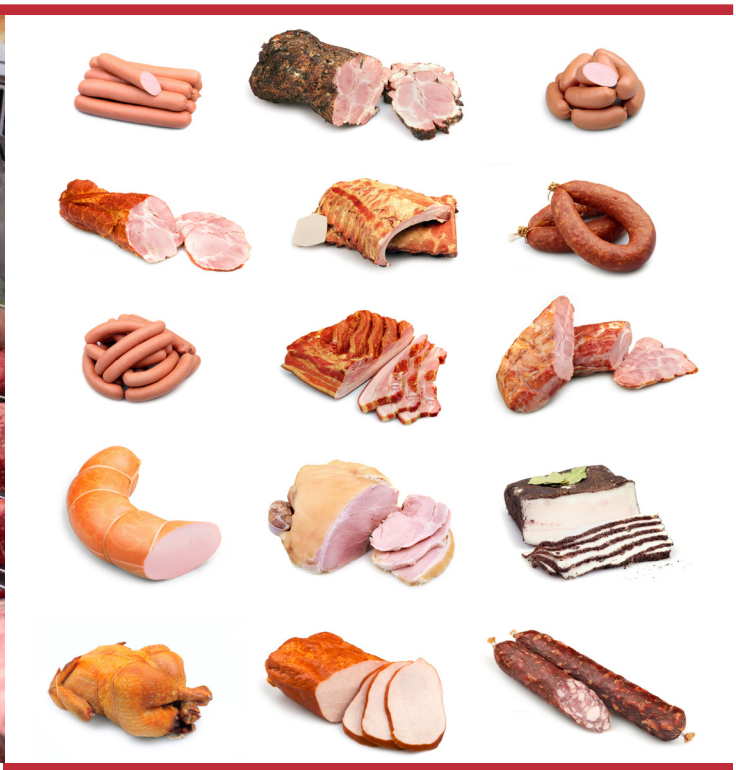
Mechanisms behind cancer risks associated with consumption of red and processed meat



DTU Food
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Content

Preface

In October 2015 The International Agency for Research on Cancer (IARC) published a summary of an expert meeting about the carcinogenicity of consumption of red meat and processed meat. The expert group classified consumption of red meat as “probably carcinogenic to humans” (group 2A) and the consumption of processed meat as “carcinogenic to humans” (group 1). The evaluation itself is not yet public but it will be published in vol. 114 of IARC Monographs.

The IARC statement made WHO announce that they would look at the results in the context of a healthy diet and recommend to the public what is an acceptable average intake of red meat and processed meat. No time limit for this task was made public.

Since the IARC evaluation is primarily based on American cohorts, the Danish Agriculture and Food Council (DAFC, Danish: Landbrug & Fødevarer) has asked the National Food Institute, Technical University of Denmark (DTU Food) to prepare a report on facts about meat in a Danish and European context. The DTU Food report does not address the IARC conclusion, neither does it contain a risk evaluation or a quantitative risk-benefit evaluation of red meat and processed meat.

The assignment from the Danish Agriculture and Food Council contained the following tasks:

1. An overview of how red meat and processed meat are defined in European and American cohort studies reporting associations between meat intake and cancer risk
2. A comparison of results from European and American/global studies on the association of meat intake and cancer risk
3. Identification of potential carcinogenic compounds in red meat and processed meat and their mechanism of action, based on scientific literature, mainly reviews
4. An indication of how the intake of potential carcinogenic compounds in red meat and processed meat may be reduced, and the possibility of eating other foods with a protective effect against colorectal cancer
5. An estimate of the dietary composition and nutritional quality in subgroups of the Danish population that most qualified comply with the nutritional recommendations and the dietary guidelines, and that have a low, average or high intake of red meat and processed meat

After the agreement about the project description DTU Food has communicated preliminary results to DAFC at a meeting prior to delivery of the report. At the meeting and after receiving the report, DAFC had the possibility to ask for clarification of results but DAFC had no influence on the scientific content and conclusions in the final report.

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Summary

The definitions of ‘red meat’ and ‘processed meat’ differ between the various scientific studies. In European cohort studies reporting associations between meat intake and risk of colorectal cancer (CRC), ‘red meat’ is defined as fresh meat from four-legged, domestic animals, while in some large American cohort studies ‘red meat’ includes ‘processed meat’ (designated ‘total red meat’ in European studies). ‘Processed meat’ is defined as meat that has undergone some form of preservation. It is mainly based on beef and pork but other types of meat, e.g. chicken may be included. Thus, the differentiation between ‘red meat’ and ‘processed meat’ expresses whether the meat is fresh or preserved, but the definitions do not take the content of potential carcinogenic compounds into account.

In a Danish cohort a reduced rectal cancer risk was associated with red beef intake. No other studies found a reduced CRC associated with red meat or processed meat intake. In the EPIC cohort involving 23 cohorts from ten European countries, increased CRC was associated with high intake of processed meat but not red meat. In the (selected) American cohorts reviewed in this report associations were found between CRC and red meat intake and processed meat intake in approximately half of the cohorts. The results may have been affected by the definition of ‘red meat’, which includes processed meat in some of the American cohorts.

A range of chemical compounds associated with red meat and/or processed meat may pose a carcinogenic risk, a risk that can be further modulated by the gut microbiota.

It is concluded that it is very likely that haem iron is carcinogenic in experimental animals in doses relevant for humans. The few mechanistic human intervention studies indicate that similar processes occur in the human colon. However, the conflicting evidence from the epidemiological studies indicates that other causes of colorectal cancer are much more important than haem iron from meat. Thus, there seem to be good evidence for the carcinogenic effect of haem iron but the potency is probably low.

In regard to dietary iron (non-haem) the results from cohort studies and nested case studies have shown an association between colon cancer risk and the intake of dietary iron. However, the evidence cannot be considered convincing, and dietary iron seems to be less carcinogenic compared to haem iron.

Meat and meat products are potential sources of numerous enteric zoonotic infections in humans. However, there has never been established a role for any viral material from beef in human colon cancer etiology.

Protein is a major constituent of meat. Intestinal bacterial catabolism of proteins from foods results in a number of different end products including ammonia, N-nitroso compounds, phenolic and indolic substances, and hydrogen sulfide. These substances are known to generate reactive oxygen species or genotoxicity, thereby contributing to carcinogenesis. It has additionally recently been shown that the gut microbiota plays a pivotal role in this increased cancer risk associated with haem iron, probably because microbial produced hydrogen sulfide disrupts the protective barrier constituted by the mucus layer covering the intestinal inner surface, and thereby exposes the intestinal epithelial tissue to the cytotoxic heam. Additionally, enzymatic activity of the intestinal

microbiota converts latent carcinogens present in cooked meat into bioactive forms such as nitrosamines, polyaromatic hydrocarbons and heterocyclic amines.

Meat may contain different preserving agents and process contaminants that affect their effect on cancer risk. Nitrite and nitrate has a long history of use in cured meat products. Their safety, particular in regard to nitrite, has often been contested. The safety concern is primarily focused on nitrite's ability to chemically react with amines forming N-nitroso compounds of which many are potent carcinogens. However, whether N-nitroso compounds to some extent can explain the results from epidemiological studies on processed meat and cancer is at presently unresolved. The scientific data do not support an association between nitrate and cancer. Cancer related health concerns in regard to nitrate are therefore limited to food matrices where nitrate can be reduced into nitrite (e.g. fermented sausages).

When foods are fried, baked, roasted, broiled, grilled or barbequed, a multitude of chemical compounds are formed in the crust and the leaking meat juices through Maillard reactions and other related reactions. Some of these compounds, notably the heterocyclic amines (HCAs), are potent carcinogens. Formation of HCAs is not restricted to red meat, and HCAs readily form in e.g. meat from poultry or fish. The formation of HCAs increases with prolonged cooking time and high temperature cooking. Several epidemiological studies have found an association between eating well-done meat and a number of cancer types including colorectal cancer. The known HCAs alone or more likely in combination with yet undetected mutagens generated during the heating of meat, would be a qualified guess as a causative factor for development of cancer among meat eaters. However, the magnitude of the risk posed by heat generated carcinogens in meat is unresolved.

The generation of polycyclic aromatic hydrocarbons (PAHs) are associated with grilling/barbequing and smoking due to the partial carbonisation of the food source or more importantly contamination from the combustion of organic matter (e.g. coal, wood). From a mechanistic point of view there is firm evidence that several of the PAHs are carcinogens. There is also some epidemiological evidence for an association between cancer and intake of grilled/barbequed meat. However, whether this association is due to intake of PAHs or it is linked to other causative factors (e.g. HCAs, haem, differences in dietary lifestyle) remains unresolved.

To elucidate whether the association between meat intake and CRC could be explained by confounding dietary factors, subgroups of the Danish populations with a diet that live up to the Nordic Nutrition Recommendations and the Danish dietary guideline, and with a low, medium or high dietary content of red and processed meat were compared. More than twice as many individuals with low dietary content of red meat and processed meat had healthy diets compared to individuals with high dietary meat content. The results showed that it is possible to eat a diet with both high, middle and low content of red meat and meet both nutrient recommendations and dietary guidelines. Having a high dietary content of processed meat makes this more difficult.

To reduce cancer risk associated with red and processed meat intake as seen in many, but not all, epidemiological studies, it is recommended that care should be taken in preparation of the meat. Meat needs to be heated sufficiently to ensure the destruction of pathogenic bacteria, but the formation of HCAs can be lowered by heating the meat crust at lower cooking temperatures, avoiding charring of the meat and in general reducing the cooking time. A range of natural constituents found in e.g. spices, wine or beer can also lower HCA formation when applied to the

meat prior to cooking. Furthermore it is possible to reduce the PAH contamination of the meat by using appropriate grilling and smoking practices. N-nitrosamine formation in cured meat can be minimised by creating an environment in the meat products that do not favour nitrosation reaction (e.g. adding ascorbic acid) and generally keeping the amount of added nitrite to a minimum. A diet with a high content of fibre rich foods such as whole-grain foods, vegetables and fruit may reduce the cancer risks associated with meat intake, both through systemic effects, and through effects on the activity of the intestinal bacteria.

Introduction

Red and processed meat is a part of many Dane's diet. Meat contributes significantly to our intake of several essential nutrients. However, the results of large cohort studies have associated the intake of red meat, and in particular processed meat, with increased risk of cancers, mainly colorectal cancer. Most of the large cohort studies have been conducted in American cohorts, and since the American dietary patterns differ from European and Danish dietary patterns, there might be differences in the cancer risk that red meat and processed meat pose, depending on the way the meat is prepared and on the foods that are accompanying the meat dishes.

The mechanistic evidence related to increased cancer risks after meat intake suggests several different compounds may be involved.

In this report we evaluated the role of meat in a healthy diet and listed the potential carcinogenic compounds in red and processed meat and their possible mechanistic effects in healthy people. Additionally, we compared results from American/global and European studies on associations between meat intake and cancer risk.

Definitions of red meat and processed meat

In this chapter we focus on definitions of red meat and processed meat from studies describing associations between meat intake and colorectal cancer risk (CRC), since the World Cancer Research Fund (WCRF) & American Institute of Cancer Research (AICR) concluded that the association to colorectal cancer is convincing, while the evidence for associations between meat intake and other cancers are less strong (World Cancer Research Fund / American Institute for Cancer Research, 2007a, 2011). Likewise, International Institute for Research on Cancer (IARC) concluded that the largest body of epidemiological data related to carcinogenicity of consumption of red and processed meat concerned colorectal cancer (IARC, 2015).

Since intake of red and processed meat may be differently associated with the risk of cancers, and since the effects may be due to different mechanisms, it is important to know how red and processed meat are defined in the studies on associations between meat intake and cancer risk.

Definitions of red and processed meat are difficult to obtain from the scientific papers reporting the associations between food intake and disease risk. Mostly the studies used food frequency questionnaires (FFQ) but even the papers describing the validation of the FFQ do not mention the exact questions that were asked related to meat intake. It was outside the time limit set for this report to contact the authors of the scientific papers to get access to the original questionnaires.

We identified individual European studies about meat and CRC from the reference lists in the IARC summary and the WCRF & AICR reports and from scientific reviews and meta-analyses on effects of meat intake on CRC. The definitions used in European studies were compared to definitions used in large American cohort studies (table 1).

In European cohort studies the designation ‘red meat’ is used relatively consistent about fresh beef/veal and pork meat. Sometimes the definition includes other four-legged, domestic animals such as mutton/lamb, horse and goat. In some studies liver and other offal are included in red meat. The red meat can be fresh or frozen and may be minced. Some studies include sausages as fresh red meat, which must mean the sausages have not undergone any form of preservation. A few papers describing cohorts from The Netherlands use the designation ‘fresh meat’ instead of ‘red meat’, and include meat from beef, pork and chicken. Poultry is defined separately in some studies and include chicken and sometimes turkey meat. Duck meat and meat from game is never mentioned in the definitions. In the European cohorts ‘red meat’ does not include meat that has undergone any form of preservation. If preserved meat is included, the designation ‘total red meat’ is used.

However, in the large American cohorts such as Nurses’ Health Study, The CPS II Nutrition Cohort and The NIH-AARP Cohort, the definition ‘red meat’ includes beef, pork and lamb from all sources, including bacon, ham and fermented/preserved sausages including hot dogs. Thus, ‘red meat’ in some American studies is comparable to ‘total red meat’ in European studies.

In both European and American studies ‘processed meat’ is defined as meat that has undergone some form of preservation, mostly smoking, fermentation, curing and/or treatment with nitrate and/or nitrite salt. It mainly includes meat from beef and pork but other types of meat, e.g. poultry, may be included. Products are typically bacon, ham, different types of sausages, cold cuts and

liver pâté. A Swedish study included blood pudding (high haem iron content), and in some studies minced meat and 'hamburgers' are defined as processed meat. Thus, the term 'processed meat' is used inconsistently. Not all types of processed meat are included in all studies.

Summary – definitions of red and processed meat

In European cohort studies reporting associations between meat intake and CRC, 'red meat' is defined as fresh meat from four-legged, domestic animals, while in some of the large American cohort studies 'red meat' include 'processed meat' (designated 'total red meat' in European studies).

'Processed meat' is defined as meat that has undergone some form of preservation. It is mainly based on beef and pork but other types of meat, e.g. chicken may be included. The term is used inconsistently.

Meat from duck and game is not mentioned in the meat definitions.

The classifications of 'red meat' and 'processed meat' are not based on content of different potential carcinogenic compounds such as haem iron, PAHs, HCAs and N-nitroso compounds. The differentiation between 'red meat' and 'processed meat' express whether the meat is fresh or preserved.

Table 1. Overview over European and American cohort studies reporting associations between meat intake and colorectal cancer risk.

Cohort	Information collected	Red meat	Processed meat	Result	Reference
The Swedish Mammography Cohort Baseline 1987-1991 Mean follow-up 13.9 years	9 of 67 questions were about red and processed meat. Multivariate analysis adjusted for age, body mass index, education level, total energy intake, alcohol, saturated fat, calcium fruits, vegetables, whole grain foods	Red meat consisted of beef and pork (whole beef, minced meat, chopped meat) Total red meat consisted of: whole beef, chopped meat, minced meat, bacon, hot dogs, ham or other lunch meat, blood pudding, kidney, liver, liver pâté	Processed meats consisted of bacon, hot dogs, ham or other lunch meat and blood pudding (high content of haem iron)	No significant association between consumption of processed meat and colorectal cancer or cancer in different parts of colon or rectum was found. Significant increased risk of colorectal cancer (rate ratio=1.32, 95% CI 1.03-1.68, p for trend=0.03) and distal colon cancer (rate ratio=2.22, 95% CI 1.34-3.68, p for trend=0.001) but not of cancers in proximal colon or rectum or of colorectal cancer when comparing low red meat intake (<50g/d) with high intake (≥94 g/d). Eating beef and pork ≥4 times/week compared to <2 times/week increased the multivariate rate ratio for distal colon cancer to 1.99 (95% CI 1.26-3.14, p for trend=0.01). No significant effect of blood pudding intake. No significant difference	(Larsson, Rafter, Holmberg, Bergkvist, & Wolk, 2005)

				between low and high poultry intake and risk of colorectal cancer but a tendency to an inverse association (p for trend=0.04)	
The Netherlands Cohort Study Baseline 1986 Follow-up 3.3 years	14 out of 150 questions were about meat with the hot meal; 5 out of 150 about meat used as sandwich filling (mainly processed meat). Variables were initially included as quintile variables, except processed meat, which was classified as non-user and three user categories. Simple model adjust for age and energy intake. Multivariate analysis adjusted for age, sex, energy intake and dietary fibre	Total fresh meat included beef, pork, minced meat, liver, chicken and other meat	Five items used as sandwich fillings (ham, bacon, lean meat products, cooked liver, other processed meats, mainly sausages) Processed meat was raw and cooked, cured meat products and sausages	Consumption of total fresh meat, beef, pork, minced meat, liver, chicken and 'other meat' was not associated with risk of colon cancer. Intake of processed meat significantly increased the risk of colon cancer (RR=1.72, 95% CI 1.03-2.87, p for trend=0.02) comparing an intake of >20 g/d with 0 g/d. For processed meat an increased risk (RR=1.17, 95% CI 1.03-1.33) was seen per increment of 15 g/d of total processed meat (equivalent to one sandwich filling)	(Goldbohm et al., 1994)
The Netherlands Cohort Study Baseline 1986	14 out of 150 questions were about meat with the hot meal; 5 out of 150 about meat products used as	Fresh meat is defined as meat that has not undergone some form of preservation, that is, smoking, fermentation	Meat products are defined as meat items that have undergone some form of preservation (mostly	No significant association was found between intake of beef, pork, minced meat, liver, chicken, 'other meat' or meat products and risk of	(Brink et al., 2005)

Follow-up 7.3 years	sandwich fillings. Simple model adjust for age and sex. Multivariate analysis adjusted for age, sex, smoking, body mass index, energy intake and family history of colorectal cancer	and/or treatment with nitrate and/or nitrite salt ('curing') and which includes beef, pork, minced meat, chicken, liver and other meat (i.e. sausages)	cured, sometimes also smoked or fermented)	colon cancer or rectum cancer in the multivariate model.	
The Netherlands Nested Case-Control Study using data from The prospective Monitoring Project on Cardiovascular Disease Risk Factors which was conducted in three Dutch towns, i.e. Amsterdam, Maastricht, and Doetinchem Study period 1987-1991 Follow-up 1998	Red meat probably includes some processed meat like sausages and meat in sandwiches. Frequency of meat consumption was assessed separately for beef, pork, poultry and fish. Consumption of four typical Dutch meat snacks, including sausage slices was assessed. Multivariate model included total energy intake, alcohol consumption and body	Frequency of consumption of fresh red meat was estimated by summation of reported beef and pork intake.	Intake of Dutch meat snacks, including sausage slices, was requested. Participants were asked how many sandwiches with meat filling they commonly consumed daily. Sandwich filling did not include smoked ham	Frequent consumption of fresh red meat (≥5 times/week vs. 0-3 times/week) increased colorectal cancer risk among men (OR 2.7; 95% CI 1.1–6.7, p for trend=0.06). No significant association to colorectal cancer was found with intake of sausage as snack or sandwich with meat filling	(Tiemersma et al., 2002)

	height (other confounders were tested)				
Norwegian National Health Screening Service Study period 1977-1983 Follow-up 1991	Study designed to identify risk factors for cardiovascular diseases (mainly to detect the main sources of fat in the diet). Multivariate analyses adjusted for attained age, height, body mass index and smoking status	Meat meals included meat stews, roasted meat, meat balls	No information	Consuming poached or fried sausages as the main meal ≥5 times per month compared to <1 time per month was associated with increased risk of colon cancer in women RR=3.5 (95% CI 1.02-11.9, p=0.03 for trend) but not in men. No association was found between intake of meat meals in general, including meat stews, pan-fried or oven-roasted meat, meat balls and colon cancer risk	(Gaard, Tretli, & Løken, 1996)
Finnish Mobile Clinic Health Examination Survey Study period 1966-1972 Baseline diet data 1967 Follow-up 1999	Multivariate analyses adjusted for age, sex, body mass index, energy intake and consumption of vegetables, fruits and cereals, smoking, occupation, geographical area	Meat products included pork, beef and other meat, sausages, offal (Ritva Järvinen, Seppanen, & Knekt, 1993)	No information	Total consumption of meat and meat products, red meat or liver was not significantly associations with the incidence of colorectal cancer. Consumption of poultry meat increased risk for colorectal cancer (RR=1.55, 95% CI 1.04-2.44), mainly due to increased colon cancer risk (RR=1.93 95% CI 1.12-3.35)	(Järvinen, Knekt, Hakulinen, Rissanen, & Heliövaara, 2001)
Finnish Alpha-	Randomized, double-	Red meat included	Sausages and	Consumption of total red	(Pietinen et

Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC Study) Study period 1985-1988 Follow-up 1995	blind, placebo-controlled study for reduction of lung cancer risk in male smokers Adjusted for smoking, body mass index, alcohol, education, physical activity at work, calcium intake	beef, pork and lamb. Total red meat consists of red meat and processed meat	prepared meats eaten as cold cuts (Pietinen et al., 1988)	meat, red meat, processed meat or poultry were not significantly associated with colorectal cancer risk	al., 1999)
Danish Diet, Cancer and Health (DCH) Baseline 1993-1997 Follow-up 2003	63 out of 192 foods and recipes covered intake of different meat items and meat dishes. Associations for intake of total meat, fried meat and processed meat adjusted for alcohol, dietary fibres, body mass index, smoking status, hormone replacement therapy. Associations for intake of red meat, fried red meat and processed red meat adjusted for intake of poultry, fish, alcohol, dietary fibres, body mass index,	Red meat included beef, veal, pork, lamb and offal. Red meat was divided into fried red meat and processed red meat. Total meat intake included red meat, poultry, fish and processed meat.	Intake of processed meat added up intake of processed red meat, including bacon, smoked ham, salami, frankfurter, Cumberland sausage, cold cuts and liver pâté and processed fish i.e. fish prepared by pickling, salting or smoking. Intake of fried meat added up pan-fried red and white meat	There were no significant associations between intake of total meat, red meat, processed meat, fried meat, fried red meat and processed red meat and risk of colorectal cancer. Participants frying their meat brown to dark had a significant higher colorectal cancer risk RR=1.36 (95% CI 1.04-1.77) compared to those frying their meat light to light brown	(Sørensen et al., 2008)

	smoking status, hormone replacement therapy				
Danish Diet, Cancer and Health Cohort Study Baseline 1993-1997 Follow-up 2009	63 out of 192 foods and recipes covered intake of different meat items and meat dishes. Adjusted for waist circumference, education, smoking status, hormone replacement therapy status (women only), sports activities, alcohol intake, dietary fibre intake, total energy intake	Red meat consisted of fresh and minced beef, veal, pork, lamb and offal	Red meat that had undergone processing such as salting, smoking or curing (bacon, smoked or cooked ham, other cold cuts, salami, frankfurter, Cumberland sausages and liver pâté)	No association was found between intake of red meat, total processed meat or subtypes (sausages, cold cuts, liver pâté), fish or poultry and risk for colon cancer or rectal cancer. Animal origin affected the cancer risk. The incidence rate ratio (IRR) for colon cancer was significantly higher for intake of red meat from lamb IRR=1.07 (95% CI 1.02-1.13, p=0.01 for trend) per 5 g/d. The risk for rectal cancer was significantly higher for red pork meat IRR=1.18 (95% CI 1.02-1.36, p=0.03 for trend) for 25 g/d. There was a significant reduced rectal cancer risk with beef intake IRR=0.83 (95% CI 0.70-0.98, p=0.03 for trend) for 25 g/d	(Egeberg et al., 2013)
The European Prospective Investigation into Cancer and Nutrition (EPIC)	Cohorts from 23 centres from ten European countries: Denmark, France, Germany, Greece, Italy, The Netherlands,	Red meat included all fresh, minced and frozen beef, veal, pork and lamb. Poultry included all	Mostly pork and beef that were preserved by methods other than freezing, such as salting (with and without nitrites),	There was no significant effect of intake of red meat, total red meat or poultry on colorectal cancer risk. Subgroup analyses of red	(Norat et al., 2005)

Baseline 1992-2000 Follow-up 1998-2002	Norway, Spain, Sweden, UK. Adjusted for age, sex, energy intake (non-fat and fat sources separately), height, weight, work-related physical activity, smoking status, fibre intake, folate intake, alcohol, centre	fresh, frozen, and minced chicken, and, in some cohorts, turkey	smoking, marinating, air drying or heating (i.e. ham, bacon, sausages, blood sausages, meat cuts, liver pâtè, salami, bologna, tinned meat, luncheon meat, corned beef and others. Lamb and poultry are rarely processed into these types of meats in Europe	meat intake showed that there was no significant difference in colorectal cancer risk for highest vs. lowest intake of meat from beef/veal, pork or lamb, but a significant trend for pork (p for trend=0.02) and for lamb (p for trend=0.03). Colorectal cancer risk was significantly associated with intake of processed meat (HR=1.42, 95% CI 1.09-1.85, p for trend=0.02) for highest (≥80 g/d) versus lowest (<10 g/d) intake. Intake of ham, bacon and other processed meats (mainly sausages) were not independently related to colorectal cancer risk	
EPIC Baseline 1992-1998 Follow-up 2005-2009	Covariates: study centre, age, sex, education, body weight, total energy intake, alcohol, smoking, physical activity	Red meat include beef, pork, mutton/lamb, horse, goat	Processed meat is all meat products including ham, bacon sausages, small part of minced meat that has been bought as ready-to-eat product. Processed meat mainly refers to processed red meat but may contain small amounts of	High (≥160 g/d) versus low (≤10 g/d) consumption of red meat was significantly associated with higher all-cause mortality (HR=1.14, 95% CI 1.01-1.28), as was consumption of processed meat (HR=1.44 95% CI 1.24-1.66). Consumption of poultry was not associated with all-cause	(Rohrmann et al., 2013)

			processed white meat as well (e.g. in sausages)	mortality. In the model corrected for measurement error, only processed meat was significantly associated to all-cause mortality HR=1.18 (95% CI 1.11-1.25); red meat or poultry was not. The increased mortality rate related to meat intake was mainly attributable to cardiovascular disease and other causes of death, not to cancer	
World Cancer Research Fund & American Institute for Cancer Research	Review of all types of cancers	'Red meat' refers to flesh from domesticated animals that have more red than white muscle fibres (beef, pork, lamb, and goat) State that some studies may have included processed meat in their classification of red meat intake	'Processed meat' refers to meat (usually red meat) preserved by smoking, curing or salting, or by the addition of preservatives: ham, bacon, pastrami, salami, sausages, bratwursts, frankfurters, 'hot dogs'. Sometimes: minced meat and 'hamburgers' are included. State that there is no generally agreed definition of processed meat' and that the term	The evidence that red meat and processed meats are a cause of colorectal cancer is convincing. There is limited evidence suggesting that red meat is a cause of cancers of the oesophagus, lung, pancreas and endometrium, and that processed meat is a cause of cancers of the oesophagus. lung, stomach and prostate. There is limited evidence that animal foods that are grilled (broiled), barbecued (charbroiled) or smoked are	(World Cancer Research Fund / American Institute for Cancer Research, 2007b)

			is used inconsistently in epidemiological studies	a cause of stomach cancer	
World Cancer Research Fund & American Institute for Cancer Research	Update on colorectal cancer only	'Red meat' refers to beef, pork, lamb, and goat from domesticated animals	'Processed meat' refers to meat preserved by smoking, curing or salting, or addition of chemical preservatives	The evidence that red meat and processed meats are a cause of colorectal cancer is convincing	(World Cancer Research Fund / American Institute for Cancer Research, 2011)
Nurses' Health Study (NHS) Baseline 1980; follow-up questionnaires every two years Follow-up 1986	Semi-quantitative 127-food-item food frequency questionnaire Adjusted for age	Red meat defined as beef, pork and lamb from all sources. *Thus, red meat means total red meat in this study.* Analyses looking at different types of meat as main meal	Not defined	Significant risk for colon cancer (RR=2.49, 95% CI 1.24-5.03, p=0.01 for trend) in women eating beef, pork or lamb as main meal every day compared to less than once a month. No significant association between colon cancer risk and intake of processed meats (RR=1.21, 95% CI 0.53-2.72, p=0.04 for trend), Significant association for intake of liver (RR=2.01, 95% CI 1.01-4.02, p=0.03 for trend) for 2-4 meals/week compared to <1 meal/month. Intake of chicken without	(Willett, Stampfer, Colditz, Rosner, & Speizer, 1990)

				skin significantly associated to reduced risk of colon cancer (RR=0.47, 95% CI 0.27-0.82, p=0.03 for trend) 5-6 meals/week compared to <1 meal/month.	
The Iowa Women's Health Study cohort Baseline 1986 Follow-up 1991	Semi-quantitative 127-food-item food frequency questionnaire. Multivariate model adjusted for age, total energy intake, height, parity, total vitamin E and supplement vitamin A intake	Not defined, but same questionnaire as in 1984 in NHS	Not defined	No significant effect on colon cancer risk of red meat intake (RR=1.04, 95% CI 0.62-1.76, p=0.08 for trend) or processed meat intake (RR=1.51, 95% CI 0.72-3.17 p=0.57 for trend) or white meat (RR=0.79, 95% CI 0.50-1.24 p=0.00 for trend)	(Bostick et al., 1994)
The CPS II Nutrition Cohort (USA, Puerto Rico and the District of Columbia Baseline 1992/1993; follow-up questionnaires every two years Follow-up 2001	Used three different models with increasing number of covariates; Model 1 adjusted for age, total energy intake, sex Model 3 adjusted for age, total energy, education, body mass index, smoking, recreational physical activity, multivitamin use, aspirin use, alcohol intake, hormone therapy (women), sex, intake of	Red meat included the following individual or grouped items on the questionnaire: bacon; sausage; hamburgers, cheeseburgers, meatloaf, or casserole with ground beef; beef (steaks, roasts, etc, including sandwiches); beef stew, or pot pie with carrots or other vegetables; liver, including chicken livers; pork, including chops, roast; hot dogs; and ham, bologna, salami,	Processed meat included bacon; sausage; hot dogs; and ham, bologna, salami, or lunchmeat	Using model 1 showed a significant effect of intake of red meat (RR=1.70 for intake in 5th quintile compared to 1st quintile, p<0.001 for trend) and processed meat (RR=1.39 for intake in 5th quintile compared to 1st quintile, p<0.001 for trend) on colon cancer risk in men but not in women (no CI given). Using model 3 showed no significant effect of intake of red meat or processed meat on colon cancer risk in men	(Chao et al., 2005)

	fruits, vegetables and high-fibre grain foods	or lunchmeat. *Thus, red meat means total red meat in this study.*		and women. Model 3 was used to calculate effect of red meat and processed meat intake on RR of cancer in proximal colon, distal colon and rectosigmoid and rectum, respectively, in men and women together. Only a significant effect of total red meat intake on rectosigmoid and rectum cancer risk RR=1.71 (95% CI 1.15-2.52, p=0.007 for trend) was shown, but no significant effect of cancer in the two part of colon. There was no significant effect of processed meat intake on risk of cancer in the three colorectal parts.	
The National Institutes of Health (NIH)-AARP (formerly the American Association for Retired Persons) Diet and Health Study Baseline 1995-1996	Adjusted for sex, education, body mass index, smoking, total energy intake, fibre intake, calcium intake	Red meat included all types of beef, pork, and lamb; this included bacon, beef, cold cuts, ham, hamburger, hot dogs, liver, pork, sausage, and steak. The meat variables also included meats added to complex food mixtures, such as	The processed meat included bacon, red meat sausage, poultry sausage, luncheon meats (red and white meat), cold cuts (red and white meat), ham, regular hot dogs, and low-fat hot dogs made from poultry	Highl red meat intake (61.6 g/1000 kcal) was significantly associated with increased risk of colon cancer (HR=1.21, 95% CI 1.03-1.41, p for trend<0.001), rectal cancer (HR=1.35, 95% CI 1.03-1.76, p for trend=0.024) and colorectal cancer (HR=1.24, 95% CI 1.09-1.42, p for trend<0.001) compared to	(Cross et al., 2010)

		pizza, chili, lasagna, and stew. *Thus, red meat means total red meat in this study.*		low meat intake (9.5 g/1000 kcal). High processed meat intake (22.3 g/1000 kcal) compared to low processed meat intake (1.6 g/1000 kcal) was significantly associated with increased risk of colorectal cancer (HR=1.16, 95% CI 1.01-1.32, p for trend=0.017), but not to colon cancer (HR=1.11, 95% CI 0.95-1.29, p for trend=0.057) or rectal cancer (HR=1.30, 95% CI 1.00-1.68, p for trend=0.145),	
International Agency for Research on Cancer (IARC)		Red meat refers to unprocessed mammalian muscle meat – for example beef, veal, pork, lamb, mutton, horse or goat meat, including minced or frozen meat	Processed meat refers to meat that has been transformed through salting, curing, fermentation, smoking or other processes to enhance flavour or improve preservation	Red meat is classified as 'probably carcinogenic to humans' (Group 2A). Processed meat is classified as 'carcinogenic to humans' (Group 1)	(IARC, 2015)

Comparison of findings of a carcinogenic effect of meat shown in European studies compared to global studies

In the reports from World Cancer Research Fund (WCRF) & American Institute for Cancer Research (AICR) (2007b, 2011) and in the summary from International Agency for Research on Cancer (IARC) (2015) the evaluations of associations between intake of red and processed meat and cancer risk are based on studies from all over the world. Large American cohort studies constitute a substantial part of these studies. Since dietary patterns and cooking methods differ widely between cultures, the results may be affected by cohort/country, because the way the meat is prepared can affect the formation of carcinogenic compounds, and because other foods eaten with the meat may interfere with the potential carcinogenic effect of meat.

Wang et al. (2015) performed a meta-analysis of seventeen relatively newly (since 1999) published prospective cohort studies looking at consumption of red meat (beef, lamb or pork) and processed meat (meat preserved by salting, curing or smoking or with the addition of chemical preservatives) and mortality. They found that consumption of each serving (50 g) of processed meat was associated with a significant higher risk of all-cause mortality (RR=1.15, 95% CI 1.11-1.19), cardiovascular mortality (RR=1.15, 95% CI 1.07-1.24) and cancer mortality (RR=1.08, 95% CI 1.06-1.11). In a subgroup analysis, positive associations between processed meat consumption and mortality were seen in both the US and the European populations, but not in Asian populations. For unprocessed red meat consumption no significant association was found with risk of all-cause (RR=1.05, 95% CI 0.93-1.19), cardiovascular (RR=1.06, 95% CI 0.88-1.28) or cancer mortality (RR=1.03, 95% CI 0.89-1.18). In another subgroup analysis, an association between unprocessed red meat consumption and mortality was found in the US populations, but such association was not seen in the European or Asian populations.

Thus, it is interesting to compare results from European studies with results from American/global studies.

It is important to note that older publications from the 1990s most often use simple statistical models with sparse corrections for confounders. Recent studies (Rohrmann et al., 2013) show that several confounders affect the results when the effect of red meat or processed meat on disease/cancer risk is determined. Factors like age, sex, education level, body mass index, smoking status, alcohol consumption, physical activity, energy intake and intake of fruits and vegetables are potential confounders that need to be taken into consideration. Most studies conducted in the 1990es used very few covariates in their adjusted models, which may have affected the results. However, newer publications include more confounders in their corrected models.

For this report, European studies on effect of meat intake on colorectal cancer risk (CRC) were identified through the reference lists from the IARC summary, the reports from WCRF & AICR and reviews and meta-analyses on effects of meat intake on CRC. The associations found in European studies were compared to results of large American cohort studies (table 1).

To put the results of the different cohort studies in perspective, when the relative risk of development of colorectal cancers are expressed for high meat intake study groups compared to low meat intake groups, it is useful to identify what the CRC is in the general population.

According to the Public Danish Healthcare Services (www.sundhed.dk [accessed 9. May 2016]), the lifetime risk for development of colorectal cancer in Denmark is 5-6%. The incidence increases with age and approximately 85% of individuals that develop colorectal cancer do so after the age of 60 years; median age is 71 years.

In The European Prospective Investigation into Cancer and Nutrition (EPIC) study population the risk of developing colorectal cancer within 10 years for a person age 50 years was 1.71% for the highest category of red meat and processed meat intake (≥160 g/d) and 1.28% for the lowest category (≤20 g/d) (Norat et al., 2005).

In the Swedish Mammography Cohort a high red meat intake (≥94 g/d) compared to low red meat intake (<50 g/d) increased CRC 32%. There was no association between processed meat intake or poultry intake and CRC (Larsson et al., 2005).

In the Netherland Cohort Study the consumption of fresh meat (including chicken and 'other meat') was not associated with risk of colon cancer. A high intake (>20 g/d) of processed meat increased the risk of colon cancer 72% compared to an intake of 0 g/d (Goldbohm et al., 1994). In an update on the same study four years later no association was found between intake of beef, pork, minced meat, liver, chicken, 'other meat' or meat products and risk of colon cancer or rectum cancer (Brink et al., 2005).

A Dutch case-control study, The Netherlands Nested Case-Control Study, found that frequent consumption (≥5 times/week) of fresh red meat compared to 0-3 times/week increased CRC among men (OR 2.7). No association was found for intake of sausages as snack or meat filling in sandwiches (Tiemersma et al., 2002).

In the Norwegian National Health Screening Service Study no association was found between intake of meat meals and colon cancer risk, but consuming poached or fried sausages as the main meal ≥5 times/month compared to <1 time/month was associated with a 3.5 times increased colon cancer risk in women but not in men (Gaard et al., 1996).

Two Finnish cohort studies, The Finnish Mobile Clinic Health Examination Survey (Järvinen et al., 2001) and The Finnish Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (Pietinen et al., 1999), both found no association between red meat, total red meat or processed meat intake and CRC. In the first study consumption of poultry meat increased the CRC 55% (Järvinen et al., 2001).

In the Danish Diet, Cancer and Health cohort no association was found between intake of total meat (including poultry and fish), red meat, processed meat and CRC (Egeberg et al., 2013; Sørensen et al., 2008). Animal origin affected the cancer risk: intake of red meat from lamb increased the colon cancer risk 7% per 5 g/d, while the rectal cancer risk was increased 18% per 25 g/d after intake of red pork meat, and the rectal cancer risk was reduced 17% per 25 g/d red beef intake (Egeberg et al., 2013).

In the EPIC study there was no effect of intake of total red meat, red meat or poultry on CRC, but the colorectal cancer hazard risk was increased 42% for highest (≥80 g/d) versus lowest (<10 g/d) intake of processed meat (Norat et al., 2005). The effect of red meat and processed meat intake on mortality was estimated using different models: 1) a simple model taking into account study centre,

age and sex, and 2) a multivariate model including study centre, age, sex, education level, body weight, body height, total energy intake, alcohol consumption, physical activity, smoking status and smoking duration (Rohrmann et al., 2013). Using the simple model, a high intake (≥160 g/d) of red meat was related to increased all-cause mortality (HR 1.37, 95% CI 1.23-1.54) compared to low intake (10-19.9 g/d). Using the multivariate model, the difference was still significant (HR=1.14, 95% CI 1.01-1.28). However, after correction for measurement error red meat intake was no longer associated with mortality. Very high red meat intake was associated with a borderline significant increase in cancer mortality (HR=1.21, 95% CI 1.00-1.46 per 100 g/d). A high consumption of processed meat (≥160 g/d) compared to low intake (10-19.9 g/d) was associated with higher all-cause mortality (HR=1.74, 95% CI 1.51-2.00) in the simple model and HR=1.44 (95% CI 1.24-1.66) in the multivariate model, and the association remained significant after correction for measurement error (HR=1.18, 95% CI 1.11-1.25 per 50 g/d). The positive association between processes meat consumption and risk of dying from cancer was HR=1.11 (95% CI 1.03-1.21 per 50 g/d). Intake of poultry was not associated to all-cause mortality or cancer mortality.

The results from European cohorts can be compared to results from four of the large American cohorts.

In the Nurses' Health Study no association was found between intake of processed meat and colon cancer risk, but the risk for colon cancer was increased 2.5 times in women eating beef, pork or lamb as main meal every day compared to less than once a month. Intake of liver (2-4 meals/week compared to <1 meal/month) doubled the colon cancer risk, while intake of chicken without skin (5-6 meals/week compared to <1 meal/month) reduced the risk 50% (Willett et al., 1990).

In The Iowa Women's Health Study no association was found between intake of red meat, processed meat or white meat and colon cancer risk (Bostick et al., 1994).

In The CPS II Nutrition Cohort intake of (total) red meat increased the colon cancer risk 70% and intake of processed meat increased the risk 39% when comparing the 5th quintile with the 1st quintile and adjusting for age, energy intake and sex; the effect was only seen in men, not in women. After adjusting for additional confounders there was no effect of intake of red or processed meat on colon cancer risk in men and women. The authors found no effect of processed meat on risk of cancers in different parts of colon and rectum, but an effect of (total) red meat on rectosigmoid and rectum cancer was seen (Chao et al., 2005).

In the NIH-AARP cohort high (total) red meat intake (median 62 g/1000 kcal) compared to low red meat intake (median 9.5 g/1000 kcal) increased the colon cancer risk 21%, the rectal cancer risk 35% and the CRC 24%. High processed meat intake (median 22 g/1000 kcal) compared to low intake (median 1.6 g/1000 kcal) increased the CRC 16%, but did not affect the colon or rectal cancer risk separately (Cross et al., 2010).

Summary – comparing European and global cohort studies

In a Danish cohort a reduced rectal cancer risk was associated with red beef intake. No other studies found a reduced CRC associated with red meat or processed meat intake.

In the EPIC cohort involving 23 cohorts from ten European countries, CRC was associated with intake of processed meat but not red meat. Intake of processed meat but not red meat was associated with increased all-cause mortality, mainly attributable to cardiovascular disease and

‘other causes of death’, not to cancer. When results from some of the individual European cohorts were evaluated, a Swedish cohort found an increased CRC associated with red meat intake, while the other cohorts showed no such association. In about half of the European cohorts an association between processed meat intake and CRC was found.

In the (selected) American cohorts reviewed in this report associations were found between CRC and red meat intake and processed meat intake in approximately half of the cohorts. The results may have been affected by the definition of ‘red meat’, which includes processed meat in some of the American cohorts

Potential carcinogenic compounds in red and processed meat

Naturally occurring compounds in meat

Iron

This chapter will focus partly on the possible carcinogenic effect of haem iron and partly on the effect of other iron species.

Haem iron

A meta-analysis of prospective cohort studies of colon cancer, in which haem intake had been estimated, indicate that the relative risk of colon cancer was slightly increased with an odds ratio of 1.18 (95% confidence interval 1.06–1.32) for individuals with the highest consumption of haem iron (Kim, Coelho, & Blachier, 2013). However, as a large Canadian epidemiological study and the Nurses' Health Study and Health Professionals Follow-up Study including 2,114 cases during a 22-year period of follow-up showed no strong correlation between consumption of haem iron and colorectal cancer risk (CRC), Kim et al. (Kim et al., 2013) concluded that the association between haem consumption and increased CRC is questionable.

In a recent review Asmore et al. (2016) assessed 9 epidemiological studies. There were significant positive associations with cancer risk in 3 of the studies, with risk estimates ranging from 1.13 to 2.18 for high compared to low haem iron intakes. Five of the remaining 6 studies reported positive, but non-significant associations between haem iron intake and colorectal cancer incidence. Based on these findings, and analysis of epidemiological studies testing the association between CRC and intake of iron from non-meat sources, Asmore et al. (2016) suggested that haem iron may play a greater role than iron from non-meat sources on cancer risk.

In a French prospective cohort of middle-aged women including 74,000 women with an age of 53.6 ± 6.6 years and a 9 year follow up, haem iron intake was associated with colorectal, especially colon, adenoma risk. Non-nitrosylated haem iron was associated with advanced distal adenoma risk, whereas nitrosylated haem iron was associated with proximal adenoma. Nitrosylated haem iron was associated with advanced distal adenoma risk, whereas nitrosylated haem iron was associated with proximal adenoma risk (Bastide et al., 2016). These findings are consistent with epidemiologic and experimental studies that show a greater carcinogenicity of processed meat, rich in nitrosylated haem iron, than of fresh red meat, which only contains non-nitrosylated haem iron (Aune et al., 2013; Bastide et al., 2015; Pegg & Shahidi, 1997).

It has been demonstrated that beef meat added to low-calcium diet promotes early stages of colon carcinogenesis in chemically-initiated rats (rats given azoxymethane to initate the carcinogenic process (Corpet, 2011). He also demonstrated a dose–response relationship between haem and tumor promotion: Tumor number was higher in black pudding-fed (blood sausage) rats than in beef meat fed rats. Tumor promotion was identical in beef meat-fed rats and in rats given a haem-equivalent diet with haemoglobin, but not in rats given the same level of inorganic iron.

Three different routes of haem or nitrosylhaem mediated carcinogenicity has been suggested: 1) formation of endogenous N-nitroso substances, 2) induction of lipid peroxidation, and 3) formation

of tumor-promoting haem species in the gut (Corpet, 2011). These routes will be described more detailed in the following sections.

A recent study aimed at determining, at nutritional doses, which is the main factor involved and proposing a mechanism of cancer promotion by red meat. The relative effect of haem iron, heterocyclic amines and endogenous N-nitroso compounds was determined and preneoplastic endpoints in chemically induced rats and validated on tumors in mice. Haem iron was the only experimental factor associated with a significant increase in precancerous lesions (mucin depleted foci) in rats. Haem iron showed no additive or synergic effects with nitrates/nitrites or with heterocyclic amines. In rats, promotion of colon carcinogenesis by dietary haemoglobin was associated with changes in noninvasive biomarkers: fecal water haem iron, lipid peroxidation products, and cytotoxic activity. An increase in the lipidperoxidation product 4-hydroxynonenal (4-HNE), cytotoxicity and genotoxicity (can damage DNA) were observed in the fecal water of the haem dosed animals. It was speculated that the cytotoxic effects of fecal water on normal and premalignant colonic cells *in vitro* mimic the *in vivo* situation with normal epithelium, where there are a low mutation frequency of the tumor suppressor gene APC and the mucin depleted foci, where the mutation frequency in this gene is high. Only fecal water from haemoglobin fed rats was more cytotoxic to normal cells than to mutated cells. It was proposed that premalignant cell selection explains the haem-induced promotion of mucin depleted foci. Aldehydes from lipid peroxidation or haem iron itself, both present at high concentration in feces from hemoglobin-fed rats, might be responsible for this differential cytotoxicity. Using a resin to specifically trap fecal aldehydes, it was shown that aldehydes alone are responsible for fecal water cytotoxicity. In addition, it was observed that 4-HNE, but not haem iron, induced differential cytotoxicity in cells similar to that observed with fecal water. Therefore, it was proposed that haem-induced lipid peroxidation in the gut explains the observed differential cytotoxicity and the colorectal cancer–promoting effects of haem that are observed *in vivo*. It was also shown that APC-mutated cells are resistant to apoptosis and can survive contact with cytotoxic and genotoxic aldehydes, which allows them to undergo further mutation and to become more malignant. It was concluded that haem iron is the main factor responsible for the promotion of colorectal cancer by red meat, and it was shown that aldehydes formed by lipidperoxidation such as 4-HNE play roles in the underlying mechanism of action. These results strongly suggest that at concentrations that are in line with human red meat consumption, haem iron is associated with the promotion of colon carcinogenesis at a preneoplastic stage (Bastide et al., 2015; 2016). This study confirms the results from a previous study with a similar design, where cytotoxicity and lipid peroxidation of fecal water, and the urinary marker of lipid peroxidation, increased dramatically in haem- and haemin-fed rats (Pierre et al., 2010).

Pierre et al. (2010) also found that haem induced more than twice the frequency of colonic mucin depleted foci in treated rats, relative to that of control rats. Also, rats given feed supplemented with of 0.5 mmol haemin/kg for 14 days induced a 10- to 50-fold increase in cytotoxicity of the fecal water compared to rats given control feed. In the supplemented rats, there was a nearly 100% increase in cell proliferation. Furthermore, the surface of the colon epithelium was injured and the crypt depth was significantly increased by compensatory hyperproliferation of crypt cells, thus leading to epithelial hyperplasia (Kim et al., 2013). Cooked and oxidized processed meat, compared to less oxidized feed, resulted in higher levels of mucin-depleted foci in azoxymethane-induced rats, thus signifying the highest potency of nitrosyl haem (Santarelli et al., 2010).

Colon microarray analysis has revealed that the expression of mucosal pentraxin is approximately 30-fold down-regulated by chloride haemin supplementation. Since pentraxin is involved in the removal of old colonic epithelial cells, down-regulation of this gene may be one explanation of the haemin-induced inhibition of apoptosis and exfoliation of the colonocytes. Haemin also down-regulate other inhibitors of proliferation (Kim et al., 2013).

Some of these routes of possible carcinogenicity have been investigated in studies with humans. In a Dutch cohote study including 4,026 persons aged 55-69 years at baseline, an increase in the mutations rate of the oncogene KRAS and the tumor suppressor gene APC with about 70% was observed with increased intake of haem. An increase cancer risk in persons with specific mutations in APC, KRAS and P53 genes was also observed in this study (Gilsing et al., 2013). Mutations in these genes are very common in all colon cancer patients (Bastide et al., 2015). Also oxidised DNA bases has been observed after intake of high amount of red meat in human intervention studies (Hammerling et al., 2016; Lewin et al., 2006). The genotoxicity of haem has been confirmed in a human tumor cell line (Oates & West, 2006).

The cytotoxic effect of haem on the colon is lost when the diet was supplemented with green vegetables (Oates & West, 2006). It was hypothesised that chlorophyll in green vegetables inhibited the formation of the haem factor by competing for solubilisation with haem in the large intestine. Alternatively, chlorophyll and haem could form a complex that blocks the site of covalent modification of the haem and reduces the formation of the haem factor (Oates & West, 2006). Calcium was also shown to protect against the effects of haem on colonic proliferation and normalising pentraxin expression, presumably because calcium precipitates haem, thereby preventing the formation of the soluble haem induced cytotoxic factor.

Mitigation

In a French prospective cohort it is suggested that dietary antioxidants could reduce some of the carcinogenic effects of haem iron on colon and possibly also on the rectum (Bastide et al., 2016). The results are consistent with a recent cross-over study, in which adding α-tocopherol to cured meat given to human volunteers decreased fat lipid peroxidation in the feces, compared with volunteers eating control cured meat without antioxidants (Serafini, Miglio, Peluso, & Petrosino, 2011). As mentioned above it has also been suggested that chlorophyll and calcium may protect from the carcinogenic effects of haem.

Summary – haem iron

Chemical carcinogenesis is a process including several steps. In general, the chemical induces DNA damage in the cells, which could result in a mutation if it is not repaired. If this initiated cell does not undergo apoptosis and if the cell proliferate it may result in a tumor, which may become malignant. As the *in vivo* and the *in vitro* experiments confirm the presence of damaged DNA, mutations in genes related to cancer, decreased apoptosis in initiated cells, increased proliferation, formation of aberrant crypt- and mucin depleted-foci, it is concluded that it is very likely that haem is carcinogenic in experimental animals in doses relevant for humans. The few mechanistic human intervention studies indicate that similar processes occur in the human colon. It must therefore be concluded that it is very likely that haem iron is also carcinogenic to humans at the same doses. The conflicting evidence from the epidemiological studies indicates that other causes of colorectal cancer are much more important than haem iron from meat. In conclusion, there seem to be good evidence for the carcinogenic effect of haem iron but the potency is probably low.

Other iron species

In most of the epidemiological studies on iron, the specific iron compound is not given. It is often dietary iron, which may also include haem iron. Iron homeostasis is strictly regulated at the level of intestinal absorption (Huang, 2003). This is a strong indication that if iron is associated with a cancer risk, it must be colorectal cancer. In a recent review a total of 10 cohort and nested case studies on dietary iron was included. Three case control studies show a statistical significant association between intake of dietary iron and colon cancer risk. One case control study show a statistical significant inverse correlation between colon cancer risk and intake of dietary iron. In the majority of the remaining cohort studies a positive, but not statistical significant effect, was observed (Ashmore et al., 2016). Although these results cannot be considered convincing it was concluded that dietary iron may play a role in modifying colorectal cancer. In an older review, epidemiological studies have examined the relationship of iron from exogenous (dietary) or endogenous (body store) sources with CRC and concluded that approximately three quarters of the larger studies supported the association of iron with CRC (Huang, 2003).

In experimental animals there is some evidence that iron may act as a co-carcinogen. In female mice, long-term administration of dextran sulfate sodium in the drinking water was shown to induce ulcerative colitis. Simultaneous feeding of those mice with an iron-enriched diet significantly increased colorectal tumor incidence and tumor multiplicity as measured by tumors/tumor-bearing mouse and tumor volume (Huang, 2003).

In a review by Fonseca-Nunes et al., an association was found between intake of iron and lung cancer risk in two studies, no association was found in two other studies and a tendency to a protecting effect in 2 studies. It was concluded that the evidence gathered, although insufficient, seems to suggest a potential association between dietary iron and lung cancer risk (Fonseca-Nunes, Jakszyn, & Agudo, 2014). In a review of the animal experiments on iron, it was concluded that although dietary iron intake in rodents increases oxidative stress and cell proliferation, in the absence of colon carcinogens, iron alone does not appear to induce colorectal cancer (Huang, 2003).

In a small human intervention study with 2 groups given either a low meat diet (60 g/d), a high meat diet (420 g/d) or an equal amount of iron it was concluded that N-nitrosation occurred after ingestion of haem but not after ingestion of inorganic iron, indicating a lower CRC from inorganic iron compared to meat (Cross, Pollock, & Bingham, 2003).

Summary – other iron species

Unspecific iron seems to be less carcinogenic compared to haem iron. A major limitation of this conclusion is that in most of the studies it is not specified which iron compound is considered. Therefore, the studies are of limited value from a mechanistic point of view.

Virus infections in beef – possible relation with the colon cancer?

Meat and meat products are the potential sources of numerous enteric zoonotic viral infections in humans.

The most important viruses that have been associated with food borne infections are Norovirus, Hepatitis A, and Hepatitis E. Especially Hepatitis E has been associated with meat products. However, the dangers of these animal-borne diseases have been greatly lessened by good practices such as pasteurization of milk and milk products and thorough cooking of meat products.

However, a recent UK survey on pigs (Betts, 2016) indicated that over 93% of pigs were seropositive for Hepatitis E virus (that means carried antibodies against it), and there is an increasing concern over a role of pork meat in transmission of the Hepatitis E to humans. It was also determined that 14% of serum samples of red deer in mainland Spain were seropositive for Hepatitis E. (Shuchin, Kitajima, Takahashi, & Mishiro, 2003). This increasing prevalence suggests a potential risk for zoonotic intestinal viral infection in humans, however, their risk with human cancer have never been established.

A controversial paper was published in 2015 by Zur Hausen and De Villiers describing a possible association between dairy cattle serum and milk factors and a risk of breast and colon cancer (Zur Hausen & De Villiers, 2015). The authors speculated that there was a risk for colon and breast cancer observed in Western countries after consumption of meat originating from the domestic cow, *Bos taurus,* which is the predominant European and North American breed. This breed is also referred to as "*taurine*" cattle.

In the core of their argument lies an epidemiological observation that the prevalence of colon cancer is very low in Mongolia, which has also the highest meat consumption in the world. However, the Mongolians eat mostly yak, mutton goat and horse meat, not the meat of the *Bos taurus* cattle. Colon cancer incidence is also very low in India, in some Arabic countries where the consumption of meat from sheep is common, as well as in Bolivia where the beef meat is derived from mixed races of beef cattle.

Therefore the authors speculate whether there is some common risk factor related to the consumption of red meat derived solely from the European breed of *Bos taurus* cattle, which can be associated with high rate of colon cancer observed in the Western countries, where this type of beef is consumed.

As a causative risk link for the observed relation between high red beef consumption and colon cancer the authors proposed the existence of an endemic virus strains present in the red meat of *Bos taurus* cattle which can promote colon cancer development.

The relations between different types of human cancers and virus are well established. At present approximately 20% of certain cancers burden can be linked to the viral infections as: Hepatitis B and C is linked to liver cancer, Epstein-Barr virus to Burkitt`s lymphoma, human herpes virus type 8 to Kaposi`s sarcoma and HPV16 and HPV 18 to the development of human cervical cancer. However, none of these instances of cancer-linked viral infections are transmitted by food. Moreover, no viral causative agent, which could be related to human colon cancer, has ever been detected in beef meat.

In their 2015 paper, Zur Hausen and De Villiers state that they isolated from cow's milk four novel types of circular DNA (Zur Hausen & De Villiers, 2015). However the origin of these DNA particles has not been established. Recently bovine polyoma viruses were also detected in red beef sold in commercial shops in San Francisco (Zhang, Li, Deng, Kapusinszky, & Delwart, 2014). Neither the circular DNAs nor the bovine polyoma viruses were shown to be pathogenic. The authors did not test whether these novel DNAs and viral particles were present in tissues from non-taurine cattle, nor did they account for the fact that such DNAs and viral particles have been increasingly common in the tissues of all animals. Thus their proposed role and infectivity in relation to the colon cancer prevalence in humans still needs to be elucidated.

It is important to understand that these authors have established merely an association between populations that do not consume milk or meat from *taurine* cattle having a lower incidence of colon and breast cancer. There is a great difference between such an association and an actual cause and effect relationship. Likewise the unrelated observation that the cow's milk contains some circular DNAs and that some of the meat from cows contains polyoma virus particles is a long way from establishing a pathogenic role for these DNAs and virus particles. Such associations, in the past, have mostly been proven either wrong or inconsequential upon further investigation. They merely serve as topics for further testing.

There has never been established a role for any viral material from beef in human colon cancer etiology. Therefore the association between human colon cancer and viral causative agents present in meat from the domestic cow breed *Bos taurus* can be regarded as weakly hypothetical.

Process contaminants in meat

Nitrite, nitrate and N-nitroso compounds

Nitrite and nitrate has a long history of use in cured meat products, defined as products added nitrite and/or nitrate along with sodium chloride (Honikel, 2008). Their safety, particular with regard to nitrite, has often been hotly contested since the 1960s, and still is today. The safety concern is primarily focused on nitrites ability to chemically react with amines forming N-nitrosamines of which many are potent carcinogens. Unlike nitrite, nitrate has a low toxicity and do not directly form N-nitroso compounds. However, under certain conditions it can be reduced into nitrite. Since many processed meat products are cured meat products, it is not surprising that focus has been directed toward nitrite and nitrosamine formation as a possible explanation for the reported positive associations between intake of processed meat and cancer.

Nitrite

To some extent nitrite inhibits the growth of the highly pathogenic bacteria, *Clostridium botulinum* that causes botulism. This is the primary argument in favour of using nitrite in meat products, especially in canned meat products. The problems with botulism arising from consumption of meat products are unevenly distributed across Europe (EFSA, 2003). This is probably due to varying hygienic production standards, but also differences in consumer preferences of processed food.

Nitrites also have several technological functions besides the preservative function. Nitrites can function as colour retention aids. Furthermore, in some products nitrites give rise to a distinct flavour (Sindelar & Milkowski, 2012).

Unrelated to its potential carcinogenic effects nitrite can cause serious acute and chronic toxicity. These toxic effects however have a threshold and are not a concern as long as the intake lies below the Acceptable Daily Intake (ADI). The current ADI for the nitrite ion of 70 µg/kg bodyweight pr. day was established by JECFA (2003) and supported in 2008 and 2010 by EFSA (2008a, 2010).

Results from an updated intake estimation for 2011-2013 on the intake of nitrite from processed meat by the Danish population has recently been conducted by Granby and Herrmann (2014). To a lesser extent the Danish population is also exposed to nitrite from vegetables most lately estimated by Petersen and Stoltze (1999). Data from these estimates show that very few, if any, Danes would be expected to have a combined intake of nitrite from food in excess of the ADI.

Another source of nitrite exposure is endogenous conversion of nitrate in saliva. It is estimated that around 5% of the total nitrate exposure is reduced to nitrite by bacteria in the oral cavity (IARC, 2010). As estimated by Leth et al. (2008) nitrite exposure from this source could potentially exceed the ADI for nitrite. Though from a toxicological point of view, this endogenously generated nitrite cannot necessarily be equated to nitrite ingested from food.

Nitrate

Nitrate can be used in meat as a curing agent in substitution or addition to nitrite. Meat is a minor contributor to the European populations intake of nitrate, with vegetables and for some citizens drinking water, being the major sources of intake (Scientific Committee for Foods, 1997). Nitrate is a substance of relatively low toxicity reflected by its ADI of 3,7 mg/kg bodyweight pr. day (EFSA, 2008a). Comprehensive scientific assessment by IARC (2010) and EFSA (2008a) on the potential carcinogenicity of nitrate finds that the evidence does not support an association between cancer and intake of nitrate. Still IARC express a concern that nitrite formed by endogenous conversion of nitrate in saliva may pose a health concern (se next section). Another concern regards meat products where nitrate can be reduced by microorganisms into nitrite (e.g. fermented sausages), in which case a concern may arise in regard to the potential formation of preformed N-nitroso compounds (see next section).

Endogenous formation of N-nitroso compounds (nitrosamines and nitrosamides)

Under acidic conditions nitrite can be activated into nitrosating agents like nitrous anhydride, dinitrogen tetroxide and the nitrous acidium ion. Nitrosating agents can react with secondary amines forming carcinogenic N-nitrosamines (IARC, 2010). Given the acidic environment in the stomach it is not surprising that there has been concern about whether nitrite is carcinogenic to humans. IARC (2010) concluded that *"Ingested nitrate or nitrite under conditions that result in endogenous nitrosation is probably carcinogenic to humans (Group 2A)"*. However, as stated by IARC themselves, the scientific evidence to support that ingested nitrate is carcinogenic, is inadequate, and neither do EFSA (2008a) find an association between nitrate and cancer. The human organism may have an effective scavenging mechanism against nitrite formed in the saliva since nitrite can react with the abundant proline rich proteins in the oral cavity. This reaction inactivates nitrite (Scientific Committee for Foods, 1997). However, nitrite originating from food will only partly be inactivated by this mechanism, as the food matrix to some extent will protect nitrite from reacting with proline in the oral cavity.

In contrast to what is found for nitrate, IARC finds that human and animal data supports an association between ingested nitrite and cancer (IARC, 2010). IARCs assessment has attracted stark criticism from other scientist which do not find the scientific data to be in support of nitrite (or nitrate) being associated to development of cancer (Bryan, Alexander, Coughlin, Milkowski, & Boffetta, 2012; Sindelar & Milkowski, 2012). A recent meta-analysis of epidemiological studies indicated that nitrite increased the risk of gastric cancer while nitrate actually lowered the risk. However if the meta-analysis for nitrate were limited to the studies that had adjusted for vitamin C, vegetable and fruit intake, the beneficial effect of nitrate disappeared. The authors stress that due to study limitations and confounding factors in the currently available studies, they were unable to fully confirm the reliability of their findings (Song, Wu, & Guan, 2015). It should be underlined that a positive association between cancer incidence and intake of nitrite may not be to due endogenous nitrosamine formation but may be related to formation of preformed N-nitroso compounds (see next section).

Concerns is not only restricted to nitrosamines but also include formation of another group of highly reactive N-nitroso compounds, the nitrosamides. It has been shown that nitrosoamides can potentially be formed in the gastric lumen given the presence of nitrite and a source rich in nitrosable compounds (Deng, 2000) (see also next section).

Whether ingested nitrite can cause cancer through endogenous formation of N-nitroso compounds is still very much debated. However, the recent findings showing associations between intake of processed meat and cancer are likely to reignite this scientific debate.

Preformed N-nitroso compounds (nitrosamines and nitrosamides)

In cured meat products nitrite can react with secondary amines forming nitrosamines. Nitrosating agents such as nitrous gases can also result in the generation of nitrosamines. Thus, in addition to cured meat products, nitrosamines can also be formed in smoked meat products or they can migrate from certain food contact materials (Lijinsky, 1999; Tricker & Preussmann, 1991). In the 1960s nitrosation was erroneously thought only to take place in acidic environments and sodium nitrite was therefore used as a conservative for fish meal that had a neutral or alkaline pH. This resulted in very high levels of nitrosamines being formed leading to farm animals dying from chronic liver toxicity. This event however sparked an explosion in scientific research into the formation and toxicity of nitrosamines in which the potent carcinogenic nature of nitrosamines was soon discovered (Lijinsky, 1999).

Nitrosamines are often divided into volatile nitrosamines and non-volatile nitrosamines. The distinction originally related to the analytical methodology that could be used for the measurement of nitrosamines. Analysis of the non-volatile nitrosamines was originally very challenging, and the scientific focus has therefore primarily been directed toward the volatile nitrosamines. Thus, while the carcinogenic nature of many of the volatile nitrosamines detected in cured meat products are well founded (NTP National Toxicology Program U.S. Department of Health and Human Services, 2011) the toxicological data on several of the non-volatile nitrosamines are lacking (Tricker & Preussmann, 1991). After the 1980s there has been very little focus on the non-volatile nitrosamines, even though several of these nitrosamines have been reported at substantially higher concentrations in cured products than the volatile nitrosamines. Nitrosamines that have been detected in cured meat products are listed below (table 2).

The ADI allocated for nitrite does not take into account its effect on the formation of preformed nitrosamines in cured products. Denmark presently upholds a more restrictive legislation on the use of nitrite than the rest of the EU. The scientific basis for this position is primarily based upon concerns relating to formation of preformed N-nitroso compounds. Nitrosamines in cured meat products has often been suggested as a potential causative factor for the reported association between consumption of processed meat and increased cancer incidence (Kim et al., 2013; Larsson & Wolk, 2012; Rohrmann et al., 2013; Santarelli, Pierre, & Corpet, 2008; Sinha, Cross, Graubard, Leitzmann, & Schatzkin, 2009). This hypothesis has been supported by a number of epidemiological studies that have reported positive associations between nitrosamines (N-Nitrosodimethylamine, NDMA) and increased incidence of stomach cancer, colorectal cancer and esophageal cancer, but also conflicting findings (Keszei, Goldbohm, Schouten, Jakszyn, & van den Brandt, 2013; Knekt, Järvinen, Dich, & Hakulinen, 1999; Larsson, Bergkvist, & Wolk, 2006; Loh et al., 2011). These studies certainly raise concern. However, it should be noted that accurately assessing the intake of NDMA using food-frequency questionnaires as used by all the conducted

studies is very difficult and this add to the uncertainty of these results. Also there is a need for additional studies to confirm or reject the reported associations. Never-the-less, the studies raise a renewed concern for nitrosamines in processed meat.

Table 2. N-Nitrosamines detected in cured meat products (Tricker & Preussmann, 1991)

Carcinogenic*	**Not Carcinogenic**
N-Nitrosodimethylamine (NDMA)	N-Nitrosoproline (NPRO)
N-Nitrosomethylethylamine (NMEA)	N-Nitroso-4-hydroxy-proline (NHPRO)
N-Nitrosodiethylamine (NDEA)	N-Nitrosothiazolidine (NTHZ)
N-Nitrosodibutylamine (NDBA)	**Carcinogenic status unknown**
N-Nitrosopyrrolidine (NPYR)	N-Nitrosothiazolidine-4-carboxylic acid (NTCA)
N-Nitroso-3-hydroxypyrrolidine (NHPYR)	N-Nitroso-2-methyl-thiazolidine-4-carboxylic acid (NMTCA)
N-Nitrosopiperidine (NPIP)	N-Nitroso-2-hydroxymethylthiazolidine-4-carboxylic acid (NHMTCA)
N-Nitrososarcosine (NSAR)	N-Nitrosooxazolidine-4-carboxylic acid (NOCA)
N-Nitrosomorpholine (NMOR)	N-Nitroso-5-methyloxazolidine-4-carboxylic acid (NMOCA)
N-Nitrosomethylaniline (NMA)	

* Carcinogenic in animal testing following oral administration

The ADI allocated for nitrite does not take into account its effect on the formation of preformed nitrosamines in cured products. Denmark presently upholds a more restrictive legislation on the use of nitrite than the rest of the EU. The scientific basis for this position is primarily based upon concerns relating to formation of preformed N-nitroso compounds. Nitrosamines in cured meat products has often been suggested as a potential causative factor for the reported association between consumption of processed meat and increased cancer incidence (Kim et al., 2013; Larsson & Wolk, 2012; Rohrmann et al., 2013; Santarelli, Pierre, & Corpet, 2008; Sinha, Cross, Graubard, Leitzmann, & Schatzkin, 2009). This hypothesis has been supported by a number of epidemiological studies that have reported positive associations between nitrosamines (N-Nitrosodimethylamine, NDMA) and increased incidence of stomach cancer, colorectal cancer and esophageal cancer, but also conflicting findings (Keszei, Goldbohm, Schouten, Jakszyn, & van den Brandt, 2013; Knekt, Järvinen, Dich, & Hakulinen, 1999; Larsson, Bergkvist, & Wolk, 2006; Loh et al., 2011). These studies certainly raise concern. However, it should be noted that accurately assessing the intake of NDMA using food-frequency questionnaires as used by all the conducted studies is very difficult and this add to the uncertainty of these results. Also there is a need for additional studies to confirm or reject the reported associations. Never-the-less, the studies raise a renewed concern for nitrosamines in processed meat.

Many of the volatile nitrosamines, notably NDMA and N-Nitrosodiethylamine (NDEA), are very potent carcinogenic substances. On the other hand, the concentrations of volatile nitrosamines reported today in western food products are normally low. Measures taken by the industry in western countries during the 1980s, such as a more restrictive use of nitrite and nitrate in cured meat products among others, has succeeded in reducing the populations exposure to these substance (Tricker & Preussmann, 1991).

A recent Danish study found that in most Danish cured meat products, the concentration of the nitrosamines known to be carcinogenic (primarily volatile nitrosamines) was very low, and overall it was estimated that the Danish populations exposure to these nitrosamines represented a low concern (Herrmann, Duedahl-Olesen, Christensen, Olesen, & Granby, 2015). A possible explanation for this outcome, could relate to a combination of a good meat quality, a restrictive use of nitrite and a widespread use of ascorbate in the Danish meat products, all factors that lowers nitrosamine formation (Herrmann, Granby, & Duedahl-Olesen, 2015). However, it should be cautioned that the study was based on a limited number of analyses (Herrmann, Duedahl-Olesen, Christensen, et al., 2015). More robust data are expected to be generated in the years to come. Furthermore the carcinogenic nitrosamines are genotoxic (damages DNA), therefore a no-effect-level for adverse outcomes cannot be established. In line with the summarized results reported by Tricker and Preussmann (1991) and in contrast to the low levels of the nitrosamines known to be carcinogenic, the two non-volatile nitrosamines, N-nitrosothiazolidine-4-carboxylic acid (NTCA) and N-nitroso-2-methyl-thiazolidine-4-carboxylic acid (NMTCA), was widely detected at relatively high concentrations in the Danish study. For comparison the mean intake of NTCA and NMTCA from Danish cured meat products was estimated to be around 100 times higher than the mean intake of all the volatile nitrosamines (Herrmann, Duedahl-Olesen, & Granby, 2015). The sparse toxicological data available and the non-carcinogenic nature of the chemically related nitrosamine NTHZ, may indicate that the N-nitroso thiazolidine acids are weakly- or non-carcinogenic (Lijinsky, 1999). However, firm conclusions about the carcinogenic nature of these and several other non-volatile nitrosamines cannot be drawn, given the presently available information.

Another important uncertainty in regard to formation of nitrosamines in processed meat products is whether we are missing a part of the picture. The nitrosamines in cured meat that we know today were discovered prior to the past three decades of revolution within analytical chemistry, notably within mass spectrometry. There is a need to carry out new exploratory studies to determine if novel nitrosamines can be discovered using modern analytical methodologies. Another longstanding concern has been the presence of another group of N-nitroso compounds; the nitrosoamides. Unlike nitrosamines, nitrosamides do not need to be metabolically activated in order damage cellular DNA (Lijinsky, 1999). Extrapolating cancer types from animals to humans (or other animals) are highly uncertain, and in most cases only very cautious predictions can be made. However, it is noteworthy that nitrosamines as a group, as reported by Lijinsky (1999) typically causes cancer in the liver, kidney, bladder, pancreas, oesophagus and tongue, but not in the colon. Nitrosamides on the other hand cause cancer in the stomach and gastrointestinal tracts, likely due to their ability to cause DNA damage on first site of contact. The presence of one nitrosamide in cured meat, methylnitrosourea, has been indirectly inferred by detection of a reaction product of the substance. However, their reactive nature makes nitrosamides very difficult to detect (Lijinsky, 1999). Nitrosamides remains a concern in cured meat products, but the presently available data cannot clarify whether this concern is warranted or not.

Mitigation – N-nitroso compounds

Reducing nitrosamines levels in cured meat can most importantly be achieved by keeping added nitrite to a minimum and adding at least 500 mg/kg of ascorbate or isoascorbate (erythorbic acid) to the product. Increasing the added amount of ascorbate or isoascorbate up to 1000 mg/kg (in the products where the legislation allows for this) may further reduce nitrosamine levels. Free iron (not haem) counteracts the beneficial effect of adding ascorbate or isoascorbate. Other factors such as meat quality, fat content, heating, smoking, maturation, packaging and storage of the product can also influence nitrosamine levels (Herrmann, Granby, & Duedahl-Olesen, 2015).

Summary - Nitrite, nitrate and N-nitroso compounds

In summary, concerns about nitrite and to a lesser extent nitrate are linked to the generation of N-nitroso compounds in cured products or endogenously. N-nitroso compounds (nitrosamines and nitrosamides) in cured meat products is a health concern to this day and the recent results from a number of epidemiological studies may well reignite research into a scientific field that has slumbered for several decades. However, whether N-nitroso compounds to some extent can explain the results from epidemiological studies on associations between intake of processed meat and cancer is presently unresolved. What we do know is that many N-nitroso compounds are potent genotoxic carcinogens, and there are serious data gaps in our present knowledge. Thus, there is a rationale to continually strive to avoid conditions in food manufacturing that favours nitrosation reactions and minimize our intake of nitrite. The scientific data does not support an association between nitrate and cancer. Cancer related health concerns in regard to nitrate are therefore limited to food matrices where nitrate can be reduced into nitrite.

In 2010 EFSA (EFSA, 2010) affirmed its support for the conclusion expressed by SCF (Scientific Committee for Foods, 1997) stating that *"exposure to preformed nitrosamines in food should be minimized by appropriate technological practices such as lowering the levels of nitrate and nitrite added to foods to the minimum required to achieve the necessary preservative effect and to ensure microbiological safety"*.

Heterocyclic amines (HCAs)

When foods are fried, baked, roasted, broiled, grilled or barbequed reactions between sugars, lipids and amino acids take place via Maillard reactions or other related reactions (Zheng & Lee, 2009). In the 1970s it was demonstrated by Dr. Sugimura and his collaborators that the charred surface of meat and fish had potent mutagenic activity. Efforts to identify the mutagenic substances led to the discovery of the HCAs (Cheng, Chen, & Wang, 2006; Wakabayashi & Sugimura, 1998). More than 20 HCAs have been detected in cooked meat since then. Formation of HCAs is not restricted to red meat and HCAs readily forms in e.g. meat from poultry (Turesky, 2007). As the name implies HCAs have at least one heterocyclic ring (composed of nitrogen and carbon atoms) and an amino group attached. An example of this chemical structure can be seen in figure 1.

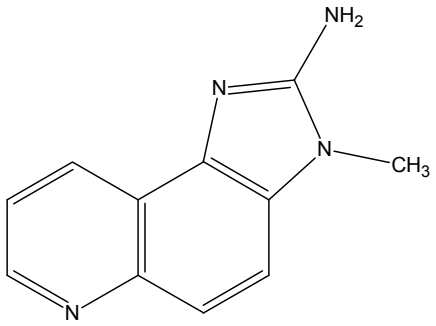

Figure 1: Amino-3-methylimidazo[4,5-*f*]quinolone (IQ).

The individual HCAs are often referred to by their abbreviation as their chemical names are rather lengthy (table 3).

Table 3. List of known heterocyclic amines (Murkovic, 2004)

Quinolines	
IQ*	2-Amino-3-methylimidazo[4,5-*f*]quinoline
MeIQ*	2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline
Quinoxaline	
IQx	2-Amino-3-methylimidazo[4,5-*f*]quinoxaline
MeIQx*	2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline
4,8-DiMeIQx	2-Amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline
7,8-DiMeIQx	2-Amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline
4,7,8-TriMeIQx	2-Amino-3,4,7,8-tetramethylimidazo[4,5-*f*]quinoxaline
4-CH2OH-8-MeIQx	2-Amino-4-hydroxymethyl-3,8-dimethylimidazo[4,5-*f*]quinoxaline
7,9-DiMeIgQx	2-Amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline
Pyridines	
PhIP*	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine
4'-OH-PhIP	2-Amino-1-methyl-6-(4-hydroxyphenyl)imidazo[4,5-*b*]pyridine
DMIP	Dimethylimidazopyridine
TMIP	Trimethylimidazopyridine
Pyridoimidazoles and -indoles	
Trp-P-1*	3-Amino-1,4-dimethyl-5H-pyrido[4,3-*b*]indole
Trp-P-2*	3-Amino-1-methyl-5H-pyrido[4,3-*b*]indole
Glu-P-1*	2-Amino-6-methyl-dipyrido[1,2-*a*:3',2'-*d*]imidazole
Glu-P-2*	2-Amino-dipyrido[1,2-*a*:3',2'-*d*]imidazole
AaC*	2-Amino-9H-dipyrido[2,3-*b*]indole
MeAaC*	2-Amino-3-methyl-9H-dipyrido[2,3-*b*]indole
Furopyridines	

MeIFP	2-Amino-(1 or 3),6-dimethylfuro-[2,3(or 3,2)-e]imidazo[4,5-*b*]pyridine
Benzoxazines	
	2-Amino-3-methylimidazo[4,5-*f*]-4H-1,4-benzoxazine
	2-Amino-3,4-dimethylimidazo[4,5-*f*]-4H-1,4-benzoxazine
Other structures	
Lys-P-1	3,4-Cyclopentenopyrido-[3,2-*a*]carbazole
Orn-P-1	4-Amino-6-methyl-1H-2,5,10,10b-tetraaza-fluoranthene
Phe-P-1	2-Amino-5-phenylpyridine

* Shown to be carcinogenic in rodents

The formation of HCAs increases with increased harshness of the thermal treatment of the meat (prolonged cooking time and high temperature cooking). Some HCAs are generated by reactions of degradation products of amino acids reacting with sugars and creatine. These HCAs can be formed even during boiling but only in extremely low levels. They begin to form in appreciable amounts when the temperature exceeds 150°C. Other HCAs are directly generated by pyrolysis of amino acids, and these require temperatures of formation in excess of 250°C. HCAs are formed in the crust of the meat and in the meat juices leaking from the meat thus potentially ending in the gravy (Murkovic, 2004; Turesky, 2007). Concentrations are highly variable and to a large extent, but not solely, depending on the harshness of the heat treatment. The highest concentrations of HCAs are linked to pan-frying, grilling/barbecuing, deep-frying and oven-broiling, while lower levels are associated with stewing, stir-frying and roasting. Besides using a more gentle thermal treatment of the meat, there are various mitigation measures that can be taken to reduce the formation of HCAs, for instance can phenolic antioxidants in certain marinades lower the formation (Meurillon & Engel, 2016; Santacana, 2012).

Carcinogenicity of HCAs

The intake of HCAs among humans predominantly originates from cooked fish and meat. The concentration of HCAs in cooked meat is small, normally ranging from around the limit of detection (~0.1 µg/kg) to a few hundred microgram pr. kg (Knize, 2006; Murkovic, 2004; Santacana, 2012). However many HCAs are extremely potent genotoxins, in particular MeIQ, IQ, and MeIQx, as shown by *in vitro* genotoxicity testing using Ames bacterial reversion assay. Genotoxicity testing using mice has further demonstrated that HCAs cause damage to DNA *in vivo*. HCAs need to be metabolically activated in order to become genotoxic. In the body they are activated by conversion into N-hydroxy-HCAs which subsequently are esterified to either acetic or sulfuric acids. It is these unstable esters that react with DNA (Turesky, 2007; Wakabayashi & Sugimura, 1998).

Several of the HCAs have been shown to be carcinogenic to rodents (see table 2). The liver is a common target organ in both rats and mice for most of the tested HCAs. But also the urinary bladder, small and large intestines, zymbal gland, clitoral gland, skin, oral cavity, mammary gland, and prostate in rats, and blood vessels, forestomach, lung, hematopoietic system, and lymphoid tissue in mice are target organs (Wakabayashi & Sugimura, 1998). The cancer types seen in rodents fed with genotoxic carcinogens can normally not be reliably extrapolated to humans. For HCAs there are clear differences in the types of cancer that HCAs causes among rats and mice, even though they are related species. One reason being is that there are important differences in

how different species metabolise HCAs, thus altering the toxicological properties of HCAs. For instance IQ has been demonstrated to be a hepatic carcinogen in cynomolgus monkeys and approximately just as potent as the highly carcinogenic nitrosamine NDMA. In contrast MeIQx was not found to be carcinogenic in cynomolgus monkeys, most likely because it was only poorly metabolised by the monkey's liver (Felton et al., 1997; Turesky, 2007). That the liver is a target organ reported for the majority of HCAs, both among mice and rats, is not surprising as the liver often is the primary site of metabolic activation of many genotoxic carcinogens (Cheng et al., 2006; Cheng, Zhang, & Wang, 2015). HCAs are multi-site carcinogens in rodents. It would be a qualified guess to assume the same is valid for humans.

MeIQx and PhIP are among the most abundant HCAs in cooked food. DNA adducts for both MeIQx and PhIP have been detected in a number of human tissues including the liver (Nagao, 1999; Nauwelaers et al., 2011). Though the presence of DNA adducts and their prevalence cannot be equated into human cancer risk, there presence demonstrate that MeIQx and PhIP in humans are metabolically activated into DNA reactive metabolites, a prerequisite for these substances being genotoxic carcinogens (Nagao, 1999).

Epidemiological studies on doneness of meat and/or HCAs and cancer

As described, the heat treatment of the food are closely linked to the generation of HCAs and potentially other yet unknown substances generated during heat treatment of meat and fish. Studies have shown that only around 30% of the mutagenic activity in a well-done beef can be accounted for by the known HCAs (Turesky, 2007). Several epidemiological studies have focused on whether there is an association between various types of cancer and the intake of well-done meat. It should be emphasised that properly conducting such studies is no easy feat. This is in particular an issue when food frequency questionnaires, as is the norm, are used to estimate the intake, whether it be the intake of well-done meat or the intake of HCAs. These challenges have been described by Knize (2006). Thus misclassification of the participant's life-time intake is a real problem, making it harder to find positive associations if they are really present.

A review by Knize and Felton (2005) summarised the results of epidemiological studies conducted prior to 2005 that investigated the relationship between meat doneness and cancer. They found 31 studies and 80% showed a positive association between the intake of well-done meat and various types of cancer. Most studies looked at breast cancer (8 studies, 1 negative study) and colorectal cancer (15 studies, 3 negative studies), both very prevalent human cancer types. Other studies reported associations to cancer in the lung, prostate, Non-Hodgkin's lymphoma, pancreas, stomach and oesophagus. Negative results were reported for cancer in the kidney and bladder (fried meat negative, barbequed meat positive).

In a more recent Danish prospective study it was found that frying the meat brown to dark was associated to an increased CRC compared to light to light brown frying (Sørensen et al., 2008).

Another approach is to investigate whether an estimated intake of all HCAs or individual HCAs are associated to development of cancer. A review of such studies published since 1996 was summarised by (Zheng & Lee, 2009). Ten studies investigated the association between cancer and total HCA content or mutagenicity, of which 7 reported positive outcomes. For the individual HCAs, studies focused on PhIP (13 studies, 8 positive), MeIQx (12 studies, 6 positive) and DiMeIQx (11 studies, 6 positive). It should be noted that it to a large extent were the same studies that were

used to investigate the effect of total and individual HCAs. Positive associations between intake of HCAs and colorectal cancer were reported in 6 out of 7 studies. A positive association between pancreatic cancer and HCAs has been reported in 3 out of 3 studies (though borderline in one study). For breast cancer 2 studies found a positive association to intake of HCAs and one study found a negative association. For prostate cancer only 1 out of 4 studies found a positive association to HCAs, despite three of the studies also looked at doneness and had found a positive association. No association was found between HCAs and stomach cancer (2 studies), but some evidence for a positive association was reported in 2 studies on oesophageal cancer. Finally 1 study showed a small borderline significant positive association for lung cancer.

From a mechanistic point there is ample evidence for the carcinogenic nature of HCAs as a group. They are potent genotoxic compounds generating DNA adducts in humans and causing *in vivo* mutations and inducing cancer in experimental animals. However, our daily intakes are small, and estimating human cancer risk from animal experiments is fraught with uncertainties. Thus, epidemiological studies might, if the effect is of a measurable size in such studies, be our best way to assess the effect of HCAs.

The overall result from epidemiological studies indicates that consumers having a high consumption of well-done meat have an increased risk of getting cancer. Also the studies focused on the estimated intake of HCAs point in this direction. Despite this, the causality for these results is not clearly established. Overcoming bias is a real challenge in such studies. People with a preference for well-done meat may well have differences in their preferred types of cooking as well as different dietary preferences compared to people who prefer their meat done or rare. Future studies are definitely warranted and epidemiologist should aim to improve their intake estimates of HCAs as outlined by Knize (2006). Also, studies should be set up to optimise the testing of a priori hypotheses and not conducted as random multiple comparison analyses as outlined by Sinha (2002).

Mitigation - HCAs

There is a range of mitigation measures that reduce the formation of HCAs. The formation of HCAs can be reduced by heating the meat more gently meaning lower cooking temperatures and shorther cooking times and avoiding any charring of the meat. This is critical for any mitigation strategy. In general, choosing gentler cooking types like stewing, stir-frying and roasting the meat instead of pan-frying, grilling/barbecuing, deep-frying and oven-broiling will lower HCA levels (Meurillon & Engel, 2016). To reduce the formation of HCAs, DMRI (2015) recommends gentle cooking, avoiding overcooking and burning the meat. Beefs should be turned every second minute and removed from the heat source (pan or grill) when the meat reaches a core temperature of 65°C. The surface colour of the meat can be used as a rough indicator of the HCA content (Aaslyng, Duedahl-Olesen, Jensen, & Meinert, 2013). Another approach that may have greater chance of success in terms of consumer acceptance is use of additives/ingredients to the meat that inhibit the formation of HCAs. Various natural phenolic antioxidants, flavonoids, terpenoids, catechins, vitamin E and the sulphur compounds allicin and diallyl disulphide have shown potential. Several of these compounds can be found in normal cooking ingredients (e.g. red pepper, rosemary, black pepper, garlic, wine and beer) and can be added to meat marinades. However, there are many variables and it is not as straightforward to mix an effective marinade as it may seem (Meurillon & Engel, 2016; Aaslyng, 2015). Finally, some studies indicate that HCAs to some extent can bind to dietary fibres and thereby lowering their bioaccessibility, thus reducing the

adverse potential of the HCAs (Meurillon & Engel, 2016). Consuming foods rich in dietary fibres such as whole grain products along with the consumption of meat could therefore potentially be a mitigation measure. However, different fibres have different potential for binding HCAs, so additional research is needed to substantiate the effectiveness of this approach.

Summary - HCAs

In summary, the known HCAs alone or more likely in combination with yet undetected mutagens generated during the heating of meat, would be a qualified guess as a causative factor for development of cancer among meat eaters. Mitigating measures to reduce our intake of HCAs are possible, but they primarily need to be directed toward consumers as most HCAs are generated during home cooking.

Polycyclic aromatic hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons or PAHs were discovered in food products in the 1960s and 1970s thus around the same time of the discovery of the nitrosamines (VKM, 2007). PAHs are a large group of rather diverse substances containing two or more fused aromatic rings. The PAHs are generated by incomplete combustion or pyrolysis of organic matter. PAHs are classical air pollutants formed by combustion of carbonaceous materials, typically originating from power plants, industrial plants, engines, furnaces, fireplaces, candle fire or tobacco smoking. PAHs of low molecular weight (2 to 3 fused ring) are sufficiently volatile to be found as freely circulating substances in the atmosphere, while PAHs of higher molecular weight (5 fused rings or more) are typically found bound to particles (Choi, Harrison, Komulainen, & Saborit, 2010). It is this latter group that would normally cause concern in food. From 400 °C to 1000 °C there is linear increase in the formation of the substances. They are chemically rather inert and therefore fairly persistent in a food source once formed. In food products, contamination with PAH are associated with grilling/barbequing, smoking, high temperature roasting due to the partial carbonisation of the food source and directly drying food using combustion gases (Duedahl-Olesen, 2013).

Carcinogenicity of PAHs

Both JECFA and EFSA has carried out risk assessments of PAHs in food (EFSA, 2008b; JECFA Joint FAO/WHO Expert Committee on Food Additives, 2006). Experts from both panels agree that the critical health related effect for PAHs is cancer. Furthermore, as several of the PAHs are genotoxic, it is not possible to determine a no adverse effect level for PAHs. Though hundreds of different PAH compounds may be generated during the incomplete combustion of organic materials the JECFA evaluation more narrowly covered 33 PAHs and focused on the 13 PAHs, which the expert panel considered to be clearly genotoxic and carcinogenic. In their evaluation EFSA focused on 15 PAH compounds that had shown clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals *in vivo*. Some PAHs are easily absorbed into the body while others are poorly absorbed. Once absorbed, they are distributed to almost all organs. PAHs are extensively metabolised in humans leading to highly reactive metabolites that can damage the DNA, thus potentially causing mutations (EFSA, 2008b).

A classical PAH is Benzo[*a*]pyrene (figure 2), which has often been used as a marker of the total exposure of PAH compounds. EFSA in 2008 stated that Benzo[*a*]pyrene alone is not a suitable indicator for the occurrence of PAHs in food and instead encouraged the use of the sum of four or eight specifically defined PAHs as a better marker for PAH exposure (PAH4: benzo[*a*]pyrene, benz[*a*]anthracene, chrysene and benzo[*b*]fluoranthene) or eight (PAH8: benzo[*a*]pyrene,

benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene). According to EFSA (2008b) barbequed meat on average contains twice as much PAH8 as grilled meat, which again contains more than twice as much PAH8 as smoked meat. The PAH concentration in roasted meat would normally be low (VKM, 2007). Thus for meat products PAH contamination are basically limited to grilled/barbequed meat and smoked meat products. According to EFSA (2008b) the average consumer gets around 10% of the total dietary PAH intake from meat products, though it should be cautioned that this percentage will markedly increase for people having a high intake of grilled/barbequed meat. Vitenskapskomitten for Mattryghet (VKM) (2007) estimate the Benzo[*a*]pyrene intake from grilled meat for consumers with a high intake of grilled food to be around 60% of the total dietary intake.

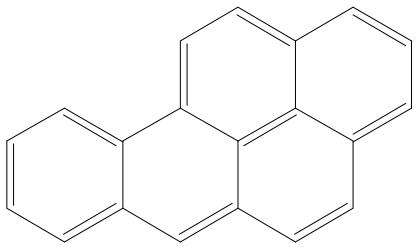

Figure 2: Benzo[*a*]pyrene.

When conducting risk assessments of substances that are carcinogenic and genotoxic, the typical approach is to calculate the margin of exposure or MOE. This is typically done by determining the benchmark dose lower confident limit for a 10% increase in the cancer incidence (BMDL10) calculated on the basis of animal experiments and dividing this dose by the estimated intake of the population. A MOE higher than 10,000 would represent a low concern, while a MOE lower than 10,000 represents a concern. Thus the lower the MOE the greater the concern and vice versa (EFSA, 2005). Using PAH8 as a marker for the exposure of the genotoxic and carcinogenic PAH compounds, EFSA (2008b) calculated the MOE for the average European consumer to be 17,000 and 9,600 for consumers with a high intake of food contaminated with PAHs (97.5 percentile). VKM (2007) calculated the total MOE (based on exposure from grilled food + the average exposure from other dietary sources) to be 8,800 for consumers with a fondness for grilled meat (30 portions a year ~ twice the average Norwegians grill meat consumption). This calculation was based on the intake of Benzo[a]pyrene alone. A calculation based on PAH8 would likely result in a lower MOE, perhaps around 8,000 extrapolating from EFSA data. It should also be stressed that 30 portions a year is most likely less than what is consumed by dedicated grill enthusiasts. Though these results demonstrate a health concern for the people most heavily exposed to PAHs, in particular people with a fondness for grilled/barbequed food, the MOE do not indicate that PAH is a major cause for cancer among Europeans. A recent Chinese study calculated that the dietary intake of PAH among Chinese urban residents would lead to a median incremental lifetime cancer risk (ILCR) around 7×10^{-5} (Duan et al., 2016) or ~ 70 lifetime cancer cases per million citizens. Though calculations of ILCR make sense for toxicologist as a risk ranking tool, they often convey the faulty misconceptions to non-toxicologists that such numbers reflect the “true” number of cases. Hence the preference to use MOE by EFSA. None-the-less, the estimated ILCR as reported by Duan et

al. (2016) indicates that it could be difficult to find associations between the dietary PAH intake and cancer in epidemiological studies, even if the estimated ILRC markedly underestimate the true ILCR.

Epidemiological studies on grilling/barbequing and/or PAHs and cancer

A number of epidemiological studies have been conducted to study whether cancer can be correlated to grilling and/or barbequing meat. Intake of grilled/barbequed meat is closely correlated with the intake of PAHs (but also HCAs) making these studies of interest for the risk assessment of PAHs.

In an US case-control study on the development of colorectal adenomas, which are precursors of colorectal cancer, a positive association was found to grilled red meat as well as the doneness of the grilled red meat (the more done the stronger the association) (Sinha, Chow, Kulldorff, & Denobile, 1999).

In a US case-control study a positive association was found between the total intake of grilled, barbequed and smoked red meat and breast cancer for postmenopausal women but not premenopausal women. Neither was the association statistically significant among postmenopausal women with a high intake of fruit and vegetables. No association was seen between breast cancer and the estimated intake of benzo[a]pyrene (Steck et al., 2007).

An US case-control study on pancreatic cancer found a positive association to consumption of grilled/barbequed red meat (Anderson et al., 2002). A US prospective cohort study by Stolzenberg-Solomon et al. (2007) also found an association between grilled or barbequed meat and pancreatic cancer in men. However, no association was found between Benzo[*a*]pyrene and pancreatic cancer. There was no effect on women, perhaps due to lower number of female participants along with the women eating less meat than the men.

The results on prostate cancer are more conflicting. A US prospective cohort study found a positive association between intake of barbequed/grilled meat and benzo[*a*]pyrene with total and advanced prostate cancer but not fatal prostate cancer (Sinha, Park, et al., 2009). However, two US prospective cohort studies did not find any association between prostate cancer and intake of grilled meat (Koutros et al., 2008) or barbequed meat (Cross et al., 2005).

A US case-control study by John et al. (2011) found a borderline significant positive association between advanced prostate cancer and intake of grilled or barbequed red meat, but no effect was seen for localized cases and neither was any association found for benzo[a]pyrene.

An Italian-Swiss case-control study found a positive association between intake of roasted/grilled meat and prostate cancer (Di Maso et al., 2013). They found associations between intake of differently cooked meat (roasted/grilled, boiled/stewed and fried/pan-fried) and several types of cancer. However, beside prostate cancer they found little effect of the cooking practice. A serious weakness of the study is the groupings of the meat preparations. Another weakness is they had no information on doneness. As shown by Knize and Felton (2005) consumption of well-done meat had frequently been associated with a variety of cancers. Several of the epidemiological studies above also found positive associations between the level of doneness and cancer, including the study by Koutros et al. (2008). A summary conducted by Zheng and Lee (2009) on six epidemiological studies on estimated intake of benzo[*a*]pyrene and cancer only found one study

reporting a positive association. This can indicate that PAHs are not a major cause for the studies reporting an association between grilled/barbequed food and cancer or it could indicate that benzo[a]pyrene is not a good marker for the total exposure of carcinogenic PAHs. Another reason may be that accurately assessing the intake of benzo[*a*]pyrene is difficult and also that the total PAH exposure may be misclassified due to exposure from non-dietary sources. This adds a high degree of uncertainty to these results.

Overall the epidemiological studies focused on studying the effect of meat cooking practices indicate a positive association between eating well-done fried, roasted, grilled/barbequed meat and the incidence of cancer. It is, however, more uncertain whether grilling/barbequing adds an additional effect on top of the effect of eating well-done meat. Future epidemiological studies should try to resolve this issue by designing studies that can better discriminate between the effects of the various types of cooking. Also, if the studies try to find associations between cancer and the estimated intake of PAHs, it will not suffice to solely estimate the intake of benzo[*a*]pyrene. Studies should be based upon estimations of the intake of PAH4 or PAH8 and the estimates should include the PAH intake from all dietary sources, not just grilled/barbequed food.

Mitigation - PAHs

There are several approaches to reduce PAH contamination of the meat during grilling. VKM (2007) has offered a range of advices besides generally limiting the intake of grilled meat:

- The heat source is placed over or beside the food so that fat cannot ignite on the heat source
- Distance between the food and the heat source is increased
- Food with lower fat content is barbequed
- Ceramic bricks in the gas barbeque are clean
- Good ventilation is provided
- Don’t cook meat in open flames

Finally, as PAH contamination primarily originates from the smoke, all types of food, such as bread and vegetables that are grilled or barbequed will be contaminated (Alomirah et al., 2011). The same mitigation measures as described above for meat products will also reduce the contamination level in other types of food.

When fat/oil ignites on the heat source, the formation of PAHs markedly increases. For instance the PAH8 contamination of grilled pita bread increased 14 times when fat was dripping from the pita breads compared to plain pita breads (Alomirah et al., 2011). Therefore ideally the fat and oils dripping from the food should not be able to come in contact with the heat source as recommended by VKM.

Grilling using an electric grill only generate little if any PAHs. Using charcoal or charcoal with wood chips for the grilling will normally result in a greater PAH contamination than using gas for the grilling while some BBQ briquettes may offer contamination levels on par with gas (Rose et al., 2015). Some types of charcoal release less PAH than other types of charcoal. Though less investigated than for the HCAs, some marinades may reduce the PAH contamination in grilled meat. Viegas et al. (2014) found a ~50 % reduction in PAH contamination in meat marinated in black beer. Pilsner beer also reduced the PAH contamination though not as effectively.

Summary – PAHs and cancer

From a mechanistic point of view there is firm evidence that several of the PAHs are genotoxic carcinogens in experimental animals and most likely also humans. MOE calculations, however, indicate a low concern for the majority of consumers, with a somewhat higher concern for people who have a fondness for grilling and barbequing. Several epidemiological studies have reported positive associations between cancer and intake of grilled/barbequed meat. However it is not clear whether this association is due to an increased intake of PAHs or to some confounding effect (e.g. HCAs, haem). Yet, PAHs from the diet cannot be ruled out as a causative factor for cancer among humans. Our present knowledge about the occurrence and toxicity of a whole range of hundreds of PAH compound are incomplete, and some of these PAHs may contribute substantially more to the total adverse impact of PAHs than the 15 well known genotoxic PAHs. Also we may generally underestimate the carcinogenicity of the known PAHs in humans. Finally it should be stressed that the reported associations between grilled/barbequed meat and cancer could be due to other carcinogenic chemicals formed from the incomplete combustion of organic material.

In conclusion, the magnitude of the adverse health impact of our intake of PAHs from the diet is highly uncertain. However we know that many PAHs are potent carcinogens that we are continually exposed to. Thus, there is a rationale in carrying out efforts to minimize our exposure to these compounds.

Role of the intestinal microbiota in meat-associated colorectal cancer risk

Generally about the involvement of the gut microbiota in colonic cancer risk

In recent years it has become evident that the complex community of microorganisms inhabiting the human gastrointestinal tract plays an important role for the risk of development of a number of diseases including (but not limited to) obesity-related disease, liver disease, inflammatory bowel disease and colorectal cancer risk (CRC) (Marchesi et al., 2015). As dietary factors affect the composition as well as the activity of the gut microbiota (Foerster et al., 2014), the implication of given dietary components for health may partly be mediated by their effects on the intestinal bacteria (Albenberg & Wu, 2014).

Increasing evidence shows a connection between the intestinal microbial community and CRC, and it has been suggested that intestinal microorganisms are involved in the onset and progression of CRC through different mechanisms including the induction of chronic intestinal inflammation, production of toxic bacterial metabolites, biosynthesis of bacterial genotoxins interfering with cell-cycle regulation, generation of secondary bile salts, and bacterially mediated activation of carcinogenic compounds originating from the diet (Nistal, Fernández-Fernández, Vivas, & Olcoz, 2015; Vipperla & O'Keefe, 2016).

Additionally, several specific bacterial pathogens seem to be directly involved in promoting CRC, but the increasing list of bacteria with potential carcinogenic activity supports the hypothesis that tumerigenesis is driven by mechanisms and pathways that are common to many bacterial groups rather than by single organisms (Louis, Hold, & Flint, 2014). An unbalance in the intestinal bacterial population is often designated *dysbiosis*, and is associated with a number of different diseases. According to the so-called 'driver-passenger model', during dysbiosis, a group of 'driver' bacteria are able to initiate disease development at the beginning of CRC progression, while after tumor formation, a change in the gut environment occurs, which results in overgrowth of 'passenger' bacteria (Nistal et al., 2015).

Specific effects of meat in relation to the microbiota

Protein is a major constituent of meat. Bacterial catabolism of proteins originating from any type of food results in a number of different end products including ammonia, N-nitroso compounds, phenolic and indolic substances, and hydrogen sulfide (Kim et al., 2013), all of which are known to result in generation of reactive oxygen species, genotoxicity, and in some cases also inflammation in the host (Louis et al., 2014), thereby contributing to carcinogenesis. In approximately 60% of CRC cases, the tumor is located in the distal colon or in the rectum, which is where bacterial protein catabolism primarily occurs. It is unclear whether the total amount of protein in the diet is more important than the source of the protein (Kim et al., 2013).

Specific carcinogenic effects are known to be related to haem, which is the pigment of red meat. It has recently been shown that the gut microbiota plays a pivotal role in this increased risk, probably because microbial produced hydrogen sulfide disrupts the protective barrier constituted by the mucus layer covering the intestinal inner surface, and thereby exposes the intestinal epithelial tissue to the cytotoxic haem (Ijssennagger et al., 2015). Additionally, enzymatic activity of the intestinal microbiota converts latent carcinogens present in cooked meat into bioactive forms such

as nitrosamines, polyaromatic hydrocarbons and heterocyclic amines (Cipe, Idiz, Firat, & Bektasoglu, 2015; Vipperla & O'Keefe, 2016).

Microbiota-based strategies to prevent meat-induced CRC risk

The listed meat-induced detrimental effects of gut microbial actions may to some extend be counteracted by interventions pushing the balance away from dysbiosis and towards a symbiotic homeostasis of the microbial community (Vipperla & O'Keefe, 2016). A diet high in fibres and complex carbohydrates escaping digestion in the small intestine will 'feed' the colonic microbiota with carbohydrate-based substrates. As bacteria ferment available carbohydrates before they switch to protein degradation, this will reduce the formation of detrimental products originating from bacterial protein catabolism. Additionally, bacterial fermentation of fibres generates short chain fatty acids including butyrate, which are known to counteract inflammation, nourish a healthy epithelium, promote expression of tumor-suppressing genes and induce apoptosis in cancer cells (Louis et al., 2014).

Strategies for prevention of meat-induced CRC, therefore comprise consumption of a generally fibre-rich diet as well as supplementation with dietary carbohydrates that lead to increased formation of luminal butyrate (Le Leu et al., 2015). Additionally, supplementation with probiotic (beneficial) bacteria may prevent CRC by scavenging toxic compounds or prevent their generation, as well as by pushing the bacterial community away from dysbiosis (Azcárate-Peril, Sikes, & Bruno-Bárcena, 2011).

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The role of meat in healthy diets

Introduction

There are many ways to compose and eat a healthy diet that meets nutrient recommendations and perhaps also dietary guidelines. This also includes the type and amount of meat e.g. red and processed meat and poultry included in the diet.

The World Cancer Research Fund (WCRF) recommended in 2007 that the dietary intake of red meat should be limited and processed meat avoided (World Cancer Research Fund / American Institute for Cancer Research, 2007b). The personal recommendation to individuals consuming meat was to consume less than 500 g red meat (prepared) per week (equivalent to 70 g *prepared*/d) with very little – if any – processed meat. This recommendation was included in the Danish dietary guidelines in 2013. The scientific evidence behind the recommendations were upgraded in 2011 (World Cancer Research Fund / American Institute for Cancer Research, 2011) and latest in 2015 (IARC, 2015).

A Nordic working group investigated the consequences of lowering the daily consumption of meat in adults, children and adolescents, from current intake to the level suggested by the WCRF through five different scenarios. The basis for the modeling was the average habitual diet and no content of processed meat in any of the five scenarios. Red meat in the diets were substituted with either white meat/fish or a proportional amount of other food products than meat (Tetens et al., 2013). The results showed that the current mean intake of meat was very similar to the levels suggested by WCRF, and that the modeling applied meant relatively low reductions of intake of red meat. Only men had daily intakes of red meat exceeding 70 g. This meant that the nutrient profile of the five scenarios were very similar. However, specific individuals have intakes of meat that differ a lot from the mean intake. The evidence behind the WCRF recommendation is based on testing dose-response between meat intake and cancer incidence/mortality, and often the increased risk of cancer is found by testing the high-intake groups against the low-intake groups. For instance, the European Prospective Investigation into Cancer and Nutrition (EPIC) study found an increased mortality risk of 14% when comparing a subgroup with an intake of 160 g red meat/day against a subgroup with an intake of 10-20 g red meat/day (Rohrmann et al., 2013). But such groups of low and high meat intake may contain subgroups with different dietary patterns and with different adherence to nutrient recommendations and dietary guidelines. Healthy eating is communicated to the population through the Danish dietary guidelines. These strive to communicate healthy eating patterns that emphasize a balanced dietary intake within energy needs, and not single foods. Another reason in favor of looking at dietary patterns instead of single foods is that it can be difficult to separate the effect of individual components of a dietary pattern in observational studies because of interrelationships between correlated dietary variables (McNeill, 2014). Therefore it is relevant to investigate the role of meat in a healthy dietary pattern as communicated by in the Danish dietary guidelines and Nordic Nutrient Recommendations instead of looking only at high meat intake or at the isolated food group.

Generally, there is a lack of good data on how the diet is composed for such groups with similar nutrient and dietary quality but so different meat intake as mentioned in the groups above from the EPIC study. Such knowledge can contribute to an increased understanding of the role of diet in health and disease and generate new hypotheses. The contribution made by red and processed

meat to high quality protein and nutrient intake probably also influence the effect of meat on health outcomes. This is frequently overlooked in epidemiological studies, but highly relevant.

This chapter first highlights the key nutrients that meat provides to the Danish diet. Next it investigates dietary patterns of persons with similar high nutrient and diet quality and low, middle and high intakes of red and processed meat. The analysis uses data from the latest Danish National Survey of Diet and Physical activity (DANSDA) 2011-13 on adults (n=3189; 15-75 years).

Beneficial nutrients in meat

A former Danish study from 2016 has shown that from a nutritional point of view, meat can be considered a food with predominantly high nutritional value that contribute positively to the intake of certain vitamins, minerals and protein in the diet of Danes. The study showed that meat provided ≥ 15% of most vitamins and minerals including vitamin D, and ≥ 30% of protein, vitamin A, thiamin, niacin, B12, B6 (29%), zinc and selenium (Biltoft-Jensen et al. 2016). Other studies further point out that the iron, zinc and vitamin D present in meat is of higher bioavailability than from other sources, and that the protein in meat contains all the essential amino acids (Binnie, Barlow, Johnson, & Harrison, 2014; Wyness, 2015). This suggests that meat qualifies as a "source" of most micronutrients and as a "good source" of 7 of these as well as of protein according to the EU Health claims regulation (The European Parliament and The Council of the European Union, 2006). In the Danish study it was predominantly red meat that provided thiamin, niacin, B6 and B12 while processed meat provided B12 and vitamin A. The contribution of vitamin A and B12 from processed meat came mainly from liver paste. Meat also provided 26% of monounsaturated fatty acids and relatively low levels of polyunsaturated fatty acids (13%). The main polyunsaturated fatty acids in red meat are the essential fatty acids, linoleic (n-6) and α-linolenic acid (n-3). When consumed, the body can convert α-linolenic acid to the long–chain n-3 fatty acids EPA and DHA. The rate of synthesis is, however, small and generally below 5% in man (Burdge & Calder, 2005). However, there were also negative contributions from meat. Meat provided 20% of the saturated fatty acids, 23% of sodium and 13% trans fatty acids. The sodium originated mainly from the processed meat (Biltoft-Jensen et al., 2016). Similar contributions of nutrients from meat are also found in other studies (McNeill, 2014; Wyness, 2015).

In the Danish study from 2016 it was furthermore found that the subgroup with the highest content of meat in the diet had higher nutrient intakes of those nutrients abundant in meat such as thiamin, riboflavin, niacin, B6, B12, phosphor, iron, zinc and selenium compared to the subgroup with lowest meat content in their diet. But they had lower intakes of the vitamins and minerals abundant in fruit, vegetables and wholegrain such as vitamin E, vitamin C, folate and magnesium, and in general they were less likely to meet macronutrient recommendations (Biltoft-Jensen et al., 2016). The subgroup with the highest meat content in the diet also had lower dietary content of fruit, vegetables, fish, wholegrain and dietary fiber and was less likely to follow dietary guidelines as a group. However, there were individuals, although very few, who both met nutrient and dietary recommendations in both the subgroup with the highest and lowest red and processed meat content in the diet. In the next section the nutrient and dietary content of those with a diet closest to nutrient recommendations and dietary guidelines in the three subgroups of low, middle and high red- and processed meat content in the diet will be described.

Methods

Sevenhundredseventyfive subjects (15-75 years) from DANSDA (2011-13) were chosen for this specific study based on their nutrient and dietary intake, and divided into three groups based on their intake of red and processed meat.

The data processing, limitation, generalizability and the formation of quartile subgroups are described elsewhere (Biltoft-Jensen et al., 2016). In this study red and processed meat is as far as possible defined according to IARC's definitions (see box below).

Red meat: Unprocessed muscle meat from mammals such as beef, veal, pork, lamb, mutton, horse or goat – including minced and/or frozen meat. It is usually eaten cooked (IARC, 2015). In this study a small intake of offal such as liver and heart is also included in red meat.

Processed meat: Meat which has been processed by salting, curing, fermentation, smoking or other processes that enhance the flavor or improve preservation. Most processed meat contain pork or beef, but also other types of red meat, poultry, organ meats (such as liver) or meat by-products such as blood (IARC, 2015). Processed meat include products such as bacon, sausages, ham, chicken nuggets, poultry deli meats and other deli meats and pâté.

In short the groups of low, middle and high meat content in the diet are based on quartiles of meat content in the whole population (n=3189), so that the 25 % with lowest and highest meat content represented the extreme intakes (high or low content) and the 2 middle quartiles were added as one group to illustrate the middle content.

Within each group the persons with a total nutrient score over and equal to 90 and a dietary guideline score over and equal to 4 was chosen. The nutrient score was determined by the ratio of nutrient intake relative to the recommended intake in the Nordic Nutrient Recommendations 2012 (Nordic Council of Ministers, 2012). For each individual a score was calculated for each nutrient. The minimum score was 0 and maximum score was 100 (100% fulfilment) for each nutrient. The higher the score, the more favorable nutrient profile. The approach was adapted after (Biltoft-Jensen et al., 2008; Thiele, Mensink, & Beitz, 2004). The diet guideline score was constructed as follows: for each individual, a dietary score was calculated for each of the five nutrients/food groups (fruit and vegetables, fish, wholegrain, energy from saturated fat and added sugar) as the ratios between the actual intake and the recommended intake of the certain nutrient or food. In individuals with intakes exceeding the cut-off values, the individual was assigned the maximum score value of 1, and no further nutritional value was added to the score. Thus, the score for each of the items included ranged from 0 to 1, with zero assigned to the intake of a food or nutrient furthest away from the dietary guideline, and one for the intake complying with the guideline (Knudsen et al., 2012). The exact calculations of the scores are described elsewhere (Biltoft-Jensen et al., 2016).

The statistical analysis is performed in SPSS program version 23.

Table 4: Overview over the statistical test used in the table 5 and 6

	Continuous variables	**Statistical tests**	**Grouped variable**	**Test**
Table 5	Age Meat content Nutrient content Dietary fiber content Alcohol content Total nutrient score and micro and macronutrient score	One Way ANOVA POST- HOC: Tukey	Gender	Chi-square
Table 6	Intake of foods Dietary Guideline score	Kruskal-Wallis and Pairwise Mann-Whitney One Way ANOVA/POST-HOC: Tukey	Percent with meatless days per week. Percent with over 1 meatless day per week.	Chi-Square

Results

Nutrient characteristics of diets with similar high nutrient and diet quality and low, middle and high content of red and processed meat

Table 5 shows that there was over twice as many individuals with low content of red and processed meat that had diets that qualified for having a high score compared to nutrient recommendations and dietary guidelines compared to those with high dietary meat content. There was a significantly higher percentage of males in the groups with high dietary contents of red and processed meat. There was no age difference among any of the groups. It is also worth noting that among the three groups of red meat, the mean content of processed meat was not statistically different. The same applies to the three groups of processed meat, where the mean content of red meat was not statistically different.

Table 5. Mean (SD) nutrient content in diets of subgroups with low, middle and high content of red and processed meat and a high total nutrient and dietary guideline score*

	Red meat			**Processed meat**		
Content	**Low**	**Middle**	**High**	**Low**	**Middle**	**High**
Number	289	377	109	283	377	115
% males	34^{AB}	31^{B}	44^{A}	27^{C}	32^{B}	54^{A}
Age	50 (16)	49 (16)	51 (16)	50 (17)	49 (16)	47 (16)
Red meat g/10MJ	29 (13)^{C}	78 (17)^{B}	150 (35)^{A}	66 (45)	71 (40)	76 (51)
Processed meat g/10MJ	31 (28)	34 (32)	38 (32)	10 (6)^{C}	34 (11)^{B}	89 (37)^{A}
Energy, MJ	8.8 (2.7)	8.7 (2.5)	8.3 (2.3)	8.4 (2.4)	8.8 (2.5)	9.0 (2.9)
Fat E% excl. alcohol	34.6 (4.6)	34.5 (4.2)	34.5 (3.8)	33.9 (4.2)^{B}	34.6 (4.3)^{B}	35.8 (4.2)^{A}
Monounsaturated fat E% excl. alcohol	12.9 (2.4)	12.8 (2.1)	13.2 (2.1)	12.5 (2.2)^{B}	13.0 (2.3)^{A}	13.5 (2.1)^{A}
Polyunsaturated fat E% excl. alcohol	6.3 (1.2)^{A}	6.0 (0.9)^{B}	5.8 (0.9)^{B}	6.0 (1.0)	6.1 (1.0)	6.2 (1.1)
Saturated fat E% excl. alcohol	12.4 (2.1)^{B}	12.8 (1.9)^{A}	12.9 (1.6)^{A}	12.5 (2.0)^{B}	12.7 (1.8)^{B}	13.2 (1.9)^{A}
Protein E% excl. alcohol	16.4 (2.4)^{C}	17.4 (2.4)^{B}	19.6 (2.1)^{A}	17.1 (2.7)^{B}	17.3 (2.4)^{B}	18.0 (2.7)^{A}
Carbohydrate E% excl. alcohol	49.0 (5.0)^{A}	48.1 (4.8)^{A}	45.8 (4.4)^{B}	49.0 (5.0)^{A}	48.0 (4.8)^{B}	46.2 (4.5)^{C}
Added sugar E% excl. alcohol	6.8 (3.1)^{A}	6.4 (2.9)^{A}	5.5 (3.1)^{B}	6.8 (3.2)^{A}	6.4 (2.9)^{A}	5.4 (2.8)^{B}
Dietary fiber g/MJ	30.9 (6.5)^{A}	29.4 (5.4)^{B}	29.6 (5.5)^{AB}	31.0 (6.2)^{A}	29.5 (5.4)^{B}	29.1 (6.1)^{B}
Alcohol E%	4.0 (4.6)	4.4 (4.4)	4.2 (4.4)	4.2 (4.7)	4.2 (4.1)	4.4 (5.0)
Total nutrient score (0=lowest; 100 =	95.7 (2.1)	95.4 (1.9)	95.7 (1.6)	95.8 (2.0)^{A}	95.6 (1.9)^{B}	95.1 (1.9)^{B}

highest)						
Macro score (0=lowest; 100 = highest)	88.6 (8.0)[A]	87.0 (7.6)[B]	87.0 (6.3)[AB]	88.1 (7.7)[A]	87.7 (7.4)[B]	86.0 (7.9)[B]
Micro Score (0=lowest; 100 = highest)	96.9 (2.2)	96.8 (2.0)	97.2 (1.5)	97.0 (2.1)	96.9 (2.0)	96.7 (2.1)

*Within each group the persons with a total nutrient score over and equal to 90 and a dietary guideline score over and equal to 4 was chosen. This equals the 10% closest to meeting total nutrient recommendations and the 20% closest meeting dietary guidelines.

[A, B, C] Values in the same row with different superscript letters are significantly different ($p<0.05$)

Red meat

The group with the high content of red meat in the diet had on average 5 times as much meat in the diet as the group with low content. There was no difference in the total nutrient score among the groups; however the middle group had significantly lower macronutrient score compared to the low content, but not the high content group, although the difference was very small.

There was no difference in content of energy, total fat, monounsaturated fat and alcohol among the 3 groups of red meat content. Furthermore, there was no difference in dietary fiber content among the groups with the lowest and highest red meat content. The middle group had a slightly lower content of dietary fiber than the low content, but not the high content group.

Not surprisingly, the group with high dietary red meat content had significantly higher content of saturated fat, and lower content of polyunsaturated fat compared to the low content group. But the differences between the high and low content groups were very small (0.5% points). The high content group had significantly higher protein content than both the middle and low group, and had significantly lower content of carbohydrate and added sugar.

To sum up, the total nutrient score supported that overall there were no differences between the nutrient profile among the three groups of low, middle and high red meat content in their diet. However, looking at individual nutrients, there were minor differences for saturated fat and protein, which were higher in the high content group, and for polyunsaturated fat, carbohydrate and added sugar, which were higher in the low content group.

Processed meat

The group with the high content of processed meat in the diet had on average 9 times as much processed meat in the diet as the group with low content. The middle and high content group had significantly lower total nutrient score compared to the low content group, although the differences were very small (0.2-0.7% point). This was due to a lower macro nutrient score among the middle and high content groups. There was no difference in micronutrient score among the three groups.

There was no difference in content of energy, polyunsaturated fat and alcohol among the 3 groups of processed meat content. The low content group had a significantly higher content of dietary fiber in the diet compared to the middle and high content group, although the difference was small (0.5-0.9 g/10 MJ).

The group with high processed meat content had significantly higher dietary content of total fat, saturated fatty acids, monounsaturated fatty acids, and protein compared to the middle and low content group, and had significantly lower content of carbohydrate and added sugar. The low content group had significantly higher dietary contents of carbohydrate and added sugar.

To sum up, there was a small difference in the nutrient score among groups, with the low content group having the highest score. Looking at individual nutrients, there were minor differences for fat, saturated fatty acids, monounsaturated fatty acids and protein which were higher in the high content group, and for carbohydrate and added sugar which were higher in the low content group.

Food characteristics of diets with similar high nutrient and diet quality and low, middle and high content of red and processed meat

Table 6. Median (P25;P75) *(mean)* food content in diets of subgroups with low, middle and high content of red and processed meat and a high total nutrient and dietary guideline score

Nutrient	**Red meat**			**Processed meat**		
Content	**Low**	**Middle**	**High**	**Low**	**Middle**	**High**
Number	289	377	109	283	377	115
Red meat g/10 MJ	30 (20;40)^{C} *29*	76 (63;90)^{B} *78*	142 (121;172)^{A} *150*	60 (34;87)^{B} *66*	65 (41;94)^{A} *71*	70 (36;103)^{A} *76*
Processed meat g/10 MJ	24 (12;43) *31*	27 (15;44) *34*	27 (14;52) *38*	10 (5;15)^{C} *10*	32 (25;43)^{B} *34*	78 (65;103)^{A} *89*
Poultry g/10 MJ	26 (4;48)^{A} *33*	15 (2;37)^{B} *24*	15 (0,1;34)^{B} *21*	25 (2;46)^{A} *31*	20 (2;40)^{A} *27*	11 (0,1;33)^{B} *11*
Total meat g/10 MJ	88 (64;120)^{C} *94*	130 (106;158)^{B} *136*	194 (171;234)^{A} *209*	100 (69;137)^{C} *107*	125 (99;158)^{B} *132*	170 (144;220)^{A} *185*
Fish g/10 MJ	63 (46;90)^{A} *71*	59 (40;79)^{B} *65*	58 (42;88)^{B} *67*	61 (44;86) *69*	59 (42;83) *67*	57 (38;86) *65*

Fruits g/10 MJ	289 (201;415) *339*	289 (187;389) *308*	280 (229;404) *318*	316 (220;442)^{A} *353*	278 (189;393)^{B} *309*	262 (181;371)^{B} *283*
Vegetables g/10 MJ	278 (208;367)^{AB} *307*	261 (195;336)^{B} *286*	293 (220;381)^{A} *323*	287 (213; 376)^{A} *311*	274 (206;348)^{AB} *300*	241 (167;310)^{B} *266*
Potatoes g/10 MJ	53 (25;100)^{B} *71*	72 (41;112)^{B} *82*	90 (36;146)^{A} *102*	68 (32;113) *80*	73 (35;116) *84*	52 (30;100) *71*
Gravy g/10 MJ	15 (6;26)^{B} *18*	14 (6;25)^{B} *18*	21 (11;32)^{A} *25*	15 (6;27) *19*	16 (7;28) *19*	14 (5;25) *17*
Whole grain g/10 MJ	80 (62;100) *83*	76 (61;99) *81*	72 (60;91) *76*	76 (58;102) *81*	76 (62;95) *80*	82 (66;102) *87*
Cheese g/10 MJ	41 (27;60) *46*	37 (25;57) *45*	39 (24;56) *43*	43 (27;58) *48*	38 (24;59) *44*	34 (25;56) *41*
Breads g/10 MJ	159 (123;197)^{A} *160*	154 (115;194)^{A} *157*	140 (111;183)^{B} *144*	148 (110;186)^{B} *150*	155 (117;195)^{B} *157*	163 (132;210)^{A} *171*
Fat spread g/10 MJ	5 (0,8;11)^{A} *9*	4 (0;11)^{A} *8*	2 (0;8)^{B} *5*	3 (0;10) *7*	4 (0;12) *8*	4 (0;9) *7*
Fast foods (total) g/10 MJ	8 (0;36) *24*	14 (0;48) *31*	0 (0;41) *31*	0 (0;34)^{B} *22*	13 (0;46)^{B} *30*	24 (0;55)^{A} *41*
'Empty calorie' sweet foods g/10 MJ	61 (36;90)^{A} *65*	61 (35;91)^{A} *63*	45 (18;80)^{B} *52*	64 (36;95)^{A} *68*	59 (34;89)^{A} *63*	47 (25;68)^{B} *48*
Milk g/10 MJ	73 (0;246) *168*	46 (0;207) *144*	54 (0;251) *146*	47 (0;199) *143*	72 (0;241) *161*	46 (0;246) *154*
Juice g/10 MJ	35 (0;116)^{A} *76*	33 (0;109)^{A} *72*	0 (0;78)^{B} *57*	30 (0;104) *67*	33 (0;111) *74*	28 (0;91) *74*

Water Kg/10 MJ	1.3 (0.7;1.9) *1.4*	1.2 (0.7;1.8) *1.4*	1.3 (0.8;2.1) *1.6*	1.4 (0.9;2.0) *1.6*	1.2 (0.6;1.8) *1.4*	1.2 (0.6;1.8) *1.3*
Sugar sweetened beverages g/10 MJ	0 (0;63) *54*	0 (0;69) *54*	0 (0;69) *53*	0 (0;63) *50*	0 (0;68) *57*	0 (0;69) *49*
Wine g/10 MJ	48 (0;140) *92*	70 (0;171) *107*	61 (0;137) *92*	66 (0;153) *106*	67 (0;147) *100*	28 (0;144) *83*
Beer g/10 MJ	23 (0;99) *89*	0 (0;128) *97*	0 (0;200) *117*	0 (0;89) *75*	38 (0;130) *96*	0 (0;226) *155*
Dietary guideline score (0= lowest; 5= highest)	4.4 (4.2;4.7) *4.4 (0.3)^{A}*	4.3 (4.1;4.5) *4.4 (0.3)^{B}*	4.3 (4.2;4.6) *4.4 (0.2)^{A}*	4.4 (4.2;4.6) *4.4 (0.3)^{A}*	4.4 (4.2;4.6) *4.4 (0.3)^{A}*	4.3 (4.1;4.5) *4.3 (0.3)^{B}*
Percent with meatless days per week	53^{A}	39^{B}	33^{C}	67^{A}	31^{B}	15^{C}
Percent with over 1 meatless day per week	38^{A}	12^{B}	3^{C}	35^{A}	8^{B}	3^{C}
Median content of consuming meals only (g/10 MJ)	79 (58;106)^{C} *88*	137 (111;171)^{B} *159*	227 (184;291)^{A} *257*	31 (17;49)^{C} *35*	87 (59;126)^{B} *98*	168 (127;232)^{A} *193*

$^{A, B, C}$ Values in the same row with different superscript letters are significantly different ($p<0.05$)

Red meat

As shown in table 6 the median total meat content in the diet was over twice as high in the group with high red meat content compared to the low content group. There was no difference in the dietary guideline score between the low and high content group, but the middle content group had significantly lower score. There were no difference in the content of processed meat, fruits, wholegrain, cheese, milk, water, sugar sweetened beverages, wine and beer among the three groups of low, middle and high content of red meat in the diet.

For vegetables there was no difference between the low and high content groups, but the middle group had a significantly lower content.

The low content group had significantly higher contents of poultry, fish, breads, fat spread, 'empty calorie' sweet foods and juice, and significantly lower contents of potatoes and gravy compared to the higher content groups. In contrast the high content group had significantly higher contents of potatoes and gravy and lower contents of poultry, fish, breads, fat spread, 'empty calorie' sweet

foods and fruit juice compared to lower content groups. Some of the differences, are however small e.g. for fish the median difference between groups was 4-5 g/10 MJ per day.

If potatoes and vegetables are added together, the total intake of vegetables is almost 20% higher in the high red meat content group compared to the low content group.

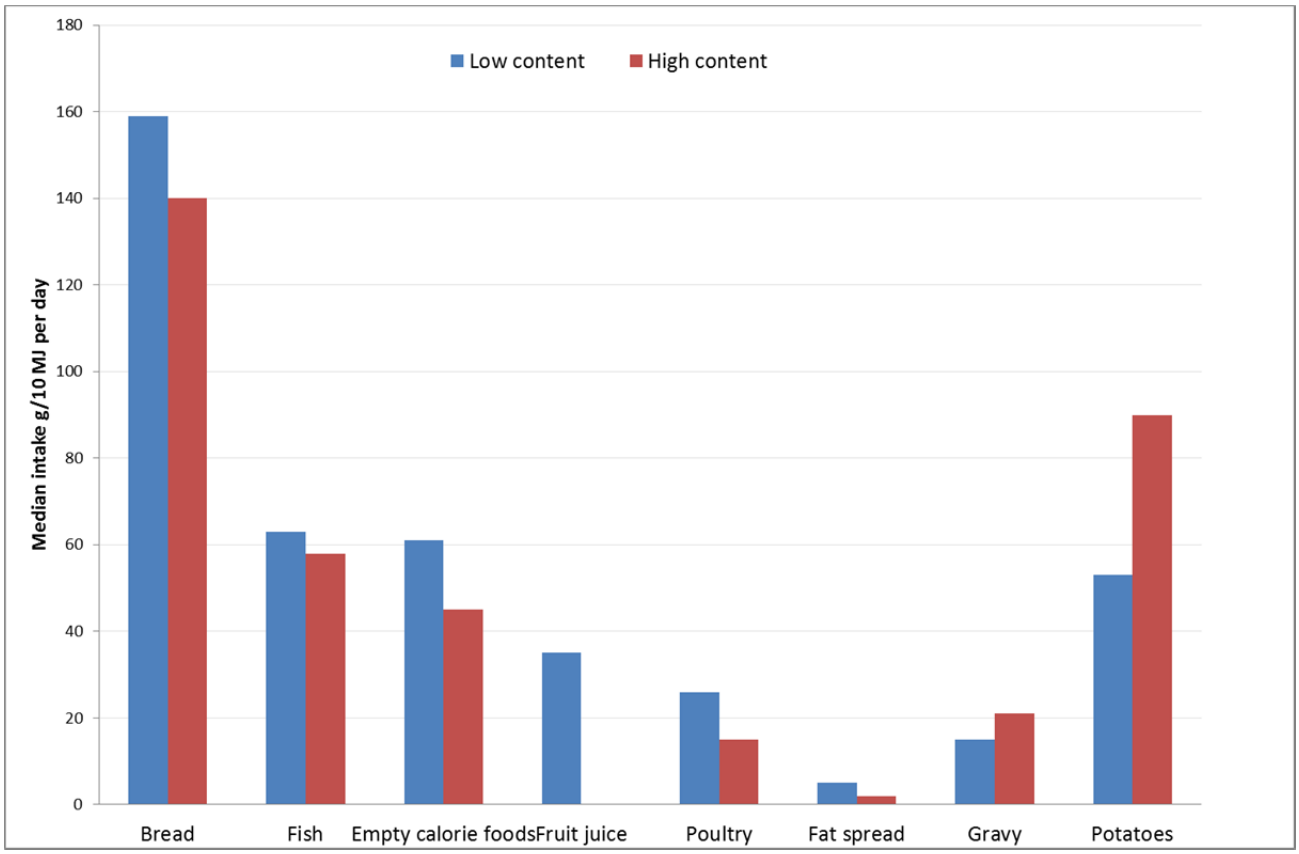

Figure 3. Food contents in the diet that was significantly different between high and low red meat content groups.

To summarize the total dietary guideline score supported that overall there were no difference between ability to meet the dietary guidelines among the three groups of low, middle and high red meat content in their diet. However, looking at individual food groups, there were differences for the content of poultry, fish, breads, fat spread, 'empty calorie' sweet foods and fruit juice which were higher in the low content group and for potatoes and gravy which were higher in the high content group as shown in figure 3.

Processed meat

As shown in table 3, the median total meat content in the diet was 70% higher in the high content group compared to the low content group. The high content group had a significantly lower dietary guideline score compared to the middle and low content group. However, the difference was small (0.1 point or 2%). There were no difference in the content of fish, potatoes, gravy, wholegrain, fat

spread, cheese, milk, juice, water, sugar sweetened beverages, wine and beer among the three groups of low, middle and high content of processed meat in the diet.

The low content group had significantly higher contents of poultry, fruits, vegetables, ‘empty calorie’ sweet foods, and significantly lower contents of red meat, bread and fast foods compared to the higher content groups. In contrast the high content group had significantly higher contents of red meat, bread and fast food, and lower contents of poultry, fruits, vegetables and ‘empty calorie’ sweet foods compared to lower content groups.

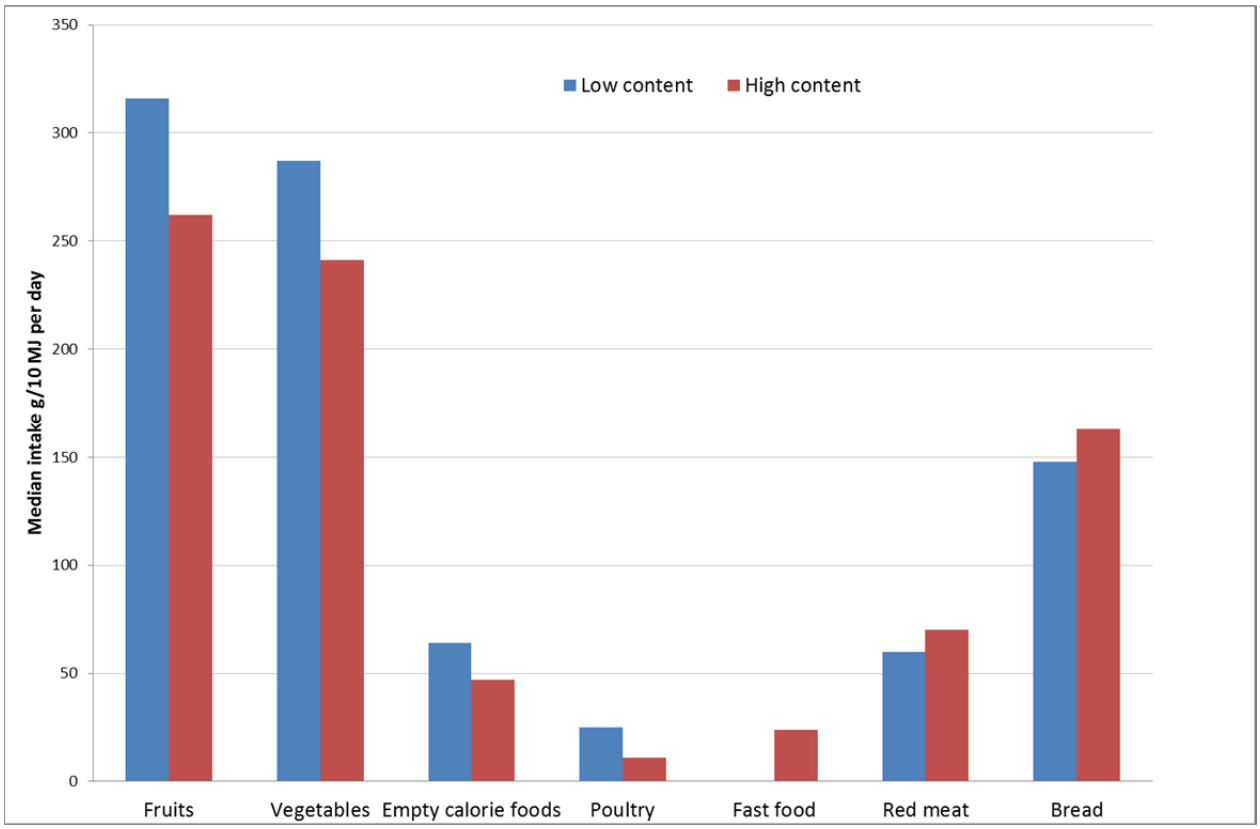

Figure 4. Food contents in the diet that was significantly different between high and low processed meat content groups.

To summarize, the total dietary guideline scores were significantly lower in the high content group compared to the middle and low content groups although the difference were small. Looking at the content of individual food groups, there were differences for the content of poultry, fruits, vegetables, ‘empty calorie’ sweet foods which were higher in the low content group and for red meat, bread and fast food which were higher in the high content group as shown in figure 4.

Finally the results showed that the low content groups of both red and processed meat were more likely to have meatless days during the week. For processed meat the group with low content was more than 4 times as likely to have a meatless day during the week compared to the high content

group. When looking at the percentages having more than one meatless day per week the low content groups of both red and processed meat were over 10 times as likely to have more than one meatless day per week compared to the high content groups. If the median content of red and processed meat is calculated based on meals with a meat intake only, the meat content is still higher in the high content groups compared to low content groups. This indicates that the small amount of meat in the low content group can't be explained by the number of meatless days, they also have a significantly lower acute meat intake.

Discussion

The results illustrated that it is possible for individuals to eat a diet with both high, middle and low content of red meat and approximately meet both nutrient recommendations and dietary guidelines (except meat) equally. However, it is obviously easier to comply with dietary guidelines and nutrient recommendations for the low content group since more individuals with low red meat content in their diet do this compared to individuals with high content of red meat in their diet. Their strategies are also different. The high content group prioritizes potatoes and gravy while the low content group prioritizes poultry, fish; breads, fat spread, and 'empty calorie' sweet foods and fruit juice. As a result the high content group had a slightly higher content of saturated fat and a higher content of protein in the diet compared to the low content group which had a higher content of polyunsaturated fat, carbohydrate and added sugar in the diet compared to the high content group. However, this did not have any influence on the total nutrient score.

Looking at the processed meat groups there were small differences in both the nutrient and dietary guideline score with the low content groups having a significantly higher score, although the differences were small. However, it seems that the high content group has more challenges including fruit and vegetables in the diet compared to the low content group. Not surprisingly they have a higher content of fast foods and breads which is often followed by processed meat. Bread based meals and fast foods have shown to be followed by lower intakes of fruits and vegetables (Fagt, 2006; Lassen, Hansen, & Trolle, 2007).

In the present study it was found that the group with a high content of red meat in the diet had a high content of vegetables and when added together with potatoes it was higher than for the low content group. This was also found in an American study where intake of vegetables was positively associated with red meat intake (Kappeler, Eichholzer, & Rohrmann, 2013). It can be speculated that the group with high red meat content prefer meals with plenty meat, potatoes, gravy and vegetables whereas the group with low meat content prefer meals with little meat, vegetables, bread and butter.

Two themes consistently emerge from studies of diet and cancer: diets rich in high-fiber plant foods such as whole grains, legumes, vegetables, and fruits and milk and foods containing vitamin D offer a measure of protection while meat, animal products, and other fatty foods are frequently found to increase risk (World Cancer Research Fund / American Institute for Cancer Research, 2011). Meat is devoid of the protective effects of fiber, antioxidants, phytochemicals, and other helpful nutrients, and it contains high concentrations of saturated fat and potentially carcinogenic compounds, which may increase one's risk of developing cancer (IARC, 2015; World Cancer Research Fund / American Institute for Cancer Research, 2007b, 2011). In the present study the three groups of low, middle and high red meat content in the diet did not differ in their dietary

content of fruits, vegetables, wholegrain and milk. The three groups of low middle and high processed meat content differed in the diet content of fruit and vegetables, but they still qualified for being close to meeting the dietary guidelines and they did not differ in their intake of wholegrain, milk and fish.

It would be relevant and new and in accordance with the Danish dietary guidelines to investigate if such groups as the high red and processed meat content groups in this study have an increased risk of developing colon cancer or if they are protected by their otherwise healthy diet. Traditionally, studies have looked at dose response relationship between meat intake and cancer risk and adjusted for confounders including single diet confounders as fruit and vegetables, but have not included the overall composition of the diet (IARC, 2015). Regarding the whole diet and the balance of food and beverages as important for health, it can be hypothesized that it might be a better approach testing dietary patterns in relation to health and disease rather than any one single food/food group.

Conditions for red meat intake resulting in reduced cancer risk

Some systematic reviews and results of cohort studies have found dietary components or foods that reduce the cancer risk associated with intake of red and processed meat.

In the WCRF/AICR update report (World Cancer Research Fund / American Institute for Cancer Research, 2011) the panel judges that the evidence that consumption of foods containing dietary fibre protects against colorectal cancer is convincing. Consumption of garlic, milk and calcium, probably protect against this cancer. There is limited evidence suggesting that non-starch vegetables, fruits and foods containing vitamin D protect against colorectal cancer, and that cheese and foods containing iron, foods containing animal fats, and foods containing sugars are causes of this cancer.

In the EPIC study population the absolute risk of developing colorectal cancer within 10 years for a person age 50 years was 1.86% for the lowest category of fish intake (<10 g/d) and 1.28% for the highest category (>80 g/d). The authors tested if it was an effect of displacement of red and processed meat by fish but found no interactions between fish and meat intake. The increased risk associated with high consumption (>129 g/d in men and >85 g/d in women) of red and processed meat versus low consumption (<30 g/d in men and <13 g/d in women) was 12-20%, independent of the levels of fish consumption. The increased cancer risk following a high intake of red and processed meat was more apparent, however not significant, if the fibre intake was low (<17 g/d) and medium (17-26 g/d in women and 17-28 g/d in men) compared to high (>26 g/d in women and >28 g/d in men) (p for interaction=0.06) (Norat et al., 2005).

In the Danish Diet, Cancer and Health cohort, substituting red meat with fish was associated with a significant lower colon cancer risk (IRR=0.89, 95% CI 0.80-0.99) for each 25 g/d but not with rectal cancer risk. However, a high fish intake was only associated with lower colon cancer risk in participants with low fruit fibre intake ($\leq$2 g/d) but not in those with high fruit fibre intake (>2 g/d). Substitution of poultry for red meat had no significant effect on colon or rectal cancer risk. Participants with lower cereal fibre intake had a significant 13% (95% CI 1.01-1.28) higher risk for colon cancer per increment of 25 g/d intake of processed meat (Egeberg et al., 2013).

According to Le Leu et al. (2015), strategies for prevention of meat-induced colorectal cancer comprise consumption of a generally fibre-rich diet as well as supplementation with dietary carbohydrates that lead to increased formation of luminal butyrate Additionally, supplementation with probiotic (beneficial) bacteria may prevent colorectal cancer by scavenging toxic compounds or prevent their generation, as well as by pushing the bacterial community away from dysbiosis (Azcárate-Peril et al., 2011).

From the present review of potential carcinogens and their mechanisms in red and processed meat it can be concluded that a possible way to reduce the cancer risk posed by intake of red and processed meat is that care should be taken in preparation of the meat. Frying the meat at lower cooking temperature will reduce the formation of HCAs. A range of natural constituents found in e.g. spices, wine or beer can also lower HCA formation when applied to the meat prior to cooking. Furthermore it is possible to reduce the PAH contamination of the meat by using appropriate grilling and smoking practices. N-nitrosamine formation in cured meat can be minimised by creating an environment in the meat products that do not favour nitrosation reaction and generally

keeping the amount of added nitrite to a minimum. A diet with a high content of fibre rich foods such as whole-grain foods, vegetables and fruit may reduce the cancer risks associated with meat intake, both through systemic effects, through effects on the activity of the intestinal bacteria and through adsorbing carcinogenic substances.

Conclusion

The definitions of ‘red meat’ and ‘processed meat’ differ in different studies, and several large American cohort studies include processed meat in the definition of red meat. In general, the difference between ‘red meat’ and ‘processed meat’ expresses whether the meat is fresh or preserved in some manner.

The associations between red and processed meat intake and CRC are not as pronounced in European cohort studies as in American studies. This discrepancy may be due to differences in definitions of red and processed meat, or it may be related to different food cultures and lifestyles confounding the results.

Cancer is a complex group of diseases that involve many risk factors. The evidence for possible mechanisms involving different compounds from red and processed meat on cancer risks is convincing. The compounds include haem iron, N-nitroso compounds (nitrite), heterocyclic amines and polycyclic aromatic hydrocarbons. However, the individual risk posed by each mechanism may be of minor magnitude compared to other causes of cancer, so that the effect is not visible in epidemiological studies.

Increasing evidence suggests that the composition of the intestinal microbiota affects the risk for colorectal cancer through different mechanisms. Thus, a high protein, low fibre diet that promotes growth of bacteria with high carcinogenic activity, increases the CRC.

Based on the available data from epidemiological and mechanistic studies it is not possible to conclude whether meat from different animal species will pose different cancer risks.

Analyses of dietary patterns in a subgroup of the Danish population showed that it is possible to have a diet that to a great extent lives up to the Nordic Nutrient Recommendations and Danish dietary guidelines with both a low, medium and high intake of red and processed meat.

To reduce the cancer risk posed by red and processed meat intake, it is recommended that care should be taken in preparation of the meat. Meat needs to be heated sufficiently to ensure the destruction of pathogenic bacteria, but the formation of HCAs can be lowered by heating the meat more gently (lower cooking temperatures and cooking times) and avoid charring of the meat. Furthermore, it is possible to reduce the PAH contamination of the meat by using appropriate grilling and smoking practices. N-nitrosamine formation in cured meat can be minimised by creating an environment in the meat products that do not favour nitrosation reaction (e.g. adding ascorbic acid) and generally keeping the amount of added nitrite to a minimum. A diet with a high content of fibre rich foods such as whole-grain foods, vegetables and fruit may reduce the cancer risks associated with meat intake.

Since ‘processed meat’ is a very heterogeneous food group that refers to meat that has been preserved in very different ways, future epidemiological studies should focus on carrying out studies that optimise the testing of a priori formulated hypothesis about the potential causative factor. Using processed meat as a singular group in epidemiological studies should be discouraged. Besides, carefully designed intervention studies in humans that test the individual mechanisms should be performed, before final conclusions on the effect of red and processed meat on cancer risks can be drawn.

Abbreviations

ADI	Acceptable Daily Intake
AICR	American Institute of Cancer Research
APC	A tumor suppressor gene
BMDL10	Benchmark dose lower confident limit for a 10% increase in the cancer incidence
CI	Confidence Interval
CRC	Colorectal cancer risk
DANSDA	Danish National Survey of Diet and Physical Activity
DAFC	Danish Agriculture and Food Council
DMRI	Danish Meat Research Institute
EFSA	European Food Safety Authority
EPIC	European Prospective Investigation into Cancer and Nutrition
FFQ	Food Frequency Questionnaire
HCA	Heterocyclic amines
HNE	Hydroxynonenal
HR	Hazard Ratio
IARC	International Agency for Research on Cancer
ILCR	Incremental lifetime cancer risk
IQ	2-Amino-3-methylimidazo[4,5-f]quinoline (a HCA)
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KRAS	An oncogene
MeIQ	2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (a HCA)
MeIQx	2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (a HCA)
MOE	Margin of Exposure
NDEA	N-Nitrosodiethylamine
NDMA	N-nitrosodimethylamine
NMTCA	N-nitroso-2-methyl-thiazolidine-4-carboxylic acid
NTCA	N-Nitrosothiazolidine-4-carboxylic acid
NTHZ	N-Nitrosothiazolidine
PAH	Polycyclic aromatic hydrocarbons
PAH4	Benzo[*a*]pyrene, benz[*a*]anthracene, chrysene and benzo[*b*]fluoranthene
PAH8	Benzo[*a*]pyrene, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene
PhIP	2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (a HCA)
RR	Relative Risk
VKM	Vitenskapskomitten for Mattryghet
WCRF	World Cancer Research Fund

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