and health-conscious non-vegetarians had a statistically significantly lower overall mortality compared with the general population<sup>(18)</sup>. The implication of these results is that perhaps the decreased mortality in vegetarians, compared with the general population, is in large part due to a healthier lifestyle, such as having lower body fat, being more physically active, and not being a smoker. However, in the large US and European cohorts that did indeed report an increased risk for early mortality among individuals with a high red and processed meat consumption, this increase in risk was largely independent of smoking, obesity and other potential confounders<sup>(19,23,24)</sup>.

## Cancer

The cancer entity that is best studied in relation to meat consumption is colorectal cancer. Numerous case-control and cohort studies have evaluated the question whether red and/or processed meat consumption is associated with risk of this disease. Many of the studies, case-control as well as cohort studies, did indeed observe positive associations between red meat consumption and colorectal cancer risk. In a summary evaluation of the studies published thus far, the World Cancer Research Fund in 2007 came to the conclusion that high consumption of unprocessed red meat and of processed meat were convincingly associated with the risk of colorectal cancer<sup>(28)</sup>. This was confirmed in the 2011 updated report. Per 50 g increase in daily processed meat consumption, the relative risk (RR) increased by 18 % (95 % CI 1.10, 1.28); per 100 g increase in daily unprocessed red meat consumption, the RR increased by 17 % (95 % CI 1.05, 1.31)<sup>(29)</sup>. Underlining this, the International Agency for Research on Cancer recently declared that there is sufficient evidence in human beings for the carcinogenicity of the consumption of processed meat<sup>(30)</sup>.

In addition to colorectal cancer, high consumption of red and processed meat might be linked to several other cancer entities such as oesophagus, lung, pancreas and endometrium (red meat) as well as oesophagus, lung and stomach (processed meat)<sup>(28)</sup>.

Based on these findings, World Cancer Research Fund recommended in their conclusions that red meat intake should be limited to <500 g/week and very little, if any, of this should be processed meat.

Several mechanisms were proposed and examined to explain an increased risk of certain types of cancer with

**Table 2.** Potential mechanisms connecting meat consumption and risk of chronic diseases

	Red or processed meat	Cancer	CVD	T2D
Haem iron	Both	x	x	x
Saturated fat	Both (higher in processed meat)	x	x	
PAH	Both	x		
HCA	Both	x		
AGE	Both	x		x
TMAO	Both	x	x	
Thermoresistant potentially oncogenic bovine viruses	Both	x		
Salt/sodium	Processed	x (stomach)	x	x
Nitrate, nitrite, NOC	Processed	x	x	x

T2D, type-2 diabetes mellitus; PAH, polycyclic aromatic hydrocarbons; HCA, heterocyclic aromatic amines; AGE, advanced glycation end products; TMAO, trimethylamine-N-oxide; NOC, N-nitroso compounds.

increased meat, particularly processed meat consumption (Table 2).

Nitrites or nitrates added to meat for preservation could increase exogenous exposure to nitrosamines, NOC and their precursors. Dietary intake of NOC was associated with cancer risk, in particular gastrointestinal cancer. For example, in the EPIC-Norfolk study, N-nitrosodimethylamine intake was associated with increased risk of gastrointestinal cancers (hazard ratio (HR) = 1.13; 95 % CI 1.00, 1.28), specifically of rectal cancer (HR = 1.46; 95 % CI 1.16, 1.84 per 1-sd increase)<sup>(31)</sup>.

High intake of salt and consumption of salted and salty foods is considered a probable risk factor for gastric cancer<sup>(28,32)</sup>. Some traditional diets include substantial amounts of salt-preserved foods, including salted meat, fish or vegetables and salted foods such as bacon, sausages and ham, which contain from 3 to 5 g salt/ 100 g<sup>(28)</sup>. High salt intake can damage the lining of the stomach, increase endogenous NOC formation, synergistically interact with gastric carcinogens, and increase the colonisation by *Helicobacter pylori*<sup>(28,32)</sup>.

Haem iron in red meat may lead to oxidative stress, which, in turn, might increase peroxidation of lipids, lead to protein modification and DNA damage<sup>(33)</sup>. Haem iron also increases endogenous NOC formation because haem in red meat can readily become nitrosylated and act as a nitrosating agent. Based on the results of the EPIC cohort, endogenous NOC formation may account for the association between red and processed meat consumption and gastric cancer risk<sup>(34)</sup>, and in a Shanghai cohort, higher endogenous NOC formation was associated with increased risk of colorectal cancer<sup>(35)</sup>.

Heterocyclic aromatic amines (HCA) and PAH are considered carcinogenic<sup>(36)</sup> and have long been hypothesised to contribute to cancer risk<sup>(37)</sup>. Several epidemiology studies did indeed observe positive

associations between dietary intake of HCA and PAH with risk of different types of cancer, in particular colorectal adenomas and colorectal cancer<sup>(38–40)</sup>. However, the study results are still rather heterogeneous, which might partly be due to crude dietary assessment methods but also differences across study populations with respect to genetic background of phases I and II enzymes responsible for HCA and PAH metabolism<sup>(37,41)</sup>.

A long-standing theory suggests that high saturated fat intake increases the risk of a variety of cancer types. In the USA, American Association of Retired Persons as well as in the EPIC cohort, high intake of saturated fats was associated with an increased risk of postmenopausal breast cancer, in particular among women who never used postmenopausal hormones<sup>(42,43)</sup>. These results support earlier findings of a pooled analysis of eight cohorts, in which high saturated fat intake tended to be associated with increased breast cancer risk (pooled RR = 1.09; 95 % CI 1.00, 1.19 for an increment of 5 % of energy from saturated fat)<sup>(44)</sup>. However, for most cancer types, results are rather heterogeneous<sup>(28)</sup> or point towards no associations, such as for prostate cancer<sup>(45)</sup>.

A new hypothesis has been proposed by zur Hausen<sup>(46)</sup>. Based on the observation that the worldwide distribution of colorectal cancer correlates with rates of beef consumption, he proposed that a specific beef factor, suspected to be one or more thermoresistant potentially oncogenic bovine viruses could contaminate beef preparations leading subsequently to latent infections in the colorectal tract. Preceding, concomitant or subsequent exposure to chemical carcinogens arising during cooking procedures can then result in increased risk for colorectal cancer synergistic with these infections<sup>(46)</sup>. So far, however, no epidemiological studies have addressed this hypothesis.

## CVD

As for colorectal cancer several studies have examined the association between red and processed meat consumption and the risk of CVD, in particular, myocardial infarction, or more broadly CHD, but also stroke. In contrast to colorectal cancer, for which both unprocessed and processed red meat appear to be important, the most recent meta-analysis showed a significantly positive association of processed meat intake with CHD (RR = 1.42; 95 % CI 1.07, 1.89 per 50 g increase in daily consumption), but not with consumption of unprocessed red meat (RR = 1.00; 95 % CI 0.82, 1.23)<sup>(47)</sup>.

Less frequently studied is the association of meat consumption with the risk of stroke. In a meta-analysis that included six cohort studies<sup>(48)</sup>, the risk of total stroke increased by 11 % (95 % CI 1.03, 1.20) for each serving per d increase in fresh red meat and by 13 % (95 % CI 1.03, 1.24) for processed meat. The authors did not detect large heterogeneity among studies ( $P > 0.16$ ). Both fresh and processed red meat were related to an increased risk of ischaemic stroke (RR = 1.13; 95 % CI 1.00, 1.27 and RR = 1.15; 95 % CI 1.06, 1.24, respectively). However, neither meat type was related to the risk of haemorrhagic

stroke (fresh red meat RR = 1.08, 95 % CI 0.84, 1.39; processed meat RR = 1.16, 95 % CI 0.92, 1.46).

Several hypotheses have been formulated to explain the associations of processed meat with the risk of CVD. Adding salt (NaCl) to red meat for conservation purposes increases the naturally low sodium content of red meat. In their meta-analysis, Micha *et al.* stated that processed meats contain about 400 % more sodium and 50 % more nitrates per g<sup>(47)</sup>, although this depends strongly on the type of meat and the methods used<sup>(49)</sup>. A high salt intake is thought to be associated with hypertension and, consequently, an increased risk of CVD<sup>(50,51)</sup>, although it is currently still unclear which amounts of salt intake do affect blood pressure and whether only certain subgroups of the population would particularly benefit from decreasing their salt intake<sup>(52)</sup>. Processed meats such as sausages, salami and bacon have a higher content of SFA and cholesterol than fresh red meat; the latter is often consumed after removing the visible fat tissue, whereas the proportion of fat in sausages often reaches 50 % of weight or even more. Although numerous studies have been conducted on the association between fat intake and risk of CHD, the association appears to be yet rather unclear. A 2010 meta-analysis came to the conclusion that both high saturated fat and cholesterol intake are related to the risk of CHD<sup>(53)</sup>, but a more recent one did not find a convincing association between dietary intake of saturated fats and coronary disease<sup>(54)</sup>. However, their effects on blood lipoproteins are well described and the latter are established causal factors in the aetiology of CVD. Nitrates and their byproducts (e.g. peroxynitrite) experimentally promote endothelial dysfunction, atherosclerosis and insulin resistance<sup>(47)</sup>.

Some other potential mechanisms do not only apply to processed meat, but to red meat in general. Firstly, haem iron in red meat may lead to oxidative stress, which, in turn, might increase peroxidation of lipids, lead to protein modification and DNA damage. Results of some studies suggested that high body iron stores (e.g. serum ferritin) could be determinants of levels of systemic oxidative DNA damage<sup>(55,56)</sup> and some, but not all epidemiological studies have shown associations between body iron stores and risk of myocardial infarction<sup>(57)</sup>. Secondly, higher intake of red meat is related to higher intake of arachidonic acid, which leads to higher plasma concentration<sup>(58)</sup>. This may cause changes in fatty acid concentration and pattern of fatty acids of platelet membranes, and eicosanoids produced from arachidonic acid promote inflammatory and prothrombotic activities. However, dietary intake of arachidonic acid does not appear to be related to the risk of stroke<sup>(59)</sup> and the association of dietary or circulating arachidonic acid with CHD is yet unclear<sup>(60–62)</sup>. More recently, US studies observed that CVD patients with higher concentrations of trimethylamine-N-oxide (TMAO) have a higher risk for major adverse cardiovascular events such as death, myocardial infarction or stroke than patients with low TMAO concentrations<sup>(63)</sup>. Intestinal bacteria metabolise the precursor of TMAO, trimethylamine, from carnitine, phosphatidylcholine (lecithin) and choline. After



absorption, in a second step, trimethylamine is oxidised to TMAO in the liver<sup>(64,65)</sup>. These trimethylamine precursors, carnitine, lecithin (phosphatidylcholine) and choline, are abundant in red meat and liver, but also fish, milk, cheese and eggs<sup>(66)</sup>. So far, however, it is unclear if and how dietary intake of red meat or any other food affects circulating TMAO concentration in healthy individuals<sup>(67)</sup>.

### Type-2 diabetes mellitus

A meta-analysis conducted by Micha *et al.* concluded that both unprocessed red meat and processed meat consumption were associated with an increased risk of type-2 diabetes. Per 50 g increase in daily processed meat consumption, the risk increased by 51 % (95 % CI 1.25, 1.81; eight cohorts), whereas the association was less strong with unprocessed red meat intake (RR = 1.19; 95 % CI 1.04, 1.37; nine cohorts; per 100 g intake)<sup>(47)</sup>. In an evaluation of the EPIC cohort, using a case-cohort design, red meat (HR: 1.08; 95 % CI 1.03, 1.13), processed meat (HR: 1.12; 95 % CI 1.05, 1.19) and meat iron intake (HR: 1.03; 95 % CI 0.99, 1.07) were associated with incident type-2 diabetes<sup>(68)</sup>.

In an analysis of the  $\alpha$ -Tocopherol  $\beta$ -Carotin Cancer Prevention cohort, the positive association between processed meat consumption and diabetes risk was explained more by dietary intake of sodium than by intake of SFA, protein, cholesterol, haem iron, magnesium and nitrate, and these results were not modified by obesity<sup>(69)</sup>. However, it is yet unclear how high sodium intake might contribute to type-2 diabetes aetiology, although salt restriction in diabetes patients might be beneficial for blood pressure control<sup>(70)</sup>.

Based on the results of animal models, some authors hypothesised that chronic exposure to nitrosamine compounds could contribute to the pathogenesis of type-2 diabetes<sup>(71)</sup>. Nitrosamines activated during metabolism may generate reactive oxygen species, which, in turn, can increase oxidative stress, DNA damage, lipid peroxidation and protein adduct formation. Oxidative stress and DNA damage lead to activation of pro-inflammatory cytokines and insulin resistance<sup>(72)</sup>.

As for cancer and CVD, haem iron appears to be an important factor in the association between red and processed meat consumption and risk of diabetes, which is supported by the EPIC-Interact results<sup>(68)</sup>. A large meta-analysis reported strong associations of serum ferritin concentration and clinically elevated transferrin saturation with an increased risk of type-2 diabetes<sup>(73)</sup>. These associations were even seen after adjusting for inflammatory factors. It is thought that higher body iron stores might impair insulin sensitivity and increase the risk of diabetes by promoting oxidative stress causing tissue damage<sup>(74)</sup>.

High amounts of advanced glycation end products are found in animal products high in protein and fat, such as meats and cheeses. In addition, higher concentrations were seen in (industrially) processed foods from animal products such as frankfurters, bacon and powdered egg

whites, compared with the unprocessed forms<sup>(75)</sup>. It is well-known that high circulating advanced glycation end products levels are associated with adverse outcomes in diabetes patients<sup>(76–78)</sup>, but so far no epidemiological study has evaluated whether dietary advanced glycation end products intake or circulating levels are associated with incident type-2 diabetes.

Inflammation appears to be involved in mediating the association between red meat consumption and diabetes (as well as CVD). In the EPIC-Potsdam study, a cohort with about 25 000 participants, a high consumption of red meat was associated with higher circulating levels of  $\gamma$ -glutamyl transferase and high-sensitivity C-reactive protein<sup>(79)</sup>. Similarly, higher red meat consumption was associated with unfavourable plasma concentrations of inflammatory and glucose metabolic markers in diabetes-free participants of the Nurses' Health Study<sup>(80)</sup>. Interestingly, BMI accounted for a significant proportion of the observed associations with these biomarkers, except for ferritin (see the next section). The authors concluded from their analysis that the substitution of red meat with other protein food would be related to a healthier biomarker profile of inflammatory and glucose metabolism.

### Methodological considerations

#### Interaction with other foods and nutrients

In EPIC, Norat *et al.* observed a strong positive association between red and processed meat intake and risk of colorectal cancer<sup>(81)</sup>. However, depending on other dietary habits, i.e. fish consumption and fibre intake, the associations were different. For example, the HR in study participants with high intake of red and processed meat was 1.09 (95 % CI 0.83, 1.42) for the group with high intake of fibre (>26 g/d in women, >28 g/d in men), but 1.50 (95 % CI 1.15, 1.97) for the group with low intake of fibre (<17 g/d) compared with participants who had low intake of red and processed meat and high intake of fibre (*P* interaction = 0.06). Similar interaction was observed by fish consumption. Also, in a study on HCA intake and colorectal adenoma risk, we observed a stronger association between the intake of 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine and adenoma risk in individuals with a flavonol intake below the median intake in the cohort, the RR progressively increased with higher 2-amino-1-methyl-6-phenylimidazo(4,5-b)pyridine intake (RR = 1.76; 95 % CI 1.17, 2.65; *P* trend 0.01; top v. bottom quartile). However, no statistically significant associations were observed for participants with a high flavonol intake (RR = 1.24, 95 % CI 0.85, 1.80; *P* trend 0.14; top v. bottom quartile)<sup>(38)</sup>. This observation is consistent with results from experimental studies in which interactive effects of phases I and II enzymes on the risk of HCA-associated cancers have been described<sup>(82)</sup>.

A third example is a potential interaction of nitrite intake from diet with intake of polyphenols on risk of gastric cancer. In a Mexican study, a high intake of total nitrite as well as nitrate and nitrite from animal sources doubled the risk of gastric cancer (OR = 1.92; 95 % CI

1.23, 3.02, top v. bottom tertile)<sup>(83)</sup>. OR about 2-fold were observed among individuals with both low intake of cinnamic acids, secoisolariciresinol or coumestrol and high intake of animal-derived nitrate or nitrite compared with high intake of the polyphenols and low animal nitrate or nitrite intake. The results of this study suggest that polyphenols may reduce gastric cancer risk through inhibition of endogenous nitrosation<sup>(83)</sup>. In a similar way, vitamin C intake modified the association between N-nitrosodimethylamine intake and risk of gastrointestinal cancers in the EPIC-Norfolk cohort<sup>(31)</sup>.

### Residual confounding

Problems in epidemiological studies are factors that act as confounders, i.e. are related to both the exposure and the outcome. Incomplete adjustment for such confounders result in residual confounding; this applies to factors that have not been assessed at all or to factors that have not been assessed in sufficient detail or precision. In many studies, incomplete adjustment for active (and passive) smoking may pose a problem. In the EPIC analysis on the association between meat consumption and mortality, we observed heterogeneity according to smoking (*P* interaction 0.019), with significant associations between processed meat intake and all-cause mortality only in former and current smokers but no significant associations in never smokers<sup>(24)</sup>.

### Heterogeneity between studies

As described earlier, several meta-analyses have been conducted using studies from a variety of settings that differ in time, place and type of dietary assessment. All these factors may contribute to heterogeneity in study results. For example, in their meta-analysis on meat consumption and all-cause mortality, Larsson *et al.* pointed out that, although most studies observed positive associations, formal tests for heterogeneity were statistically significant<sup>(26)</sup>. This heterogeneity might be due to different meat consumption habits and, thus, differences in the range of red and processed meat consumption in a population (e.g. higher in the USA than in East Asia), to differences in foods that contribute to meat consumption categories (e.g. different types of processed meats consumed in different populations) and to the length of follow-up<sup>(26)</sup>. Another factor that contributes to heterogeneity in study results is differences in adjustment variables as shown in a meta-analysis of meat consumption and colorectal cancer risk<sup>(84)</sup>.

### Conclusions

Processed meat, which is mostly processed red meat, but might also include white meat, is associated with increased all-cause mortality and also with increased risk of some types of cancer (such as colorectal and gastric cancer), CVD and type-2 diabetes. Although most epidemiological studies point towards such an association, the strength of the association appears to be unclear. For example, the associations between processed meat consumption and

all-cause mortality appear to be much stronger in the USA<sup>(19,23)</sup> than among European<sup>(24)</sup> studies. The reasons for this discrepancy are still unclear.

Factors that are associated with total meat and in particular with processed meat consumption, and can act as confounders need to be addressed carefully in epidemiological studies as lifestyle differs between individuals with high and low processed meat consumption. Information from other types of studies in human subjects are needed to support a causal role of processed meat in the aetiology of chronic diseases. Using the Bradford Hill criteria simply based on epidemiological studies is not sufficient because it does not preclude misinterpretation due to confounding or bias<sup>(85)</sup>. However, trials that randomise individuals into a low consumption v. control group are difficult to conduct, in particular if the outcome is a 'hard' endpoint, such as cancer, myocardial infarction or diabetes. Using intermediate endpoints, such as changes in blood lipids, concentration of advanced glycation end products or DNA adducts is difficult, too, because the link between these markers and incident disease or mortality is not unique, such that high cholesterol concentrations might be linked to CHD but also some types of cancer. Other approaches, such as studies using the Mendelian randomisation approach, may help to establish causal associations between processed meat consumption and risk of chronic diseases.

If, however, after careful evaluation of existing studies, it will turn out that the processed meat consumption is indeed causally associated with chronic diseases, it needs to be addressed which factors are responsible for these associations and how the risk might be reduced.

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### Conflicts of Interest

None.

### Authorship

S. R. drafted the manuscript. J. L. reviewed and revised the manuscript.

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