Aetiological and clinical aspects of symptomatic gallstone disease and pancreatic cancer

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Introduction

This work investigated in a UK prospective cohort study, firstly, the aetiology of gallstone disease, and secondly, that of pancreatic cancer, with a focus on physical activity and diet. The epidemiological studies benefitted from the accuracy of measurement tools, namely a validated physical activity questionnaire and a seven-day food diary (7-DFD). These novel methods aided the improved definition of risk factors thus highlighting biological mechanisms leading to disease and methods of prevention. The third investigation was a clinical survey evaluating benefits for patients of a Pancreatic Support Service (PASS), which screened and treated nutritional and depressive symptoms in patients with pancreatic cancer.

Methods

The European Prospective Investigation into Cancer-Norfolk enrolled 25 639 men and women, aged 45-74 years, between 1993-1997, measuring anthropometrics, lifestyle factors, diet with 7-DFDs, physical activity and collecting serum samples at baseline. The cohort was followed up until 2010, with multi-variate hazard ratios calculated for incident symptomatic gallstones and pancreatic cancer according to risk factors. The clinical survey, compared survival, doses of chemotherapy and clinical parameters in a retrospective group of 16 patients and then in a prospective group of 19 patients who were also reviewed by PASS.

Results

For gallstone disease, positive associations were found for obesity, serum triglycerides, dietary calcium and *trans* fatty acids, with inverse associations for serum HDL, physical activity, alcohol, caffeinated coffee and dietary niacin, cholesterol and iron intake. Pancreatic cancer had inverse associations detected for physical activity, dietary docosahexaenoic acid, dietary vitamin E and selenium, and serum vitamin C. The survey found those reviewed by PASS had fewer and shorter hospital admissions with no effects on survival or doses of chemotherapy.

Conclusion

This work found associations between various dietary factors and physical activity for both symptomatic gallstones and pancreatic cancer. These findings have implications in understanding biological mechanisms and could lead to preventative public health measures for both diseases. The survey reported the introduction of PASS was associated with a reduced number and duration of hospital admissions and the reasons for this should be explored in future work.

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Acknowledgments

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CHAPTER ONE

THE AETIOLOGY OF GALLSTONE DISEASE

Abstract

Obesity, physical activity, alcohol, serum lipids and dietary nutrients in the aetiology of gallstones - a UK prospective cohort study.

INTRODUCTION: Gallstones are a common gastroenterological disease with their formation involving cholesterol saturation, aggregation of crystals and gallbladder stasis. These mechanisms are influenced by obesity, physical activity, alcohol and diet, all of which were evaluated in a UK prospective study using novel methods of assessing exposures. Data from serum lipids was also analysed to clarify the potential mechanisms for how lifestyle factors may affect gallstone formation.

METHODS: A total of 25 639 men and women, aged 45-74 years were recruited between 1993-1997 into the European Prospective Investigation into Cancer-Norfolk. Participants attended a health check at enrolment which recorded their anthropometrics, alcohol intake, serum lipids with a physical activity questionnaire which had been previously validated against detailed physiological measures. A seven-day food diary (7-DFD), the most accurate pragmatic form of measuring diet in large scale epidemiological studies, was completed recording all food eaten, detailing brands and portion sizes. Nutrient intakes were calculated in those diagnosed with gallstone disease and in a random sample of 3 970 controls, using a computer program with information on 55 000 foods. Sex specific hazard ratios (HR) were calculated of developing incident symptomatic gallstone disease after 14 years for body mass index, waist circumference, serum lipids and alcohol intake. To minimise a regression dilution effect, physical activity was analysed after 5 years and nutrient intakes after 10 years follow-up. Analyses were adjusted for age, gender, body mass index, alcohol intake and total energy intake.

RESULTS: In men and women, each unit in body mass index and each inch in waist circumference were associated with an 8% increased risk of gallstones. Increased serum triglycerides were positively associated (men, highest vs lowest quartile HR=2.02, 95% CI=1.03-3.98; women HR=2.43 95% CI=1.52-3.90), with negative associations for physical activity ("active" vs "inactive" category >65% reduction) and HDL (men, highest vs lowest quartile of HDL, HR=0.22, 95% CI=0.09-0.52; women, HR=0.55, 95% CI=0.36-0.85). In men only, increased dietary calcium intake was associated with disease (highest vs lowest quintile of intake, HR=2.31, 95% CI=1.00-5.35), with inverse associations for alcohol (3% reduction per unit/week. 95% CI=1%-5%) and caffeinated coffee (23% reduction per cup/day, 95% CI=5%-38%). In women only, increased dietary *trans* fatty acids were positively associated (HR=1.94, 95% CI=1.06-3.54), with inverse effects dietary cholesterol (highest vs lowest quintile HR=0.59 95% CI=0.35-0.99), iron (highest vs lowest quintile HR=0.35 95% CI=0.19-0.66) and niacin (HR=0.54 95% CI=0.32-0.90).

CONCLUSION: This is the first large European prospective study to investigate gallstones and has confirmed and defined the effects of BMI, waist circumference, physical activity, alcohol and coffee. The use of detailed 7-DFDs has provided novel inverse associations in women for dietary iron, niacin and cholesterol. Positive association with disease were reported for dietary *trans* fatty acids in women and calcium intake and in men with all findings supported by plausible biological mechanisms. If future aetiological work confirms causal associations, then population-based dietary and lifestyle recommendations may help prevent a significant proportion of symptomatic gallstones.

Introduction

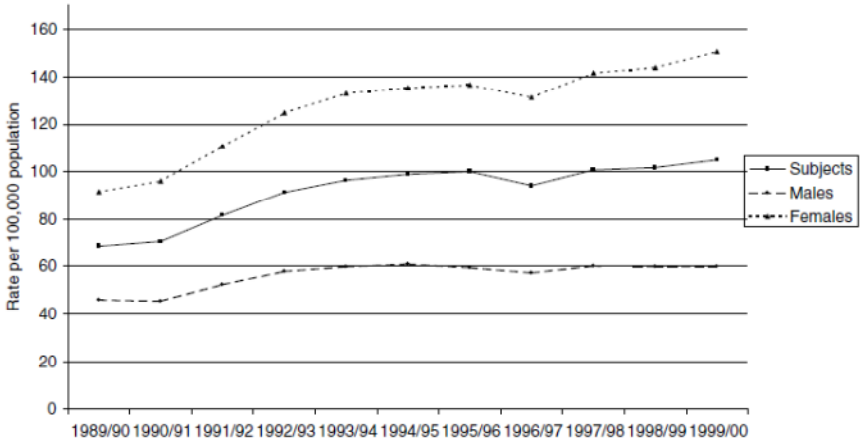
Gallstone disease represents a major health problem worldwide. In the United Kingdom, 49 000 cholecystectomies are performed annually[1] with 700 000 in the United States, where the treatment of such patients costs $6.2 billion.[2] In England, data collected from Hospital Episode Statistics for admissions from the Department of Health between 1989/1990 and 1999/2000, showed the age-standardised annual hospital admission rates for cholelithiasis increased by 30% for males and 64% for females (figure 1).[3]

Asymptomatic gallstones are present in up to 20% of the European adult population[4-6] and of these an estimated 2% develop symptoms each year.[7] The commonest symptoms are abdominal pain but complications such as pancreatitis and cholangitis can be fatal. This chapter reviews the descriptive epidemiology of gallstone disease, biological mechanisms leading to gallstone formation, clinical presentations, and aetiological factors. To identify the relevant literature, searches of Medline (OVID and PubMed) were performed identifying English language articles using terms related to each section in this thesis and the keywords "gallstones" and "gallstones disease". Papers were identified between 1950 and March 2011. The bibliographies of retrieved articles were reviewed to identify additional relevant references.

Definition of gallstone disease

Gallstones are calculi formed in the gallbladder or less commonly in the biliary tree. The term cholelithiasis (derived from the Greek: *chol-*, "bile" + *lith-*, "stone" + *iasis-*, "process") describes the presence of gallstones, whilst cholecystolithiasis describes the presence of stones in the gallbladder and choledocholithiasis is stones in the bile ducts. Gallstones may cause symptoms, and hence gallstone disease, either within the gallbladder, or if they migrate, the biliary tree or small bowel.

Figure 1. The age-standardised hospital admission rates for cholelithiasis per 100,000 population, by sex, in England between 1989/1990 and 1999/2000 (source; Kang *et al, Aliment Pharmacol Ther 2003*[3]).



1. Descriptive Epidemiology

Prevalence

The prevalence of gallstones has been assessed in epidemiological studies using either trans-abdominal ultrasonography (USS) in live subjects and necropsy studies in the deceased. The burden of gallstone disease is similar in the Western countries, with the median prevalence in large population, as detected by trans-abdominal ultrasonography, ranging between 5.9% to 21.9%.[8] In the UK, the prevalence of gallstones in a stratified random sample of 1 896 British adults using ultrasonography was 6.8% in male and 8.0% in females.[9] Although there has been an increase in the age-standardised hospital admission rates for gallstone disease, a study of the prevalence of gallstones at necropsy in England reported between 1998 and 2008, gallstones in men fell slightly from 20.2% to 19.1% (p=0.022), and in women fell from 30.4% to 29.0% (p=0.03), with a gallstone related mortality of 0.7%.[10] Prevalence studies using necropsy should be interpreted with caution, as the population are mostly elderly or have died prematurely and are more likely to have co-morbidities than the general population which could predispose to gallstone disease.

Age

Gallstone disease is rare in children with the frequency of disease rising markedly after the age of 40 years. A cross-sectional survey of 15 910 men and 13 674 women showed that age is a strong risk factor in both sexes for both prevalent asymptomatic gallstones and gallstone disease.[11] Compared to men aged 30-39 years, those aged 60-69 had an odds ratio (OR) for gallstones of 4.48 (95% CI=3.59-5.59) and for gallstone disease OR=5.63 (95% CI=4.65-6.83) and in women, OR 3.07 (95% CI=2.58-3.65) for gallstones and OR=3.95 (95% CI=3.43-4.54) for gallstone disease. Increasing age predisposes to gallstone formation due to declining activity of cholesterol 7α-hydroxylase, the rate limiting enzyme for bile acid synthesis, a process which leads to increased biliary cholesterol saturation.[6] The elderly are also more likely to have lifestyle risk factors, such as decreasing physical activity, which may promote gallstone formation.

Ethnicity

Ethnicity influences gallstone prevalence with the highest rates been described in Pima Indians, who are native to Arizona, in the United States. Female Pima Indians have a prevalence of gallstones of 64.1% and for men it is 29.5%.[12] Similar rates occur in native South Americans, particularly in Chile with rates of

49.4% in women and 12.6% in men.[13] A prevalence study in the United States using ultrasonography in a population over 14 000, found white Americans have prevalence rates of 16.6% in women and 8.6% in men, black Americans have prevalence rates of 13.9% in women and 5.3% in men, and Mexican Americans have rates of 26.7% in women and 8.9% in men.[14] The lowest rates of gallstone prevalence and disease occur in Africa and Asia with necropsy rates of 3%.[6]

2. Clinical presentations of gallstone disease

Gallstones can produce a variety of symptoms and syndromes, dependent on their anatomical site, with approximately 2% becoming symptomatic each year.[7] However, most patients with gallstones remain symptom free, with the risk of symptoms and complications receding 15 to 20 years after developing of prevalent stones. [15-16] In a Swedish population of 739 men and women aged 35-85 years, during the first 5 years after detection of asymptomatic gallstones the cumulative risk of requiring treatment was 7.6%.[17] A Cochrane Review in 2010 concluded that only patients with symptomatic gallstones should undergo surgery as complications of elective cholecystectomy are high, at approximately 17% for surgury.[18] These complications are usually mild, although can occasionally be serious including biliary leakage, peritonitis, fistula formation and the inherent risks of a general anaesthetic.

Biliary colic

Up to three-quarters of patients presenting with gallstone disease experience episodes of severe abdominal pain due to biliary colic. Biliary colic most commonly occurs when a gallstone becomes lodged in the cystic duct, although can rarely occur in the absence of gallstones in patients with gallbladder polyps or cholesterolosis of the gallbladder.[15] Biliary colic typically produces moderate to severe right-upper quadrant pain, around 15 minutes after a meal, although the attacks can also occur at random. The pain can be associated with nausea and vomiting and the symptoms rarely last longer than 3 to 4 hours, which corresponds with the time for the gallstone to pass through the biliary tree or become dislodged from the cystic duct. Prolonged symptoms raise the possibility of a complication of gallstone disease or an alternative diagnosis. Mild inflammatory change of the gallbladder wall can occur with biliary colic, with recurrent episodes leading to chronic cholecystitis.[15] Biliary colic usually responds promptly to analgesics, in particular non-steroidal anti-inflammatory drugs.

Recurrent biliary colic is usually treated by removal of the gallbladder (cholecystectomy), performed by a laparoscopic approach, although open procedures are occasionally required for more complex cases. Non-surgical approaches to treat gallstones are limited in patients unfit for surgery but include oral litholysis (dissolution of gallstones) with ursodeoxycholic acid but 50% of gallstones recur. Extracorporeal shockwave lithotripsy has been abandoned due to the success of laparoscopic surgery.[19]

Acute cholecystitis

Acute cholecystitis is acute inflammation of the gallbladder wall, which in 95% of cases is due to complete obstruction of the cystic duct by a gallstone with the remainder due to acalculous cholecystitis. The resulting inflammation causes oedema, infection, vascular compromise with serious cases complicated by gallbladder empyema (a pus filled gallbladder). Here, gallbladder wall necrosis occurs with perforation and abscess formation and peritonitis. Acute cholecystitis is initially treated with intravenous fluids and antibiotics which leads to a resolution of the acute inflammation in 70-80% of patients.[15] The timing of when to offer laproscopic cholecystectomy in patients with acute cholecystitis remains controversial, with early surgery within 48 hours of onset of symptoms associated with reduced complications and lower conversion rates to open procedures than delayed (>5 days) or interval (>6 weeks) surgery.[15] In those patients with significant co-morbidities, conservative treatment with intravenous antibiotics and fluids is often the preferred treatment whilst monitoring for complications which can be treated with percutaneous cholecystostomy if a gallbladder empyema develops.

Ascending cholangitis

Ascending cholangitis is inflammation of the bile ducts due to bacterial infection, characterised by Charcot's triad of fever, jaundice and right upper abdominal pain. This is caused by obstruction of biliary drainage due to a migrated gallstone occluding the common bile duct. This potentially life-threatening complication requires urgent supportive care, antibiotics and drainage of the biliary tree, usually by endoscopic retrograde cholangiopancreatogram (ERCP) with either removal of the stone or placement of a stent.

Acute biliary pancreatitis

Gallstones are the commonest cause of acute pancreatitis, accounting for approximately 65% of cases,[20] where the stone occludes the pancreatic duct preventing the outflow of pancreatic enzymes. Autodigestion occurs, in which the pancreatic enzymes cause pancreatic damage with both local and systemic inflammatory response. Acute gallstone pancreatitis usually presents with rapid onset upper abdominal pain and vomiting. Gallstone pancreatitis can be a life-threatening disease and requires correction of hypovolaemia, antibiotics and the early consideration of endoscopic sphincterotomy via ERCP in severe cases.[20]

Gallbladder cancer

Cancer of the gallbladder is rare, with around 620 cases diagnosed each year in the UK.[21] Gallbladder cancer usually arises in the setting of chronic inflammation, with most patients (75%) having pre-existing gallstones and cholecystitis. The presence of gallstones increases the risk of gallbladder cancer by 4 to 5 fold.[22] Although gallstone disease is associated with a significantly increased risk of developing gallbladder cancer, the aetiology is likely to be multi-factorial. Other risk factors for gallbladder cancer include inflammatory diseases such as primary sclerosing cholangitis, ulcerative colits and helicobacter infection. Medications (methyldopa, oral contraceptives), chemical exposures (pesticides, vinyl chloride) heavy metals and radiation have also been implicated in disease.[22]

Rare presentations of gallstone disease

Impaction of gallstones in the gallbladder neck (Hartmann's pouch) can lead to compression of the common hepatic duct producing jaundice, which is termed Mirizzi's syndrome. This presentation can be further complicated by fistula formation (cholecystocholedochal fistula) which usually requires surgical repair. Gallstones can rarely erode through the gallbladder wall and into the stomach (cholecystogastric fistula) or small bowel (cholecystoenteric fistula). Gallstone impaction in the stomach or duodenum leads to gastric outlet obstruction, known as Bouveret's syndrome, while stone impaction in the small bowel causes gallstone ileus.

3. The pathogenesis of gallstones

Bile and cholesterol

The formation of gallstones is a complex biochemical process, involving the interaction of bile contents, cholesterol concentration and gallbladder motility. Bile is a complex aqueous colloidal fluid which has several physiological functions, including the excretion of lipids and facilitating intestinal fat absorption. Bile is formed in the hepatic canaliculi (spaces between the tight junctions of hepatocytes) before being transported into the bile ducts. Bile consists of water, electrolytes and lipid solutes dispersed in mixed micelles (aggregates of surfactants in a colloid) and vesicles which can emulsify other fats. The lipid solutes consist of bile salts, phospholipids (96% phospatidylcholines), cholesterol, proteins and bilirubin conjugates. Phospholipids and bile salts are essential for removal of insoluble cholesterol molecules.[23]

In health, half of the secreted bile is stored, concentrated and slightly acidified in the gallbladder in between meals. The gallbladder mucosa concentrates the bile by active absorption of water and electrolytes in exchange for hydrogen and bicarbonate which acidify the bile. The mucosa also secretes proteins and mucus glycoproteins which influence the composition of bile and play a role in gallstone pathogenesis. In the gallbladder water is reabsorbed leading to increased cholesterol saturation and explains why most stones form in the gallbladder rather than the biliary tree.[24] Bile remains in the gallbladder for several hours until it is excreted into the intestine. Several hormones influence gallbladder function, the primary hormone being cholecystokinin (CKK) as well as secretin, gastrin and pancreatic polypeptide.[25] Vagotomy and inflammation disrupt neural input into the gallbladder which promotes gallbladder hypotonia and biliary stasis leading to gallstone formation.

Cholesterol is a sterol, also classified as a steroid alcohol, which is a subgroup of steroids. The overall molecule is flat with a polar hydroxyl group at the 3-position of the A-ring. Cholesterol is synthesized from one molecule of acetyl CoA (also known as acetyl-coenzyme A) and one molecule of acetoacetyl-CoA via the mevalonate pathway, which includes the enzyme 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMG-CoA). Cholesterol is solubilised in bile within the micelles, and in particular by bile salts and phospholipids (i.e. phosphatidylcholine), with their concentration determining the degree of cholesterol saturation within the bile.[19]

Bile salts

Bile salts, also termed bile acids, are one constituent of bile, and are anionic detergents synthesized from cholesterol in the liver and represent a major pathway for the excretion of cholesterol and other waste products. Bile salts serve several other biological functions including the emulsification of lipids and activation of digestive lipases. They are largely (95%-99%) reabsorbed in the distal small bowel, taken up by the liver, and re-secreted into the bile, a process known as enterohepatic circulation.[26]

The rate-determining enzyme of bile salt formation is cholesterol-7α-hydroxylase (CYP7A1), which is highly regulated. It is a member of the cytochrome P450 superfamily, which is a large and diverse group of enzymes. CYP7A1 catalyzes the formation of 7-alpha-hydroxycholesterol from cholesterol, with low activity of CYP7A1 causing increased cholesterol secretion and decreased bile salt excretion in humans which predisposes to gallstone formation.[27] CYP7A1 is down-regulated by Sterol Regulatory Element Binding Proteins (SREBP) when plasma cholesterol levels are low and is up-regulated by the nuclear receptor LXR (liver X receptor) when cholesterol (specifically oxysterol) levels are high.[28] Gene expression of CYP7A1 is strongly repressed by insulin, and results in low levels of CYP7A1 expression in the human liver and increases the risk of gallstone disease.[29]

The primary bile salts, cholate and chenodeoxycholate, are hydrophilic bile salts which solubilise cholesterol in the bile and prevent gallstone formation. The pharmacological use of hydrophilic bile salts such as ursodeoxycholate can dissolve stones and prevent cholesterol crystallization. However, secondary bile salts (i.e. deoxycholate) formed from the deconjugation of primary bile salts in the intestine by bacterial CYP7A1 activity are hydrophobic and strongly promote cholesterol crystallization. Humans have the most hydrophobic bile salt composition of all animals.[30] Some gallstone patients have more bacteria with CYP7A1 activity. Administering antibiotics and suppressing these bacteria reduces biliary deoxycholate concentration and normalises biliary cholesterol saturation which prevents of the formation of gallstones.[31] Prolonged small and large bowel transit times promote absorption of deoxycholate into the enteropathic circulation. Slow intestinal transit increases secondary biliary acid formation (deoxycholic acid) which leads to increased gallstone disease.[32]

Gallstone formation and genetics

Gallstones are formed from the precipitation of bile and a mixture of particulate matter within the gallbladder. Gallstones are broadly categorised into four groups; cholesterol gallstones; mixed type, brown pigment stones and black pigment stones with a considerable overlap existing between these groups.[33] In western societies, 80-90% gallstones are cholesterol gallstones with the remainder being brown or black pigment stones.[19, 34-35] Cholesterol gallstones are composed mainly of cholesterol crystals (70%) held together by a matrix of glycoproteins, calcium salts and bile pigments.[19]

Genetic factors are involved in the development of gallstones. Gallstone susceptibility is a "complex trait" with genetic factors estimated to contribute to 25% of the risk of disease.[36] Studies in both human and mouse models have identified multiple cholesterol gallstone susceptibility genes (*Lith* genes) and contributed to the understanding of the pathophysiological mechanisms of cholesterol gallstone formation.[37] The genetic predisposition to gallstones can either arises from a monogenic defect though more commonly polymorphisms in multiple genes, with each one contributing to the risk of subsequent disease. The mechanisms involve either gene-gene interactions, or interactions with the environment including diet, obesity, drugs and pregnancy. This has lead to a new view that hepatic hypersecretion of biliary cholesterol could be induced by multiple *Lith* genes and insulin resistance in the metabolic syndrome, which interact with environmental factors to produce the disease phenotype.[37]

Inbred mice have been used to identify over 80 cholesterol gallstone susceptibility (*Lith*) genes using the technique of quantitative trait locus (QTL) analysis.[37] These genes exert their effects either in the liver, gallbladder or small intestine. An example of the gene effects include altered hormone receptors function (i.e. oestrogen receptor, cholecystokinin), lipid membrane transporters, lipid regulatory enzymes and altered mucin production.[37] By evaluating the effects of *lith* genes, the pathogenic model of cholesterol gallstone formation suggests the primary factor is the hepatic hypersecretion of cholesterol into the bile which may be accompanied by further alterations in the hepatic secretion of bile salts or phospholipids leading to cholesterol supersaturation of the bile. Alterations in the proportions of constituents in bile can lead to "phase separation" of cholesterol from solution in bile. All the following changes in bile composition promote cholesterol crystallization; 1) increased cholesterol concentration; 2) increased bile salt hydrophobicity; 3) increased phospholipids containing unsaturated acyl chains. Preventative factors are 1) dilute cholesterol saturation; 2) hydrophilic bile salts; 3)

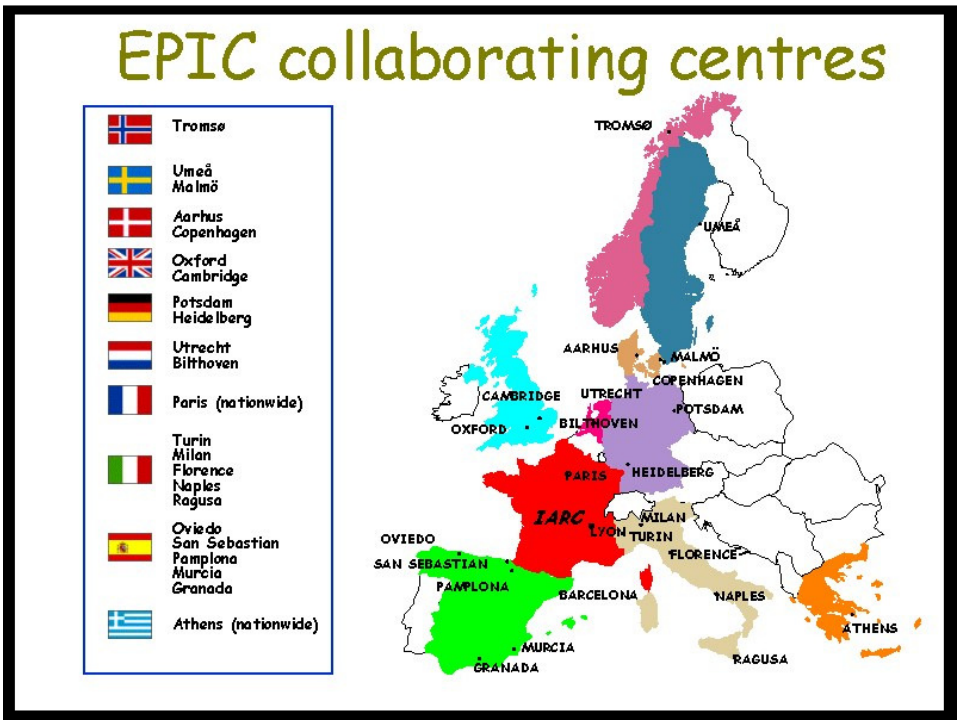
saturated phospholid acyl chains.[30] With phase separation there is formation of unilamellar vesicles which aggregate to form multilamellar vesicles and eventually micro-crystals. These micro-crystals aggregate within the gallbladder where cholesterol monohydrate crystals are able to nucleate in the mucin gel glycoprotein scaffolding enabling stone formation.[23] Excess mucin secretion, which aids accelerated phase transitions of cholesterol and dysfunctional gallbladder motility both promote gallstone formation.[38] Occasionally monogenic defects lead to gallstone disease, such defects of ABCB11 (adenosine triposphate-binding cassette transporter B11) which controls bile salt export and underlies benign recurrent intrahepatic cholestasis (BRIC), 65% of BRIC patients developing gallstones.[39]

4. The study cohort population

EPIC

The baseline study population used to investigate the aetiology of gallstone disease and pancreatic cancer was the European Prospective Investigation into Cancer and Nutrition – Norfolk (EPIC-Norfolk). EPIC was conceived in the 1980's to principally define more clearly the relationship between nutrition and the aetiology of common cancers and chronic diseases. EPIC-Norfolk is part of a wider prospective cohort investigation in 10 European countries collecting data on diet, lifestyle and environmental factors in approximately 520 000 middle-aged European adults.[40] These subjects are being followed up to investigate the incidence of illnesses in relation to both epidemiological data and biochemical markers recorded at baseline. EPIC-Europe recruited participants at 26 centres in the European countries of Denmark, France, Germany, Greece, Italy, The Netherlands, Norway, Spain, Sweden and the United Kingdom (Figure 2). In the UK there are two sub-cohorts, one co-ordinated from Oxford of a nationwide population of around 57 500 men and women over the age of 35 years, with a high proportion of vegetarians. The second sub-cohort, EPIC-Norfolk, is co-ordinated from Cambridge, with a population of 30 447 men and women living in Norfolk, East Anglia.

Figure 2. Countries collaborating in the European Prospective Investigation into Cancer and Nutrition (EPIC) (source; EPIC-Norfolk website).

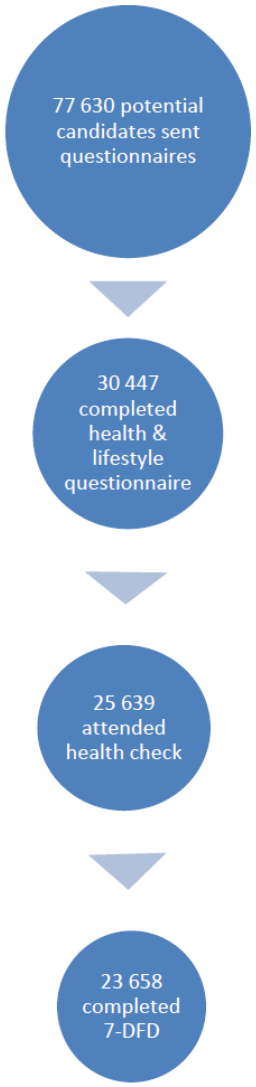


EPIC-Norfolk

EPIC-Norfolk was designed with the intention of recruiting a cohort of approximately 25 000 men and women from the general population, aged 45-74 years. Norfolk is a geographically distinct area with little outward population migration and served by three hospitals. The stability of the Norfolk population was an advantage as this would facilitate more complete case ascertainment of future disease end-points. The initial estimate of the cohort size was chosen as a balance, to firstly generate a sufficient number of clinical end-points and secondly to aid the practicality of using accurate methods for measuring exposures, including biological assays.[41] The city of Norwich and the surrounding area were chosen as this population was derived from a mix of city, suburban and rural lifestyles (Figure 3). In total, 77 630 individuals were identified between 1993 and 1997 who were registered with 35 general practices and were sent invitations to participate. Of these, 30 447 (39.2%) gave signed consent for participation and completed a baseline health and lifestyle questionnaire. All those who returned the health and lifestyle questionnaire were sent an appointment to attend a health check, to which 25 639 (84.2%) participants attended with most completing a seven-day food diary (Figure 4). The Norwich District Health Authority Ethics Committee approved the study and all volunteers gave signed consent for their medical notes to be reviewed if they developed illnesses.

Figure 3. Location of study population EPIC-Norfolk.

Figure 4. The number of participants involved in different phases of recruitment for EPIC-Norfolk and the data collection methods used.



General practitioners identified 77 630 potential candidates who were sent invitations to participate in EPIC-Norfolk.

30 447 gave signed consent and completed a 24-hour dietary recall and the health and lifestyle questionnaire.

25 659 participants attended the 1st health check which recorded anthropometrics and took blood and urine samples.

23 658 completed the 7-day food diary.

5. Measurement of lifestyle and diet

At recruitment the following characteristics and exposures were recorded;

i. Basic demography
ii. Anthropometrics
iii. Physical activity
iv. Alcohol consumption
v. Dietary intake
vi. Blood and urine analysis

Each of these is now described in greater detail.

Basic demography

The health and lifestyle questionnaire recorded information on age, gender, social class as determined by occupation,[42] family history of illness, previous medical history, medication, parity, alcohol, consumption, cigarette smoking and physical activity. Smoking status was classed as "never smoker", "previous smoker" or "current smoker". Type 2 diabetes status, was classified either as present or absent at baseline.

Anthropometry

At the baseline health check, a nurse recorded anthropometric assessments including height (nearest millimetre without shoes using a free-standing stadiometer) and weight (nearest 0.2kg without shoes in light clothing using digital scales) from which body mass index (BMI, kg/m^2) was calculated. Hip and chest circumferences were measured using a D-loop non-stretch fibreglass tape (to the nearest millimetre) [41].

Physical activity

Physical activity was assessed within the health and lifestyle questionnaire sent to participants at the initial enrolment. The questionnaire recorded participants' physical activity at work (one of four categories), home and during recreation (including the intensity and duration) and finally the number of flights of

stairs they climbed per day (appendix 1). The EPIC physical activity questionnaire had been previously validated by comparing it to a 3-day activity diary.[43] However, using a validating measure of the same fundamental type as the one to be validated increases the risk of correlated error.[44] Therefore, a second validation study of the EPIC physical activity questionnaire used objectively measured energy expenditure as the validating measure.[45] In this work, conducted over one year, 173 volunteers completed four separate assessments of cardiorespiratory fitness (as measured by sub-maximal oxygen consumption whilst cycling) and secondly 4-day energy expenditure (as determined by heart rate monitoring). Concurrently, participants completed the EPIC physical activity questionnaire which recorded their activities over the past year. In the analysis, the only questions on physical activity that correlated with energy expenditure related to occupational activity (p for trend <0.001) and certain recreational activities (a combination of cycling and other physical activity i.e. keep fit, aerobics, swimming and jogging).[45] Questions on low-intensity activities (i.e. gardening, walking and housework) and stair climbing did not significantly correlate with energy expenditure and hence were not used in the derivation of a four-level physical activity index, which combined physical activity at work with the time taken performing recreational activity. These four categories were "inactive", "moderately inactive", "moderately active" and "active" (Table 1). Within each individual category, the summations of different amounts of occupational and recreational physical activity levels were similar. There were positive associations between the 4-level physical activity index, derived from the physical activity questionnaire and the measures of the ratio of daytime energy expenditure to resting metabolic rate ($p=0.003$) and cardiorespiratory fitness ($p=0.001$). The repeatability of the questionnaire was high (weighted kappa=0.6, $p<0.001$) when evaluated in 2 271 participants who completed the questionnaire on two occasions, 18-21 months apart [45]. Therefore, the relevant information on physical activity was extracted from the baseline questionnaire.

Table 1. The 4-level physical activity index (Source; Wareham *et al, Public Health Nutr 2003*[45]).

Category of Physical Activity	**Description of activity**
Inactive	Sedentary job and no recreational activity.
Moderately Inactive	Sedentary job with <0.5h recreational activity per day or Standing job with no recreational activity.
Moderately Active	Sedentary job with 0.5 to 1.0 hr recreational activity per day or Standing job with 0.5 hr recreational activity per day or Physical job with no recreational activity.
Active	Sedentary job with > 1.0 hr recreational activity per day or Standing job with >0.5 hr recreational activity per day or Physical job with at least some recreational activity or Heavy Manual Job.

Dietary assessments

EPIC-Norfolk was unique amongst EPIC centres, as 7-day food diaries were used to assess dietary intake in its participants. However, similar to other EPIC centres, a food frequency questionnaire (FFQ) and a 24-hour recall record were also completed. The 7-day food diary (7-DFD) allowed finer between-individual discrimination with validation studies demonstrating improved correlations with dietary intakes compared to FFQs.[46-47] This reduces regression dilution that may otherwise prevent the detection of true associations between diet and disease. Therefore, EPIC-Norfolk has unique advantage compared to other large prospective cohort studies by virtue of the use of 7-day food diaries.

A total of 23 658 participants completed seven-day food diaries (7-DFDs) in the week after the baseline health check (response rate of 92.2%). A nurse explained how to complete the diary, the first day of which was filled in with the nurse, as a 24 hour recall of their previous day's dietary intake. The remaining six days were completed by the participants themselves at home. They recorded their entire dietary intake, including portion sizes, brands and cooking methods in eight separate meal times daily. The names of commercially prepared foods or packaging from products were included in the diary to allow more accurate nutritional assessments. Homemade foods were described in detail using recipes supplied by the participants. Portion sizes were estimated by either weighing the food or comparing each item with photographs supplied of different foods of varying quantities. At the end of the week's record, supplementary questions were asked on important contributions to nutrient intake, including cooking oils and milk consumption.

After completion, the diaries were returned to the research centre where they were interpreted and coded by trained nutritionists with the data inputted into a specially designed computer programme called DINER (Data Into Nutrients for Epidemiological Research). Each data enterer worked according to an extensive data entry reference manual (DINER_derm) to ensure consistency between coders. Each item in the food record is entered by making references to a series of windows of nutritional options to produce the final line of data. A food item, portion size and number of portions are entered for all reported foods (Figure 5). The program was designed to ask for the information necessary for each specific food list item, such as fat used in cooking or brand name. Food diaries were entered according to each individual meal slot. Reasons for missing meals were

documented, and factors affecting usual intake such as illness or special diet were recorded. Every entry in the diary was allocated to one of both 11 000 food items and 55 000 portion sizes within DINER, by selecting the food item which best described it. Where a description of the food was lacking, the item was assigned the average composition for that food type. DINER facilitated the translation from participant reported free text of food into lines of structured data (Figure 5) which was then converted into nutrient values or food groups.[48] Each line of data in each food diary was converted into a weight of that food and then the nutrient database in DINER calculated the nutrients contained in the weight of food. Intake for each day was summated to give the total intake over 7 days and then each nutrient was then divided by 7 to provide the average daily intake. The nutrient database in DINER is based on foods in the United Kingdom Food Composition Database, the nutrient database of the Royal Society of Chemistry and from food manufacturer's databases. Each 7-DFD took a nutritionist approximately 4 hours to code with an average of 220 food and drink items reported in each diary. An example of the accuracy of their nutrient assessment was that 337 specific types and brands of breakfast cereals were included in DINER. The computer program was checked for potential errors in the coded diaries such as unexpectedly large portion sizes or duplication of entries. If any anomalies were detected they were further assessed by the nutritionist. As most, but not all, of the returned 23 658 diaries are coded in EPIC-Norfolk, a random sample of 3 970 (16.8%) have been coded to use in the gallstone study in a case-cohort analysis. The size and nature of this subset of the 3 970 subjects was derived by the EPIC-Norfolk co-ordinating centre and was not specific to this study. It was considered to be a large enough sample to allow statistically significant comparisons to be a calculated against relatively common diseases. The subset was designed to have similar characteristics and demographics as the whole EPIC-Norfolk population. Although not used in this study, repeated 7-DFDs were completed by 21 000 participants 18 months after enrolment, 16 000 participants at 3 years and 10 000 participants at 13 years. These food diaries will be coded by nutritionists in the future.

Figure 5. An example of data entry using DINER

A participant has recorded eating a homemade apple pie, size coded as 3B.

The data coder enters apple pie into DINER using a drop down menu which details the type of pastry used.

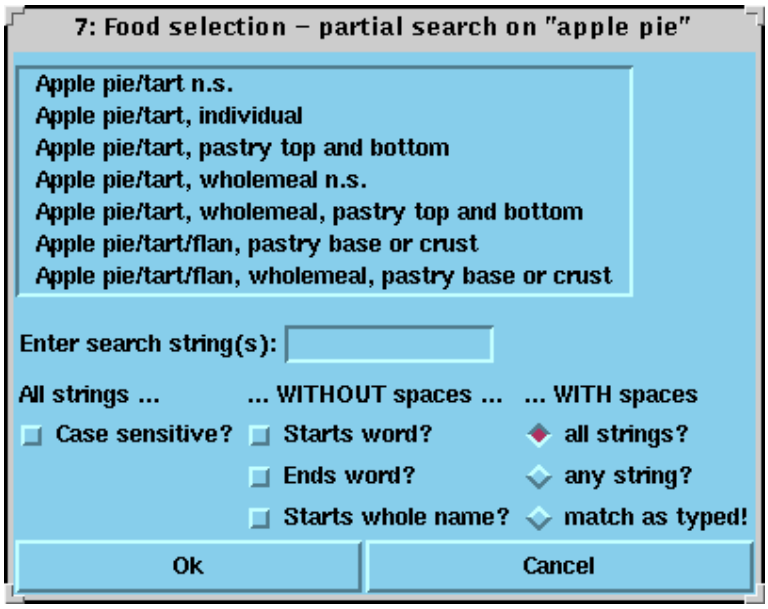


Figure 5 continued. The portion size can be selected from a large number of options. In this example participant has used picture references to code the portion size as "3b".

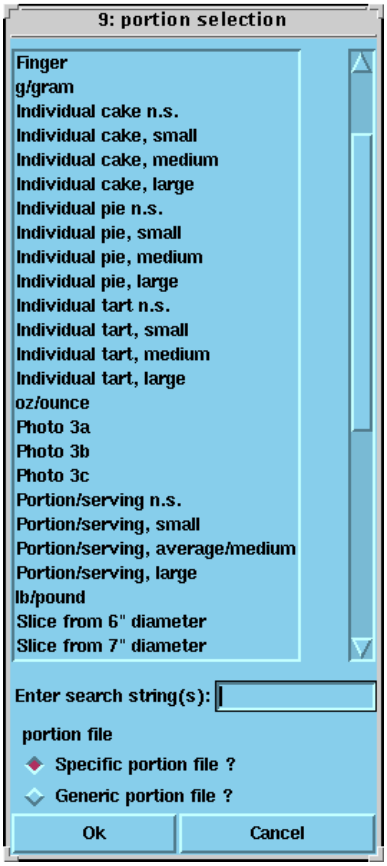
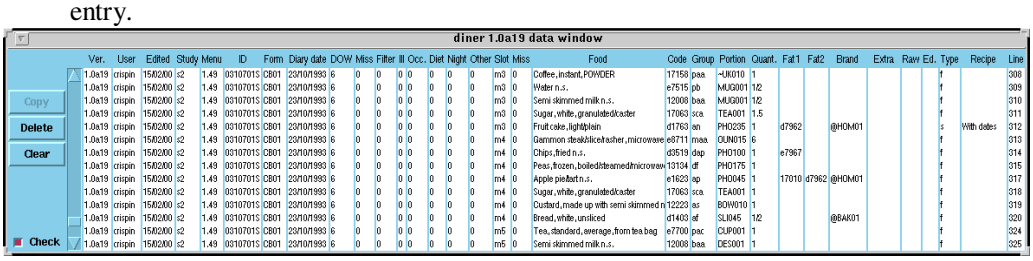


Figure 5 continued. An example of the lines of data produced with each diary entry.



Each food item in the 7-DFD is represented by one line of data in the final structure. A sandwich is entered as bread, spread and filling, a cup of tea is entered as black tea and milk (and sugar where taken). After a coder has made the initial entry for a food diary, they run a checking program. The program checks that the correct number of days and meal slots have been entered (or noted as empty) and identifies potentially unreasonable amounts of foods and that the right portion types have been used for different food items (source; EPIC-Norfolk website).

Supplement use

The use of vitamin and mineral supplements was also recorded in the 7-DFD by participants. A label-based database was developed containing information on 2 066 supplements, with 16 586 ingredients. This vitamin and mineral supplements (ViMiS) database contained manufacturers' information to allow calculation of each micronutrient intake.[49] To simplify the analysis, a binary variable of "user" or "non-user" for each vitamin or mineral was classed by whether the dose exceeded 5% of food-sourced intake as defined by the cohorts completed 7-DFD's. For example, the average daily intake of vitamin C from the diet was 89mg, with 5% of daily intake 4.45mg; therefore a participant supplementing with 60mg of vitamin C a week (average 8.6mg/day) would be classified a vitamin C supplement user whereas a supplement of 30mg vitamin C a week would be recorded as a non-user.[49]

Alcohol consumption

Alcohol intake was assessed from the food frequency questionnaire (FFQ) as data was collected all the participants who completed the baseline questionnaire and average alcohol intake is the only dietary variable that is recalled with greater accuracy using FFQs than 7-DFD [50-51]. The FFQ recorded how many drinks were consumed each week of: i) beer, cider or lager, ii) wine, iii) sherry or fortified wine and iv) spirits and from this alcohol was estimated in UK units. An example of the estimates used are that an average pint of beer will have 2.2 units of alcohol and one glass of small wine has 1.5 units (1 UK unit = 7.9 grams or 10 millilitres).

Blood and urine samples

At baseline, non-fasting blood samples were taken and immediately transported to the laboratory to measure full blood count, serum triglycerides, total cholesterol, HDL cholesterol, LDL cholesterol, glycosylated haemoglobin A1c (HbA1c), Thyroid-Stimulating Hormone (TSH), free Thyroxine (T4) and serum vitamin C. The remainder of the serum was stored for potential analyses in the future. Urinalysis was performed using Multistix 8SG testing for blood, leucocytes, nitrite, specific gravity, glucose, protein, ketone, and pH with a specimen kept for storage.

6. Risk factors for gallstone disease

The rise in the prevalence of gallstone disease in the last century suggests life-style factors are of paramount importance in the aetiology of gallstones. The potential risk factors that are involved will be discussed in this chapter.

Gender and female sex hormones

Gallstone disease is commoner in women who are twice as likely to form gallstones.[8] However, the prevalence in males rises towards that in females with increasing age so that the ratio changes from 1:2 in subjects under 50 years, to 1:1.2 in those over 70 years.[10] The mechanism for the increased risk of gallstone disease in women is probably related to female sex hormones as parity, oral contraceptive use and hormone replacement therapy (HRT) all increase the risk of gallstone disease.[52-53] There are biological mechanisms to explain these associations with Lith gene studies demonstrating that oestrogen enhances cholesterol cholelithogenesis by augmenting functions of the hepatic estrogen receptor-α (ER-α). In the liver, the ER-α receptor stimulates the SREBP-2 (sterol regulatory element binding proteins pathway) promoting cholesterol biosynthesis and hepatic secretion of biliary cholesterol.[54] Furthermore, progesterone and oestrogen receptors have been identified in human gallbladder tissue with 3 months of oestrogen therapy increasing residual gallbladder volumes and reduce gallbladder emptying both of which promote stone formation.[55]

There is robust aetiological epidemiological data confirming female sex hormones are a risk factor for gallstone disease. Compared to being nulliparous, each additional pregnancy increases the risk of gallstone disease by approximately 10%,[11] whilst HRT use for greater than 1 year has been associated with a 4x greater risk of gallstone disease (OR = 4.05, 95% CI = 1.12-14.76).[52] Randomised placebo controlled trials of oestrogen use designed initially to assess the secondary prevention of coronary heart disease in post-menopausal women, showed that oestrogen supplementation increased the risk of gallstone disease with results of OR=1.38 (95% CI=1.00-1.92)[56] and OR=1.59 (95% CI=1.28-1.97) [53]. The consistency of the experimental, epidemiological and trial evidence implies oestrogen is a causal risk factor for gallstone disease.

Underlying chronic diseases and medical conditions

Liver Cirrhosis

Liver cirrhosis is a well-established risk factor for gallstone disease with the prevalence of gallstone disease 25-30% higher than the general population.[57] Most stones in cirrhotic patients are composed of black pigment type the mechanism of formation related to altered pigment secretion, abnormal gallbladder motility and increased oestrogen levels.[8] There may be confounding with non-alcoholic fatty liver disease which is associated with obesity and dyslipidaemia. As cirrhosis is uncommon, it only contributes to a small proportion of all gallstone disease.

Terminal ileal disease and Crohn's disease

Terminal ileal disease, usually caused by small bowel Crohn's disease, leads to a 2 to 3 fold increased risk of mostly pigment gallstones.[58] Here normal reabsorption of bile salts in the terminal ileum is prevented, allowing bile salts to enter the colon where they solubilise unconjugated bilirubin, which is passively absorped in the colon, reconjugated, and resecreted into bile. This process leads to excess bilirubin secretion and predisposes to black pigment gallstone formation.[59] Again, as Crohn's disease is uncommon, it has little effect on the total burden of disease, although the increased risk of gallstone disease is an important consideration when evaluating a patient with Crohn's disease and abdominal symptoms.

Rapid weight loss

Weight loss exceeding 1.5kg per week from dieting predisposes to gallstone formation.[60-62] Patients undergoing bariatric surgery are particularly prone to gallstone formation (prevalence pre-operatively of 21.6% vs 1 year post-operative incidence of 52.8%) which is believed to be induced by a lack of gallbladder stimulation and hence biliary stasis. Decreased calorie intake and rapid bowel transit alter gut hormone secretion, with reduced cholecystokinin release, leading to gallbladder hypomotility.[63] Prophylactic cholecystectomy at the time of bariatric surgery is sometimes considered or the routine use of ursodeoxycholic acid post-operatively.[64]

Obesity

Increasing obesity is a risk factor for gallstone disease although the mechanism is unknown and data from European studies is minimal. The World Health Organisation defines obesity as "abnormal or excessive fat accumulation that may impair health".[65] Body mass index (BMI; expressed in kg/m2) is often used as a measure of obesity and gives an estimate of relative weight for height. The World Health Organisation categories BMI as follows; BMI 20-25 kg/m^2 "normal", 25-<30 kg/m^2 "overweight", 30-<35 kg/m^2 "obese class I", 35-<40 kg/m^2 "obese class II", >40 kg/m^2 "obese class III".[65] However, BMI is not an accurate measure of total body fat or the distribution of body fat.[66] Waist circumference gives a better estimation of central adiposity, particularly in advancing age when there is attrition of muscle volume[67] and a tendency for fat to accumulate intra-abdominally.[68] Abdominal obesity is associated with hyperinsulinaemia and insulin resistance which leads to dyslipidaemia, namely elevated serum triglycerides and reduced HDL.[69-72] These lipid alterations raise biliary cholesterol saturation[73-74] and increase mucin production, both of which enhance the aggregation of cholesterol into microcrystals.[75] Obesity and hyperinsulinaemia also contribute to gallstone formation by causing gallbladder hypomotility which promotes cholesterol crystal aggregation.[76-78]

Epidemiological studies have confirmed the association between obesity and gallstones. Cohort studies have methodological advantages over case-control studies, in that the anthropometry recorded is prior to the development of disease and more likely to be related to aetiology. In case-control work patients may have difficulty accurately recalling their pre-symptomatic weight and use of their current weight may have altered due to disease. Large prospective cohort studies in the US have found BMI, waist circumference or waist-hip-ratio increase the risk of symptomatic gallstones in both genders,[79-80] with smaller European prospective studies confirming the association in both incident asymptomatic and symptomatic gallstones.[81-82] Further work is needed to clarify the association in a large European study and to assess biological mechanism.

Type 2 Diabetes

Type 2 diabetes is positively associated with gallstone disease although the association is complex as it may be confounded by obesity, dyslipidaemia, and a family history of gallstones. Type 2 diabetes is characterised by insulin resistance

which increases the risk of gallstone formation.[83-84] Supportive epidemiological data includes a large case control study where men with diabetes had OR=1.54 (95% CI=1.24-1.91) and women OR=1.92 (95% CI=1.60-2.31).[11] The same population was then followed up prospectively for 10 years to identify those developing incident gallstone disease, and failed to show that diabetes was an independent risk factor for gallstones in women (men OR=2.72, 95% CI 0.89-8.33, women OR=1.00 95% CI 0.22-4.49).[81] Further studies that have adjusted for the presence of obesity also did not find diabetes an independent risk factor for gallstone disease.[85-87] Therefore, it appears that diabetes is unlikely to be an independent risk factor for gallstone disease though is related to anthropometric risk factors.

Dyslipidaemia

Although most gallstones consist of cholesterol, no definite association has been shown between serum hypercholesterolaemia and gallstone disease.[85, 88-89] However, there is evidence that the dyslipidaemia associated with obesity, diabetes and the metabolic syndrome, i.e. low serum high-density lipoprotein-cholesterol (HDL) and raised serum triglycerides increase risk.[88-89] Patients with hypertriglyceridaemia are known to have both cholesterol supersaturation of the bile and decreased gallbladder motility with an increased risk of gallstone formation.[90] Increased serum HDL may be important in preventing stone formation. HDL plays a critical role in the reverse cholesterol transport by removing cholesterol from the peripheral tissues and delivering it to hepatocytes for excretion into the bile. HDL also accepts a significant amount of excess cholesterol from the liver and plays an important role maintaining cellular cholesterol homeostasis.[91] In rodents and humans, cholesterol from plasma HDL is a key source of cholesterol for biliary secretion either as unesterified cholesterol or after transformation into primary bile acids.[92] In human studies, using radiolabelled cholesterol, HDL cholesterol rather than LDL cholesterol represents the main source of biliary lipid cholesterol secretion[93] with a much larger fraction of cholesterol carried by HDL secreted in the form of bile acids compared to that of LDL.[94] These findings may explain why raised serum HDL has an inverse relationship with biliary cholesterol saturation[74] and gallstone disease although the exact pathophysiological mechanisms are not defined. Mouse models deficient of the binding proteins apoA-I (apolipoprotein A-I) or ABCA1 (ATP-binding cassette

transporter A1), which control HDL levels do not change biliary cholesterol secretion.[95-96] However, a particular polymorphism (TaqBI) of cholesteryl transfer protein (CETP) which transfers HDL to non-HDL lipoproteins for further uptake by the LDL pathway in the liver, lowers plasma HDL levels and is associated with gallstone disease.[97-98] Raised triglycerides could influence gallstone formation by increasing biliary cholesterol saturation[73] and bile viscosity via enhanced mucin production promoting aggregation of cholesterol into microcrystals, the precursor of stones.[75]

The biological hypotheses described above need to be supported by epidemiological work. These would need to show that serum lipids measured before the development of disease influence the risk of gallstones. Cohort studies are the preferred methodology for studying serum markers of disease as they are measured before diagnosis, while case-control studies could lead to bias if lipid levels are altered by the disease itself. There are two previous prospective cohort studies which investigated serum lipids and their association with gallstone disease. A US study found that increased serum HDL and decreased triglycerides were associated with a decreased risk of gallstones in men and women.[88] An Italian prospective study (MICOL) evaluated the presence of gallstones using ultrasonography at enrolment and again 10 years later and found that men, but not women, had a negative association with HDL and total cholesterol and a positive association with triglycerides. These prospective studies suggest that serum lipids are predictive of the risk of developing gallstone disease, although there are inconsistencies particularly in females, and whether total cholesterol is associated. Lipid profile data from case-control[99-101] and cross-sectional work[11, 89, 102-103] also showed inconsistent associations and have less validity as it is unknown if alterations in the lipid profile precede the development of disease. A consistent effect of decreased HDL and increased triglycerides leading to gallstone disease would suggest that obesity and the metabolic syndrome may in part lead to gallstone disease by inducing this pattern of dyslipidaemia. More data is required from European populations to help clarify the inconsistencies. Data from the same population measuring both lipid biomarkers and anthropometry is needed.

Physical activity

There are several plausible biological mechanisms for how physical activity may prevent gallstone formation. Exercise reduces plasma triglycerides[104]

and insulin levels[105], both of which lead to a lower cholesterol saturation of the bile[74, 106]. Triglycerides also increase bile viscosity by stimulating mucin secretion from gallbladder mucosal cells which promotes the aggregation of cholesterol into microcrystals[75]. Regular exercise raises High-Density Lipoprotein–Cholesterol (HDL-C) levels[107-108] which are inversely associated with gallstone prevalence.[89] HDL-C is a precursor of bile acids[94] which reduce its lithogenicity. Exercise, by increasing cholecystokinin levels, has a prokinetic effect on the gut[109] which stimulates gallbladder contractility and prevents bile stasis.[110] To support the experimental data for a protective effect of exercise, large population based epidemiological studies are required showing that those who exercise are at a lower risk of developing gallstones. Both case-control and prospective cohort studies can be used to investigate this potential association. However, prospective investigations provide more accurate information on pre-symptomatic physical activity, as this is measured before the development of disease and consequently is not subject to the recall biases inherent in case-control studies. The published cohort studies have reported that higher levels of physical activity reduced the risk of symptomatic gallstones by approximately a third.[111-115] However, a limitation of all these investigations was that the method for measuring physical activity, namely questionnaires had not been validated against detailed physiological measures of physical activity, including energy expenditure and cardiorespiratory fitness. Similar data on exercise is also needed in European populations to clarify the association and define the magnitude of effect. To address these limitations, physical activity questionnaires assessed against energy expenditure (repeated 4-day heart monitoring) and cardiorespiratory fitness (repeated measures of sub-maximal oxygen uptake)[45] are important since they allow the accurate categorisation of physical activity levels. Demonstrating an association of physical activity with gallstones should lead to it being accurately measured and included in aetiological models of gallstone formation. It would also be important as encouraging increased physical activity levels may help to reduce the numbers developing symptomatic gallstones.

Alcohol

Alcohol may prevent the development of gallstones by several biological mechanisms. Alcohol stimulates cholecystokinin release[116] and therefore gut motility[117] which prevents biliary stasis and cholesterol crystal aggregation.[32]

Alcohol intake also increases HDL levels[118-120] by reducing cholesteryl ester transfer protein (CETP) activity which prevents the conversion of HDL into LDL.[121] HDL is then metabolised to primary bile acids which help to solubilise biliary cholesterol. Alcohol may also increase lecithin cholesterol acyl transferase (LCAT) activity (an enzyme that converts free cholesterol into cholesteryl ester), which further increases HDL.[122]

The role of alcohol is best investigated by prospective cohort investigations. Cohort studies which record alcohol intake before the development of symptoms have methodological advantages over case-control work and avoid protopathic bias which occurs if alcohol intake is reduced following the development of symptoms.[123] Prospective cohort studies of health professional from the United States reported inverse associations between alcohol intake and gallstones in both men [124] and women.[125] A further prospective US study of 12 773 people reported an inverse association in women, although none in men.[88] Smaller case-control studies using ultrasonography to assess outcome for prevalent silent stones also found an inverse association with alcohol.[126-127] However, prospective data in a European population is required investigating alcohol in this population, describe the effect size, and assess if the effects are in both males and females. Alcohol intake is more reliably recalled than other dietary intakes, and as such, it is one of the few dietary factors that is evaluated with greater accuracy using a food frequency questionnaire, rather than a food diary.[47] Hence, in EPIC-Norfolk, alcohol intake is the only dietary value derived from the FFQ rather than the 7-DFD.

Drug and medical treatments

Statins

Statins inhibit 3-hydroxy 3 methylglutaryl (HMG) coenzyme A (CoA) reductase which diminishes cholesterol synthesis in the liver, resulting in reduced biliary cholesterol saturation in humans.[128] Statins also increase plasma HDL levels and decrease plasma triglycerides[129] which are associated with a decreased risk of gallstone disease. Data on statin use may be obtained from both prospective and retrospective work as potential recall bias for medication use should be minimal in retrospective work. The largest prospective cohort study in this field used the UK General Practice Research Database (GPRD) and identified 27 035 patients with a history of cholecystectomy who were matched against 106 531 controls. The adjusted odds ratio for requiring cholecystectomy after 20 or more

prescriptions of statins was 0.64 (95% CI 0.59-0.70).[130] Another large prospective study was the US Nurses' Health study which identified 2 479 incident case of cholecystectomy after 8 years follow-up from the baseline population of 90 302 women aged 34-59 years. Users of statins, compared to non-users, had a multivariate relative risk of disease of 0.82 (95% CI=0.70-0.96).[131] The results from these two large epidemiological studies suggest that statins do prevent gallstone disease, although there is a risk of residual confounding. People prescribed statins are often advised by their doctors to make alterations to their diet and lifestyle, leading to changes in their behaviour that could reduce the risk of developing gallstones. Future work needs to include all potential aetiological agents in the models investigating statins.

Fibrates

Fibrates may potentially alter the risk of developing gallstone disease due to their effects on biliary cholesterol secretion. Fibrates are the first choice drug to treat hypertriglycerdiaemia and reduce the risk of cardiovascular disease and can also be used in the therapy for hypercholesterolaemia if statins are not tolerated.[132] Fibrates are hypolipidemic drugs that lower the progression of atherosclerotic lesions mainly through activation of the nuclear receptor peroxisome-proliferator activated receptor-alpha (PPAR-α) which is a subgroup of a nuclear receptor gene family. PPAR-α activation mediates changes in lipoprotein metabolism leading to an increased hepatic uptake and esterification of free fatty acids, as well as increasing mitochondrial free fatty acid uptake promoting free fatty acid oxidation.[133] Fibrates also significantly reduce cholesterol-7-alpha-hydroxylase (CYP7A1) activity.[27, 134] CYP7A1 is the rate limiting enzyme for bile acid biosynthesis and hence cholesterol elimination, with low activity of CYP7A1 causing increased cholesterol secretion in humans.[27] Fibrates increase biliary cholesterol secretion [135] and in a randomised controlled trial to evaluate the effects of clofibrate in cardiovascular disease, 1 103 participants were treated with clofibrate vs 2 789 with placebo. The rate of any gallbladder disease was higher in the treatment group (3.3% vs 2.0%, p=0.018).[136] This finding was confirmed in a case-control study which reported an increased risk for the presence of gallstones (diagnosed with ultrasonography) with a history of fibrate use (multivariate RR=1.7, 95% CI 1.0-2.7).[137] Since there is strong biological data relating fibrate use to increased gallstone formation with supporting data from RCTs and case-control studies, fibrates are regarded as causal agents for the development of gallstone disease.

Aspirin

Aspirin could prevent gallstone formation by reducing the volume of mucin secreted into the gallbladder. Mucin release is partly mediated by prostaglandins formed from arachidonic acid via the cyclo-oxygenase pathway, which aspirin inhibits. Animal models have demonstrated that aspirin inhibits mucin secretion [138] with human studies in obese participants confirming this effect [139]. Although some clinical investigations and one observational study have reported that aspirin use is associated with reduced gallstone formation [11, 140] these findings are not supported by other investigations. A RCT of 4 524 patients who received either 1 000mg of aspirin a day or placebo found no difference in the rate of hospitalization for gallstone disease.[141] A case-control study assessed gallstone prevalence between users and non-users of aspirin or NSAIDs and again reported no differences.[142] However, aspirin has been effective in preventing gallstone formation in the obese undergoing rapid weight loss[143] and future prospective studies examining the timing and dose of aspirin use with respect to gallstone diagnosis are needed to clarify if it has an effect.

Total parenteral nutrition, octreotide and ceftriaxone

Total parenteral nutrition and the drug, octreotide, both suppress the release of cholecystokinin leading to gallbladder stasis and stone formation. Octreotride also predisposes to gallstones by slowing colonic transit times which increases the absorption of the hydrophobic secondary bile salt, deoxycholate, which is re-secreted into the bile and promotes cholesterol precipitation.[144] More than 50% of patients treated with octreotide will develop cholelithiasis [145] with the same proportion forming biliary sludge after 6 weeks of TPN treatment.[8] As the number of people receiving these two therapies is relatively small, they have a minimal significance on the total burden of gallbladder disease. Ceftriaxone is a third generation cephalosporin which is secreted unmetabolised into the bile and is associated with the production of biliary sludge.[146] However, its short term use rarely leads to gallstone disease.

7. Diet and gallstones disease

The geographical variation in gallstone disease prevalence, with increased disease in Westernised countries, suggests that environmental factors, and in particular diet, are likely to be involved in the pathogenesis of gallstone disease. Over the past 40 years, within the same population group in the UK, there has been a dramatic increases in both gallstone prevalence[147] and disease requiring hospitalisation.[3] To assess diet and the risk of developing incident gallstones or gallstone disease extensive epidemiological and experimental work has been performed. There are a multitude of nutrients which could either increase or decrease the risk including macronutrients, vitamins and minerals. Prospective epidemiological studies which evaluate diet prior to the development of symptoms are methodologically superior to case-control studies and cross-sectional surveys of gallstone disease, as they eliminate protopathic and recall biases. Studies that use ultrasonography to detect silent gallstones in case-control studies should also eliminate protopathic bias.

Measuring dietary intake

Diet is a plausible environmental factor to investigate in the aetiology of gallstone disease, although nutritional epidemiology has many methodological limitations. If the aim is to measure current dietary habits, the Heisenberg uncertainty principle may occur, namely, by stopping something to measure it, its behaviour changes, which in practice means when people are asked to record current dietary intake they may be inclined to alter their eating habits.[148] If the aim is to measure past dietary intake, then difficulties in recalling food intake and conceptual abilities lead to measurement error. Even if the diet is accurately recorded, further error will be introduced by the use of food composition tables which convert dietary data to nutrient values. Food composition tables and nutrient databases give average values of a limited number of samples of each food type. Food composition table are subject to sampling errors, missing values, nutrient losses and gains during processing, and altered bioavailability which all contribute to error and variation in findings from nutritional studies. To minimise the error from measuring diet an appropriate assessment method should be selected which has been thoroughly validated.

The ideal DAM would be quick and easy to complete and cheap but both accurate and reproducible. There is no single best DAM which can be applied as a standard in all epidemiological studies and all the available options have advantages and disadvantages. Various dietary assessment methods (DAMs) are available with varying degrees of accuracy and cost. These range from (cheapest and least accurate first) national food supply data, household surveys, 24-hour recall to food frequency questionnaires, food diaries, weighed records and laboratory assessments such as urinary nitrogen as a biomarker of protein intake, and doubly labelled water to evaluate energy expenditure.

Dietary assessments in populations

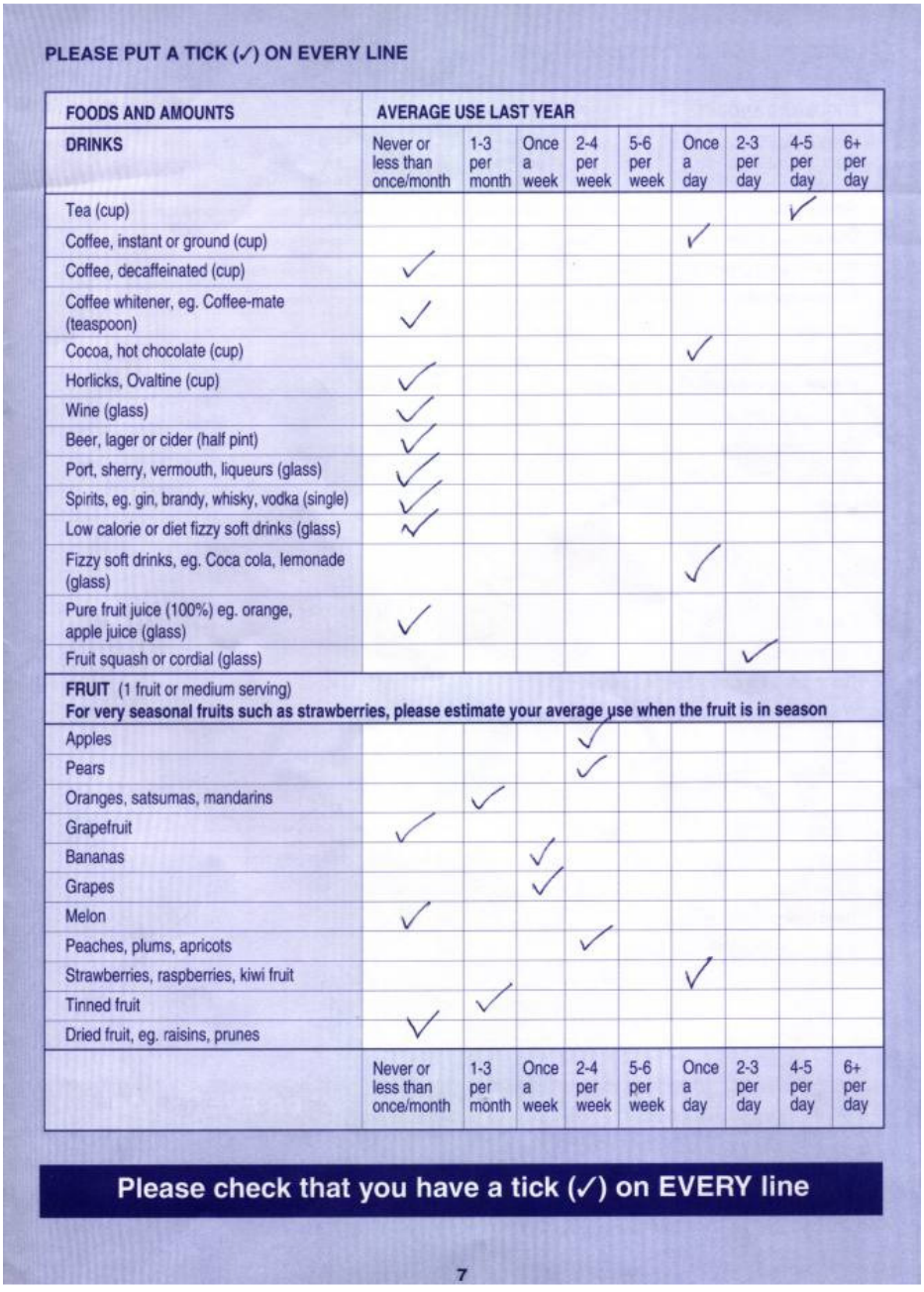
Diet can be assessed in either populations or individuals. This section will discuss methods of measuring dietary intake in groups, by national food supply data and 24-hour recalls obtaining national dietary data and the use of the food account method in household surveys. Estimating a nation's dietary intake can be made from national food supply data which is usually collected commercially and can be used in ecological studies. One example is the Food and Agricultural Organization of the United Nations (FAO) which calculates the quantity of food

produced in a country. This is added to the food imported, and then a subtraction of the food exported, lost in storage, fed to animals, and used for non-dietary purposes to calculate a measure of consumption. This gives an estimate of the per capita consumption of dietary intake, by dividing by the size of the total population.[149] The data collected can be used to compare differences between the incidence of disease and diet in different countries to generate hypotheses on aetiology. There are errors inherent in this approach which makes interpretation difficult, principally as there is no correction for co-variates associated with disease risk. National population surveys have been used to collect more detailed dietary information on subgroups of this population. Commonly, in the population setting 24-hour recall diaries are used to investigate the associations between diet and disease and can provide a reasonable estimate of the diet in a given group. Household surveys provide information on the average dietary consumption and are most often undertaken using the "food account method" where all the food entering the household is recorded, usually in the form of shopping purchases. The longest running household survey using this method is the British Household Food Consumption and Expenditure Survey (The National Food Survey), which is conducted annually.[148] Household surveys allow comparisons of different sub-groups within a population, for example the geographical variation of dietary fibre intake and colon cancer mortality within the UK.[150] Such work is hypotheses generating rather than hypothesis testing and for more detailed assessments evaluations of diet in individuals is required. There are three principle methods of assessing diet in individuals, namely food frequency questionnaires, 24-hour recall and food diaries.

Food frequency questionnaires (FFQs)

Food frequency questionnaires are the most frequently used dietary assessment methods (DAM) in case-control studies and cohort studies.[148] They are designed to measure usual eating habits over a defined period of time and consist of lists of food types and items, together with options on the frequency of consumption, ranging from never to many times per day (Figure 6). FFQs can either be self or interviewer administered and benefit from being quick, easy to undertake and cheap. They have advantages if the sample is geographically dispersed, when they can be posted. They are suitable for certain nutrients which are readily recalled e.g. alcohol, particularly if the aim is to rank participants into broad groups of intake rather than precise qualitative amounts. Considerable work is required to develop and validate FFQs against recognised standards.

Figure 6. An example of a completed food frequency questionnaire (source; EPIC-Norfolk website).



PLEASE PUT A TICK (✓) ON EVERY LINE

FOODS AND AMOUNTS	AVERAGE USE LAST YEAR								
DRINKS	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day
Tea (cup)								✓	
Coffee, instant or ground (cup)						✓			
Coffee, decaffeinated (cup)	✓								
Coffee whitener, eg. Coffee-mate (teaspoon)	✓								
Cocoa, hot chocolate (cup)						✓			
Horlicks, Ovaltine (cup)	✓								
Wine (glass)	✓								
Beer, lager or cider (half pint)	✓								
Port, sherry, vermouth, liqueurs (glass)	✓								
Spirits, eg. gin, brandy, whisky, vodka (single)	✓								
Low calorie or diet fizzy soft drinks (glass)	✓								
Fizzy soft drinks, eg. Coca cola, lemonade (glass)						✓			
Pure fruit juice (100%) eg. orange, apple juice (glass)	✓								
Fruit squash or cordial (glass)							✓		
FRUIT (1 fruit or medium serving) **For very seasonal fruits such as strawberries, please estimate your average use when the fruit is in season**									
Apples				✓					
Pears				✓					
Oranges, satsumas, mandarins		✓							
Grapefruit	✓								
Bananas			✓						
Grapes			✓						
Melon	✓								
Peaches, plums, apricots				✓					
Strawberries, raspberries, kiwi fruit						✓			
Tinned fruit		✓							
Dried fruit, eg. raisins, prunes	✓								
	Never or less than once/month	1-3 per month	Once a week	2-4 per week	5-6 per week	Once a day	2-3 per day	4-5 per day	6+ per day

Please check that you have a tick (✓) on EVERY line

7

24-hour food recall

The 24-hour food recall method records the previous day's intake, and is commonly used in cross-sectional investigations, although they may also be employed in case-control and cohort studies. The participant is asked to report all their food and drink consumed in the 24-hours prior to the interview by a researcher or by self-completing the questionnaire. Subjects should not be given prior warning of the interview to prevent alteration of their behaviour. The actual foods consumed are described together with estimated or the known weights or portion sizes. 24-hour recalls are quick and easy to administer with good compliance as the information is relatively easy to recall. This method was used in the US National Health and Nutrition Examinations Survey (NHANES) III.[151] The limitations are that they only provide a snap-shot of dietary intake and no estimate of the day-to-day variation of an individual's diet. When used in epidemiological studies to rank individuals into categories of nutrient intake, this inherent measurement error will reduce the ability to describe significant associations between diet and disease, as many will be misclassified.[148] There is also a tendency for subjects with a high intake to under-report and those with a very low intake to over-report leading to a "flat-slope" syndrome.[152] Hence, the use of 24-hour recalls in large scale nutritional epidemiological research may result in no significant differences being detected between dietary intakes of a nutrient and disease, although one may actually exist (type II error). For differences to be detected then large variations in diet between cases and control would need to exist.

Food diaries

Food diaries require an individual to accurately record, over a set time period, usually 3 to 7 days, all food and drink consumed. The individuals are taught how to describe and estimate the type and weight of food to be eaten, including brands, individual recipes and to record any leftover. Detail regarding the volume of food consumed can be improved by asking participants to weigh the food, use the packaging, or compare the product to pictures or photos of portion sizes. The text recorded in the diary is interpreted by a nutritionist and entered into a computer program to produce average daily nutrient values of intake. Historically, food diaries have been used to validate FFQ and 24-hour recalls. They have not been used as methods to record dietary intake in large cohort studies due the length of time and tuition required to complete and interpret them successfully.

Validity of dietary assessment methods

All dietary assessments methods (DAMs) incur measurement error, so validation of DAMs enables an understanding of the relationship between what is measured and the truest measures of intake. To establish the validity of a DAM it needs to be compared against the best standard or reference measure, even though this may itself have inherent errors.[153] Therefore, it is only possible to evaluate DAMs relative to a previously established "best" reference measure. The latter should ideally have an error independent of that which might be recorded in the DAM. Ideally, a valid external measure, such as a biochemical marker of intake, should be used rather than an internal measure, such as a detailed diary, which could lead to a bias in one aspect of measurement being carried over to another.[148] Biomarkers of dietary intake are an unbiased reference measure in nutritional validation studies because their measurement error is independent of those of DAMs.[51] Examples of biomarkers used in validation studies include doubly labelled water (measure of energy expenditure), urinary nitrogen excretion (protein intake) and serum concentrations (e.g. vitamin C and carotenoids).[51] However, not all biomarkers reliably reflect dietary intake (i.e. serum iron levels and dietary iron intake) and they do not exist for most nutrients, so other reference methods are required such as weighed records.

Dietary assessment and validation in EPIC-Norfolk

The EPIC cohort in Norfolk is unique amongst EPIC centres, as 7-day food diaries were used to assess dietary intake in its participants. However, as in other EPIC centres, a food frequency questionnaire (FFQ) and a 24-hour recall record were also used. The 7-day food diary used in the Norfolk cohort allows finer between-individual discrimination and validation studies, against weighed records, have demonstrated improved correlations with dietary intakes.[51] Extensive work was carried out to select the validation methods to be used in EPIC-Norfolk. Studies conducted in a metabolic suite, established a validation protocol to provide the most feasible accurate measure of usual dietary intake over a one year period. This protocol was a minimum of 16 days of weighed records (4x4 days over one year) and eight 24-hour urinary collections for nitrogen and potassium (4x2 days over one year).[51] Validation studies for daily intakes of nutrient compared to 16-day weighed records were conducted on 24-hour recalls, FFQs and 7-day food diaries. These reported that 7-day food diaries had better correlation coefficients for nearly all nutrients (17 of 18) than FFQ and 24-hour recall when using weighed

records as the standard (Table 2). Compared to 16-day weighed records, examples of correlations achieved for 7-DFDs, 24-hr recall and FFQs were;

	7-DFD	24-hr recall	FFQ
energy intake	r=0.59	r=0.42	r=0.52
sugars intake	r=0.77	r=0.63	r=0.51
iron intake	r=0.83	r=0.53	r=0.43
vit C intake	r=0.70	r=0.54	r=0.54
alcohol intake	r=0.88	r=0.60	r=0.90

The only dietary variable which had a higher correlation when measured with the FFQ compared to the 7-DFD was alcohol. This was partly due to alcohol intake often being zero and alcohol is readily recalled.[51]

The validity of the different dietary methods has also been compared with 24-hour urine biomarkers for nitrogen and potassium excretion which are used to estimate dietary protein and potassium intake, respectively.[46, 51] Participants were classed into quintiles of dietary intake to allow comparisons with the urinary measurements. 7-day food diaries achieved a correlation for protein intake of r=0.65, compared to FFQs r=0.24, and 24-hour recall r=0.10. Similar findings were reported for potassium consumption. The results from validation studies using external measures of 16-day weighed records and 24-hour urine excretion studies, highlight that 7-DFD give the most accurate measure of dietary intake when compared to FFQs and 24-hour recall. Nutritional epidemiological studies will only detect diet-disease relationships, particularly small effects, if the DAMs are sufficiently accurate. Inaccuracies in DAMs may explain the difficulties in defining associations when using FFQ and 24-hour recall data which has been used in all previous epidemiological studies. Aetiological studies of diet and disease risk are therefore need using data from food diaries.

Table 2. Means, standard deviations and Spearman correlation coefficients for daily intakes of nutrients compared between 16-day weighed record and three different dietary methods (source; Bingham SA *et al Int J Epidemiol 2001*[51]).

	16-day weighed records		FFQ			24-hour recall			7-day estimated food record (food diary)		
	Mean	SD	Mean	SD	r	Mean	SD	r	Mean	SD	r
Energy MJ	7.83	1.50	***8.81	2.40	0.52	7.57	2.2	0.42	7.89	1.49	0.59
Fat g	76	19	***87	31	0.55	77	34	0.40	*80	23	0.63
Protein g	69	12	***82	22	0.43	70	24	0.21	69	12	0.66
Carbohydrates g	223	52	**245	77	0.55	*206	68	0.60	215	47	0.71
Sugars g	109	35	***135	55	0.51	102	42	0.63	106	33	0.77
Starch g	108	27	***93	33	0.53	*98	41	0.56	104	25	0.70
NSP g	16	5	***19	7	0.57	15	6	0.61	*15	4	0.74
'Fibre' g	22	7	***27	10	0.55	21	10	0.58	22	6	0.73
Potassium g	3.2	0.6	***4.0	2.2	0.39	3.1	0.9	0.56	3.2	0.6	0.76
Calcium mg	952	245	***1308	453	0.50	879	360	0.28	*864	248	0.67
Iron mg	12.9	3.8	13.6	7.0	0.43	12.3	5.9	0.53	12.4	2.9	0.83
Carotene mg	3.4	1.9	***5.1	3.2	0.45	3.5	3.7	0.28	3.2	1.8	0.66
Retinol µg	797	773	1010	952	0.55	1070	4178	0.54	740	821	0.35
Vitamin C mg	99	41	122	53	0.54	**87	56	0.54	106	48	0.70
Alcohol g	9	13	8	11	0.90	**7	14	0.60	9	10	0.88
Fat % energy	35.9	4.6	36.3	6.1	0.64	36.5	8.9	0.49	*37.0	5.7	0.77
Protein % energy	15.2	2.3	***16.1	2.7	0.70	16.5	4.9	0.45	15.2	2.7	0.81
Carbohydrate % energy	45.6	5.8	44.6	6.5	0.69	*44.0	9.0	0.57	***43.8	5.7	0.81

* $P < 0.01$ Significantly different from weighed records.
** $P < 0.001$ Significantly different from weighed records.
*** $P < 0.0001$ Significantly different from weighed records.
95% confidence intervals for n = 150 are −0.06–0.26 for r = 0.1; −0.04–0.36 for r = 0.2; 0.16–0.44 for r = 0.3; 0.26–0.54 for r = 0.4; 0.38–0.62 for r = 0.5; 0.50–0.70 for r = 0.6; 0.68–0.78 for r = 0.7; 0.74–0.86 for r = 0.8; 0.86–0.94 for r = 0.9.

Reproducibility of dietary assessments

Reproducibility is a measure of the ability of the dietary assessment method to obtain the same result at repeated testing. The terms reproducibility, reliability and repeatability have been used synonymously, although they define subtly different aspects. Repeatability is the ability to repeat the method in the same manner and reliability is the accuracy of a measure. Reproducibility is the ability of a dietary assessment method to obtain the same result again.[153] Potential variations in dietary observations in individuals may be due to the normal variation in diet or due error in the DAM used. To reduce the variability due to dietary variation, a study can be undertaken over a longer period of time, with repeated measures and in large populations to balance out normal or seasonal variations. Inaccuracy can also be incurred by the assessment tool used (measurement error), which can be reduced by using robust and simple assessment tools, which are not dependent upon observer reporting.

To investigate whether dietary intake from a single measurement was representative of longer term dietary habits, a Dutch study used food frequency questionnaires in a cohort of 400 participants to record dietary intake at baseline with repeat assessment each year for five years. The single baseline FFQ ranked subjects according to into quintiles of nutrient intake, and there was little variation over 5 years, with an average decline in the correlation coefficient for a nutrient over this time of r=0.07.[154] These results indicate that a single baseline measure of dietary intake places most participants in the appropriate quintile for at least 5 years, although there is a lack of data to clarify how long a single baseline measure remains reliable.

Overview of nutritional assessment methods

Nutritional epidemiology is a complex discipline which can involve several different types of study design and dietary assessment methods. Although randomised controlled trials would be the ideal methodology they are not practical and therefore cohort studies are used. These are preferable to case-control work as there are less recall and selection biases. To measure diet, the most accurate and pragmatic method is required. 7-day food diaries are the most accurate pragmatic measure to use if there is the infrastructure and finance to support their use, although there are no published reports using these in the investigation of gallstones and pancreatic cancer.

8. Diet as a risk factor for gallstone disease

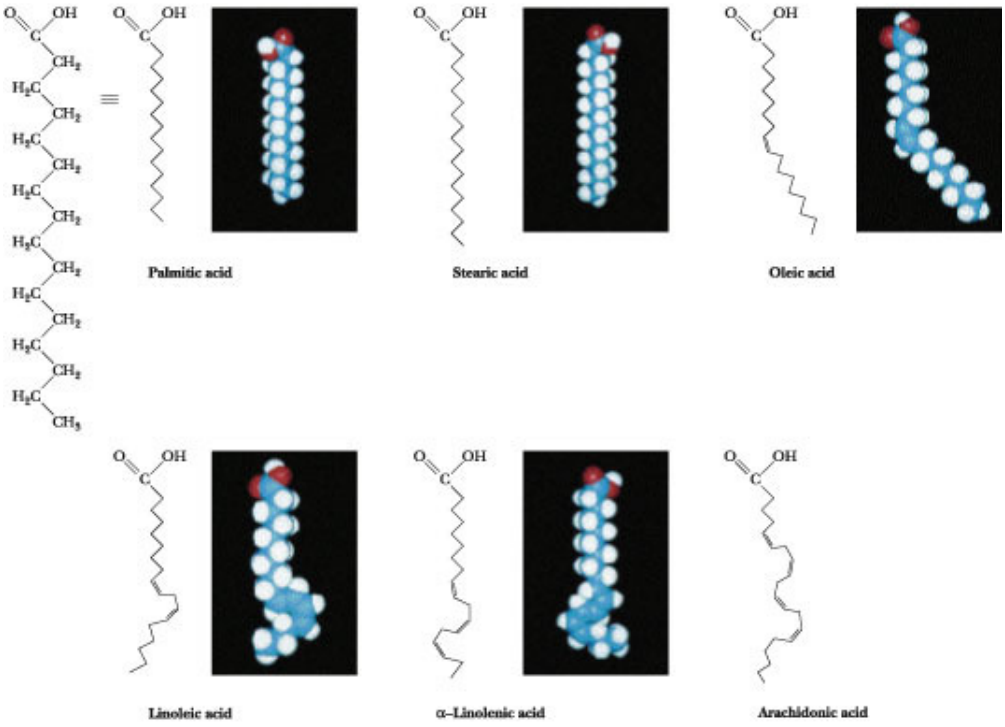
Total energy intake

Total energy intake may lead to gallstone disease by contributing to weight gain and obesity, although it is unclear if total energy intake leads to weight gain, with previous prospective epidemiological studies reporting no effect of total energy intake on the risk of weight gain of a population.[155-156] [157] The causes for obesity are complex and not yet fully understood, with excess energy intake and a sedentary lifestyle probably being important factors with host genetics, colonic flora, and environmental conditions also involved.[158] Epidemiological studies assessing total energy intake in gallstone disease have reported mixed results. Prospective studies of symptomatic gallstone disease have found both a positive [159] and inverse association with total energy intake [160] with cross-sectional and case-control studies reporting similar results, though these studies are limited by study numbers and their inherent biases. Further prospective studies in different populations are required to clarify if total energy intake is involved in the aetiology of gallstone disease.

Dietary fat and fatty acid groups

Dietary fat consists of fatty acids, cholesterol or sterols. Naturally occurring fatty acids are by far the largest component of the dietary fat, and they are grouped depending on the presence of double bonds on the carbon chain, being either saturated (no double bonds), monounsaturated (one double bond) or polyunsaturated fatty acids (more than one double bond)(Figure 7).[161] Another group of fatty acids are the *trans* fatty acids (or *trans*-fats) that are polyunsaturated fatty acids formed by the partial hydrogenation of unsaturated oils. During this process, hydrogen binds to some of the double bonded carbons, changing them into single bonds, which solidifies the oil which can be a useful property in the commercial preparation of foods. They are found in small concentrations in dairy products but nearly all dietary *trans* fatty acids produced industrially.

Figure 7. Diagrams and examples of the three main fatty acid classes.



A saturated fatty acid, stearic acid (18:0); A monounsaturated fatty acid, oleic acid (18:1n-9 cis); The polyunsaturated fatty acids, linoleic acid (18:2n-6,9 all cis) and arachidonic acid (20:4n-6,9,12,15 all cis) (source; http://web.virginia.edu/Heidi/chapter8/Images/8883n08_01.jpg).

Total fat intake

Total dietary fat could contribute to gallstone disease by promoting weight gain which stimulates several pathogenic mechanisms. However, prospective cohort studies including the Nurses' Health Study of over 90 000 women [162] and the Health Professionals Follow-up Study of 50 000 men [163] found no association between the overall percentage of calories derived from fat and weight gain. There were similar findings for other important health outcomes, including cancer, heart disease, and weight gain. Supporting these findings, a large randomised trial of 49 000 post-menopausal women from the US found that those on a low-fat diet didn't lose or gain weight any more weight than women who followed their normal diet [164] and there was no effect on cardiovascular outcomes.[165] However, these same studies did report that the composition of dietary fat consumed did alter outcomes with a strong positive association with weight gain with the percentage of energy derived from animal fat, saturated fat, and *trans* fat.[166] The manner in which different fatty acids are metabolised may lead to differing contributions to weight gain with monounsaturated, and polyunsaturated fatty acids more likely to be oxidised, rather than stored as is the case with saturated fatty acids.[167]

Epidemiological studies have reported mixed findings for total fat intake and the development of gallstones. Three prospective studies of symptomatic gallstone disease did not find any associations with total dietary fat.[159-160, 168] Studies using ultrasonography to detect silent stones found an inverse association in an Italian population [169] and a positive though non-significant association with total fat in a Danish population.[170] The mixed results for total dietary fat probably reflect the mixed biological effect of fatty acid groups and it is possible that total dietary fat is not associated with gallstone disease. Further inconsistencies in the data may be due to errors in the methodology for recording dietary fat intake. However, no previous study has used 7-day food diaries to evaluate dietary intake which gives a higher correlation for fat intake compared to FFQs (correlation using 16-day weighed records of fat intake against FFQ=0.55 and 7-DFD=0.63[51]). Therefore, clarification of the role of total fat in can be achieved in the EPIC-Norfolk cohort using 7-DFDs to estimate dietary fat intake with the prospective design a further methodological advantage.

Saturated fatty acids

Long chain saturated fatty acids are known to contribute to raised serum triglycerides,[171-172] and increase insulin secretion and decrease insulin sensitivity[173-176] which are mechanisms which promote gallstone disease. Both epidemiological and intervention studies of fatty acid classes suggest that saturated fat worsens insulin sensitivity, while monounsaturated and ω-6 polyunsaturated fats improve it.[174] Raised insulin levels cause gallbladder hypomotility and dyslipidaemia leading to increased biliary cholesterol saturation and mucin production, all of which promote stone formation. In studies on hamsters, saturated fatty acids, and in-particular long chain ones, have been shown to increase cholesterol gallstone formation.[177-178] A prospective cohort study in the US Health Professionals study found that short and medium chain saturated fatty acids were not associated with gallstone risk although long-chain saturated fatty acids did increase the risk of disease (highest quinitle vs lowest relative risk=1.24, 95% CI=1.02-1.50).[179] Further confirmatory prospective cohort studies are required to clarify if total saturated fatty acid intake or individual fatty acids increase the risk of gallstone disease.

Monounsaturated and Polyunsaturated fatty acids

Monounsaturated and polyunsaturated fatty acids could reduce the formation of gallstones via their effects on insulin sensitivity.[174] In hamster models of gallstone disease, diets rich in mono and polyunsaturated fatty acids prevented the formation of gallstones.[180-181] There is limited epidemiological data in this area. A study using US Health Professionals cohort reported the highest intake of monounsaturated fatty acids had a relative risk of disease of 0.83 (95% CI=0.70-1.00, p for trend=0.01) whilst for polyunsaturated fatty acids RR=0.84 (95% CI 0.73-0.96, p for trend=0.01).[182] Further aetiological studies in this area are required to clarify if there is an association with mono and poly-unsaturated fatty acid intake and the use of 7-day food diaries will provide a more accurate assessment of the dietary intake.

Trans fatty acids

Trans fatty acids (TFAs) are formed during the process of partial hydrogenation of mono- or polyunsaturated fatty acids. They occur naturally in the

milk and the animal fat of ruminants such as sheep and cows in the form of conjugated linoleic acid and vaccenic acid, although they only contribute to around 0.5% of total energy intake.[183] However, after the industrial hydrogenation of oils was developed to produce *trans* fatty acids in the early 20th century, industrially produced TFAs became the largest contributor to dietary *trans* fatty acids and a significant part of the Western diet providing 2 to 5% of total energy intake and approximately 5% of total fat in the United States.[184] *Trans*-fatty acids solidify and preserve foods and are mostly consumed in fast food, snack food and baked foods.[185] During the 1990's studies began to report the potential negative health outcomes associated with trans-fatty acids consumption. In randomised trials, TFA consumption lowered HDL-cholesterol and raised LDL-cholesterol, triglycerides and total cholesterol,[186] with these lipid changes known to be associated with gallstone disease.[88, 187] TFAs act via several biological mechanisms to cause cardiovascular disease and potentially gallstones, namely by promoting systemic inflammation, insulin resistance and visceral adiposity.[185, 188] *Trans* fatty acid consumption is associated with a greater risk of cardiovascular disease than any other nutrient per calorie consumed.[189] Due to these negative cardiovascular outcomes associated with TFAs, measures were taken to reduce industrially made TFAs in the diet. In the UK, major advances have been made with all major supermarkets ceasing the use of TFAs in their own branded food in 2007. In 2010, an editorial in the British Medical Jounal[183] and a statement from the National Institute for Health and Clinical Excellence (NICE)[190] called for the complete removal of industrial trans-fatty acids from the diet.

There is currently limited experimental and epidemiological work evaluating TFAs and gallstone disease. The main mechanism by which TFAs may cause gallstone disease is via serum lipid changes. A dietary intervention study in healthy subjects demonstrated that a diet with 10% of energy derived from TFAs lead to raised LDL-cholesterol and decreased HDL-chloesterol [191]. TFAs increase in plasma trigylcerides.[186, 192] These lipid changes are known to be associated with increased biliary cholesterol saturation and an increased gallstone disease incidence.[88, 187] Only one epidemiological study has evaluated *trans* fatty acids and symptomatic gallstone disease namely the Health Professionals Follow-up Study in men which found a small increased risk with increased TFA intake (higest vs lowest quintile HR=1.23, 95% CI=1.04-1.44).[193] These results need to be confirmed in further epidemiological studies, particularly in women and in a European population.

Dietary cholesterol

Since the majority of gallstones are mostly composed of cholesterol, it has been hypothesized that dietary cholesterol predisposes to gallstone formation, with many reviews on aetiology listing increased dietary cholesterol as a risk factor.[8, 19, 194] This has been supported by studies in some, but not all animal models. Increased dietary cholesterol lead to gallstones in the prairie dog, [195] squirrel monkey[196] and hamster[197] although, in chickens, rabbits and rats, a month of a high cholesterol diet had no effect on the biliary composition.[198]

Human intervention studies evaluating the effects of dietary cholesterol have given mixed results. A study of 10 men fed a high cholesterol diet (750mg/day) for 3 weeks reported the mean biliary cholesterol saturation increased.[199] Another study fed 12 patients with asymptomatic gallstones and 7 healthy women, 500mg, 750mg and 1000mg of dietary cholesterol for 3 weeks. In both groups the cholesterol saturation increased with increased dietary cholesterol intake, with those with prevalent gallstones having increased biliary cholesterol secretion compared to the healthy controls.[200] A Danish study of nine healthy female students assessed their biliary compostion before and after the addition egg yolk to the diet (1-2g cholesterol daily) while keeping macronutrients unchanged. They found no increase in biliary cholesterol concentration, with some individuals actually decreasing their levels.[201] These findings were replicated in another study of six normolipidemic and six hypertriglyceridaemic subjects, where high dietary cholesterol feeding had no consistent effects on the molar cholesterol concentration in duodenal bile.[202] However, they did find that in normolipidemic subjects, a high dietary intake of cholesterol lead to changes in bile acid composition with an increased production of chenodeoxycholic acid. This caused a reduced cholic acid to chenodeoxycholic acid ratio which reduces cholesterol saturation of the bile. Chenodeoxycholic acid can be used to treat gallstones as it solubilises the bile. However, cholic acid increases cholesterol super-saturation by down-regulating cholesterol-7-α-hydroxylase (the rate-limiting step in bile acid synthesis).[202] Hence, it appears in experimental human studies that dietary cholesterol may alter the biliary composition, although clarification is needed. The inconsistencies may be due to cholesterol metabolism varying between different populations, with short-term dietary cholesterol supplementation leading to increased cholesterol saturation of the bile in some groups. In others there may be an increase of chenodeoxycholic acid secretion which could prevent gallstones. However, long-term increased

dietary cholesterol may lead to further adaptive responses not evaluated in these interventional studies.

The epidemiological evidence for the role of dietary cholesterol has largely taken place in case-control and cross-sectional studies which are vulnerable to bias and in particular protopathic bias. The results varied between an increased risk of gallstones with increased dietary cholesterol[203-204] to a decreased risk[127, 205] with the only large prospective cohort study, which used food frequency questionnaires to assess dietary cholesterol, not finding any association (highest vs lowest quintile RR=1.0 95% CI=0.8-1.3).[206] Hence, to clarify the role of long term dietary cholesterol in the risk of gallstone disease prospective studies are required using an accurate measure of dietary intake which can be achieved with 7-day food diaries.

Protein

A high protein diet affects the lipid profile with an increased HDL and decreased triglycerides as well as improved insulin sensitivity,[207-208] which decrease the risk of gallstone diease.[88] Hamster models have supported the role of a high protein diet in protecting against gallstone disease.[209-210] In epidemiological work, the US Nurses' Health study examined the effect of protein in 121 700 women with 7 831 cases of cholecystectomy over a 20 year follow-up period. Total dietary protein was not associated with cholecystectomy (highest quintile of intake vs the lowest RR=1.00, 95%=0.93-1.08), although vegetable protein was associated with a decreased risk with a RR= 0.79 (95% CI=0.71-0.88). These results suggest that vegetable based proteins may reduce the risk of gallstones although there could be a residual confounding effect from other dietary components of a high vegetable diet. However, overall there is no compelling evidence of a direct effect of protein intake on gallstone risk, and hence it is not included as a covariant in the analysis.

Carbohydrates

Carbohydrates have varying physical forms, chemical structures and particle sizes that produce different physiological responses, including on glucose homeostasis and insulin action. A simple classification separates the smallest carbohydrates into monosaccharides and disaccharides, which are commonly referred to as sugars, with the larger polysaccharides and oligosaccharides referred to as complex carbohydrates. The glycaemic index is used as a measure of how

quickly food glucose is absorbed, while glycaemic load is a measure of the total absorbable glucose in foods. High measures of each, correspond with increased insulin demands and insulin resistance.[211] A high intake of carbohydrates has been associated with the dyslipidaemia found in gallstone disease,[212] and the substitution of unsaturated fatty acids for carbohydrates can improve the lipid profile [213] The effects of carbohydrates on insulin and lipids may be a mechanism though which they could cause gallstone.

Epidemiological studies have found that dietary sugars are associated with an increased risk of gallstone disease in both case-control [204] and prospective studies.[168] Dietary carbohydrates and gallstone disease have been assessed prospectively in both the US Health Professionals Study (men) and the Nurses' Health Study (women). In men, after adjusting for known risk factors, the highest vs the lowest quintile of total carbohydrate had relative risk of 1.59 (95% CI 1.25-2.02; p for trend=0.002) with positive associations for glycaemic load and glycaemic index.[214] Similar results were reported in women, with total carbohydrate intake showing a positive association (highest vs lowest quintile, RR=1.35, 95% CI=1.17-1.55; p for trend<0.0001) as did glycaemic load and glycaemic index.[215] These finding suggest that carbohydrates are associated with gallstone disease although they need to be investigated prospectively in a European population using an accurate measure of diet and do not yet justify the inclusion of carbohydrates as a covariant of gallstone disease.

Iron

The availability of iron affects the function of several enzyme systems which could alter the risk of developing gallstones via several different mechanisms. Perhaps the most important in gallstone formation is cholesterol-7α-hydroxylase (CYP7A1) which regulates bile salt excretion and maintains cholesterol in solution. CYP7A1 requires a reducing agent (electron donor) for effective functioning, which is a role fulfilled by iron.[28] Animal studies of the effects of iron deficiency have been carried out in 40 male prairie dogs, with no previous evidence of anaemia or iron deficiency, who were fed either a iron supplemented or iron deficient diet for 8 wks.[216] The bile of dogs on an iron deficient diet had more cholesterol crystals (80% vs 20%, $p<0.05$) and a higher cholesterol saturation index (1.27 vs 0.91, $p<0.05$). The measured CYP7A1 levels were lower in dogs fed an iron deficient diet, suggesting that iron deficiency

promotes cholesterol gallstone formation due to alterations in the activity of this hepatic enzyme. Another mechanism involving iron deficiency inducing gallstone formation is through raised biliary transferrin levels, which have been found in the gallbladder of iron deficient prairie dogs. Transferrin acts as a powerful pronucleating agent promoting stone formation [216]. Iron also alters gallbladder motility and bile flow since it is a co-factor for nitric oxide synthase (NOS) which plays a key role in bile flow regulation and the normal relaxation of the gallbladder. This was demonstrated in 24 female prairie dogs fed either a normal or iron deficient diet for 8 weeks [217]. Fasting gallbladder volumes were measured and gallbladder muscle strips were harvested to measure NOS. They found that dogs fed an iron deficient diet had greater gallbladder volumes and diminished NOS levels both of which contribute to gallbladder stasis and gallstone formation. A similar study also evaluated sphincter of oddi function and found that after 8 weeks of an iron deficient diet dogs had reduced NOS concentration in the sphincter of oddi and increased cholesterol crystal formation.[218] However, conversely iron may also promote the formation of gallstones via the oxidisation of proteins which then become less soluble and precipitate. Pigment stones are rich in iron and excess iron easily forms aggregates to promote both cholesterol and pigment stones.[219]

It is possible that both iron deficiency and iron excess may contribute to gallstone formation with men more susceptible to iron overload and women to iron deficiency due to menstruation and pregnancy. Epidemiological studies in humans evaluating the role of iron in gallstone disease are limited with the only prospective study conducted in male in the US Health Professionals study which found a higher intake of dietary iron was associated with an increased risk of disease.[220] A Turkish case-control study of 111 cases (80% female) and 81 controls (84% female) found that iron deficiency was associated with a higher prevalence of gallstones disease and impaired gallbladder motility.[221] An Indian study of male and female patients admitted with gallstone disease found iron deficient patients had increased biliary cholesterol compared to those with normal serum iron levels[222] with a similar study in both men and women finding gallbladder cholesterol concentrations were higher in patients with a low serum iron.[223] Therefore the available evidence suggests that excess dietary iron is a risk factor for men although in men and women iron deficiency is a risk factor for gallstones. Despite the potential biological effects of iron in the formation of gallstones there is a lack of studies investigating the role of dietary iron intake in gallstone disease. No previous cohort studies have investigated the effect of dietary intake in women and only one in males. For these

reasons, this study in the EPIC-Norfolk cohort will evaluate whether dietary iron alters the risk of gallstone disease.

Niacin

Niacin is a B vitamin found in a large variety of foods though particularly in cereals, meat, vegetables and mushrooms. Niacin could reduce gallstone disease by altering cholesterol metabolism. Niacin was originally shown to alter the serum lipid profile in 1955,[224] firstly lowering total cholesterol, serum triglycerides, very-low-density lipoprotein (VLDL) and low density lipoprotein (LDL) and secondly, increasing high-density lipoprotein (HDL) levels.[225-226] Niacin exerts these effects by inhibiting the enzyme hepatocyte diacylglycerol acyltransferase-2 which is important in triglyceride synthesis. The inhibition of triglyceride synthesis results in accelerated degradation of apolipoprotein B (a protein that forms, and binds to LDL) decreased VLDL, LDL secretion.[227] Niacin is the most potent agent at increasing HDL levels, which it achieves by increasing the half-life of HDL via inhibition of the expression of a surface protein on the hepatocyte, thus preventing hepatic uptake of HDL.[227]

The serum lipid changes that niacin achieves at pharmacological doses are associated with a decreased risk of gallstone disease, particularly raised HDL and lowered triglycerides [88] Few studies have evaluated the effects of niacin on gallstone disease. Experimental models in animals showed that rabbits and quails fed a diet rich in niacin had reduced plasma and biliary cholesterol levels.[228-229] However, niacin supplementation in rats lead to an increased biliary cholesterol saturation though this did not lead to an aggregation of cholesterol crystals, increased bile acid secretion protected against gallstone formation.[230] No epidemiological studies have assessed the impact of dietary niacin on the risk of developing gallstones in humans, which is merited in view of the biological actions of this nutrient.

Fibre

A high fibre diet may prevent gallstone disease by shortening intestinal transit times which reduces the formation of secondary bile salts. Secondary bile salts are hydrophobic and promote cholesterol precipitation and gallstone formation[231-232]. Fibre may also have an effect on serum lipids, by reducing serum

cholesterol, although it may not alter serum HDL and triglyceride levels.[233-234] Studies in the prairie dog, found that fibre supplementation of a lithogenic diet reduced biliary cholesterol concentration.[235] Two prospective cohort studies have evaluated the association between dietary fibre and gallstone disease in women with both reporting an inverse association.[160, 236] Further case-control and cross-sectional studies have also found an inverse association of dietary fibre intake.[169-170, 203-204] However, of the current work only one study was a large cohort,[236] and the effect of fibre in men is unknown. The Italian MICOL study used ultrasonography to detect silent gallstones reported no association of fibre to gallstone disease in 14 272 men with 787 cases of disease [169]. To clarify if there is an association between fibre and gallstone disease we have reported a prospective cohort study for the first time using 7-day food diaries to assess dietary intake which has better correlations for dietary fibre intake than FFQs which were used in previous studies (correlation using 16-day weighed records of fat intake against FFQ=0.55 vs 7-DFD=0.74 [51]).

Calcium

Calcium is the major chemical constituent of gallstones and is found in both "pure" cholesterol gallstones at low concentrations and in brown and black pigment stones at high concentrations.[33] Calcium salts (either bilirubinates, carbonates, fatty acylates and bile salts) play a fundamental role in the formation of gallstones and it has been proposed that gallstone formation requires both cholesterol and calcium salt precipitation.[237] Microscopic examination of gallstones reveal that cholesterol crystal and calcium salt precipitates are organised in a structured manor, often with alternating rings of cholesterol and pigment deposits,[238]. This is similar to the biomineralization process of structures including bone and teeth.[33] Patients with gallstones of any type tend to have gallbladder bile containing a higher calcium concentration than those without gallstones.[239-240] Patients with a history of primary hyperparathyroidism which causes hypercalcaemia, have increased prevalence rates of cholelithiasis in some [241-242] but not all surveys.[243]

The effect of dietary calcium on gallstone disease has not been extensively investigated in epidemiological or human intervention studies. Biliary calcium has also been recognized to play a central role in the formation of pigment gallstones. Calcium supplementation in the prairie dog has been shown to increase biliary

calcium and long term supplementation promotes gallbladder sludge and pigment gallstone formation in the prairie dog.[244] Epidemiological studies in a small prospective study in men [168] and a case-control study reported calcium was associated with a reduced risk of gallstone disease.[203] However, large prospective trial data is lacking to clearly define the relationship between calcium and gallstone disease.

Coffee

Both caffeinated coffee and decaffeinated coffee have biological affects which alter hepatobiliary processes involved in cholesterol lithogenesis. Gallbladder function was assessed in a study of six healthy volunteers after the consumption of either caffeinated, decaffeinated coffee or sodium chloride. Caffeinated coffee lead to increased cholecystokinin (CCK) release and gallbladder contraction in a dose dependent fashion.[245] Decaffeinated coffee compared to isosmotic and isothermic sodium chloride solution also increased CCK release and gallbladder contraction. These results suggest caffeinated coffee, and to a lesser degree, decaffeinated coffee, stimulate gallbladder function which could prevent gallstones. These findings were supported by a RCT of caffeine added to the diet of sixteen prairie dogs. None of those fed caffeine developed gallstones whilst all dogs not given caffeine did form stones, with gallbladder function tests reporting increased bile flow, improved GB motility and reduced gallbladder bile protein levels.[246] Caffeine may also have effects on inhibiting gallbladder fluid absorption.[247] Apart from caffeine, coffee also contains cafestol and kahweol which are derived from the lipid fraction and are members of the diterpene family. These two compounds can alter lipid metabolism down regulate 3-HMG-CoA reductase activity which diminishes cholesterol synthesis in the liver,[248] with these action leading to reduced biliary cholesterol saturation in humans.[128]

Epidemiological studies investigating the effects of coffee intake and gallstone disease have been undertaken in the US Nurses' Health Study and the US Health Professionals' Study which both reported inverse associations for increased caffeinated coffee intake, although no effects were found for either tea or decaffeinated coffee intake. Not all studies have reported a negative association of coffee with gallstone. A US cross-sectional survey used ultrasonography to screen 13 938 US citizens for prevalent gallstones, with dietary information collected in the Third National Health and Nutrition Survey, and did not report an association

with either men and women with coffee consumption.[249] A Danish and German cross-sectional survey,[250-251] also reported no effect of coffee intake. However, neither of these European studies was prospective and coffee avoidance may occur in patients with symptomatic upper gastrointestinal disease. Therefore, further studies, particularly in European populations, are required to confirm that coffee and caffeine intake is inversely associated with gallstone disease.

9. Summary of introduction

Gallstones are a common clinical problem and their formation is complex involving many pathophysiological and biochemical mechanisms. These include cholesterol saturation, aggregation of crystals and stasis of the gallbladder. Many factors can affect these processes including the well established risk factors of gender, obesity, physical activity, parity and hormone replacement therapy. The epidemiological data is consistent for obesity but there is little information from European populations and the precise biological mechanisms are unknown. The effect of exercise also needs to be investigated in a European population using validated instruments for recording physical activity. Many nutrients including food groups, vitamins and minerals may impact upon stone formation, although the current epidemiological literature is either limited prospective cohort work or uses less accurate measures of dietary intake. This is the first cohort study to use 7-day food diaries to evaluate dietary exposures, which are the most accurate pragmatic dietary assessment method in large scale epidemiological work. The aim of this study was to assess in a large European prospective study the effects of lifestyle factors including obesity, physical activity, diet and alcohol on the risk of developing symptomatic gallstone disease in both genders. Data from serum lipids was also analysed to clarify the potential biological mechanisms for how lifestyle factors may affect gallstone formation. The prospective design of the study is essential to reduce the selection and measurement biases associated with previous case-control studies. Confirmation and quantification of potential risk factors associated with symptomatic gallstones will further our understanding of gallstone aetiology and could influence public health policy to help prevent biliary stone disease.

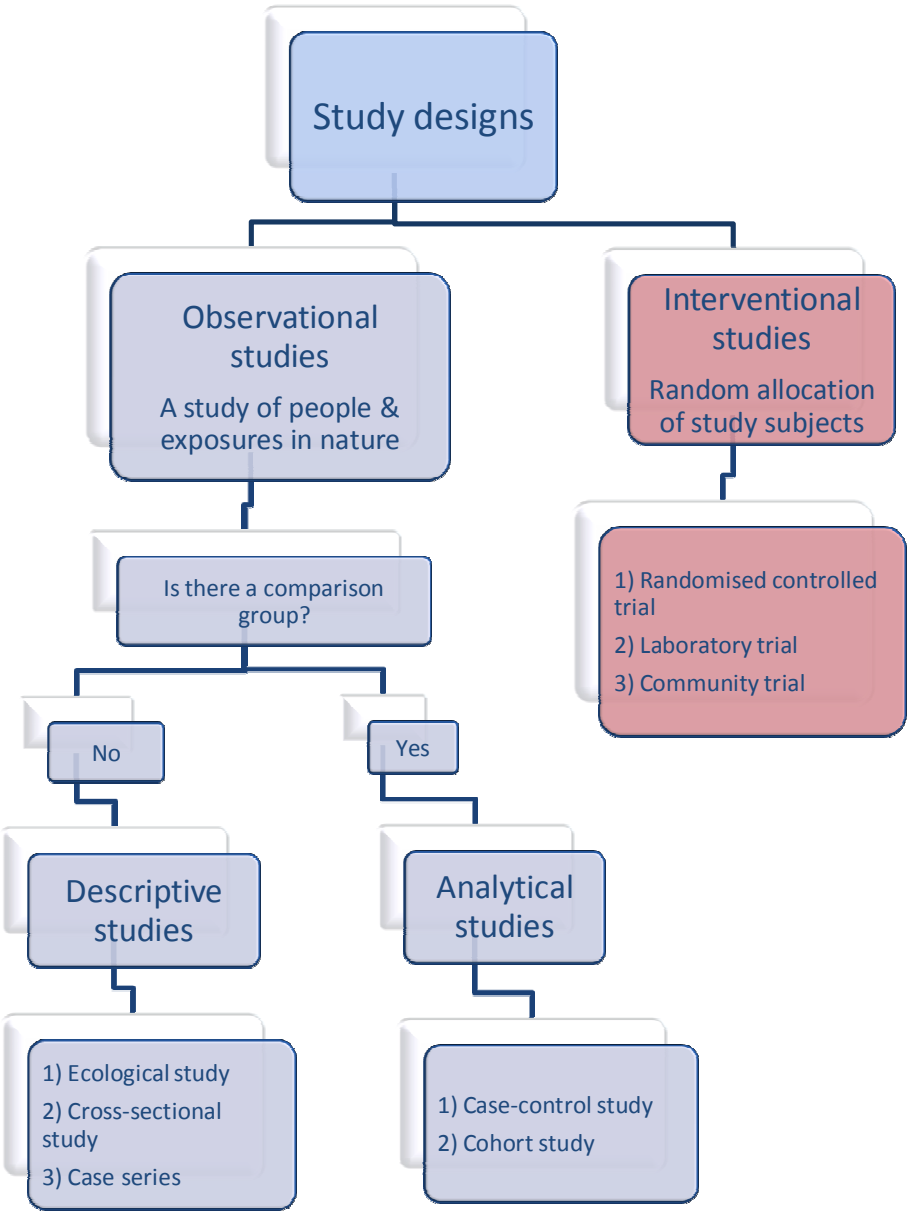
Methods

1. Selection of epidemiological study evidence

To investigate potential risk factors of disease, there are several clinical and epidemiological study designs available, each with inherent advantages and disadvantages. Studies are either observational, where the investigator does not assign the subjects their exposure, or interventional (experimental), where the exposure is assigned. Observational studies are sub-divided again into either descriptive investigations, including ecological, cross-sectional and case series, or to analytical studies, namely case-control or cohort studies (Figure 8). Each of these methodological designs is relevant to investigating the aetiology of a disease.

The selection of the study design is dependent on the stage of development of the hypothesis, logistics and the exposure being studied. Descriptive studies are often the initial investigations used to develop hypotheses, as they are relatively quick and inexpensive to conduct. The findings can then be developed in more complex study designs comparing different levels of exposures or interventions. Since the allocation of a nutritional intervention to an individual can be both pragmatically and ethically difficult, cohort studies are often employed to provide robust information. Prospective cohort investigations remove both recall and selection biases associated with case-control studies. The degree of recall bias for an exposure varies according to the one being studied. Recalling past diet is difficult, whereas exposures such as smoking and parity are readily recalled with accuracy. The following section describes study design in greater detail.

Figure 8. Overview of study designs.



Ecological and cross-sectional studies

Both ecological and cross-sectional studies are descriptive studies which generate hypotheses and benefit from being less expensive and time-consuming to perform and can utilise routinely collected data. However, they cannot assess a temporal relationship and may not be generalisable to other populations and hence other study designs are required to more accurately assess the aetiology of disease. Ecological studies are conducted at a population level, rather than in individuals, and allow comparison between populations or changes in their characteristics over time. An example would be the decline in smoking prevalence and the decreased rates of lung cancer in a given population over a period of time. Ecological investigations identify potential risk factors for further investigation in other types of study, although they are unable to explore causality. Cross-sectional (prevalence) studies measure the frequency of an exposure and outcome, at a given point in time. This measures the number of individuals with a disease in that population and the proportion who are exposed to that risk factor. Cross-sectional studies identify prevalent rather than incident disease which can lead to associations being made with factors that prolong survival or occur as a result of the disease rather than associations with aetiology.

Case-control studies

Ecological and cross-sectional investigations generate hypotheses which can be investigated in analytical work, which includes case-control studies. Case-control studies identify people with a disease (cases) who are then compared with those who do not have the disease (controls). They are used to study a wide variety of diseases and exposures and benefit from being able to use accurate measurement tools as the numbers studied are often relatively small. Case-control studies have advantages when studying rare diseases and exposures as they can recruit from many sites and require relatively small sample sizes compared to cohort studies. However, a major problem they have is recall bias where it is difficult to ensure the information collected truly represents that before the onset of symptoms i.e. that involved in aetiology of the disease. If patients have had symptoms for long periods recalling the pre-symptomatic exposure is difficult. Consequently cases tend to report their current exposure in the symptomatic period which is not reflective of that involved in the aetiology. This recall bias is of particular relevance when recording dietary intake. Another limitation of case-control studies

is that there can be substantial selection biases, particularly if control groups are selected which are unrepresentative of the general population. Ideally both cases and controls should be drawn from the same population and are therefore comparable. However, the relative simplicity of case-control studies means that they are often the first study design used to compare differences in risk factors between groups, although more complex study designs may be utilised to advance the hypothesis.

Prospective cohort studies

The second method of analytical study design is the prospective cohort study which recruits a defined group of well people who are subsequently followed-up, of whom a small number are diagnosed with the disease under investigation. Cohort studies allow a comparison of baseline risk factors between people who subsequently develop disease, with those who do not. Their strength is that the exposure data is collected before the onset of symptoms and disease and hence truly representative of that which may be involved in aetiology. Recall bias is eliminated with this study design which can be high in case-control work, particularly for exposures such as diet. Also there is less selection bias as both those who develop the disease and those who remain well are drawn from the same base-line population. Furthermore, advantages of cohort studies are that they both allow study of many diseases developing in the baseline population and the calculation of incidence. However, due to their large size which is required to acquire sufficient cases for analysis, they are expensive to set up and manage, requiring a large amount of logistical support. There is also a time lag between creating a cohort for study and having the data available for analysis. Finally, the representativeness of the cohort compared to the general population needs to be considered in terms of the population demographics, level of exposure and nature of the disease.

Randomised control trials

A limitation of all observation work is that there may be unknown factors associated with aetiology which exist in different proportions between case and controls. This methodological problem can be overcome in randomised controlled trial (RCTs). In RCTs, the study subjects are allocated by random to receive the

treatments under study or no treatment, which should ideally be a placebo or sham treatment. Following randomisation, the two or more groups of subjects are followed up in exactly the same way, and therefore the only differences between them are the intervention being assessed. Randomisation minimises selection biases which should ensure that the characteristics between the study groups are very similar. Also it allows an equal distribution of confounders in that both known and unknown prognostic factors should occur at the same rate in both groups. RCTs are usually performed to assess clinical treatments, such as new drugs, although they can also provide evidence on aetiology. For example, studies initiated in the 1980s to investigate aspirin in the prevention of cardiovascular disease are now providing additional data regarding aspirin lowering the risk of developing cancer.[40-41] However, not all exposures can be assessed in RCTs; for example it is not possible or ethical to randomise participants to an intervention which may be harmful i.e. smoking or high *trans*-fatty acid diet. Furthermore, it is also difficult and unethical to assess factors which cannot be excluded from the control group i.e. an intervention trial of vitamin C supplementation as both controls and cases will consume vitamin C in their diet.

Hierarchy of study design

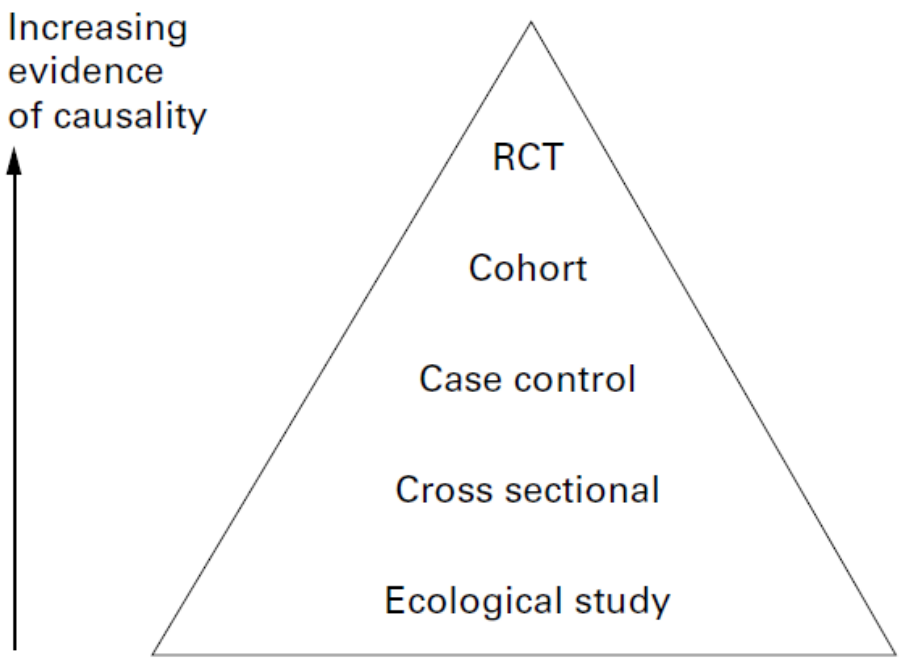
In evaluating the evidence for risk factors in the review of pancreatic cancer, priority has been given to interventional work, namely randomised controlled studies which minimises both bias and confounding. However, interventional trials of diets do not exist for many nutrients as they are both impractical to conduct and unethical. Information from observational epidemiological studies is therefore required. Although both case-control and cohort studies can be used to investigate aetiology, cohort studies provide stronger evidence of associations than case-control studies, as the former have decreased recall and selection biases. For diet, recalling past diet prior to the onset of disease can be difficult, hence cohort studies are preferred for nutritional studies and have been utilised within this study. However, for variables such as smoking, parity and medication use, recall bias is lower and case-control work is valid and pragmatically easier to conduct. The order of hierarchy used when reviewing study evidence is RCTs, cohort studies which provider stronger evidence of associations than case-control studies and finally descriptive investigations (Figure 9).

However, all study types are important in the process of developing and investigating hypotheses.

Choice of study design

This study has used the cohort design to investigate the aetiology of both gallstone disease and pancreatic cancer to minimise recall and selection bias. This is particularly important in dietary enquiries as diet is liable to change over time and it is unrealistic to expect people to remember their dietary pattern several years previously. Both gallstone disease and pancreatic cancer may affect the subject's diet and hence introduce protopathic bias if the dietary history is measured after the onset of symptoms. A cohort design allows the detection of unexpected effects factors in the aetiology of disease rather than restriction to selected factors defined when designing a case-control study or RCT. Difficulties undertaking a cohort study are the inherent time-lag between initiation of the study and derivation of study findings and the expense of conducting a cohort study. However, with EPIC-Norfolk the study has already been ongoing for over 17 years and the considerable expense in designing, coordinating and managing the cohort has also already been borne by funders, the Medical Research Council, UK, and Cancer Research, UK.

Figure 9. The hierarchy of study design in determining causality.



2. Case ascertainment

After recruitment and completion of questionnaires, the cohort was monitored to identify those participants who developed new incident gallstone disease up to June 2007, i.e. a maximum follow-up time of 14 years after recruitment. The definition of incident symptomatic gallstones was made if the participant developed clinical evidence of new symptoms suggesting gallstone disease at least 18 months after recruitment, along with either radiological and/or surgical evidence of gallstones. Participants with symptomatic gallstone disease were identified by matching the EPIC database with the Norfolk Health Authority computer records of hospital admissions and procedures. The International Classification of Diseases-10 (ICD-10) codes used were K80.0 (biliary colic, cholecystitis, cholangitis and pancreatitis secondary to gallstones)(table 2).[252] The notes of all potential cases were retrieved by requesting them from Norfolk and Norwich University Hospital records. The clinical notes were reviewed by a medical gastroenterologist to ensure that the symptoms recorded were suggestive of gallstone disease and reports were sought confirming the presence of gallstones on ultrasonography, CT scan, surgical and pathological specimens. Cases were excluded if participants recorded a history of gallstone disease or cholecystectomy at recruitment in the health and lifestyle questionnaire (figure 8). Cases were also excluded if they developed symptoms within 18 months of recruitment into EPIC-Norfolk to ensure the baseline data were truly representative of that prior to symptoms. The presence of "silent" (asymptomatic) gallstones at recruitment was not assessed as to do this, abdominal ultrasonography of the whole cohort would be required which was unfeasible. Therefore all confirmed cases of new gallstone disease, diagnosed after at least 18 months after entry into EPIC-Norfolk were identified with their case notes reviewed before being determined suitable for inclusion in the study.

Table 3. The ICD-10 codes of used to identify clinical cases which could be attributable to gallstone disease.

ICD-10 code	**Clinical diagnosis**
K80	Cholelithiasis
K80.0	Calculus of gall bladder with acute cholecystitis
K80.1	Calculus of gallbladder with other cholecystitis
K80.2	Calculus of gall bladder without cholecystitis (e.g. biliary colic, gallstone (impacted) of cystic duct)
K80.3	Calculus of bile duct with cholangitis
K80.4	Calculus of bile duct with cholecystitis
K80.5	Calculus of bile duct without cholangitis of cholecystitis
K80.8	Other cholelithiasis
K85	Acute pancreatitis
K86.1	Chronic pancreatitis

Figure 10. Recruitment questions to define gallstone and gallbladder status

Gallstones	Yes	____
Age first diagnosed?		____
Have you had your gallbladder removed?	Yes	____
If ***yes***, please state at what age	Age	____

3. Statistical analyses

The statistical analysis was performed using the computer program STATA Version 10 (Stata, College Station, Texas, USA). The data for men and women were analysed separately, as the current known covariates differ between the genders. Baseline characteristics and risk factors were compared between those with and without incident gallstone disease using a t-test for normally distributed continuous variables, a Mann-Whitney U test for non-parametric continuous variables, and a chi-squared test for categorical variables. Known risk factors for gallstone disease and study exposures were defined and divided into categories (table 3). Cox proportional hazards regression models estimated the hazard ratios (with 95% confidence intervals) of developing incident gallstone disease according to each category of exposure, using the lowest level of exposure as the baseline value, with further analyses of the trends across categories.

Hazard ratios are used to allow hypothesis testing and reflect the analysis of time survived to an event such as development of a disease, death or cure. A hazard is the rate at which an event happens, so that the probability of an event happening in a short time interval is the length of time multiplied by the hazard. Although the hazard may vary with time, the assumption in proportional hazard models for survival analysis is that the hazard in one group is a constant proportion of the hazard in the other group with this proportion the hazard ratio. Hazard ratios differ from relative risk ratios in that the latter are cumulative over an entire study, using a defined endpoint, while the former represent instantaneous risk over the study time period. Hazard ratios are less prone to selection bias with respect to the endpoints chosen, and can indicate risks that happen before the endpoint.

A cohort analysis was made for variables available in the whole cohort, namely BMI, waist circumference, serum lipids, physical activity and alcohol intake derived from the FFQ. For alcohol, the FFQ was used rather than the 7-DFD as it is one of very nutrients that the FFQ can measure with equal accuracy to the 7-DFD[51] and also the data was complete for the whole cohort. The cohort analyses were made after 14 years follow-up, except physical activity where the primary analysis was made after 5 years to minimise the effects of regression dilution bias. For dietary variables a case-cohort analysis was performed (see dietary analysis section below), as all food diaries are yet to be coded, with a follow-up period of 10 years to minimise regression dilution bias.

Table 4. Characteristics and exposures used in analysis, with units and cut-points.

Characteristic	**Units**	**Cut-points**	
		Men	Women
Age at recruitment	Years	continuous	Continuous
Parity	number of children	--	0
		--	1-2
		--	≥3
Hormone replacement therapy		--	never used
		--	previous use
		--	current use
Body mass index	kilograms/metre^2	<25 (normal)	<25
		25-<30 (overweight)	25-<30
		30-<35 (obese class I)	30-<35
		≥35 (obese class II & III)	≥35
Waist circumference	inches	<34	<28
		34-<36	28-<30
		36-<38	30-<32
		38-<40	32-<34
		40-<42	34-<36
		≥42	≥36
Serum lipids	Mmol/litre	Quartiles	Quartiles
Alcohol intake	units per week (1 UK unit = 7.9 grams or 10 mls)	0	0
		>0-<7	>0-<7
		7-<14	7-<14
		14-<21	14-<21
		≥21	≥21
Physical activity	Derived from physical activity index (table 2)	Inactive	Inactive
		Moderately inactive	Moderately inactive
		Moderately active	Moderately active
		Active	Active
Dietary nutrients	Variable	Quintiles	Quintiles
Coffee and Tea	Cups per day	0, 1, 2, ≥3	0, 1, 2, ≥3

Obesity analysis

Two different measures of obesity were analysed; body mass index and waist circumference with the categories for each shown in table 3. In the multivariate analysis, each was corrected for known risk factors for gallstone disease which in men were age at recruitment[253], alcohol[124] and physical activity[254] with the addition of parity and HRT use in women.[52] A further analysis assessed the effect of each additional unit of BMI and inch of waist circumference on the risk of disease.

Serum lipid analysis

Serum lipids were analysed in sex-specific quartiles of triglycerides, total cholesterol, HDL and LDL and adjusted only for age at recruitment. These HRs were not adjusted for other factors such as obesity and alcohol, as previous studies suggest that obesity[69-72] and alcohol[118-120] modify the lipid profile and hence could be on the same causal pathway as lipids and are therefore not true confounders. However, where significant associations with gallstone disease have been found, a further analysis has been performed with stratification of body mass index and alcohol intake categories.

Alcohol analysis

Alcohol was analysed both as a categorical variable (table 3) as well as a continuous variable to estimate its unit effect. These analyses were adjusted for age at recruitment, BMI and physical activity, with the addition of parity and HRT use in women.

Physical activity analysis

Physical activity was analysed using the four levels of physical activity (table 2). The hazard ratios were adjusted for age at recruitment, BMI and alcohol in men with the addition of parity and HRT use in women. An analysis was performed of a binary variable comparing the highest level of physical activity against a combination of the lowest three. The primary outcome was the risk of developing symptomatic gallstones after 5 years of follow-up. Five years was considered the time over which a single measure of baseline physical activity would be representative of that in the future, therefore minimising regression dilution bias. The secondary outcome was the risk of gallstones at the full follow up time of up to 14 years after recruitment. The combined population attributable risk (PAR) of increasing physical activity by one level was calculated using the formula; Combined PAR = 1 – (1–PAR1)x(1–PAR2)x(1–PAR3), where PAR1 =

the reduction in incidence that would be observed (after five years) if "inactive" participants increased their activity by one level. PAR2 and PAR3 are similar calculations for the "moderately inactive" and "moderately active" groups, if the activity level is increased by one category.

Dietary analysis

For dietary variables, a case-cohort analysis was performed using a representative subset of 3 970 randomly selected participants from the cohort who did not develop gallstones. This approach was required as not all of the completed diaries had been coded. Each nutrient was divided into gender specific fifths of intake across the distribution of the whole cohort. Multi-variate analyses adjusted for age at recruitment, physical activity, total energy intake, alcohol intake and BMI, as well as parity and HRT in women. The primary analysis was performed after 10 years of follow-up. Ten years was considered the time during which a single measure of dietary intake at recruitment from the 7-DFD would be representative of long-term nutritional intake. This approach would reduce regression dilution bias potentially caused by a proportion of the cohort altering their diet during follow up.

Energy adjustment of intakes was made for dietary nutrients as it helps to control for several factors including body size, metabolic rate and physical activity. For example, a positive association with a food type may not be a true aetiological factor, just that it is related to larger body sizes or greater physical activity (and hence energy expenditure). Adjusting for energy intake may also reduce the errors from estimating dietary intake of nutrients.[255] Energy intake will also have measurement error, which is highly correlated to the intake of nutrients. By adjusting for energy intake the errors of nutrient intake are partially corrected, which has been demonstrated for protein.[255] There are significant differences in absolute macronutrient intake between individuals who give valid records, and those who do not, and these differences are reduced after adjusting for energy expenditure.[148]

Coffee and tea analysis

Caffeinated coffee and tea data was derived from the 7-DFD and was analysed by the number of cups consumed a day, where one cup was the equivalent of 250ml. There were four categories, namely zero intake, up to one cup a day (<250mls), 2 cups (250-500mls) or ≥3cupsable. The hazard ratios were adjusted for

age at recruitment, BMI and physical activity, with the addition of parity and HRT use in women.

Results

1. Obesity, physical activity, alcohol use and serum lipids

Baseline characteristics of the cohort used in the analysis of obesity, alcohol, physical activity and serum lipids.

From the initial cohort of 25 639 participants, 1 376 (5.4%) were excluded from the analysis who reported a cholecystectomy (1 000 participants) or a medical diagnosis of gallstones (376) at recruitment, which left a cohort of 24 263 participants (13 075 women and 11 188 men). During the 14 years of follow-up (279 504 person-years), a total of 201 women (1.56% of women) and 95 men (0.86% of men) developed incident symptomatic gallstones. The incidence of symptomatic gallstones in women was nearly double that of men (1.34 per 1 000 P-Y vs 0.74 per 1 000 P-Y, $p<0.0001$). In women, the mean age at diagnosis was 65.9 yrs (SD=9.4yrs) with the interval between enrolment to diagnosis of 6.0 yrs (SD=2.9yrs). In men, the mean age at diagnosis was 69.1 yrs (SD=9.2yrs) with the interval to diagnosis of 5.9 yrs (SD=3.0yrs). The baseline characteristics of the cohort by gallstone status are shown in Table 5. In women, cases had more children and greater HRT use than controls. The clinical diagnoses were: biliary colic (53.7%), cholecystitis (23.6%), obstructive jaundice (10.2%), acute pancreatitis (10.1%), empyema (1.4%) and ascending cholangitis (1.0%). The baseline data were 100% complete for the whole cohort for physical activity, alcohol intake, BMI and waist circumference. Data on serum cholesterol and triglycerides were available on 93.2% of the cohort and 90.1% for HDL and LDL, with similar proportions for both cases and controls.

Table 5. Baseline characteristics of the study population according to incident gallstone disease status after 14 years of follow-up.

	Men			Women		
	Incident disease	Non-incident disease	p value	Incident disease	Non-incident disease	p value
Number	86	11 188		201	12 874	
Age at recruitment	63.2	59.4	<0.0001	60.0	58.7	0.050
(years, mean (SD))	(9.1)	(9.3)		(8.8)	(9.3)	
Body mass index	27.5	26.5	0.0036	28.0	26.1	<0.0001
(kg/m2, mean (SD))	(2.9)	(3.3)		(4.8)	(4.3)	
Alcohol intake, median	5.0	6.0	0.037	2.0	2.5	0.16
(units/wk, (IQR))	(1.5-10.5)	(2-14.5)		(0.5-6.0)	(0.5-6.5)	
Physical activity index score			0.095			0.079
Inactive	40.0%	30.5%		31.3%	29.8%	
Moderately inactive	27.4%	24.5%		37.3%	32.2%	
Moderately active	16.8%	23.1%		21.9%	22.3%	
Active	15.8%	21.9%		9.5%	15.7%	
Parity category						0.038
0 children	-	-		9.0%	14.2%	
1-2 children	-	-		54.7%	55.8%	
≥3 children	-	-		36.3%	30.0%	
Hormone Replacement Therapy use						0.011
Never taken	-	-		58.7%	68.6%	
Former user	-	-		14.9%	11.2%	
Current used	-	-		26.4%	20.2%	

SD=standard deviation, IQR=inter-quartile range,

Obesity

The mean BMI at recruitment was significantly higher in cases than control for both genders (Table 5). Increased body mass index significantly increased the risk of developing gallstones in both genders (Table 6). For each additional unit increase of BMI in men, the adjusted hazard ratio (HR)=1.08 (95% CI=1.02-1.14, p=0.005) and in women, the HR=1.08 (95% CI=1.06-1.11, p<0.001) i.e. an 8% increased risk of developing gallstone disease in both genders. Higher categories of BMI were also positively associated with disease, for men a BMI 25-<30 vs BMI <25 the adjusted HR=2.31 (95% CI=1.35-3.97) and in women HR=1.60 (95% CI=1.14-2.24) with significant trends across categories. The population attributable fraction of incident gallstone disease with a BMI greater than 25kg/m^2 was 38% in the whole population (46% in men and 35% in women). Waist circumference was positively associated with the risk of developing gallstone disease in both genders (Table 7). In men, for each one inch increase in waist circumference the HR=1.08 (95% CI=1.03-1.14, p=0.002) and in women the HR=1.08 (95% CI=1.05-1.12, p<0.001). Most categories of waist circumference were found to at least double the risk of incident gallstone disease. In men, for a waist circumference of 40-<42 inches vs <34 inches the HR=3.94 (95% CI=1.45-10.68). In women, for a waist circumference of 34-<36 vs <28 inches the HR=2.88 (95% CI=1.59-5.21). Waist circumference measurements correlated strongly with BMI in both men (r=0.85) and in women (r=0.85).

Table 6. The effect of body mass index (BMI) on the risk of developing symptomatic gallstones.

	Category of Body Mass Index (kg/m^2)				p for
Men	<25	25-<30	30-<35	≥35	trend
Number of participants	3 764	5 920	1 302	177	
% of cohort	33.7	53.0	11.7	1.6	
Number of P-Y	43 649	68 175	15 052	2 019	
Number of cases	17	63	13	2	
Cases per 1000 P-Y	0.39	0.92	0.86	0.99	
Hazard ratio[1]	1.00	2.29	2.08	2.53	0.012
(95% CI)		(1.34-3.92)	(1.01-4.30)	(0.59-11.00)	
Hazard ratio[2]	1.00	2.31	2.12	2.62	0.010
(95% CI)		(1.35-3.97)	(1.03-4.37)	(0.60-11.42)	
Women	<25	25-<30	30-<35	≥35	
Number of participants	5 941	5 020	1 589	499	
% of cohort	45.5	38.5	12.2	3.8	
Number of PY	68 295	57 847	18 221	5 656	
Number of cases	58	83	41	18	
Cases per 1000 P-Y	0.85	1.43	2.25	3.18	
Hazard ratio[1]	1.00	1.64	2.59	3.70	<0.001
(95% CI)		(1.17-2.30)	(1.73-3.86)	(2.18-6.28)	
Hazard ratio[3]	1.00	1.60	2.57	3.60	<0.001
(95% CI)		(1.14-2.24)	(1.73-3.89)	(2.11-6.14)	

[1] Adjusted for age at recruitment.

[2] Adjusted for age and categories of physical activity & alcohol intake.

[3] Model as 2 with hormone replacement therapy use & parity.

P-Y = person-years

Table 7. The effect of waist circumference on the risk of developing symptomatic gallstones.

Men	<34 inches	34" - <36"	36" - <38"	38" - <40"	40" - <42"	≥42"	p for trend
Participants, n	1 760	2 007	2 547	2 120	1 340	1 395	
% of cohort	15.8%	18.0%	22.8%	19.0%	12.0%	12.4%	
Number of P-Y	20 438	23 299	29 398	24 530	15 357	15 940	
Number of cases	5	12	26	18	18	16	
Cases per 1000 P-Y	0.24	0.52	0.88	0.73	1.17	1.00	
Hazard ratio[1]	1.00	1.96	3.28	2.63	3.92	3.35	0.008
(95% CI)		(0.69-5.57)	(1.26-8.57)	(0.98-7.13)	(1.45-10.63)	(1.22-9.19)	
Hazard ratio[2]	1.00	1.95	3.31	2.66	3.94	3.40	0.008
(95% CI)		(0.69-5.55)	(1.27-8.84)	(0.99-7.21)	(1.45-10.68)	(1.23-9.37)	
Women	**< 28"**	**28" - <30"**	**30" - <32"**	**32" - <34"**	**34" - <36"**	**≥36"**	
Participants, n	1 980	2 401	2 680	2 194	1 540	2 251	
% of cohort	15.2%	18.4%	20.5%	16.8%	11.8%	17.3%	
Number of P-Y	22 711	27 638	30 896	25 318	17 648	25 771	
Number of cases	16	19	37	37	38	54	
Cases per 1000 P-Y	0.70	0.69	1.20	1.46	2.15	2.06	
Hazard ratio[1]	1.00	0.97	1.68	2.04	2.99	2.91	<0.001
(95% CI)		(0.50-1.87)	(0.93-3.03)	(1.13-3.70)	(1.65-5.42)	(1.65-5.13)	
Hazard ratio[3]	1.00	0.94	1.60	1.94	2.88	2.77	<0.001
(95% CI)		(0.49-1.84)	(0.89-2.88)	(1.07-3.52)	(1.59-5.21)	(1.56-4.89)	

[1] Adjusted for age at recruitment.

[2] Adjusted for age and physical activity & alcohol intake

[3] Model as 2 with adjustment for hormone replacement therapy & parity.

P-Y = person years

Physical activity

Physical activity was analysed after 5 years of follow-up to reduce regression dilution error, with increased level of physical activity associated with a reduced risk of gallstone disease in both men and women (Table 8). In men, the "active" category vs the "inactive" category the HR=0.18 (95% CI=0.04-0.80) with a significant trend across categories (p=0.008). After 14 years of follow-up, the results in men were not significant (active vs inactive HR=0.73, 95% CI=0.40-1.35, p for trend=0.20). In women after 5 years, the "active" vs "inactive" category HR=0.34 (95% CI=0.14-0.83) with a trend across categories p=0.041. In women after 14 years of follow-up the results were not significant ("active" vs "inactive" HR=0.66, 95% CI=0.39-1.12, a p for trend of 0.24).

Table 8. Physical activity and the risk of developing symptomatic gallstones.

	Categories of physical activity				p for
Men	Inactive	Moderate inactive	Moderate active	Active	trend
Participants, n	3 416	2 740	2 581	2 450	
% of cohort	30.5%	24.5%	23.1%	21.9%	
5 years follow-up					
Number of P-Y	17 037	13 675	12 888	12 246	
Number of cases	22	12	6	2	
Cases per 1000 P-Y	1.29	0.88	0.47	0.16	
Hazard ratio[1]	1.00	0.79	0.46	0.17	0.005
(95% CI)		(0.39-1.61)	(0.18-1.14)	(0.4-0.74)	
Hazard ratio[2]	1.00	0.82	0.49	0.18	0.008
(95% CI)		(0.40-1.68)	(0.20-1.22)	(0.04-0.80)	
14 years follow-up					
Number of P-Y	39 181	31 701	29 918	28 374	
Number of cases	38	26	16	15	
Cases per 1000 P-Y	0.97	0.82	0.53	0.53	
Hazard ratio[1]	1.00	0.96	0.67	0.70	0.14
(95% CI)		(0.58-1.59)	(0.37-1.22)	(0.38-1.29)	
Hazard ratio[2]	1.00	0.99	0.70	0.73	0.20
(95% CI)		(0.60-1.64)	(0.39-1.27)	(0.40-1.35)	

Women	Inactive	Moderate inactive	Moderate active	Active	
Participants, n	3 902	4 215	2 915	2 043	
% of cohort	29.8%	32.2%	22.3%	15.6%	
5 years follow up					
Number of P-Y	19 447	21 014	14 537	10 203	
Number of cases	35	33	22	6	
Cases per 1000 P-Y	1.80	1.57	1.51	0.59	
Hazard ratio[1]	1.00	0.89	0.86	0.34	0.033
(95% CI)		(0.55-1.45)	(0.50-1.50)	(0.14-0.81)	
Hazard ratio[3]	1.00	0.87	0.89	0.34	0.041
(95% CI)		(0.53-1.43)	(0.51-1.54)	(0.14-0.83)	
14 years follow up					
Number of P-Y	44 677	48 401	33 560	23 676	
Number of cases	63	75	44	19	
Cases per 1000 P-Y	1.41	1.55	1.31	0.80	
Hazard ratio[1]	1.00	1.16	1.00	0.62	0.13
(95% CI)		(0.82-1.63)	(0.68-1.49)	(0.37-1.06)	
Hazard ratio[3]	1.00	1.19	1.07	0.66	0.24
(95% CI)		(0.84-1.68)	(0.72-1.60)	(0.39-1.12)	

[1]Adjusted for age at recruitment.

[2] Adjusted for age and categories of body mass index and alcohol intake.

[3] Model as 2 with adjustment for hormone replacement therapy & parity.

P-Y = person-years

Alcohol

In men after 14 years of follow-up, alcohol had an inverse association for each extra unit consumed per week with a HR=0.97 (95% CI=0.95-0.99, p=0.016) i.e. a 3% reduction in the risk of gallstone disease for each extra unit of alcohol per week. In women, there was no unit effect (HR=0.99, 95% CI=0.97-1.02). There were no associations between the individual categories of alcohol intake and the risk of developing gallstones in either gender (Table 9), although in men the trend across categories was significant (HR=0.82, 95% CI=0.68-1.00, p=0.044).

Table 9. Alcohol intake and the risk of developing symptomatic gallstones.

	Alcohol category (units* per week)					p for
Men	0	0.1-<7	7-<14	14-<21	≥21	trend
Participants, n	1 098	4 911	2 375	1 236	1 568	
% of cohort	9.8%	43.9%	21.2%	11.1%	14.0%	
Number of P-Y	12 740	57 071	27 263	14 135	17 969	
Number of cases	11	48	24	6	6	
Cases per 1000 P-Y	0.86	0.84	0.88	0.42	0.33	
Hazard ratio[1]	1.00	1.09	1.18	0.59	0.47	0.056
(95% CI)		(0.56-2.10)	(0.58-2.42)	(0.22-1.61)	(0.17-1.29)	
Hazard ratio[2]	1.00	1.10	1.20	0.58	0.46	0.044
(95% CI)		(0.58-2.14)	(0.58-2.46)	(0.21-1.58)	(0.17-1.25)	
Women	0	0.1-<7	7-<14	14-<21	≥21	
Participants, n	2 277	7 995	1 935	611	257	
% of cohort	17.4%	61.1%	14.8%	4.7%	2.0%	
Number of P-Y	26 468	91 965	22 049	6 979	2 854	
Number of cases	38	128	22	9	4	
Cases per 1000 P-Y	1.44	1.39	1.00	1.29	1.40	
Hazard ratio[1]	1.00	1.01	0.73	0.94	1.04	0.49
(95% CI)		(0.70-1.45)	(0.43-1.23)	(0.45-1.94)	(0.37-2.92)	
Hazard ratio[3]	1.00	1.01	0.72	0.99	1.10	0.57
(95% CI)		(0.70-1.46)	(0.42-1.24)	(0.48-2.05)	(0.39-3.11)	

* one unit = 10 mls or 7.9 grams of alcohol

[1] Adjusted for age at recruitment.

[2] Adjusted for age and categories of physical activity & BMI.

[3] Model as 2 with adjustment for hormone replacement therapy & parity.

P-Y =person-years

Lipids

After 14 years follow-up, increased serum triglycerides were associated with a higher risk of symptomatic gallstones in both genders (Table 10). In men, the highest vs the lowest quarter HR=2.02 (95% CI=1.03-3.98) with the trend across quarters HR=1.29 (95% CI=1.05-1.57, p=0.009). Similarly, in women, the highest vs lowest quarter HR=2.43 (95% CI=1.52-3.90) and the trend across quarters HR=1.30 (95% CI=1.13-1.48, p<0.001). An increasing serum HDL was inversely associated with symptomatic gallstones in both genders (Table 10). In men, comparing the highest vs the lowest quarter of HDL, the HR=0.22 (95% CI=0.09-0.52) and the trend across quarters HR=0.62 (95% CI=0.49-0.77 p<0.001). In women, comparing the highest vs the lowest quarter of HDL, the HR=0.55 (95% CI=0.36-0.85), and the trend across quarters HR=0.84 (95% CI=0.74-0.96 p=0.010). Total cholesterol and LDL cholesterol levels were not associated with the risk of developing gallstone disease in either sex.

Table 10. Serum lipids and the risk of developing symptomatic gallstones.

	Quarter of the distribution				p for
Men	1	2	3	4	trend
Triglycerides					
Cutpoints (mmol/l)	0.30-1.20	1.28-1.80	1.90-2.50	2.60-18.90	
Hazard ratio	1.00	1.37	2.18	2.02	0.013
(95% CI)	-	(0.70-2.68)	(1.14-4.15)	(1.03-3.98)	
Total cholesterol					
Cutpoints (mmol/l)	2.05-5.30	5.35-6.0	6.05-6.70	6.75-15.10	
Hazard ratio	1.00	0.92	0.70	0.68	0.145
(95% CI)	-	(0.55-1.62)	(0.38-1.30)	(0.36-1.25)	
HDL-cholesterol					
Cutpoints (mmol/l)	0.20-1.00	1.10-1.20	1.30-1.40	1.49-3.20	
Hazard ratio	1.00	0.87	0.35	0.22	<0.001
(95% CI)	-	(0.54-1.40)	(0.17-0.72)	(0.09-0.52)	
LDL-cholesterol					
Cutpoints (mmol/l)	0.50-3.28	3.29-3.87	3.88-4.51	4.52-8.66	
Hazard ratio	1.00	1.47	0.89	1.05	0.686
(95% CI)	-	(0.81-2.63)	(0.64-1.72)	(0.56-1.95)	
Women	1	2	3	4	
Triglycerides					
Cutpoints (mmol/l)	0.19-1.00	1.09-1.40	1.50-2.00	2.05-26.00	
Hazard ratio	1.00	1.97	2.40	2.43	<0.001
(95% CI)	-	(1.24-3.15)	(1.52-3.80)	(1.52-3.90)	
Total cholesterol					
Cutpoints (mmol/l)	2.60-5.40	5.5-6.20	6.30-7.0	7.10-18.00	
Hazard ratio	1.00	1.13	1.29	1.14	0.483
(95% CI)	-	(0.72-1.69)	(0.81-1.91)	(0.70-1.72)	
HDL-cholesterol					
Cutpoints (mmol/l)	0.50-1.10	1.15-1.40	1.50-1.70	1.90-5.90	
Hazard ratio	1.00	0.85	0.82	0.55	0.010
(95% CI)	-	(0.57-1.26)	(0.56-1.20)	(0.36-0.85)	
LDL-cholesterol					
Cutpoints (mmol/l)	0.44-3.24	3.25-3.90	3.91-4.69	4.70-10.30	
Hazard ratio	1.00	1.11	1.43	1.13	0.419
(95% CI)	-	(0.72-1.74)	(0.93-2.18)	(0.72-1.77)	

Hazard ratios were adjusted for age.

Since serum triglycerides and HDL were both found to have significant effects upon the risk of gallstone disease, a further analysis was made stratifying categories of body mass index (BMI). In Table 11, the trend across category hazard ratio has been calculated for increased quarters of serum triglycerides for each category of body mass index. In men, only in those with a BMI <25kg/m2 was there a significant effect (trend HR=1.71, p for trend=0.030). In women no significant effects were found in any BMI category. A similar analysis was performed for HDL and BMI, detailed in Table 12. In men significant trend hazard ratios were found in all categories except in those with a BMI >35 and in women, no significant effects were found.

Table 11. Stratified analysis of serum triglycerides by body mass index category and the trend hazard ratio of developing symptomatic gallstones.

	Quarter of triglycerides distribution				Trend	p for
Men	1	2	3	4	HR	Trend
BMI category						
<25 kg/m^2 Number of cases (controls)	3 (1351)	3 (1086)	4 (628)	4 (399)	1.71	0.030
25 to <30 kg/m^2 Number of cases (controls)	9 (1200)	16 (1546)	19 (1287)	13 (1175)	1.16	0.22
30 to <35 kg/m^2 Number of cases (controls)	1 (139)	2 (282)	4 (306)	4 (367)	1.19	0.57
> 35 kg/m^2 Number of cases (controls)	1 (12)	1 (27)	0 (36)	0 (50)	0.27	0.17
	Quarter of triglycerides distribution				Trend	p for
Women	1	2	3	4	HR	Trend
BMI category						
<25 kg/m^2 Number of cases (controls)	16 (2304)	13 (1435)	17 (1102)	6 (646)	1.11	0.63
25 to <30 kg/m^2 Number of cases (controls)	9 (1042)	22 (1092)	22 (1137)	26 (1174)	1.19	0.19
30 to <35 kg/m^2 Number of cases (controls)	4 (198)	8 (272)	11 (374)	14 (489)	1.03	0.89
> 35 kg/m^2 Number of cases (controls)	0 (35)	4 (70)	4 (115)	5 (163)	1.07	0.82

Hazard ratios were adjusted for age.

Table 12. Stratified analysis of serum HDL by body mass index category and the trend hazard ratio of developing symptomatic gallstones.

	Quarter of HDL distribution				Trend	p for
Men	1	2	3	4	HR	trend
BMI category						
<25 kg/m^2 Number of cases (controls)	4 (766)	9 (848)	1 (800)	0 (1050)	0.50	0.011
25 to <30 kg/m^2 Number of cases (controls)	27 (1801)	16 (1422)	8 (1058)	6 (946)	0.74	0.020
30 to <35 kg/m^2 Number of cases (controls)	7 (500)	4 (308)	0 (158)	0 (128)	0.47	0.092
> 35 kg/m^2 Number of cases (controls)	2 (65)	0 (33)	0 (10)	0 (17)	N/A	N/A
	Quarter of HDL distribution				Trend	p for
Women	1	2	3	4	HR	trend
BMI category						
<25 kg/m^2 Number of cases (controls)	12 (1289)	12 (1024)	14 (1431)	14 (1743)	0.95	0.66
25 to <30 kg/m^2 Number of cases (controls)	31 (1643)	18 (938)	18 (1010)	12 (854)	0.93	0.44
30 to <35 kg/m^2 Number of cases (controls)	21 (590)	5 (299)	9 (281)	2 (163)	0.81	0.21
> 35 kg/m^2 Number of cases (controls)	7 (226)	3 (79)	2 (48)	1 (30)	1.13	0.65

N/A = not able to calculate

Hazard ratios were adjusted for age.

Further analysis was made for serum triglycerides and HDL stratified for categories of alcohol intake. Due to the small numbers in the highest category of alcohol (>21 units per week) this category was combined with those consuming >7 units per week. In Table 13, the trend across category hazard ratio has been calculated for increased quarters of serum triglycerides for categories of alcohol intake. In men, those with an alcohol intake >7 units per week, the trend effect of increased triglycerides was significant (trend HR=1.58, p for trend=0.006) with a greater magnitude of effect in those with zero intake although the result was only of borderline significance (trend HR=1.81, p for trend=0.053). In women no significant effects were found in any of the alcohol categories for serum triglycerides.

A similar analysis was performed for HDL and alcohol, detailed in Table 14. In men, a similar magnitude of effect was found for both 0 to <7 units of alcohol per week (trend HR=0.63, p for trend =0.008) and for >7units per week (trend HR=0.64, p for trend=0.006). In women, only found in those consuming 0 to <7 units per week had a significant effect (HR=0.82, p for trend=0.016).

Table 13. Stratified analysis of serum triglycerides by alcohol category and the trend hazard ratio of developing symptomatic gallstones.

	Quarter of triglyceride distribution				Trend	p for
Men	1	2	3	4	HR	trend
Alcohol category						
0 units/week Number of cases (controls)	2 (234)	1 (267)	2 (203)	6 (215)	1.81	0.053
> 0 to <7 units/wk Number of cases (controls)	10 (1183)	11 (1301)	13 (983)	6 (912)	1.00	0.99
≥ 7 units/wk Number of cases (controls)	2 (1298)	10 (1398)	12 (1073)	9 (867)	1.58	0.006
	Quarter of triglyceride distribution				Trend	p for
Women	1	2	3	4	HR	trend
Alcohol category						
0 units/week Number of cases (controls)	5 (449)	7 (437)	8 (508)	10 (580)	1.20	0.28
> 0 to <7 units/wk Number of cases (controls)	15 (2233)	32 (1756)	36 (1665)	34 (1491)	1.36	0.24
≥ 7 units/wk Number of cases (controls)	7 (609)	5 (470)	6 (399)	4 (294)	1.04	0.86

Hazard ratios were adjusted for age.

Table 14. Stratified analysis of serum HDL by alcohol category and the trend hazard ratio of developing symptomatic gallstones.

	Quarter of HDL distribution				Trend	p for
Men	1	2	3	4	HR	Trend
Alcohol category						
0 units/week Number of cases (controls)	8 (390)	1 (252)	2 (161)	0 (116)	0.48	0.073
> 0 to <7 units/wk Number of cases (controls)	20 (1621)	15 (1226)	3 (801)	2 (722)	0.63	0.008
≥ 7 units/wk Number of cases (controls)	12 (1126)	13 (1140)	4 (1064)	4 (1306)	0.64	0.006
	Quarter of HDL distribution				Trend	p for
Women	1	2	3	4	HR	Trend
Alcohol category						
0 units/week Number of cases (controls)	13 (760)	5 (411)	10 (474)	2 (329)	0.88	0.46
> 0 to <7 units/wk Number of cases (controls)	50 (2442)	28 (1475)	21 (1670)	18 (1558)	0.82	0.016
≥ 7 units/wk Number of cases (controls)	6 (419)	1 (342)	8 (435)	7 (576)	1.05	0.80

Hazard ratios were adjusted for age.

2. Dietary outcomes using 7-day food diaries

Baseline characteristics of the cohort used in the analysis of dietary nutrients

All dietary analyses were made after 10 years of follow-up to reduce the effects of regression dilution bias. From the initial cohort 23 658 (92.3%) who completed the 7-day food diary (7-DFD), after excluding those with a previous history of cholecystectomy or gallstone, 166 women and 82 men developed incident gallstone disease. Not all food diaries from the cohort are currently coded, so a random sample 2 066 women and 1 660 men were used as the comparison population. The total length of exposure over 10 years, was 21 555 person-years for women and 17 050 for men. The baseline characteristics were compared between participants with and without incident gallstone disease and are listed in Table 15 which shows in both genders BMI was higher in cases and controls. In men, cases had a lower alcohol intake. In women, cases were more likely to use HRT.

Table 15. Baseline characteristics of the study population, after 10 years of follow-up, according to incident gallstone disease status.

	Men			Women		
	Incident Disease	Non-incident disease	p value	Incident disease	Non-incident disease	p value
Number	82	1 660		164	2 066	
Age at recruitment (years, mean (SD))	64.0 (8.4)	59.5 (9.3)	<0.0001	60.0 (8.9)	58.7 (9.4)	0.006
Body mass index (kg/m2, mean (SD))	27.7 (2.9)	26.5 (3.3)	0.0011	27.9 (4.5)	26.1 (4.2)	<0.0001
Alcohol intake (units/wk, median (IQR))	5.0 (1.5-10.5)	6.0 (2-14.0)	0.044	2.0 (0.5-5.8)	2.5 (0.5-7.0)	0.11
Physical activity index score			0.055			0.094
Inactive	40.2%	30.4%		35.4%	29.0%	
Moderately inactive	26.8%	23.2%		34.8%	32.9%	
Moderately active	19.5%	23.3%		21.3%	22.5%	
Active	13.4%	23.2%		8.5%	15.6%	
Parity category						0.11
0 children	-	-		7.9%	14.0%	
1-2 children	-	-		56.7%	54.7%	
≥3 children	-	-		35.4%	31.3%	
Hormone Replacement Therapy use						0.028
Never taken	-	-		59.9%	68.9%	
Former user	-	-		15.9%	12.2%	
Current used	-	-		26.2%	18.9%	

SD=standard deviation, IQR=interquartile range

Total energy intake and macronutrient

Quintiles of total energy intake and the dietary macronutrients, fat, protein and carbohydrate, had no association with symptomatic gallstone disease after 10 years in either men or women (Table 16 and Table 17).

Fatty acid classes

None of the naturally occurring fatty acid classes (saturated, monounsaturated and polyunsaturated fatty acids) were associated with gallstone disease in men (Table 18), or women (Table 19) after 10 years of follow up. However, trans-fats were associated with an increased risk of gallstones in women, but not in men. In women, the highest quintile of trans-fat intake compared to the lowest intake had a HR of 1.94 (95% CI=1.06-3.54) with the trend across fifths, HR=1.16 95% CI=1.00-1.33, p=0.051) (Table 19).

Cholesterol

In women, increased dietary cholesterol was associated with a reduced risk of gallstone disease after 10 years of follow-up. The highest quintile of cholesterol intake compared to the lowest had a HR of 0.56 (95% CI=0.35-0.99) with a significant trend across fifths (HR=0.86 95% CI=0.76-0.97, p=0.015) (Table 19).

Table 16. Total energy and macronutrient intake and the risk of developing symptomatic gallstones in men.

	Quintile				
Men	1	2	3	4	5
Number of participants	350	350	350	350	350
Total energy intake					
Cut points (kcal/day)	322 to 1813	1814 to2111	2112 to 2337	2339 to 2640	2643 to 6050
Cases	22	19	14	12	15
HR (95% CI)[1]	1.00	0.86 (0.47-1.62)	0.71 (0.36-1.39)	0.64 (0.31-1.29)	0.97 (0.49-1.91)
HR (95% CI)[2]	1.00	0.93 (0.50-1.73)	0.81 (0.41-1.60)	0.75 (0.37-1.53)	1.19 (0.60-2.39)
Total fat intake					
Cut points (grams/day)	16 to 65	65 to 77	78 to 90	91 to 107	107 to 340
Cases	22	18	12	16	14
HR (95% CI)[1]	1.00	0.80 (0.43-1.49)	0.58 (0.29-1.17)	0.81 (0.43-1.55)	0.81 (0.41-1.61)
HR (95% CI)[2]	1.00	0.75 (0.37-1.53)	0.58 (0.23-1.42)	0.79 (0.30-2.07)	0.61 (0.19-1.98)
Total carbohydrate					
Cut points (grams/day)	20 to 215	216 to 253	254 to 286	287 to 330	330 to 647
Cases	21	15	16	18	12
HR (95% CI)[1]	1.00	0.68 (0.35-1.31)	0.80 (0.42-1.53)	1.00 (0.53-1.89)	0.69 (0.34-1.42)
HR (95% CI)[2]	1.00	0.74 (0.35-1.55)	0.91 (0.39-2.13)	1.08 (0.41-2.83)	0.62 (0.19-2.00)
Total protein					
Cut points (grams/day)	26 to 67	67 to 77	77 to 85	85 to 95	96 to 175
Cases	17	24	8	15	18
HR (95% CI)[1]	1.00	0.68 (0.35-1.31)	0.80 (0.42-1.53)	1.00 (0.53-1.89)	0.69 (0.34-1.42)
HR (95% CI)[2]	1.00	1.50 (0.77-2.90)	0.59 (0.24-1.48)	1.17 (0.51-2.73)	1.56 (0.63-3.87)

HR=hazard ratio, CI=confidence interval.

[1] Adjusted for age at recruitment.

[2] Adjusted for age and categories of BMI, physical activity, alcohol and quintiles of total energy intake.

Table 17. Total energy and macronutrient intake and the risk of developing symptomatic gallstones in women.

	Quintile				
Women	1	2	3	4	5
Number of participants	449	449	449	449	449
Total energy intake					
Cut points (kcal/day)	588 to 1373	1373 to 1598	1598 to 1782	1782 to 2020	2020 to 3527
Cases	37	33	31	26	37
HR (95% CI)[1]	1.00	0.90 (0.57-1.44)	0.84 (0.52-1.36)	0.75 (0.46-1.23)	1.09 (0.69-1.72)
HR (95% CI)[2]	1.00	1.02 (0.63-1.64)	0.94 (0.58-1.51)	0.86 (0.52-1.44)	1.31 (0.82-2.09)
Total fat intake					
Cut points (grams/day)	13 to 48	49 to 59	59 to 68	69 to 80	80 to 176
Cases	42	23	29	29	41
HR (95% CI)[1]	1.00	0.53 (0.32-0.88)	0.69 (0.43-1.10)	0.70 (0.43-1.11)	1.06 (0.69-1.63)
HR (95% CI)[2]	1.00	0.54 (0.31-0.95)	0.78 (0.42-1.44)	0.88 (0.43-1.80)	1.35 (0.60-3.04)
Total carbohydrate					
Cut points (grams/day)	58 to 168	168 to 199	199 to 224	224 to 257	258 to 418
Cases	39	25	36	35	29
HR (95% CI)[1]	1.00	0.65 (0.40-1.08)	0.97 (0.61-1.52)	0.92 (0.59-1.46)	0.78 (0.48-1.26)
HR (95% CI)[2]	1.00	0.67 (0.38-1.18)	1.02 (0.55-1.92)	0.91 (0.45-1.86)	0.62 (0.27-1.42)
Total protein					
Cut points (grams/day)	24-54	54-61	61-68	69-76	77-145
Cases	32	49	22	30	31
HR (95% CI)[1]	1.00	0.68 (0.35-1.31)	0.80 (0.42-1.53)	1.00 (0.53-1.89)	0.69 (0.34-1.42)
HR (95% CI)[2]	1.00	1.50 (0.94-2.41)	0.64 (0.36-1.15)	0.79 (0.44-1.41)	0.73 (0.39-1.37)

HR=hazard ratio, CI=confidence interval.

[1] Adjusted for age.

[2] Adjusted for age and categories of BMI , alcohol, physical activity, HRT use, parity and quintiles of total energy intake.

Table 18. Fatty acid class and cholesterol intake and the risk of developing symptomatic gallstones in men.

	Quintile				
Men	1	2	3	4	5
Number of participants	350	350	350	350	350
Total energy intake					
Cut points (kcal/day)	322 to 1813	1814 to 2111	2112 to 2337	2339 to 2640	2643 to 6050
Cases	22	19	14	12	15
HR (95% CI)[1]	1.00	0.86 (0.47-1.62)	0.71 (0.36-1.39)	0.64 (0.31-1.29)	0.97 (0.49-1.91)
HR (95% CI)[2]	1.00	0.93 (0.50-1.73)	0.81 (0.41-1.60)	0.75 (0.37-1.53)	1.19 (0.60-2.39)
Total fat intake					
Cut points (grams/day)	16 to 65	65 to 77	78 to 90	91 to 107	107 to 340
Cases	22	18	12	16	14
HR (95% CI)[1]	1.00	0.80 (0.43-1.49)	0.58 (0.29-1.17)	0.81 (0.43-1.55)	0.81 (0.41-1.61)
HR (95% CI)[2]	1.00	0.75 (0.37-1.53)	0.58 (0.23-1.42)	0.79 (0.30-2.07)	0.61 (0.19-1.98)
Total carbohydrate					
Cut points (grams/day)	20 to215	216 to 253	254 to 286	287 to 330	330 to 647
Cases	21	15	16	18	12
HR (95% CI)[1]	1.00	0.68 (0.35-1.31)	0.80 (0.42-1.53)	1.00 (0.53-1.89)	0.69 (0.34-1.42)
HR (95% CI)[2]	1.00	0.74 (0.35-1.55)	0.91 (0.39-2.13)	1.08 (0.41-2.83)	0.62 (0.19-2.00)
Total protein					
Cut points (grams/day)	26 to 67	67 to 77	77 to 85	85 to 95	96 to 175
Cases	17	24	8	15	18
HR (95% CI)[1]	1.00	0.68 (0.35-1.31)	0.80 (0.42-1.53)	1.00 (0.53-1.89)	0.69 (0.34-1.42)
HR (95% CI)[2]	1.00	1.50 (0.77-2.90)	0.59 (0.24-1.48)	1.17 (0.51-2.73)	1.56 (0.63-3.87)

HR=hazard ratio, CI=confidence interval.

[1] Adjusted for age.

[2] Adjusted for age and categories of BMI, physical activity and alcohol.

Table 19. Fatty acid class and cholesterol intake and the risk of developing symptomatic gallstones in women.

	Quintile					
Women	1	2	3	4	5	
Number of participants	449	449	449	449	449	
Saturated fats						
Cut points (grams/day)	3.6 to 17.3	17.4 to 21.5	21.6 to 25.8	25.9 to 31.7	31.7 to 84.4	
Cases	41	28	24	34	37	
HR (95% CI)[1]	1.00	0.66 (0.42-1.05)	0.52 (0.31-0.85)	0.84 (0.55-1.30)	0.92 (0.60-1.40)	
HR (95% CI)[2]	1.00	0.62 (0.38-1.04)	0.57 (0.28-0.92)	0.84 (0.46-1.47)	0.87 (0.44-1.63)	
Monounsaturated fats						
Cut points (grams/day)	3.7 to 16.5	16.5 to 20.3	20.3 to 23.9	23.9 to 27.9	27.9 to 59.4	
Cases	41	18	40	23	42	
HR (95% CI)[1]	1.00	0.44 (0.26-0.74)	0.94 (0.62-1.43)	0.52 (0.31-0.86)	1.16 (0.77-1.74)	
HR (95% CI)[2]	1.00	0.46 (0.26-0.82)	1.18 (0.64-1.92)	0.68 (0.31-1.22)	1.31 (0.69-2.74)	
Polyunsaturated fats						
Cut points (grams/day)	1.9 to 8.7	8.7 to 10.7	10.8 to 12.8	12.9 to 15.8	15.9 to 39.6	
Cases	42	26	26	31	39	
HR (95% CI)[1]	1.00	0.61 (0.38-0.97)	0.64 (0.40-1.02)	0.74 (0.47-1.16)	1.02 (0.67-1.56)	
HR (95% CI)[2]	1.00	0.60 (0.37-0.99)	0.64 (0.39-1.10)	0.82 (0.44-1.32)	1.05 (0.59-1.90)	
***Trans* fats**						
Cut points (grams/day)	0.2 to 1.6	1.6 to 2.1	2.1 to 2.6	2.6 to 3.3	3.3 to 9.6	
Cases	33	28	31	27	45	
HR (95% CI)[1]	1.00	0.84 (0.51-1.38)	0.96 (0.59-1.56)	0.81 (0.49-1.34)	1.40 (0.49-2.19)	
HR (95% CI)[2]	1.00	0.96 (0.58-1.61)	1.17 (0.68-2.00)	1.07 (0.60-1.91)	1.94 (1.06-3.54)	*
Dietary cholesterol						
Cut points (mg/day)	2.1 to 119	120 to 153	154 to 188	189 to 233	233 to 684	
Cases	40	36	34	24	30	
HR (95% CI)[1]	1.00	0.90 (0.57-1.41)	0.84 (0.53-1.32)	0.57 (0.35-0.95)	0.76 (0.47-1.21)	
HR (95% CI)[2]	1.00	0.82 (0.52-1.30)	0.73 (0.46-1.18)	0.54 (0.32-0.91)	0.59 (0.35-0.99)	**

HR=hazard ratio, CI=confidence interval, mg=milligram.

*p for trend=0.05, **p for trend=0.015

[1] Adjusted for age.
[2] Adjusted for age, BMI category, physical activity category, alcohol category, HRT use, parity and quintiles of total energy intake.

Iron

In women, after 10 years of follow-up, increased dietary iron was associated with a reduced risk of developing symptomatic gallstones with the highest quintile of intake vs the lowest HR=0.35 (95% CI=0.19-0.66) with a significant trend across fifths (HR=0.82, 95% CI=0.71-0.94, p=0.004) (Table 21). No effect was found for dietary iron intake in men (Table 20).

Niacin

Increased dietary niacin was associated with a reduced risk of gallstone disease in women, for each of the four higher quintiles of niacin intake ($p<0.05$) (Table 21). The highest quintile of niacin intake compared to the lowest, had a hazard ratio of 0.54 (95% CI=0.32-0.90) with a significant trend across fifths (HR=0.86, 95% CI=0.76-0.97, p=0.004). Since the effect sizes were similar for each of the four higher intake of niacin, this could suggest that there could be a threshold effect for dietary niacin, with women in the lowest fifth of intake at a higher risk of disease. In men, there were negative associations with each of the four higher quintiles of dietary niacin intake, though none were statistically significant (Table 20) and no effect across quintiles HR=0.97(95% CI=0.81-1.16, p=0.70).

Fibre

In women, after 10 years of follow-up, there was a negative association between the four higher quintiles of dietary fibre and symptomatic gallstone disease, however, none of the quintiles reached statistical significance (Table 21) and the trend across categories was also non-significant (HR=0.91, 95% CI=0.81-1.03, p=0.14). In men, there was no association between fibre intake and disease (Table 20).

Dietary calcium

Dietary calcium was associated with an increased risk of gallstone disease in men after 10 years of follow-up, with the highest quintile of intake vs the lowest HR=2.31 (95% CI=1.00-5.35) and the trend across categories HR=1.25 (95% CI=1.03-1.52, p=0.023) (Table 20). In women, no effects were found for dietary calcium intake (Table 21).

Table 20. Dietary iron, niacin, fibre and calcium intake and the risk of developing symptomatic gallstones in men.

	Quintile					
Men	1	2	3	4	5	
Number of participants	350	350	350	350	350	
Dietary iron						
Cut points (mg/day)	3.4 to 10.2	10.2 to 11.9	11.8 to 13.8	13.8 to 16.1	16.1 to 42.2	
Cases	22	21	15	16	16	
HR (95% CI)[1]	1.00	0.90 (0.48-1.69)	0.67 (0.33-1.33)	0.87 (0.45-1.70)	0.86 (0.44-1.67)	
HR (95% CI)[2]	1.00	0.98 (0.50-1.90)	0.70 (0.33-1.51)	1.00 (0.46-2.17)	0.97 (0.44-2.15)	
Dietary niacin						
Cut points (mg/day)	5.6 to 15.9	15.9 to 19.1	19.2 to 22.2	22.2 to 26.1	26.1 to 60.1	
Cases	25	16	19	17	13	
HR (95% CI)[1]	1.00	0.69 (0.36-1.32)	0.94 (0.50-1.76)	0.80 (0.41-1.55)	0.72 (0.35-1.48)	
HR (95% CI)[2]	1.00	0.75 (0.37-1.53)	0.58 (0.23-1.42)	0.79 (0.30-2.07)	0.61 (0.19-1.98)	
Dietary fibre						
Cut points (grams/day)	3.6 to 11.4	11.4 to 13.8	13.9 to 16.5	16.5 to 20.0	20.1 to 61.2	
Cases	20	16	19	20	15	
HR (95% CI)[1]	1.00	0.64 (0.31-1.32)	1.03 (0.54-1.97)	1.08 (0.57-2.07)	0.91 (0.46-1.80)	
HR (95% CI)[2]	1.00	0.64 (0.31-1.35)	1.11 (0.55-2.22)	1.16 (0.58-2.31)	1.01 (0.48-2.13)	
Dietary calcium						
Cut points (mg/day)	152 to 677	677 to 816	817 to 963	963 to 1152	1153 to 2788	
Cases	18	18	11	22	21	
HR (95% CI)[1]	1.00	1.08 (0.54-2.16)	0.71 (0.32-1.57)	1.48 (0.75-2.88)	1.63 (0.83-3.20)	*
HR (95% CI)[2]	1.00	1.29 (0.62-2.67)	0.92 (0.39-2.15)	2.12 (0.97-4.61)	2.31 (1.00-5.35)	**

HR=hazard ratio, CI=confidence interval, mg=milligram.

*p for trend=0.089, **p for trend=0.023

[1]Adjusted for age.

[2]Adjusted for age and categories of BMI, physical activity, alcohol intake and quintiles of energy intake.

Table 21. Dietary iron, niacin, fibre and calcium intake and the risk of developing symptomatic gallstones in women.

	Quintile					p for
Women	1	2	3	4	5	trend
Number of participants	350	350	350	350	350	
Dietary iron						
Cut points (mg/day)	1.9 to 8.3	8.3 to 9.7	9.7 to 11.2	11.2 to 13.2	13.2 to 39.5	
Cases	47	38	32	40	21	
HR (95% CI)[1]	1.00	0.81 (0.52-1.26)	0.69 (0.43-1.10)	0.85 (0.55-1.32)	0.44 (0.25-0.76)	0.012
HR (95% CI)[2]	*□*□*0.7	0.72 (0.45-1.16)	0.59 (0.35-1.00)	0.68 (0.46-1.14)	0.35 (0.19-0.66)	0.004
Dietary niacin						
Cut points (mg/day)	1.8 to 12.9	12.9 to 15.5	15.5 to 17.9	17.9 to 21.0	21.1 to 44.8	
Cases	51	35	29	34	29	
HR (95% CI)[1]	1.00	0.81 (0.52-1.26)	0.69 (0.43-1.10)	0.85 (0.55-1.32)	0.44 (0.25-0.76)	0.033
HR (95% CI)[2]	1.00	0.72 (0.45-1.16)	0.59 (0.35-1.00)	0.68 (0.46-1.14)	0.35 (0.19-0.66)	0.014
Dietary fibre Cut points (grams/day)	1.3 to 10.3	10.2 to 12.5	12.5 to 14.7	14.7 to 17.6	17.6 to 51.4	
Cases	49	31	39	26	33	
HR (95% CI)[1]	1.00	0.71 (0.45-1.13)	0.85 (0.54-1.32)	0.62 (0.38-1.00)	0.71 (0.45-1.14)	0.12
HR (95% CI)[2]	1.00	0.70 (0.44-1.13)	0.83 (0.52-1.31)	0.63 (0.37-1.05)	0.68 (0.41-1.13)	0.14
Dietary calcium						
Cut points (mg/day)	120 to 588	558 to 680	681 to 800	800 to 943	943 to 2871	
Cases	32	45	38	32	31	
HR (95% CI)[1]	1.00	1.35 (0.84-2.14)	1.13 (0.70-1.84)	0.91 (0.55-1.52)	1.00 (0.61-1.66)	0.47
HR (95% CI)[2]	1.00	1.40 (0.85-2.29)	1.21 (0.71-2.06)	0.92 (0.52-1.66)	0.97 (0.53-1.77)	0.44

HR=hazard ratio, CI=confidence interval, mg=milligram.

[1]Adjusted for age.

[2]Adjusted for age and categories of BMI, physical activity, alcohol intake and quintiles of energy intake.

Coffee and tea

Caffeinated coffee was associated with a decreased risk of gallstone disease in men with three or more cups a day compared to zero intake associated with a 57% reduced risk (HR=0.43, 95% CI=0.22-0.83) (Table 22). There was a significant effect for each additional cup drank per day (HR=0.77 95% CI=0.62-0.95, p=0.013). No effects were found for caffeinated tea, decaffeinated coffee in men and women and also no effect was found for caffeinated coffee in women (Table 23).

Table 22. Caffeinated and decaffeinated coffee and caffeinated tea and the risk of symptomatic gallstones in men.

	Cups per day				
Men	0 (none)	1 (<250mls/day)	2 (250-500mls/day)	3+ (>500mls/day)	
Caffeinated coffee					
Number of participants	438	518	342	453	
% of cohort	25.0%	29.5%	19.5%	26.0%	
Number of P-Y	4 809	5 864	3 902	5 242	
Number of cases	34	25	16	15	
HR (95% CI)[1]	1.00	0.61 (0.35-1.04)	0.63 (0.34-1.17)	0.44 (0.23-0.85)	*
HR (95% CI)[2]	1.00	0.60 (0.35-1.04)	0.61 (0.33-1.14)	0.43 (0.22-0.83)	**
Caffeinated tea					
Number of participants	176	138	245	1 196	
% of cohort	10.0%	7.9%	14.0%	68.1%	
Number of P-Y	1 982	1 544	2 767	13 524	
Number of cases	5	8	11	66	
HR (95% CI)[1]	1.00	1.78 (0.56-5.60)	1.18 (0.39-3.51)	1.58 (0.64-3.95)	
HR (95% CI)[2]	1.00	1.75 (0.55-5.51)	1.15 (0.38-3.44)	1.58 (0.63-3.96)	
Decaffeinated coffee		>30mls/day			
Number of participants	1 511	244			
% of cohort	86.1%	13.9%			
Number of P-Y	17 056	2 761			
Number of cases	79	11			
HR (95% CI)[1]	1.00	1.02 (0.54-1.93)			
HR (95% CI)[2]	1.00	1.03 (0.54-1.94)			

P-Y=person years, HR=hazard ratio, CI=confidence interval.

*p for trend=0.015, **p for trend=0.013

[1]Adjusted for age.

[2]Adjusted for age, BMI category, physical activity, alcohol category and quintiles of total energy intake.

Table 23. Caffeinated and decaffeinated coffee and caffeinated tea and the risk of symptomatic gallstones in women.

	Cups per day			
Women	0 (none)	1 (<250mls/day)	2 (250-500mls/day)	3+ (>500mls/day)
Caffeinated coffee				
Number of participants	545	681	438	583
% of cohort	24.3%	30.3%	19.5%	25.9%
Number of P-Y	5 998	7 583	4 887	6 555
Number of cases	51	46	40	41
HR (95% CI)[1]	1.00	0.72 (0.47-1.08)	0.98 (0.64-1.51)	0.79 (0.51-1.21)
HR (95% CI)[2]	1.00	0.78 (0.52-1.19)	1.06 (0.68-1.65)	0.85 (0.55-1.31)
Caffeinated tea				
Number of participants	262	210	309	1 466
% of cohort	11.5%	9.5%	13.8%	65.2%
Number of P-Y	2 885	2 318	3 463	16 357
Number of cases	20	18	21	119
HR (95% CI)[1]	1.00	1.15 (0.60-2.19)	0.82 (0.44-1.55)	0.94 (0.57-1.53)
HR (95% CI)[2]	1.00	1.18 (0.62-2.26)	0.86 (0.46-1.63)	0.95 (0.58-1.57)
Decaffeinated coffee	0		>30mls/day	
Number of participants	1 828		419	
% of cohort	81.4%		18.6%	
Number of P-Y	20 280		4 744	
Number of cases	389		30	
HR (95% CI)[1]	1.00		0.92 (0.62-1.38)	
HR (95% CI)[2]	1.00		0.98 (0.65-1.46)	

P-Y=person years, HR=hazard ratio, CI=confidence interval.

*p for trend=0.015, **p for trend=0.013

[1]Adjusted for age.
[2]Adjusted for age, BMI category, physical activity category, alcohol category, HRT use, parity and quintiles of total energy intake.

Discussion

The epidemiological study of anthropometry, diet, physical activity and lipid biomarkers found that in both men and women, body mass index, waist circumference and serum triglycerides were positively associated with the development of symptomatic gallstone disease, with physical activity and HDL negatively associated. Additionally, in men only, alcohol and caffeinated coffee consumption were negatively associated with the development of disease with increased dietary calcium positively associated. In women only, dietary *trans*-fatty acids were positively associated with disease, with dietary cholesterol, iron and niacin negatively associated. Each of these risk factors will now be discussed.

1. Obesity, physical activity, alcohol use and serum lipids

Obesity

In both men and women, increasing obesity was positively associated with the development of gallstones, with each extra unit of BMI, or additional inch of waist circumference, significantly increasing the risk by 8%. A BMI greater than 25kg/m^2 (overweight or obese) was associated with at least a doubling in risk compared to one less than 25kg/m^2 (normal BMI) with 38% of incident gallstone disease attributable to a BMI greater than 25kg/m^2. There was a trend across categories with both an increased BMI and waist circumference associated with a greater risk of disease. The epidemiological data supports experimental data as obesity has several biological mechanisms contributing to gallstone disease predominantly by promoting hyperinsulinaemia, insulin resistance and dyslipidaemia. These pathophysiological changes lead to increased biliary cholesterol concentration, greater mucin secretion and gallbladder hypomotility, all of which are important processes in gallstone formation.

Our results support the findings from many other studies that obesity increases the risk of gallstone disease, including the largest prospective studies conducted in US cohorts. The Nurses' Health study initially evaluated the effect of BMI on the risk of newly diagnosed symptomatic gallstone disease in 90 302 women aged 34-59 years.[60] After 8 years of follow-up, 2 122 cases were diagnosed and the multivariate analysis, a BMI≥35 compared to a BMI<24 had a RR of gallstone disease of 4.64 (95% CI=3.86-5.57). In EPIC-Norfolk, our results were

similar, with a BMI≥35 vs <25 associated with a HR of 3.60 (95% CI=2.11-6.11). The Nurses' Health later reported the effect of waist circumference on the risk of cholecystectomy in 42 312 women, followed-up for 14 years, in which 3 197 cases were identified. A waist circumference of ≥36 inches vs <26 inches had a multivariable RR of 3.40 (95% CI 2.84-4.07)[80], a result of similar magnitude to that obtained in women this study (≥36 inches vs <28 inches, HR=2.77 95% CI 1.56-4.89). However, the rate of gallstone disease in Nurses' Health study was 6.2 cholecystectomies per 1000 person years (PYs) compared to 1.3 cases of symptomatic gallstone disease per 1000 PYs in females in EPIC-Norfolk, which highlights the potential differences in the populations studied, and the importance of verifying that waist circumference is also significant risk factor in a UK population. In studies of men, the US Health Professional Follow-up Study identified 1 117 incident cases of gallstone disease, in a cohort of 51 529 participants, and assessed the effects of both BMI and waist circumference.[79] Those with a BMI≥28.5 compared to a BMI<22.2 had a multivariate RR=2.30 (95% CI=1.76-3.00) which are similar results from men in EPIC-Norfolk (BMI≥35 vs BMI<25 HR=2.62 95% CI=0.60-11.42). The Health Professional Follow-up Study analysis of waist circumference reported a waist circumference of >40.4 inches compared to <34 inches had a multivariate RR=2.45 (95% CI=1.94-3.11) which again was of similar magnitude to that found in men in EPIC-Norfolk (≥42 inches vs <34 inches, HR=3.40 95% CI 1.23-9.37).

This is the first UK prospective study to quantify the effect of obesity on the risk of developing gallstone disease. By demonstrating that each unit of BMI, or additional inch of waist circumference, increases the risk of gallstone disease by 8%, it provides a simple and concise measure to enable public health planning to prevent the burden of disease. The calculated population attributable fraction from EPIC-Norfolk, estimates that 38% of symptomatic gallstone disease are due to a BMI over 25. The evidence supports a causal role for obesity in gallstone disease, since experimental evidence and aetiological work consistently report large effect sizes, with a dose effect.

Physical activity

This study reported after 5 years of follow-up and adjusting for covariates, the highest level of physical activity was associated with a 82% reduced risk of developing symptomatic gallstones in men, with a 66% reduced risk in women.

After 14 years of follow-up the negative association remained, but of smaller magnitude and not statistically significant. The amount of physical activity in the highest category is equivalent to either exercising for one hour a day if employed in a sedentary job, exercising for 30 minutes a day if working in a standing job, or finally a heavy manual job without any additional activity (Table 1). There are plausible biological mechanisms to explain the protective effect found with regular exercise increasing HDL and reducing plasma triglycerides.[104, 256] Both these lipid changes are inversely associated with the prevalence of gallstones.[88] Exercise also decreases biliary cholesterol,[74], mucus secretion,[75] and improves gallbladder motility,[110] all of which prevent gallstone formation.

The finding of a significant reduction in risk after five years of follow-up, but not after the full follow-up period, may be attributable to regression dilution bias. The study used a single measure of physical activity, taken at recruitment, although repeated assessments to account for variation over time would give more accurate data. Prospective studies which analyse disease rates from just one initial baseline survey of a risk factor generally underestimate the real associations of disease after longer periods of follow up [257]. This random measurement error occurs as some of the population will change their level of physical activity over time. If this happens in both those who may become cases or non-cases there is an under-estimate of the true association. Physical activity is likely to vary over time, particularly as a population ages. Hence, it is likely that the analysis after five years, rather than fourteen years of follow-up, gives a more accurate assessment between physical activity and incident gallstone disease from baseline data.

The protective effect of physical activity is supported by work from five other aetiological prospective cohort studies which demonstrated an inverse association with gallstones [111-115]. However, none of these used a physical activity questionnaire validated against physiological parameters which is a more accurate assessment of physical activity. A study of 7 831 American men of Japanese ancestry [111], reported a relative risk (RR) between the highest and lowest quartiles of physical activity of 0.7 (95% CI=0.6-1.0). In the US Health Professionals Follow-Up Study of 45 813 men [112], the RR of gallstone disease between the highest and lowest quintiles of physical activity was 0.63 (95% CI=0.51-0.79). Similarly, in the US Nurses' Health Study of 60 290 women [113], those in the highest quintile of activity had a RR of cholecystectomy of 0.69 (95% CI=0.61-0.78). A US study of 8 010 postmenopausal women [114] reported participants in the two lowest quartiles of physical activity had an odds ratio (OR) of 1.59 (95% CI=1.11-2.29) and 1.57 (95% CI=1.11-2.23) of developing gallstones compared

with the highest quartile. The same study randomised 182 women to a walking intervention programme with follow up for 14 years. Women in the lowest tertile of physical activity had a 13% higher risk of developing gallstones (OR=1.13, 95% CI=1.01-1.28). Two other prospective studies failed to demonstrate associations, although they had significant methodological weaknesses including the use of an unvalidated method of recording physical activity and a prolonged follow-up period of up to 50 years.[85, 258]

The results from EPIC-Norfolk and previous studies demonstrate that physical activity reduces the risk of developing symptomatic stones. A prospective study using trans-abdominal ultrasonography helped to clarify whether physical activity exerted this effect by reducing symptoms or by preventing stone formation.[115] A cohort of 2 130 American Indian men and women, a population with a high risk of developing gallstones, underwent ultrasonography examination at baseline to exclude prevalent gallstones and then repeated the ultrasonography after four years of follow-up. Physical activity was recorded at baseline using an questionnaire assessing leisure and occupational activity.The authors found that 650 participants developed gallstones which were either silent or symptomatic. The median baseline physical activity levels were lower in both women ($p<0.01$) and men ($p<0.10$) with new gallstone formation. These results suggest that exercise reduces gallstone formation rather than reducing symptoms from existing stones. In summary, data from experimental and aetiological work suggests that increased physical activity reduces gallstones by a causal mechanism. The results in epidemiological studies are consistent and with large effect sizes with a dose response.

Alcohol

We detected inverse association between increased alcohol intake and symptomatic gallstone disease in men, but not women. In men, each additional unit of alcohol (10mls or 7.9grams) significantly reduced the risk of symptomatic gallstones by 3% and there was a significant trend across categories (HR=0.82 95% CI 0.68-1.00, p=0.044) although no individual categories reached statistical significance. Larger numbers of cases and controls in each category may have allowed detection of a small effect. There are several biological mechanisms which could account for the protective effect of alcohol. Alcohol stimulates cholecystokinin release[116] and gut motility[117] which prevents biliary stasis and

cholesterol crystal aggregation.[32] Alcohol also increases serum HDL levels [118-120] by reducing cholesteryl ester transfer protein (CETP) activity which prevents the conversion of HDL into LDL,[121] with increased HDL leading to reduced cholesterol concentration in the bile.[74]

Prospective cohort studies from the United States have reported inverse associations between alcohol intake and gallstones in the Health Professional Follow-Up Study of men (1.9-3.8 units/day alcohol vs no intake RR=0.75, 95% CI=0.60-0.93)[124] and the Nurses' Health Study in women (1.9-3.8 units/day alcohol vs no intake RR=0.80, 95% CI=0.72-0.89).[125] The Atherosclerosis Risk in Communities (ARIC) Study involving 12 773 men and women from four different US states (North Carolina, Minnesota, Mississippi and Maryland) who were either white (9478 participants) or African American (3295) reported a negative association in women (>7 drinks/week RR=0.53, 95% CI=0.3-0.9), although no effect in men.[88] The Italian MICOL study used ultrasonography in 14 272 men and identified 787 participants with asymptomatic gallstones (hence avoiding protopathic bias) which found a protective effect of alcohol in men (chi-square=10.9, p=0.001). However, in 11 850 females of which 1 014 had silent gallstones, alcohol was not significantly protective (chi-square=1.4, p=0.24).[169] Our study also failed to detect an effect of alcohol in women which is a finding replicated in two other case-control studies.[103, 205] The lack of effect in women in this study could be due to the lower rates of alcohol consumption in women with only 21.5% drinking more than 7 units (56g) of alcohol a week compared to 46.4% of men. Therefore, a lack of subjects and hence power may cause a small inverse association to be undetected. Also, we used a single food-frequency questionnaire at recruitment to estimate alcohol intake, unlike the US Nurses' Health Study which used repeated assessments of alcohol intake during follow-up. A single recording of alcohol intake will lead to measurement error and an underestimation of any effects, if one is present, which may explain the lack of effect found in women.

There is accumulating evidence to support a causal role for alcohol preventing gallstones, including biochemical and experimental data, although its effect size is likely to be modest. However, before it can be deemed a causal factor, the inconsistency of reports needs to be clarified. This could be achieved by using more accurate assessments of alcohol intake in future aetiological work to reduce measurement error, as well as the larger cohort sizes.

Lipids

The three risk factors previously discussed, namely obesity, physical activity and alcohol may all exert their effects through alteration of the lipid profile. Raised triglycerides could influence gallstone formation by increasing biliary cholesterol saturation[73] and mucin production[75] and decreasing gallbladder motility[90] all of which increase the risk of gallstone formation. Increased serum HDL may prevent gallstone formation as it is the major source of cholesterol for biliary secretion with a much larger fraction secreted in the form of bile acids compared to that of LDL[94] leading to reduced biliary cholesterol saturation.[74] This study is the largest prospective study evaluating serum lipids and the risk of gallstone disease and reported that raised serum high density lipoprotein-cholesterol (HDL) and decreased triglycerides were associated with a reduced risk of symptomatic gallstone disease.

In the stratified analysis, an elevated serum triglyceride increased the risk of gallstones only in men with a BMI <25kg/m2, with no effects in women, suggesting an increased BMI may not exert its effects via elevation of triglycerides. The stratified analysis of BMI and HDL showed a decreased risk of gallstone disease with increased serum HDL in all BMI categories for both men and women which neither supports nor contradicts the theory that obesity increases gallstone risk by suppressing serum HDL. In the stratified analysis of alcohol intake and triglycerides, men consuming the most alcohol (>7 units/week) with high triglycerides had the greatest risk of gallstone disease suggesting that alcohol may predispose some the gallstone disease by elevating serum triglycerides. In the stratified analysis of alcohol intake and HDL, in men, a similar protective effect of increased HDL was found in those consuming alcohol, suggesting that alcohol could protect against gallstone disease by increasing HDL.

There are two previous prospective cohort studies that have evaluated the relationship between gallstone disease and serum lipids, the largest being the Atherosclerosis Risk in Communities (ARIC) Study in the US of 12 773 men and women[88] which is similar to our work in Norfolk and found the same trends and magnitudes of associations. Elevated HDL was negatively associated with gallstone disease in men (highest vs lowest quarter HDL, RR=0.42, 95% CI=0.3-0.7) and in women (highest vs lowest quarter HDL, RR=0.64, 95% CI=0.5-0.9). Raised triglycerides were positively associated with disease in men (highest vs lowest quartile RR=1.65, 95% CI=1.0-2.7) and in women (highest vs lowest quartile RR=2.57, 95% CI=1.7-3.9). An Italian study evaluated the gallbladder

with ultrasonography at enrolment and repeated it 10 years later to assess for new gallstone formation.[81] Here 232 men and 253 women were identified with new incident gallstone disease. In men there was a negative association with HDL cholesterol (regression coefficient (RE)= -0.0118, $p<0.040$) and total cholesterol (RE= -0.0034, $p<0.030$) and a positive association with triglycerides (RE= 0.0004, $p<0.007$). In women no associations were found with any of the serum lipids. A limitation of the lipids data was the use of non-fasting blood sample to measure serum lipids. Serum HDL and total cholesterol are not significantly different in the fasting and non-fasting state.[259] However, serum triglycerides remain elevated for several hours after eating and the calculation of serum LDL is dependent on serum triglycerides and hence for accurate measurement of these two lipids a 12-hour fast is recommended.[259] The lack of fasting samples in EPIC-Norfolk would cause measurement error in serum triglyceride and LDL level with an underestimate of the magnitude of effects found. Overall, consistent findings from the previous American and Italian studies, and now the largest prospective study to be conducted, helps to support a role for the dyslipidaemia found in obesity and metabolic syndrome in the aetiology of gallstones. These findings are consistent with the epidemiological associations we found with obesity, physical activity and alcohol and may explain the route through which they act.

2. Diet

The results from this study are the first to be reported in a prospective investigation using 7-day food diaries to measure dietary intake in a large cohort. Diet is a plausible environmental factor to investigate in the aetiology of gallstone disease cancer, with different nutrients having effects on mechanism involved in gallstone formation. When assessing the role of diet in disease there are limitations to an epidemiological studies. Measurement of diet lacks precision and specificity, causing small effect sizes to be difficult to detect. Further measurement error is incurred if the physical attributes of a food are not taken into consideration i.e. cooking style and preparation, freshness etc. which can affect nutrient values. Finally, nutrient intakes are highly correlated, and therefore attribution of causation to one nutrient considered to be acting on its own may be misleading. [260] Each of these limitations should be considered when drawing conclusions from results obtained.

Total energy intake

There was no effect of total energy intake after either age adjustment or in the multi-variate model. There is a lack of plausible biological mechanisms to explain how total energy intake may contribute to gallstone disease beyond a possible contribution to obesity. Our findings are consistent with previous prospective studies which reported mixed findings. Results from the US Nurses' Health study revealed that among the 59 306 women whose BMI was less than 25 kg/m^2, a high energy intake (>8200 J per day), as compared those with a low energy intake (<4730 J per day), was associated with an increased incidence of symptomatic gallstones (RR=2.1; 95% CI=1.4-3.3).[159] However, in those with a BMI>25 energy intake did not increase the risk of developing gallstones. The results were only adjusted for age and alcohol intake with none for physical activity, parity or HRT use and BMI was only defined into two categories. Hence, although this prospective study has been conducted using appropriate methodology, the failure to adjust for many confounders and to find an association in those with a BMI> 25 suggests that the importance of total energy intake is uncertain. A smaller US prospective study of 4 730 women used data recorded in the National Health and Nutrition Examination Survey collected between the year 1971-75, who were then followed-up for 10 years. The authors reported that in women below 50 years of age there was a decreased risk of developing hospitalised

gallstone disease with an increased energy intake (HR 75th percentile vs 25th percentile =0.69 95% CI=0.53-0.88), although no adjustment was made for physical activity.[160] The Italian MICOL study using ultrasonography to assess for asymptomatic gallstone disease found that in men an increased total energy intake was associated with a reduced risk of gallstones (highest vs lowest quintile of intake RR=0.79, 95% CI=0.63-0.99, p for trend =0.004) although no effect was seen in women.[169] The study design of MICOL negated recall and protopathic bias but not adjust was made for physical activity. A variety of cross-sectional and case-control studies have found positive, negative and no associations, although they are limited by study numbers and design.[127, 203-204, 261] Results from this study suggest that total energy intake is not directly associated with the development of gallstone disease after adjusting for known co-variates including obesity and physical activity. This revives the hypothesis that the composition of the diet, rather than the absolute intake of energy, could determine gallstone risk.

Total fat

This work found that total dietary fat was not associated with gallstone disease in either men or women. Previously total dietary fat was thought to contribute to weight gain and hence promote gallstone formation, although it is now recognised that total dietary fat is not a risk factor for weight gain.[162-164] The lack of a plausible biological mechanism for total dietary fat is supported by the epidemiological data which has failed to find a consistent association between this macronutrient and gallstone disease. The largest prospective cohort to assess dietary fat was the Nurses' Health study of 88 837 women, followed-up for 4 years and identified 433 participants undergoing cholecystectomy. The highest vs lowest quintile of fat intake had a RR of cholecystectomy = 0.9 (95% CI=0.7-1.1, p for trend=0.8).[206] Data from other epidemiological studies has also not supported an effect of total dietary fat on gallstone disease. The data from this study, previous epidemiological studies and the lack of supporting experimental evidence suggests that total fat intake is not associated with gallstone disease. However, fat composition and individual fatty acids, rather total dietary fat, need to be considered when reaching conclusions about dietary fat and the risk of developing gallstone disease.

Trans-fatty acids

This study found that an increased dietary intake of *trans*-fatty acids (TFAs) were associated with an increased risk of gallstone disease in women, but not in men. TFAs are known to alter the serum lipid profile, causing raised triglycerides and decreased HDL[191], both of which are associated with an increased risk of gallstone disease. TFAs also increase insulin resistance [185, 188] which is also associated with gallstone disease. Only one previous epidemiological study has been published which has evaluated the association of trans-fatty acids and symptomatic gallstone disease. The US Health Professionals Follow-up Study of 45 912 men monitored for 14 years identified 2 356 new cases of symptomatic gallstone disease.[193] After adjusting for co-variates, men in the highest compared to the lowest quintile had a RR of disease of 1.23 (95% CI=1.04-1.44, p for trend=0.03). The results reported from EPIC-Norfolk for women after 10 years follow-up are of a higher magnitude (HR=1.95, 95% CI 1.06-3.54, p for trend=0.051). The greater effect size in EPIC-Norfolk could be due to the use of 7-day food diaries rather than food frequency questionnaires which were used in the Health Professional study. FFQs have greater measurement error for nutrient intake causing an underestimation of the true effect size. EPIC-Norfolk failed to detect an effect for men, although this might be due to the small number of cases in men. There are consistent results from this work, the Health Professionals Study and experimental investigations that suggest *trans*-fatty acids promote gallstone disease. Measures already undertaken to reduce TFAs consumption in the diet over the past decade could therefore lead to a fall in gallstone incidence and the industrial eradication of TFAs from the diet should be encouraged. Further research into this area is may be difficult to undertake due to lower levels of TFA in the diet and ethical issues concerning the introduction of TFAs into the diet.

Dietary cholesterol

This study found that the highest quintile of dietary cholesterol intake, after 10 years of follow-up in women, was associated with a 41% (HR=0.59 95% CI=0.35-0.99) reduction in symptomatic gallstone disease (p for trend=0.015). No associations were found in men. Dietary cholesterol may influence gallstone formation through complex regulatory affects in the liver. The conversion of cholesterol into bile salts is a major pathway for its elimination from the body, along with direct hepatic excretion.[262] The exact serum lipid source of the

cholesterol excreted into the bile is unclear, although it is likely to be derived from an increased uptake of HDL and LDL as well as the decreased conversion of cholesterol into bile salts.[37] Cholesterol is involved in two negative feedback mechanisms which could influence gallstone formation. The first involves the rate determining enzyme in bile salt synthesis, cholesterol 7-alpha hydroxylase (CYP7A). Dietary cholesterol is known to up-regulate CYP7A which leads to increased bile salt production, with less cholesterol available for excretion in the bile, both of which reduce the risk of gallstone formation.[26] *Lith* gene analysis has identified a further cholesterol negative feedback mechanism. A family of transcription factors called sterol regulatory element binding proteins (SREBP) regulate the synthesis of cholesterol, especially SREBP-2. When cholesterol levels are low, SREBP-2 is released and activates genes for HMG-CoA reductase, as well as other enzymes involved in cholesterol synthesis.[263] In summary, raised dietary cholesterol intake could lead to a reduced risk of gallstone formation by up-regulating CYP7A which increases bile salt excretion, and also by inhibiting the release of SREBP-2, which down-regulates hepatic cholesterol synthesis, with both these effects reducing biliary cholesterol concentration. Therefore, the previous hypothesis that dietary cholesterol could lead to stone formation may have been too simplistic.

Previous epidemiological studies evaluating the role of dietary cholesterol have shown inconsistent results. The only large prospective cohort study, which used food frequency questionnaires to assess dietary cholesterol intake in 88 837 women, did not find any association (highest vs lowest quintile RR=1.0, 95% CI=0.8-1.3).[206] The results from case-control and cross-sectional work include an increased risk of gallstones with increased dietary cholesterol[203-204] and a decreased risk.[127, 205] However, a randomised control trial in men from Los Angeles of a diet designed to lower plasma cholesterol (high in unsaturated fat, low in saturated fat, low in cholesterol, and high in plant sterol) reported that those receiving the experimental diet versus those on a normal diet had a higher rate of gallstone prevalence (34% vs 14%, $p<0.01$).[264] Furthermore, in those on the experimental diet, the prevalence of gallstones correlated with the number of trial meals eaten ($p<0.05$). [264] These study findings lead to an editorial in the New England Journal of Medicine suggesting that a diet designed to lower plasma cholesterol may promote gallstone formation,[265] although it is unclear which element of the diet may have lead to the increased gallstone prevalence.

There are several cautions to interpreting the findings for dietary cholesterol in women, particularly since these findings were not replicated in men.

Dietary cholesterol intake could also be correlated with an altered lifestyle or dietary behaviour. For example, those who are overweight may have been told to reduce total fat and cholesterol in their diet and hence have a low cholesterol intake, although in the analysis adjustment was made for BMI. Alternatively, participants with high serum cholesterol may have been following a low cholesterol diet and may also have been treated with a fibrate or statin. However, serum cholesterol is not associated with gallstone disease,[88] and although fibrates promote stone formation, current evidence suggest that statins reduce the risk of gallstones. Overall, the finding that dietary cholesterol protects against gallstone disease is supported by a plausible mechanism and the previous similar finding in the study of men from Los Angeles.[264] However, further epidemiological research into the association is needed to look for a consistent effect before increased dietary cholesterol could be regarded as reducing the risk of gallstone disease.

Iron

Dietary iron was associated with a highly significant decreased risk of gallstone disease in women, but not men, after 10 years of follow-up. The highest quintile of intake had a HR of 0.35 (95% CI=0.71-0.94) with an 18% reduction for each increased fifth of intake (p for trend=0.004). The lack of effect in men may be due to the low rates of iron deficiency in men. Iron has several biological effects which could account for a reduced risk of gallstone disease. Iron is required for the effective function of the enzyme CYP7A1, enabling cholesterol conversion into bile salts which maintains biliary cholesterol in solution.[28] Iron is also required for nitric oxide synthase function, with iron deficiency associated with decreased gallbladder motility [217] and impaired sphincter of Oddi dysfunction [218] which both promote gallstone formation. Furthermore, there is a pro-nucleating effect of raised biliary transferrin levels found in iron deficiency.[216]

Only one previous prospective study has evaluated dietary iron intake, and this was conducted solely in men. The US Health Professionals study followed-up 44 758 men for six years with 2 468 developing symptomatic gallstones. Those with a higher intake of haem iron were at an increased risk of developing gallstones (highest quintile vs lowest RR=1.21, 95% CI=1.03-1.42), although non-haem iron intake was not associated (highest quintile vs lowest RR=1.14, 95% CI=0.99-1.31).[220] This study was in men aged 40-75 years, which is a group vulnerable to iron overload, since iron stores accumulate in linear fashion in men with increasing

age [266] with excess iron stores possibly contributing to stone formation. The authors suggested that elevated iron stores can induce lipid peroxidation, generating hydroxyl radicals that stimulate mucus glycoprotein secretion into the gallbladder and promote cholesterol crystal formation. They commented that a diet with a high iron content may be correlated with increased meat intake and hence increased dietary saturated fat and triglyceride intake, which could promote gallstone formation, although the study did correct for saturated fat intake.

Several small aetiological studies have been published suggesting that iron deficiency promotes gallstone formation. A Turkish case-control study compared gallstone prevalence in 111 iron deficient patients against 81 controls using ultrasonography.[221] The prevalence of gallstones or previous cholecystectomy was higher in the iron deficient patients (13.5% vs 6.2%, p=0.048). They also assessed gallbladder motility in both groups using gallbladder emptying studies, and reported a higher residual volume of the gallbladder in those with iron deficiency (4mls vs 2.8ml, p=0.035) indicating impaired gallbladder motility. An Indian study of 100 patients admitted with gallstone disease measured serum iron and biliary cholesterol concentrations. Patients with iron deficiency had increased biliary cholesterol compared to those with normal serum iron levels (biliary cholesterol 375mg/dl vs 214mg/dl, p<0.0001).[222]. A similar study of 50 patients with gallstone disease divided patients into two groups dependent on whether they had normal or low serum iron levels and reported gallbladder cholesterol concentrations were significantly higher in the low serum iron group (0.7g/dl vs 1.2g/dl, p<0.0001).[223]

In this work from EPIC-Norfolk, increased dietary iron was associated with a decreased risk of gallstones in women with the magnitude and dose-effect supportive of a causal effect suggesting this is a true association. This novel finding could partly account for the increased rates of gallstone disease seen in women of child bearing age who are prone to iron deficiency and gallstone disease. Further epidemiological studies are needed to confirm this association and imply causation, although it may be possible to use data collected from randomised controlled trials of iron supplementation in women to assess if such therapy is associated with lower rates of gallstone disease.

Niacin

I believe this is the first epidemiological study to evaluate the effect of dietary niacin intake on the risk of developing gallstone disease. The study found

that in women, after 10 years of follow-up, dietary niacin significantly reduced the risk of disease, with those in the top quintile 46% less likely to develop gallstones compared to those in the bottom one. Furthermore, there was a 14% reduced risk for each increased quintile of intake (p for trend=0.004). No effects were found in men, although all four higher quintiles had a non-significant inverse association with gallstone disease. Niacin could prevent gallstone disease by its known biological effects on lipid metabolism and is already used by cardiologists to prevent vascular disease.[267] Niacin increases serum HDL and lowers serum triglycerides, with these lipid changes associated with a reduced risk of gallstone disease. However, cardiologists prescribe niacin at doses of 1 to 2 grams a day whereas the average dietary intake in EPIC-Norfolk was 17 mg per day in women and 21 mg per day in men.

There are no human studies which have directly investigated the effects of dietary niacin on gallstone disease or biliary composition. However, in the 1970's a large randomised controlled trial in 8 341 men with a previous myocardial infarct, assigned participants to one of several treatments, including either niacin 3grams/day (1 110 participants), clofibrate 1.8 grams per day (1 103) or placebo (2 789). The primary end-points were vascular events with secondary end-points including incident gallbladder disease (either cholecystectomy or symptomatic gallstones). In those treated with niacin the five year rate of new gallbladder disease was 2.7% vs 2.0% (p=0.18) in the placebo group. This placebo controlled trial suggests that niacin supplementation may not prevent gallstone disease in men, although similar studies have not taken place in women who had an inverse association with niacin in this study. Further epidemiological and clinical studies on the effect of dietary niacin, particularly in women, would need to be undertaken before advocating that niacin has a direct effect in preventing gallstone disease.

Fibre

Dietary fibre was not associated with symptomatic gallstone disease in either gender after 10 years of follow-up which was defined as the primary end-point. Women did have negative associations for each higher quintile of intake, although none reached statistical significance (highest vs lowest HR=0.68, 95% CI=0.41-1.13). There are biological mechanisms to account for fibre preventing gallstone formation. These include shortening intestinal transit times which reduces the formation of hydrophobic secondary bile salts which may otherwise promote

gallstone formation. Fibre also has some modest effects on serum lipid, although these appear to mostly be to decrease serum total cholesterol and LDL-cholesterol rather than raising HDL or decreasing triglycerides.[234]

Dietary fibre has been assessed in the US Nurses' Health Study which followed-up 69 778 women for 16 years identifying 5 771 cases of cholecystectomy. Women in the highest quintile compared to the lowest intake of fibre, had a relative risk of cholecystectomy of 0.87 (95% CI=0.78-0.96, p for trend=0.005) with the effect maintained for insoluble fibre, but not soluble fibre. In the analysis, adjustments were made for many lifestyle habits which could be correlated with increased fibre intake (i.e. smoking, physical activity and alcohol) which lead to a strengthening of the association. However, the authors also adjusted for factors not proven to be risk factors for gallstone disease including dietary protein and saturated fat.[236] The modest inverse effect reported in the Nurses' Health Study may concur with the borderline results found in EPIC-Norfolk. If EPIC-Norfolk had had more cases of disease, an significant effect may have been identified. Although EPIC-Norfolk had the benefit of using 7-DFDs to measure diet, detecting a statistically significant small effect size with only 201 cases identified would be difficult. The only other prospective cohort study of women found an inverse association between fibre and gallstone disease.[160] No studies have reported an effect of fibre in men[169] which reflects the findings in this study.

Calcium

In men, dietary calcium was associated with a significant increased risk of gallstone disease after 10 years of follow-up (highest quintile of intake vs the lowest HR=2.31, 95% CI 1.00-5.35, trend across categories HR=1.25, 95% CI 1.03-1.52, p=0.023). In women, no effects were found for dietary calcium intake. There are biological mechanisms by which calcium intake could both increase and decrease the risk of gallstone disease. Calcium salt precipitation plays a fundamental role in the formation of all types of gallstones,[237] with the bile of patients with gallstones containing a higher concentration of calcium then those without gallstones.[239-240] Patients with a history of primary hyperparathyroidism, which causes hypercalcaemia, have been found in some but not all surveys to have an increased prevalence rate of cholelithiasis.[241-243] Through these mechanisms increased dietary calcium could raise the risk of gallstone disease, as reported in

this study. However, the previous but sparse epidemiological data suggested a protective role for calcium, possibly due to the ability of this mineral to bind to secondary bile salts in the gut lumen which prevents their re-absorption. The prospective Dutch Zupten study of 860 men identified 54 cases of symptomatic gallstone disease and reported that the top tertile of calcium intake was associated with a reduced risk of gallstones (HR=0.3, 95% CI 0.1-0.7).[168] A case-control study of only 54 cases and 46 controls also reported calcium was associated with a reduced risk of gallstone disease in women, but not in men.[203] However, until this investigation in Norfolk there was a lack of a large prospective study. The results from EPIC-Norfolk indicate that dietary calcium intake needs to be investigated further as a potential risk factor for gallstone disease. There are limitations to our study, which include the null finding in women, which increases the likelihood that the increased risk found in men was a chance finding. The study does not include an assessment of calcium supplementation, which could explain the null effect in females who are more likely to use calcium supplements. Future research could use the populations studied in previous randomised controlled trials of oral calcium supplementation undertaken in the fields of osteoporosis and hypertension, to evaluate if calcium supplementation lead to increased rates of gallstone disease.

Coffee

In men, but not women, caffeinated coffee had a strong inverse association with symptomatic gallstone disease, with each cup drank per day reducing the risk by 23%. No effects were found for tea or decaffeinated coffee, for men or women. Caffeinated coffee has metabolic effect that influence gallstone formation including, increased CCK release and gallbladder contraction,[245] increased bile flow and reduced gallbladder bile protein levels,[246] and reduced gallbladder fluid absorption.[247]

The results from EPIC-Norfolk are supported by the US Health Professionals study of 51 529 men. Over a 10 year period, 1 081 participants were identified with symptomatic gallstone disease.[268] The consumption of caffeinated coffee was associated with a dose dependent negative association with ≥4 cups a day RR=0.67 95% CI=0.53-0.84. No effect was reported for tea or decaffeinated coffee., although the Nurses' Health study did report an inverse association. In over 80 000 women, 7 811 cases of cholecystectomy were reported during 20 years of follow-up. In those who consumed caffeinated coffee there was a statistical inverse

association with a dose effect for 1, 2-3 or ≥4 cups a day compared to no intake (≥4 vs 0, multivariate RR=0.77, 95% CI=0.71-0.83, p for trend<0.001).[269] Caffeinated drinks of any type were also found to have an inverse association, although decaffeinated coffee was not associated with risk. EPIC-Norfolk did not find an association in women, which could either be due to a reduced effect of coffee in women or that over risk factors i.e. parity, HRT use, override the effects of coffee.

Several studies have reported no effect of coffee intake,[249-251] but they are limited by being cross-sectional surveys of prevalent disease which may incur bias due to the avoidance of coffee, particularly since it stimulates gallbladder contraction which could provoke biliary colic. EPIC-Norfolk is the first European study to report a protective effect of caffeine in men but no effects of decaffeinated coffee. This is likely to be a true association, as there is a large dose-dependent effect, it has been replicated in previous cohort studies and there are supporting biological mechanisms and experimental work.

3. Strengths and limitations of the study design

There are several strengths and limitations to the study which need to be considered when interpreting the findings. A large population cohort was used in EPIC-Norfolk which reduces the possibility of chance findings in our results. However, with only 86 cases of incident gallstone disease after 10 years of follow-up available for analysis in the male population, chance findings become more likely. However, for all the associations found in this study there are plausible biological mechanisms to support their role and usually either supporting animal or human intervention studies.

Internal validity

Chance

The internal validity of a study is dependent on chance, bias, confounding and measurement error. There are several advantages and limitations to this study which need to be considered when interpreting the findings. A relatively large population cohort was used in EPIC-Norfolk, of over 25 000, with 166 female & 82 male incident cases of gallstone after 10 years and 201 women and 95 men after 14 years which is of sufficient magnitude to minimise chance findings, although this was more likely to occur for men. For all the associations found in this study there are plausible biological mechanisms to explain their action, with either supporting animal or human intervention studies.

Chance finding are minimised in larger studies such as the US Nurses' Health Study which included >60 000 women, with >1 000 000 person-years follow-up and identified over 6 000 cases of cholecystectomy,[131, 270-271] and the US Health Professionals Study which included >40 000 participants, with >500 000 P-Ys follow-up identifying >2 000 incident cases of symptomatic gallstones.[193, 220, 272] However, these large cohort studies did not benefit from utilising the more accurate methods of measuring risk exposures used in EPIC-Norfolk, such as the 7-day food diaries. The size of the US studies will have compensated for some of the inaccuracies of measurements, but they may still be unable to detect the effect of specific nutrients if the dietary assessment method was not of sufficiently detailed.

Selection bias

A major advantage was the prospective design of this study which minimises several potential sources of bias. The anthropometric, serum lipid, physical activity and dietary data were collected at baseline prior to the development of known gallstone disease. The prospective study design also reduced selection bias as cases and non-cases are drawn from the same population. If cases and non-cases are compared at the time disease is identified, there is the potential for differential reporting of exposures, particularly dietary intakes, which is a limitation of the case-control design. Also, symptoms may alter behaviour in those with gallstone disease, in particular they may alter their diet by decreasing foods which precipitate symptoms causing a "protopathic" bias[123] and leading to type 1 error. To minimise bias introduced by a disease altering behaviour prior to its diagnosis, no cases were included if they were diagnosed within 18 months of enrolment into EPIC-Norfolk.

Regression dilution bias

All the analyses relied on a single baseline measurement, which after prolonged follow-up, can lead to regression dilution bias due to participants altering their diet. Prospective studies which analyse disease risk from just one initial baseline survey of an exposure, may underestimate the magnitude of risk of disease after longer periods of follow-up. This effect is amplified if the analysis includes many co-variates, all of which become less accurate over time.[257] This random measurement error occurs as some of the population will change their magnitude of exposure to the risk over time. In EPIC-Norfolk, we considered that physical activity and dietary intake were particularly vulnerable to variation over time. Ideally, the analysis should be made after the shortest period possible prior to the development of symptoms to minimise the effects of regression dilution bias, whilst allowing an appropriate follow-up period to acquire a significant number of incident cases of disease. For physical activity, the primary analysis was performed after five years to allow the accumulation of a significant number of incident cases, and the secondary analysis after fourteen years of follow-up to give a more accurate assessment between physical activity and incident gallstone disease from baseline data, and indeed a stronger association was seen at 5 years than 14 years. In dietary analysis, the analysis was performed after 10 years follow-up and in future work we intend to include data from diaries completed by participants after

18 months and 5 years of follow-up which will help to minimise regression dilution bias.

Follow-up bias

Follow-up bias could occur if those with a specific characteristic were more likely to move away from the catchment area of the local hospital where incident cases of gallstone disease were identified. For example; if more participants with a high level of physical activity moved outside the catchment area of the local hospital compared to inactive participants, then our study would conclude a higher level of physical is associated with a lower risk of disease. However, this is unlikely to occur on a large scale as the study population chosen had little outward migration.[41] Follow-up bias could also occur due to a limitation in the method of identifying cases of potential gallstone disease. All patients were identified at the Norfolk and Norwich University Hospital (NNUH) using the hospital database records, although EPIC-Norfolk participants treated at surrounding NHS hospital such as James Paget Hospital in Great Yarmouth or local private hospitals would not be identified. It is unlikely that participants treated at NHS hospitals would have different characteristics or behaviour to those treated at the NNUH. Follow-up bias may occur by not identifying patients treated in the private sector, as they are likely to have different lifestyle and diet characteristics. However, we suspect that numbers solely treated in the private sector are small and would not significantly alter results.

Lack of asymptomatic stones and stone type

A limitation of this study was that no evaluation was made of prevalent asymptomatic stones either at recruitment or during the follow-up period. Previous studies have used ultrasonography to evaluate the presence of "silent" gallstones at both enrolment and follow-up which provides information on whether both asymptomatic and symptomatic stones have similar risk factors, which they do.[81-82, 126] However, this study was not originally designed to evaluate prevalent gallstone disease and screening a cohort of over 25 000 people with ultrasonography would be costly and unfeasible. From the outset, this study did not attempt to estimate the incidence of gallstone formation but rather the incidence and risk factors for new symptomatic gallstones, which are of direct clinical and public health importance. No analysis was made of whether the cases of gallstone disease were due to cholesterol stones, brown, black or mixed pigment stones. This would have been particularly useful in clarifying which biological mechanism may be involved and

is probably of particular relevance to the findings for dietary iron and calcium since they are more likely to be involved in the pathogenesis of pigment stones. However, the majority of gallstones in the Western population are cholesterol stones.

Adjustment for known risk factors

Importantly, in the analysis we adjusted for the majority of known risk factors for gallstones, although there was no information on family history of gallstones, rapid weight loss and prolonged fasting which can affect stone formation.[60-61,160, 169] A family history of gallstones and associated genes are estimated to contribute around 25% of the total gallstone risk.[36] However, this deficiency may be less important as the genes need to interact with environmental factors to cause disease. The genetic predisposition to gallstones arises from polymorphisms in multiple genes with each making a small contribution to the risk of developing disease.[37] Genes can lead to disease via either gene-gene interactions or interactions with the environment including diet, obesity, drugs or pregnancy. It would have been useful to assess if those with a family history of disease had an interaction with any of risk factors in this study.

External validity

The study is generalisable to a UK population aged between 45-74 years of age who are most susceptible to gallstones. However these results cannot be extrapolated to younger adults and children as they are not included in the study population and may have different risk factors. The disease presentation in this study was representative of that seen clinically, being predominantly biliary colic. The previous justification for the role of several nutrients in gallstone disease have been based upon animal models or on the acute feeding of human subjects with the volumes or doses of the nutrient given much higher than that achieve normally in the diet. However, both of these models are likely to be unreliable when interpreting the chronic effects lower levels of dietary intake. For this reason, the evidence collected from EPIC-Norfolk will have much greater validity, as it has been demonstrated in a large, diverse UK population.

Concluding remarks of the discussion

This large UK prospective cohort study investigating the aetiology of gallstone disease has for the first time reported significant effects of dietary *trans*-fatty acids, cholesterol, iron and niacin in women and calcium in men. The discovery of these novel risk factors was aided by the use 7-day food diaries which provided the most accurate measure of dietary intake undertaken in a large scale study. These new findings are supported by biological mechanisms but need to be explored in future studies to look for consistent effects, and if confirmed, could provide information to modify the diet or behaviour to reduce the risk of developing gallstone disease or the recurrence of symptoms. These novels findings may have clinical implications such as identifying high risk populations which may benefit from the use of appropriate preventative measures. One such population is pregnant females who are susceptible to iron deficiency as well as gallstone disease and may benefit from iron supplements at an early stage of pregnancy to prevent gallstones. Another high risk population are patients with a history of gallstone disease who are currently recommended to follow a low fat/low cholesterol diet which may actually be contributing to the future development of gallstones or bile duct calculi rather than consuming a cholesterol containing diet which may help prevent stone recurrence. This scenario is analogous to those who suffer from calcium oxalate renal stones who require an appropriate calcium intake to prevent recurrent renal stones. Hence the new understanding of the dietary nutrients which alter the risk of developing gallstone disease will lead to an improved understanding of the biological mechanisms involved in the pathogenesis of gallstone disease as well as improved management of those susceptible to stone formation.

This study has also defined the precise effect of previously defined risk factors for gallstone disease. The lifestyle risk factors obesity and physical activity were estimated for the first time in a UK population, with a supportive mechanism of effect from the serum lipids data. Our results confirmed a protective effect for alcohol and caffeine intake that in men, but not women. Evidence from EPIC-Norfolk and other studies will allow a predictive model of gallstone disease to be devised, which could lead to public health measures to prevent this common gastrointestinal disease.

CHAPTER TWO

THE AETIOLOGY OF PANCREATIC CANCER

Abstract

Pancreatic cancer: physical activity, dietary nutrients and serum vitamin C in the aetiology of disease: data from a UK prospective cohort study using information from detailed and validated questionnaires.

INTRODUCTION: The aetiology of pancreatic cancer is largely unknown, although physical activity and dietary nutrients may prevent carcinogenesis via improved insulin sensitivity and prevention of DNA damage. The aim of this prospective study was to investigate physical activity and dietary nutrients, particularly fatty acids and anti-oxidants, and the risk of developing pancreatic cancer. The study benefitted from accurate methods of measuring exposures, namely, a physical activity questionnaire, validated against detailed physiological measures, and a seven-day food diary (7-DFD), the most accurate pragmatic form of measuring diet in large scale epidemiological studies.

METHODS: A total of 25 639 men and women, aged 45-74 years were recruited between 1993-1997 into the European Prospective Investigation into Cancer-Norfolk. Participants attended a health check at enrolment which recorded their anthropometrics, alcohol intake, serum lipids with a questionnaire recording occupational and recreational physical activity. A 7-DFD was completed recording all food eaten, detailing brands and portion sizes. Nutrient intakes were calculated in those diagnosed with pancreatic cancer and a random sample of 3 970 controls, using a computer program with information on 55 000 foods. Hazard ratios (HR) were calculated, using Cox regression, for developing pancreatic cancer for categories of physical activity, nutrient intakes and serum vitamin C, adjusted for age, gender, smoking status, body mass index and diabetes, with the addition of total energy intake for dietary nutrients. The primary analysis was made after 10 years follow-up, with a total follow-up period of 17 years.

RESULTS: During the 10 year follow-up period, 53 participants (41.5% women) developed pancreatic cancer (69.7 years (SD=8.6 years). The main findings in the primary analysis were statistically significant inverse associations for increased physical activity in participants younger than 65 years ("active" vs "inactive" HR=0.11 95% CI=0.01-0.88), increased dietary DHA intake (trend across quintiles HR=0.80 95% CI=0.65-0.98), a threshold effect for dietary vitamin E and selenium and increased serum vitamin C levels (highest vs lowest quintile HR=0.16, 95% CI=0.04-0.73, p for trend=0.008). Borderline statistically significant negative associations were found for total n-3 fatty acid intake (highest vs lowest quintile HR=0.30, 95% CI=0.07-1.21) and the threshold effect of dietary vitamin C.

CONCLUSION: There were inverse associations for physical activity, serum vitamin C and dietary antioxidants and n-3 fatty acids. They are all supported by plausible biological mechanisms and justify measuring these factors in future aetiological work. If consistent associations are confirmed in future epidemiological studies, implying causality, then population-based dietary and physical activity recommendations may help prevent a significant proportion of pancreatic cancer cases.

Introduction

Exocrine pancreatic cancer is a devastating disease causing approximately 230 000 deaths annually worldwide, representing approximately 2% of cancers overall, but 6% of cancer deaths.[273] The cancer has a very poor prognosis, with only 16% of patients surviving beyond 1 year and just 0.2-3.0% longer than 5 years.[274-275] The poor survival times highlight the need to identify modifiable risk factors to prevent the incidence of this lethal disease and to guide developments for future treatments.

The study of the epidemiology of pancreatic cancer has three aims i) to describe the distribution and burden of the disease; ii) to elucidate the aetiology; iii) to provide information necessary to prevent the disease as well as to help understand the biology to aid treatment.[276] So far only a few risk factors for pancreatic cancer have been clearly defined which hinders the prevention of this tumour. The recognised environmental risk factors are tobacco smoking,[277] diabetes [278] and obesity.[279-280] Diet is a possible risk factor for pancreatic cancer, although not yet confirmed, and could account for some of the differences in incidence between countries with different diets and the increased prevalence in countries adopting Westernised eating habits.[281] However, the results from most studies investigating diet have been inconclusive and inconsistent.[282-283]

This introductory chapter will review both the descriptive and aetiological epidemiology of pancreatic cancer. To identify the relevant literature, searches of Medline (OVID and PubMed) were performed identifying English language articles, between the years 1950 and March 2011, using terms related to each section in this thesis and the keywords "pancreatic cancer" and "pancreatic carcinoma". The bibliographies of retrieved articles were reviewed to identify further relevant references.

Definition of pancreatic cancer

Tumours of the pancreas gland arise from either endocrine or exocrine cells, with exocrine tumours accounting for around 97% of all such cancers.[284] This review concerns pancreatic exocrine cancer, commonly referred to in the literature as "pancreatic cancer". Exocrine tumours are classically ductal adenocarcinomas (80%) which show ductal differentiation often with an intense desmoplastic reaction in the surrounding stroma. Histological variants of ductal adenocarcinoma contribute a further 10% of exocrine pancreatic tumours, namely serous or

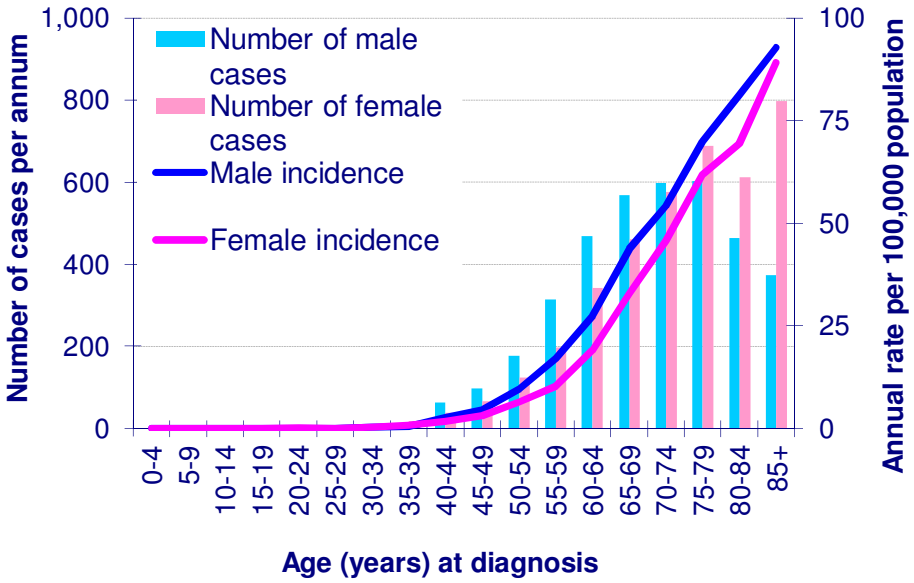
mucinous cystadenocarcinoma (4%), intraductal papillary-mucinous neoplasms (3%) or acinar cell carcinoma (2%).[285]

1. Descriptive Epidemiology

Incidence

In the year 2008, 7 781 deaths from pancreatic cancer were reported in the United Kingdom (UK).[21, 273] The lifetime risk of developing pancreatic cancer in the UK is 1 in 86, with most patients dying of the illness, making it the 6th commonest cause of death from any cancer.[21] The incidence increases rapidly with age in both genders, with less than 5% of cases occurring before the age of 50 years. Between the ages of 50-54 years, the annual incidence of disease is 9.6 cases per 100 000 population per year, which rises to 70.0 cases per 100 000 per year in people aged 75-79 years (Table 15).[21] The largest absolute number of cases occurs in those between the ages of 70-79 years, although the rate continues to rise in older people.

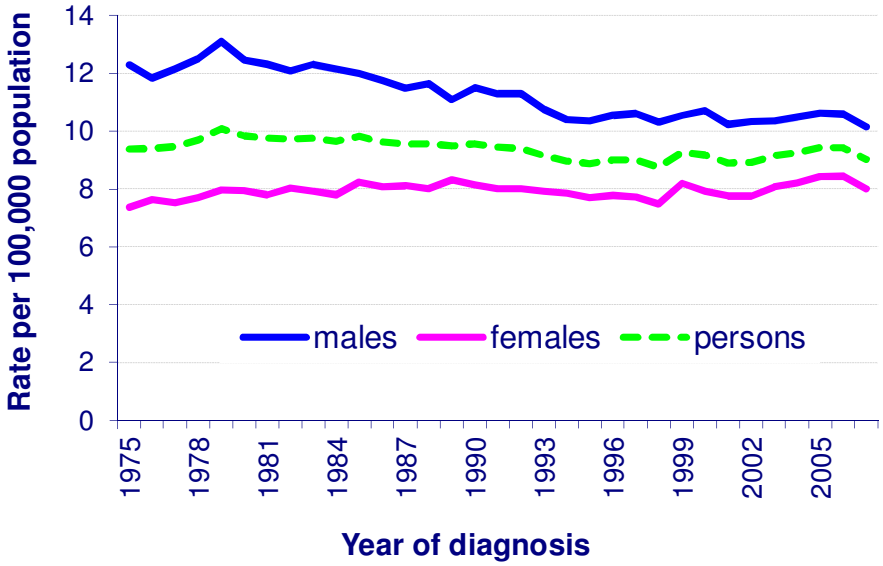
Figure 11. Number of new cases and age specific incidence rates of pancreatic cancer by sex, in the United Kingdom 2007 (Source; Cancer Research UK, 2009[21]).



Sex

Worldwide there are approximately 120 000 deaths each year in men and 107 000 in women.[281] In the UK, the age-standardised rates of pancreatic cancer are higher for males, although they have declined since the 1970s (12.3 cases per 100 000 in 1975, 10.1 per 100 000 in 2007), whereas the rates in women have remained fairly constant (9.4 cases per 100 000 in 1975, 9.0 per 100 000 in 2007) (Figure 12).[21] The decrease in men is likely be due to the decline of tobacco smoking in males, as this probably accounts for up to 20% of pancreatic cancer cases in the UK.[277] Although men have a higher age-standardised incidence than women, in 2007, females accounted for more cases of disease due to their increased longevity (men 3 748 cases vs 3 936 in women).[21]

Figure 12. Age standardised incidence rates of pancreatic cancer for the UK, 1975-2007 (Source; Cancer Research UK, 2009[21]).



Geographic and socioeconomic variation

Pancreatic cancer is commoner in developed countries where the rates are nearly three times greater than in low and middle income ones.[283] The highest incidence of disease has been reported among Maoris in New Zealand[281] and in South Koreans[286] which may reflect their high rates of smoking. In Western Europe (UK, Germany, France, Belgium, Netherlands) the mortality due to pancreatic cancer fell towards the end of the last millennium, whilst there were increased rates in Southern Europe (Greece, Italy, Macedonia, Portugal).[286] The rise in incidence in Southern Europe may have occurred due to changes in lifestyle and diet, or the increased use of cross sectional imaging, leading to increased diagnosis. This initially occurred in the 1980's in Western Europe and in the 1990's in Southern Europe. In the United States the incidence is stable, although it is noticeable that the US black population has a higher annual incidence than whites (10.2 *vs* 6.6 per 100 000, 1996-2000).[287] Japan experienced a significant increase in age-standardized mortality rates between 1950 (1.4 per 100 000 population) and 1995 (12.5 per 100 000 population) [288] which again could be partly due to improved diagnostic test or the adoption of a westernised lifestyle.

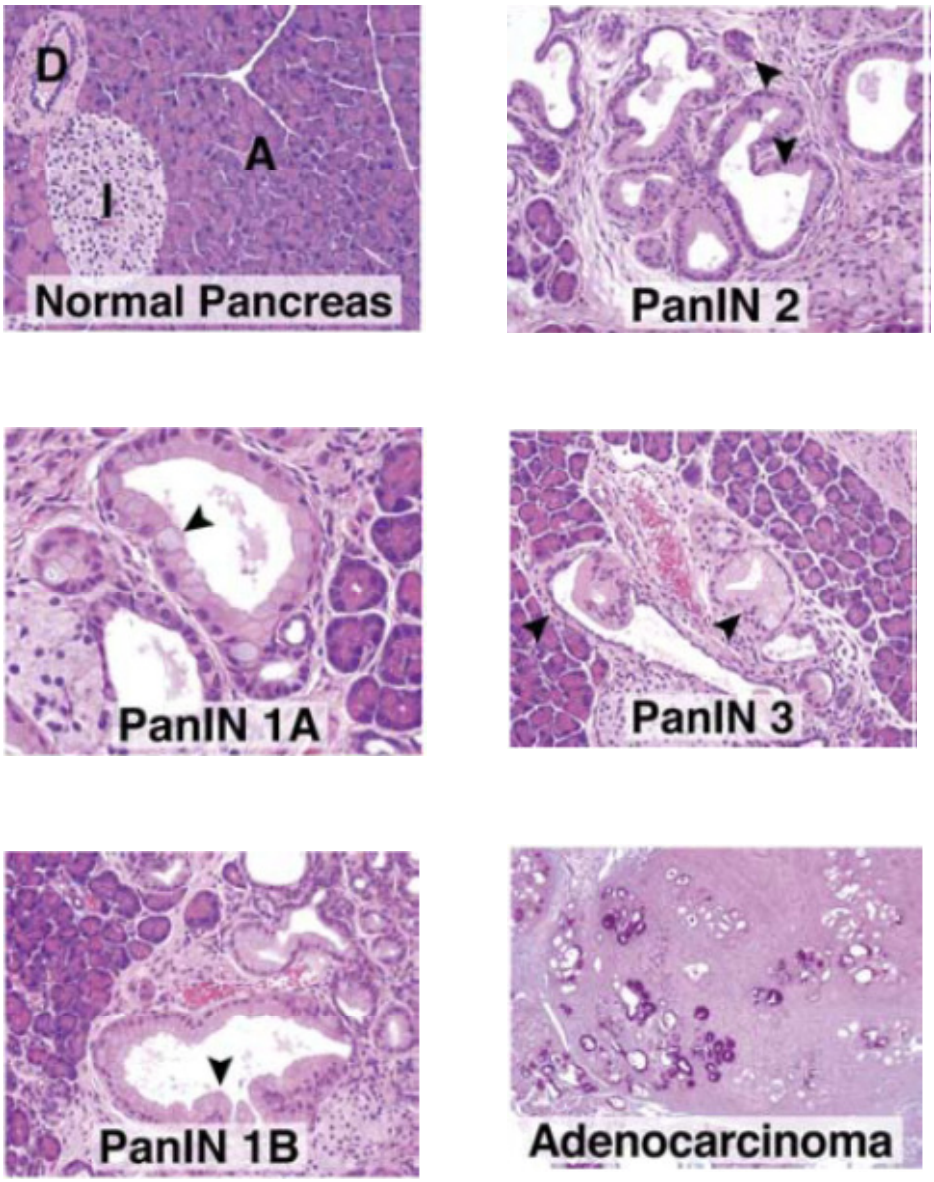
Data on the impact of social class on pancreatic cancer risk is limited with only two cohort studies published.[289-290] The prospective UK Whitehall Study of 19 019 male government employees reported no effect of socioeconomic status (RR=0.95, 95% CI=0.59-1.51), although importantly the cohort did not include manual or industrial workers.[289] A Norwegian cohort study of 63 374 men and women identified 166 incident cases of pancreatic cancer.[290] They reported an increased risk in women of higher compared to lower socioeconomic status (RR=2.5; 95% CI=1.2-5.2), and among men employed in farming, agriculture or forestry compared to those with a lower socioeconomic status (RR=2.1; 95% CI=1.1-4.0). However, cohort studies have limitations for assessing the impact of socioeconomic status on the risk of developing disease, as the population studied will not reflect the spectrum of socioeconomic statuses in society. Therefore, currently there is insufficient evidence to suggest that socioeconomic status is a risk factor for pancreatic cancer.

2. Biology of pancreatic cancer

Pancreatic precursor lesions

Pancreatic ductal adenocarcinoma is the commonest histological variant of this cancer and has precursor lesions, termed pancreatic intraepithelial neoplasia or PanIN. PanIN lesions are classified as PanIN-1a, PanIN-1b, PanIN-2 and PanIN-3 (Figure 13) and are associated with progressive genetic alterations, with mutations of oncogenes and tumour suppressor genes such as Ki-RAS, p53 and BRCA2.[291] These genetic alterations cause cytological and architectural atypia, leading ultimately to *carcinoma in situ* as found in PanIN-3.[292] PanIN lesions are often detected in patients undergoing pancreatectomy for chronic pancreatitis and also in post-mortem examinations of normal pancreas glands, although there are likely to be other precursor lesion yet to be described.[293]

Figure 13. Histology of normal pancreas and histopathology of pancreatic intraepithelial neoplasia (PanIN) lesions and adenocarcinoma from a mouse model of pancreatic cancer (source; Tinder et al. *J Immunol 2008*[294]).

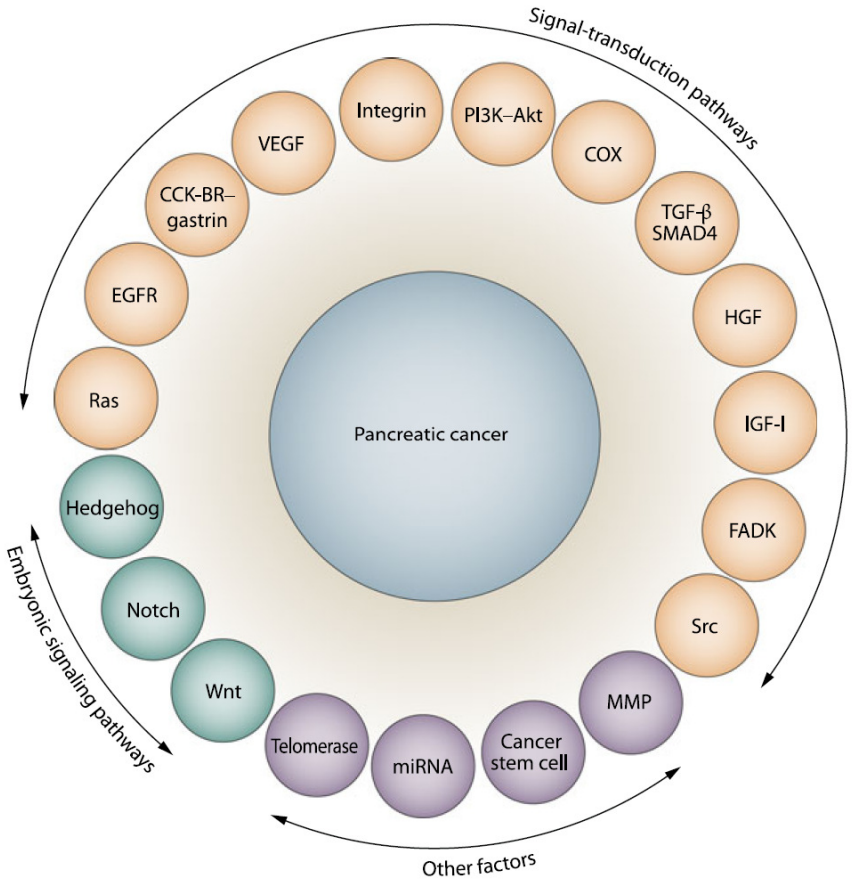


Haematoxylin and eosin (H & E) staining of pancreatic tissue from a mouse model of preinvasive and invasive ductal pancreatic cancer. Representative images are shown of the various stages of PanIN lesions and adenocarcinoma. The arrows indicate the foci of the PanINs. D: duct, I: Islet and A: Acinar.

Molecular pathogenesis

Pancreatic cancer is fundamentally a genetic disease, due either to somatic (acquired) or inherited mutations of cancer-associated genes, with environmental factors (including dietary factors) promoting somatic mutations. A worldwide collaboration performed a comprehensive genomic analysis on 24 samples of pancreatic adenocarcinoma, sequencing 20 661 genes and identifying 63 genetic alterations.[295] These genetic mutations are involved in a core set of 12 cellular signalling pathways that become dysregulated and are key processes in pancreatic carcinogenesis.[295] These molecular pathways are now the target of research into potential future treatments, and have diverse roles in the promotion of tumour growth, resistance to apoptosis, invasion, metastasis and angiogenesis (Figure 14). One of the cellular signalling pathways identified is the *Ras* family of genes which can initiate carcinogenesis and are critical DNA targets for chemical carcinogens. *KRAS* is a member of the *Ras* family and encodes membrane bound GTP-binding proteins. Somatic mutations in the *KRAS* oncogene are an early and fundamental event in the pathogenesis of most exocrine pancreatic cancer.[296] These somatic mutations are the most frequent oncogene alterations in human cancer, and a prime example of activation by point mutation. Mutations in *KRAS* are very frequent in pancreatic cancer (present in up to 95% of pancreatic cancer cases), resulting in impaired cell signalling and triggering a variety of cellular processes such as transcription, translation and enhanced cell survival that initiate the early phases of pancreatic carcinogenesis.[296]

Figure 14. The multiple molecular pathways and processes involved in pancreatic carcinogenesis (source; Wong *et al. Nat Rev Gastroenterol Hepatol 2009*[296])



The pathways and processes involved in pancreatic carcinogenesis. Entities involved in the "*signal-transduction pathways*" have diverse roles in the promotion of tumour growth, resistance to apoptosis, invasion, metastasis and angiogenesis. Reactivation of physiological "*embryonic signalling pathways"* are also important. *"Other factors"* involve MMP (matrix metalloproteinase) which are important for tumour invasion and neovascularisation. Telomerase is involved in the maintenance of telomeres and is activated in the majority of pancreatic cancers. The miRNAs (microRNAs) regulate gene expression post-transcriptionally.

3. The aetiology of pancreatic cancer

In assessing the evidence for pancreatic cancer risk factors, the same process was followed as detailed in the previous chapter, "Selection of epidemiological study evidence".

Genetic syndromes predisposing to pancreatic cancer

There are several genetic and family syndromes predisposing to pancreatic cancer that can be classified into three broad categories: i) patients diagnosed with a syndrome associated with pancreatic cancer, ii) those with a gene mutation susceptible for pancreatic cancer on genetic profiling and iii) those with a family history of pancreatic cancer. Inherited syndromes have been identified which significantly increase the risk of developing pancreatic cancer, although no common genetic abnormality has been identified in all these conditions. Hereditary pancreatitis is characterised by the familial occurrence of pancreatitis with an early age of onset due to mutations in the cationic trypsinogen gene (PRSS1) in 75% of cases with the remaining 25% unknown genetic aetiology.[297] This results in a gain in function of the digestive enzyme trypsin which induces persistent inflammation and provides a mitogenic stimulus.[298] Patients with hereditary pancreatitis have an estimated 53 times greater risk (95% CI=23-105) of developing pancreatic cancer with a lifetime risk of 1 in 2.5.[299] In Peutz-Jeghers syndrome (also known as hereditary intestinal polyposis syndrome), a germ line mutation in the STK 11 gene prevents the action of this tumour suppressor gene in the earliest steps in the progression of hamartomas into adenocarcinomas.[300]. This leads to a marked increased risk of developing pancreatic cancer with an estimated relative risk of 132 (95% CI= 44-261) and a cumulative lifetime risk of 36%.[301] Patients with familial atypical multiple mole melanoma (FAMMM) syndrome have a significantly elevated risk of developing pancreatic cancer, with a cumulative lifetime risk of 17%.[302] Further cancer syndromes which increase the risk of pancreatic cancer include hereditary non-polyposis colorectal cancer (HNPCC) with an 8.6 fold increased risk,[303] and familial breast-ovarian cancer (*BRCA1* mutations with a 2-fold increased risk and *BRCA2* mutations with 5% lifetime risk [304-305]). A German study investigating families with two or more members diagnosed with pancreatic cancer reported that 19% of the studied families had a *BRCA2* gene mutation.[306]

Family history of pancreatic cancer

A family history of pancreatic cancer is present in 10% of patients,[307] with the presence of a first degree relative with disease associated with an increased relative risk of between 2.5 to 5.3.(20;22;84;91)[308] Familial pancreatic cancer includes patients with a strong family history but without an identified genetic syndrome. Although the definition of familial pancreatic cancer is debated, it is generally defined as at least two first-degree relatives with pancreatic cancer, without meeting criteria for one of the above syndromes. The risk in familial pancreatic cancer increases with the more relatives affected, with a relative risk of 6.4 (95% CI=1.8-16.4) in those with two affected relatives, rising to 32.0 (95% CI=10.2-74.7) in those with three affected relatives.[309] The increased risk to family members could be due to genetic factors, lifestyle habits or an interaction of both, which are similar in different generations, and example being smoking habits. A case-control study, conducted in Michigan US, addressed this possibility in an investigation involving 247 incident patients and 420 population-based controls which assessed the family history of pancreatic cancer in both groups.[308] Pancreatic cancer in a first degree relative (parent, sibling, offspring) was associated with a statistically significant increased risk of cancer in an individual, with a RR of 2.5 (95% CI=1.3-4.7) after adjusting for smoking in the patient's relative. An interaction was found between the two factors, with the risk 6.0 times greater (95% CI = 2.0 – 18.3) in those with an affected relative who also smoked.

Screening high risk individuals

Genetic conditions are rare, so the absolute contribution they make to all cases of pancreatic cancer are small. Potentially these groups of patients could be screened with the aim to identify an early stage of disease allowing more effective treatment and improved survival. Recent studies investigating the benefits of screening in high risk populations have shown mixed results.[310-311] A German study screened 76 high-risk individuals over a 5 year period, performing a total of 182 examinations of both MRI and EUS. They detected 3 PanIN lesions (one PanIn1 and two PanIN2) and 4 low grade neoplasm (1 Intraductal papillary mucinous neoplasm and 3 serous oligocystic adenomas). However, the natural history for PanIN lesion is not yet established and most may not progress to cancer. The authors concluded that considering the cost and psychological stress incurred, screening had not produced a justifiable benefit.[310] The most recent study screened

51 high-risk individuals in New York, US, (50% of Ashkenazi Jewish descent) with radiology and endoscopic ultrasonography, detecting six pancreatic cancers (11.8%), with one having metastatic disease and five others who underwent resection surgery (one total pancreatectomy for pancreatic cancer, three distal and one central pancreatectomy for pancreatic intraepithelial neoplasia 2 and IPMN). A further four cases of non-pancreatic tumours were identified through the screening programme (a retroperitoneal carcinoid, thyroid, uterine and ovarian cancer) with all patients surviving during the follow-up period of between 1 to 4 years.[311] The findings in this New York population suggest that screening has benefits, although this cannot be extrapolated to different high-risk population groups. Currently, screening high-risk individuals should only take place within a research programme which hopefully will lead to the future development of evidenced based guidelines. Further work in high-risk individuals could also determine if any environmental risk factors interact to affect their risk of developing disease.

Concomitant illness predisposing to pancreatic cancer

Pancreatitis

Acute and chronic pancreatitis are both associated with an increased risk of pancreatic cancer. Chronic pancreatitis is rarely inherited in an autosomal-dominant pattern (1% of all chronic pancreatitis patients) when it is described a hereditary pancreatitis (discussed previously on p145) although most commonly chronic pancreatitis is due to alcohol (75-80% of patients), sporadic (20%) or familial (3%) which may all be associated with mutations of the PRSS1 gene as well as SPINK1 (pancreatic secretory trypsin inhibitor) or CFTR (cytic fibrosis transmembrane conductance regulator). Those who are heterozygous for both SPINK1 and CFTR carry a 20-40 increased risk of developing chronic pancreatitis.[297]

Investigations have clarified the risk chronic pancreatitis incurs on the development of pancreatic cancer. A large multi-national collaboration from four countries (Australia, Canada, the Netherlands, and Poland) of 823 cases of chronic pancreatitis and 1679 controls reported that acute or chronic pancreatitis was associated with an increased risk of cancer (OR=4.68, 95% CI=2.23-9.84) with a markedly increased risk observed within the first year of being diagnosed with pancreatitis (OR=13.8, 95% CI=2.52-75.5).[312] If a patient had pancreatitis for more than 1 year the effect was attenuated (OR=3.93, 95% CI=1.64-9.46) with a similar risk if pancreatitis was diagnosed more than 10 years previously (OR=3.82, 95%

CI 1.26-11.6). These results suggest that pancreatitis can be induced by pancreatic cancer as well as being a risk factor for developing cancer. A previous large cohort study also found a history of chronic pancreatitis increased the risk of pancreatic cancer. After excluding patients that developed pancreatic cancer within the first 2 years, pancreatitis was associated with an odds ratio of 14.4 (95% CI=8.5 to 22.8).[313] In a French prospective study of 373 consecutive patients (86% men) with proven chronic pancreatitis (85% alcohol related), followed-up for a median of 9.2 years, four cases of pancreatic cancer were identified. The relative risk compared to the normal population was 19.0 (95% CI=7.3-68.3), although no adjustment was made for cigarette smoking or diabetes (which were present in 2 of the four cases). This result is therefore likely to be an overestimate of the effect of chronic pancreatitis.[314] Pancreatitis and pancreatic cancer could share similar aetiological factors, which was investigated in the multi-national collaboration from Australia, Canada, the Netherlands and Poland of 823 cases of pancreatic cancer matched with 1679 controls. They reported that in those with a history of both pancreatitis and smoking there was a significantly increased risk of cancer (OR=15.4, 95% CI=3.2-74.9) implying an interaction between pancreatitis and smoking in the aetiology of pancreatic cancer.[312]

Type 2 Diabetes mellitus

Type 2 diabetes mellitus is known to be associated with pancreatic cancer, although previously it was uncertain if diabetes increased the risk of pancreatic cancer or if was a manifestation of pancreatic cancer due to islet cell destruction. Diabetes could act via three different mechanisms in the carcinogenic process. Firstly, pancreatic cancer and diabetes could have a shared aetiology, secondly the metabolic effects of diabetes could promote pancreatic cancer and thirdly treatments of diabetes could hypothetically lead to pancreatic cancer. Type 2 diabetes, like obesity, is characterised by insulin resistance and hyperinsulinaemia.[315] Raised serum insulin levels could promote pancreatic cancer by stimulating the proliferation of pancreatic cancer cells.[316-317] Insulin in animal models increases pancreatic cancer risk by activating the IGF receptor, although these effects occur at supraphysiologic levels of insulin.[318] The hormone can also modify intermediate pathways, possibly by reducing levels of insulin-like growth factor binding protein-1 (IGFBP-1) which is associated with an increased risk of pancreatic cancer.[319]

Many epidemiological studies have investigated type 2 diabetes and pancreatic cancer with a meta-analysis published in 1995 of 20 case-control and

cohort studies reporting an overall estimated relative risk of diabetes of 2.1 (95% CI=1.6-2.8).[320] This did not change significantly when the analyses were restricted to diabetes with a duration of at least 5 years (RR=2.0, 95% CI=1.2-3.2). A second meta-analysis in 2005 of type 2 diabetes, included 17 case-control and 19 cohort studies published between the years 1996 to 2005 and reported an similar odds ratio of 1.8 (95% CI=1.7-1.9).[278] These two meta-analyses reporting consistent findings support a modest association between type 2 diabetes and pancreatic cancer. Recent onset diabetes, i.e. within one year of developing pancreatic cancer, is particularly associated with an increased risk and is probably secondary to pancreatic cancer destroying islet cells and decreasing insulin production (reverse causality). Recent onset diabetes is associated with a 4 to 7 fold increase in risk, such that 1% to 2% of patients with recent-onset diabetes will develop pancreatic cancer within 3 years.[321] The epidemiological data is supported by biological marker studies which are positively associated with pancreatic cancer which include pre-diagnostic elevations in post-load plasma glucose,[322-323] serum and plasma glucose, [324-325] insulin[326] and plasma C-peptide.[327] A Swedish cohort study of 33 293 women and 31 304 men identified 62 cases of incident pancreatic cancer and found that fasting glucose, but not post-load glucose, was associated with an increased risk of developing pancreatic cancer (top vs lowest quartile, RR=2.49, 95% CI=1.23-5.45, p for trend=0.006).[324] A US study combined data from four large prospective studies and assessed C-peptide levels and the risk of developing pancreatic cancer. C-peptide formed by the cleavage of pro-insulin to form insulin and C-peptide, is a more reliable marker of insulin secretion, than insulin itself, as it has a longer half life and a more predictable metabolic clearance. The North American study identified 179 cases of pancreatic cancer after a maximum of 20 years follow-up. Pre-diagnostic plasma C-peptide was positively associated with pancreatic cancer risk (OR=1.52; 95% CI=0.87-2.64, highest compared with the lowest quartile, P for trend = 0.005), with the association not modified by body mass index or physical activity. The results from these biological markers of glucose and insulin homeostasis indicate that poor glycaemic control and raised insulin levels are associated with an increased risk of pancreatic cancer. The consistency of the evidence from both epidemiological and biomarker data suggests diabetes and hyperinsulinaemia are a causal agents in promoting pancreatic cancer.

Gallstones and cholecystectomy

Epidemiological studies have evaluated the relationship between gallstone disease, cholecystectomy and pancreatic cancer, and there are plausible biological mechanisms to account for a possible carcinogenic effect. Cholecystectomy elevates the levels of the gut hormone cholecystokinin-pancreozymin which can induce pancreatic hyperplasia and hypertrophy.[328-329] In the only large cohort study (104 856 women from the US Nurses' Health Study and 48 928 men from the US Health Professionals Follow-up Study) to investigate this relationship, 349 cases of pancreatic cancer were identified after 16 years of follow-up. The adjusted (including smoking, BMI, physical activity and diabetes) relative risk of developing pancreatic cancer was RR=1.11 (95% CI=0.78-1.56) suggesting gallstones and cholecystectomy are not significant risk factors.[330] In the largest case-control study to investigate the association, a modest increase was seen (OR=1.42 95% CI=1.09-1.84), although no adjustment was made for diabetes and obesity which are probably confounders for gallstone disease and pancreatic cancer. Gallstones and cholecystectomy have been reported as a risk factor in several previous case-control studies, with estimates in the range of 1.3 to 2.8,[331-334] although again, none of these studies adjusted for potential confounders. Therefore, due to the inconsistent findings and lack of adjustment for co-variates in some studies, gallstone disease is not established as a causal factor for pancreatic cancer and more work is required.

Helicobacter Pylori infection

Helicobacter *pylori* (H. *Pylori*) has been implicated in the pathogenesis of pancreatic cancer by inducing atrophic gastritis which promotes hypergastrinaemia and increased secretin levels, both of which can stimulate pancreatic cancer cell growth.[335-336] The ideal epidemiological study to investigate this association would be a cohort study where H. *Pylori* status is measured before the development of cancer. In a case-control study it would be unclear whether H. *Pylori* infection preceded the development of pancreatic cancer or occurred as a consequence of it, although infection with H. *Pylori* usually occurs in childhood.[337] In 2011, a meta-analysis of all previous major epidemiological studies, included three case-control studies and three nested case-control studies, with a total of 2 335 pancreatic cancer patients and which reported a statistically significant adjusted odds ratio of 1.38 (95% CI=1.08-1.75).[338] However, the adjustment for potential confounders varied between studies, with one not adjusting for smoking, two studies failing to adjust for age and three not adjusting for sex. This is important as all these co-variates for

pancreatic cancer are associated with the risk of H. *Pylori* infection. Only one study corrected for the potential confounders of BMI and alcohol intake and its results were not significant (OR=1.25, 95% CI=0.75-2.09).[339] Therefore, the current evidence cannot confirm an effect of H. *Pylori* and further studies measuring potential confounders are required.

Pregnancy

There are biological hypotheses which could account for a protective effect of pregnancy and increased parity against the development of pancreatic cancer. Pregnancy reduces total body iron stores and induces changes in the IGF (insulin-like growth factor) axis, both of which may lower the risk of pancreatic cancer. Elevated IGF promotes cellular proliferation and inhibits apoptosis which could predispose the individual to pancreatic cancer,[318, 340] and women with four or more births, compared to nulliparous women, have a lower concentration of IGF-1 (180ng/ml vs 212ng/ml, p for trend= 0.003).[341] Free iron induces DNA damage by causing oxidative stress [342] with case-control studies reporting raised serum iron concentration and increased iron consumption are positively associated with pancreatic cancer.[343-344] Several epidemiological studies have investigated parity and pancreatic cancer and found increased parity reduces the risk of pancreatic cancer [345-348] which could explain why women have a lower incidence of the cancer. These studies recorded a reduction in risk of at least 20% in women who had 4 or more children. Two Scandinavian studies [349-350] and a Japanese study [351] found no association, although the Scandinavian reports did not adjust for the possible confounding effect of smoking. Overall, despite plausible biological mechanisms, the epidemiological evidence is inconsistent and should be clarified by future work considering all potential confounders.

Smoking

Cigarette smoking is the most consistent risk factor for developing pancreatic cancer.[283] Smoke contains *N*-nitrosamines which cause lung and pancreatic adenocarcinomas in animal models, and these are largely responsible for cancers in smokers.[352] Both case-control and cohort studies can be used to assess smoking exposure since recall bias in the former should be low. Two recent meta-analysis reported that cigarette smoking is associated with a 75% increased risk of

pancreatic cancer, and that there is a dose-dependent effect, with the excess risk from smoking persisting for at least 10 years after cessation.[353] [354] The European Prospective Investigation into Cancer (EPIC) study used a large cohort of 465 910 participants to assess the risk of smoking.[355] A total of 524 incident cases of pancreatic cancer were identified with current smokers having a hazard ratio of 1.71 (95% CI=1.36-2.15) compared to never smokers. Exposure to environmental tobacco smoke, or passive smoking, at work or at home also increased the risk (HR=1.54, 95% CI=1.00-2.39).[355] Exposure to environmental tobacco smoke in childhood was also associated with an increased risk of disease in EPIC (HR=2.61, 95% CI=0.96-7.10) with the positive association replicated in one other cohort study of US women,[356] but not in another US study.[357] The consistency of experimental and epidemiological evidence suggests both active and passive smoking are associated with an increased risk of pancreatic cancer. Hence, smoking should always be measured and adjusted for in aetiological epidemiological studies of pancreatic cancer and encouraging a population to never smoke or stop smoking should reduce the incidence. Currently it is estimated that the worldwide population attributable risk of smoking is 25% of all cases of pancreatic cancer.[277, 358]

Alcohol

Alcohol is a known risk factor for many cancers including breast, colon, oesophagus and liver.[359-360] A high alcohol intake can cause pancreatitis, which pre-disposes to pancreatic cancer, although alcohol could exert an effect independent of pancreatitis through several mechanisms. Firstly, acetaldehyde is the first metabolite of alcohol, and is a well established carcinogen.[361] Secondly, the breakdown products of ethanol are fatty acid ethyl esters which accumulate in the pancreas and may induce cell injury. Alcohol also increases the production of reactive oxygen species, resulting in oxidative DNA damage and alters the effect of dietary antioxidants. Alcohol may also cause gene mutations in enzymes related to cytochrome P450, glutathione S-transferase, aldehyde dehydrogenase, cationic trypsinogen, and pancreatic secretory trypsin inhibitor. Gene mutations lead to a loss of function in these enzymes which regulate normal pancreatic homeostasis and in particular inhibit localised damage from pancreatic digestive enzymes.[362]

Many epidemiological studies have investigated whether alcohol is positively associated with the development of pancreatic cancer. In the EPIC

(European Prospective Investigation into Cancer), 478 000 participants were followed-up for 9 years and 555 cases of exocrine pancreatic cancer identified. An alcohol intake of >30grams per day at recruitment compared to 0.1-4.9g/day, used as the reference value, did not affect the risk (RR=0.94, 95% CI=0.69-1.27) and no effect was found between abstainers and the reference category (RR=1.06, CI=0.79-1.41).[363] The PanScan collaborative study of twelve cohort and one case-control investigation, with 1 530 cases and 1 530 controls, did not report an association between total alcohol intake and pancreatic risk in multi-variate analysis (OR=1.38, 95% CI=0.86-2.23 comparing >60g alcohol/day vs >0-<5g alc/day).[364] However, in men consuming > 45g/day (5.7 units) of alcohol from liquor/day there was a doubling in risk compared to abstainers (OR=2.23, 95% CI=1.02-4.87). Results from another large cohort study of 470 681 American men and women also reported that those consuming high amounts of alcohol from liquor (spirits) had a 62% increased risk (HR=1.62, 95% CI=1.24-2.10) of pancreatic cancer compared to abstainers.[365] A high alcohol intake is associated with several lifestyle factors which could be linked to pancreatic cancer, such as increased exposure to environment tobacco smoke which was not adjusted for in any of the analyses. Furthermore, alcohol causes pancreatitis, a known risk factor for pancreatic cancer. Overall, the evidence indicates that moderate alcohol intake does not affect the risk of developing pancreatic cancer, although a high intake, particularly of liquor, could be positively associated but this may be mediated by inducing pancreatitis. Alcohol data from EPIC-Norfolk was already presented within the multicentre EPIC study discussed previously.[363]

Obesity

There are plausible biological mechanisms for how obesity may promote pancreatic cancer. Obesity increases insulin resistance, insulin levels and the risk of developing type 2 diabetes, all of which are associated with the development of pancreatic adenocarcinoma.[278] Obesity may increase the risk via hyperinsulinaemia and activation of the insulin-like growth-factor (IGF) axis. Both excess insulin and IGF axis activity can stimulate carcinogenesis by altering cell division and preventing the programmed death of defective cells (apoptosis).[366] Over the past 10 years, many epidemiological studies have reported an elevated risk of pancreatic cancer with obesity.[279, 367-373] Obesity may need to be present for many years to influence pancreatic carcinogenesis, and for this reason prospective studies, which

collect anthropometric data many years before the development of symptoms are required to investigate the relationship. Data collected from EPIC-Norfolk participants has already been evaluated within the main EPIC cohort, with a total of 438 405 men and women followed-up for approximately 6 years with 324 incident cases identified.[374] There was a non-significant increased risk of pancreatic cancer with increasing body mass index (RR=1.09, 95% CI=0.95-1.24 per 5 kg/m^2) with an increased waist circumference significantly associated (RR per 10 cm increase=1.13; 95% CI=1.01-1.26; P for trend=0.03). The two largest studies have performed pooled analyses from multiple large cohort studies.[280, 375] The largest pooled analysis of 12 cohort studies and 1 case-control study of 2 170 cases and 2 209 controls reported a 33% increased risk of developing pancreatic cancer in those within the highest cohort specific quartile of BMI.[280] However, although adjustments were made for age, smoking and diabetes none were made for physical activity and energy intake which could be confounders. Evidence from a large US case-control study of 841 cases and 754 controls showed that obesity not only increased the risk of developing pancreatic cancer, but that more young patients with pancreatic cancer were overweight and had shorter survival times.[279] The authors noted that a raised body mass index aged 40 years was more important than that in later life in influencing the risk of pancreatic cancer. Current evidence suggests obesity is a risk factor for pancreatic cancer and hence it should be adjusted for as a co-variate when analysing other risk factors. The rising trend of obesity in most westernised nations may therefore lead to an increased incidence of pancreatic cancer and is a concern.

Physical activity

Physical activity may decrease the risk of developing pancreatic cancer by improving both glucose tolerance and insulin sensitivity, by increasing insulin-stimulated glycogen synthesis and enhancing skeletal muscle glucose transport.[376-377] Raised serum insulin levels have been implicated in the proliferation of pancreatic cancer cells,[316-317] activation of the insulin-like growth factor (IGF) receptor,[318] and modification of intermediate pathways of glucose metabolism, such as insulin-like growth factor binding protein-1 (IGFBP-1), which has been associated with an increased risk of pancreatic cancer.[319]

To investigate physical activity, cohort studies are a more robust methodology than case-control work as the former report *current* activity rather

than *recalled* activity levels. Two meta-analyses have been published which included similar studies. One in the year 2008 included 16 cohort studies, 1 nested case-control and 2 retrospective case-control studies[378] and a second meta-analysis in 2010 assessed, 22 prospective studies and 6 retrospective case control studies.[379] Neither reported an effect from increased leisure activity with the second meta-analysis reporting a RR=0.96, 95% CI=0.89-1.03, although occupational activity did have a protective effect. Both meta-analyses quoted a reduction in risk for the highest vs the lowest of four categories of physical activity at work of a quarter (2010 meta-analysis RR=0.75, 95% CI=0.59-0.96).[379] A case-control study which evaluated physical activity during teenage years, early adult years and mid-life found no association with the risk of developing pancreatic cancer.[380] These studies were limited by the method used to assess physical activity, with none using a questionnaire which had been accurately and comprehensively validated against physiological measures of energy expenditure. Before a final conclusion on physical activity and disease risk can be reached, further investigations are required using such validated questionnaires to accurately measure all forms of physical activity in representative populations. Physical activity is difficult to measure, and hence imprecisions in its assessment (measurement error) will lead to an underestimate of an effect if one truly exist. Furthermore, physical activity may be associated with many confounders for pancreatic cancer, such as obesity and diet, and this must be considered in the evaluation of physical activity. We aim to evaluate the physical activity in EPIC-Norfolk using a questionnaire which has been validated against physiological measures of cardiorespiratory function and energy expenditure. Since physical activity is mostly derived from the level of activity at work, we aim to investigate the effect of physical activity in the whole cohort, but also just in participants younger than 65 years of age, when they are more likely to be in employment.

Drugs

Aspirin

There is emerging evidence that aspirin, statins and metformin may reduce the risk of pancreatic cancer. Aspirin is a cyclo-oxygenase inhibitor that reduces the incidence or growth of several cancers in animal models.[381] These biological effects are believed to be mediated by a reduced production of prostaglandins and other inflammatory mediators which have carcinogenic properties. Case-control studies have failed to show an association,[382] which may be due to inaccuracies in

the measurement of aspirin use before symptoms. Randomised controlled trials (RCTs) of aspirin use, originally designed to investigate aspirin in preventing cardiovascular disease, are now available to provide data on its potential role in cancer. Since most RCTs are on populations too small to evaluate the risk of developing pancreatic cancer, a study has combined data from eight RCTs of daily aspirin use (75mg–1200mg) for at least 4 years, to a maximum of 9 years, including 25 570 patients taking either aspirin or placebo.[383] During the period of intervention, 45 participants died from pancreatic cancer. No effect was found for aspirin taken for 0-5 years on the risk of pancreatic cancer death (HR=0.88 95% CI=0.44-1.77), but aspirin use for more than 5 years did reduce pancreatic cancer deaths by 75% (HR=0.25 95% CI=0.07-0.92, p=0.04). After aspirin was stopped there was no continued protective effect for up to 20 years' follow-up (HR for pancreatic cancer death=0.81 95% CI=0.51-1.26). The results from both experimental studies and a single pooled RCT provide evidence that aspirin used for longer than 5 years prevents pancreatic cancer deaths, although further trial evidence is required before recommending the routine use of aspirin as prophylaxis against pancreatic cancer.

Statins

Statins (HMG-CoA reductase inhibitors) reduce serum cholesterol by inhibiting the rate limiting step in cholesterol synthesis and are used in the primary and secondary prevention of cardiovascular disease. In addition to cholesterol, there are several other products derived from HMG-CoA reductase, including farnesyl pyrophosphate and geranylgeranyl pyrophosphate and by reducing these metabolites statins may also have effects on the carcinogenic process. These metabolites affect GTPase signalling proteins whose functions can influence cell proliferation.[384] Therefore it is plausible that patients prescribed statins may have a reduced risk of pancreatic cancer. The strongest methodology for demonstrating a protective effect of statins would be a RCT which removes the biases and confounding associated with observational epidemiological work. A recent meta-analysis of 3 RCTs, 4 cohort studies, and 5 case-control studies found no evidence of an association between statin use and pancreatic cancer among either the RCTs (RR=0.99, 95% CI=0.44-2.21) or the observational work (RR=0.86, 95% CI=0.60-1.24).[385] However, the largest cohort study to be undertaken in this area of nearly half a million US Veterans, reported statin use of more than 6 months was associated with a reduction in pancreatic cancer risk, with an odds ratio of 0.33 (95% CI=0.26-0.41, p<0.01).[386] A nested case-control study using the world's

largest prescribing database of around 5 million people, the UK General Practice Research Database (GPRD), identified 1 141 cases of pancreatic cancer. There were no associations with either any previous statin use (OR=0.93, 95% CI=0.76-1.14) or long-term use (OR=0.71, 95% CI=0.42-1.20).[387] The inconsistencies of these reported results may be due to the different populations studied, the types and doses of statins used and the differing study methodologies. Despite the chemopreventive potential of statins demonstrated in experimental studies, current evidence does not support the using these drugs to reduce the risk of pancreatic cancer although future work is required to clarify if they have an effect in preventing pancreatic cancer.

Metformin

There is emerging evidence that metformin may prevent pancreatic cancer. Metformin is a biguanide that interacts with the enzyme AMPK (AMP activated protein kinase) and induces muscles to take up glucose from the blood. The upstream regulator of AMPK is the protein kinase, LKB1, which has tumour suppressive activity.[388] AMPK is activated via LKB1, achieved by both metformin and exercise, and could explain why exercise is beneficial in the primary and secondary prevention of certain cancers.[389] In hamster models of pancreatic cancer, metformin had a significant protective effect against tumour development.[390] Epidemiological studies have reported that diabetic patients treated with metformin were less likely to develop cancers of any type,[391-392] although only one investigation has assessed the association between metformin and pancreatic cancer. This was a case-control study which recruited 973 patients with pancreatic cancer (259 were diabetic) and 863 matched controls (109 diabetic). Diabetic patients prescribed metformin had a significantly lower risk than diabetics who were not prescribed metformin of pancreatic cancer (OR=0.38, 95% CI=0.22-0.69).[393] These findings require clarification in further investigations, particularly in large scale cohort studies. If metformin is confirmed to protect against pancreatic cancer, the drug may offer a method of reducing the incidence of disease in all patients with type 2 diabetes, a group at increased risk.

4. Diet as a risk factor for pancreatic cancer

Diet is a plausible environmental factor to investigate in the aetiology of pancreatic cancer, although nutritional epidemiology has many methodological difficulties. These have already been discussed in chapter one, in the section "Measuring dietary intake". The risk of developing pancreatic cancer may be modulated by diet, as different nutrients have potential causative and protective effects on the process of carcinogenesis. Dietary factors which may induce these effects include food groups, macronutrients, micro-nutrients, beverages and cooking methods. Many cohort studies have investigated dietary factors although several studies were not included in this review due either to not using a validated questionnaire [394] or the populations were not generalizable (e.g. a population of Californian Seventh-day Adventists[395] and of Japanese atomic bomb survivors[396]). The prospective cohort studies reviewed (Table 24) all used food frequency questionnaires (FFQs) to measure dietary intake which had been validated against internal methods, namely 24-hour recall or diary histories.

Table 24. Cohort studies investigating the diet and pancreatic cancer.

	Study name and acronym	Country	Number of participants	Sex	Age (years)	Dietary assessment	Validation method
1	Alpha-Tocopherol, Beta-Carotene Cancer prevention study						
	ATBC	Finland	27 111	Male	50-69	FFQ	2-DFD
2	Swedish Cohort Study						
	SCS	Sweden	77 797	Both		FFQ	7-DFD
3	Multiethnic Cohort Study						
	MEC	US	215 000	Both	45-75	FFQ	24-hr recall + FFQ
4	US Nurses' Health Study						
	NHS	US	88 794	Women	30-55	FFQ	24-hr recall
5	US Health Professionals Follow-up Study						
	HPFS	US	49 364	Men	40-75	FFQ	24-hr recall
6	Singapore Chinese Health Study						
	SCHS	Singapore	60 524	Both	45-74	FFQ	24-hr recall + biomarkers
7	National Institutes of Health (NIH)-American Association of Retired Persons(AARP) Diet & Health Study						
	NIH-AARP		>500 000	Both	50-71	FFQ	24hr recall
8	Netherlands Cohort Study						
	NLCS	Holland	120 853	Both	55-69	FFQ	3-DFD
9	European Prospective Investigation into Cancer						
	EPIC	Europe	521 000	Both	35-70	FFQ	24-hr recall
10	The Iowa Women's Health Study						
	IWHS	US	41 837	Women	55-69	FFQ	24-hr recall
11	Prospective study of pancreatic cancer in the elderly						
		US	13 979	Both	65-85	FFQ	24-hr recall

Total energy intake

Total energy intake may contribute to the risk of pancreatic cancer via weight gain and obesity, although surprisingly previous prospective epidemiological studies have failed to demonstrate that total energy intake promotes weight gain[155-156] [157] There are no other plausible biological mechanisms for how increased energy intake *per se* may alter the risk of pancreatic cancer, although it may reflect the intake of other nutrients or could be due to increased energy expenditure and hence increased physical activity.[397] Previous epidemiological studies in this area have reported mixed results between energy intake and the risk of pancreatic cancer. In the alpha-tocopherol beta-carotene (ATBC) cancer prevention study, total energy intake, adjusted for smoking and age, had a negative association with pancreatic cancer risk (highest vs lowest quintile HR=0.62, 95% CI=0.36-1.07, p for trend=0.05).[398] These findings may represent a confounding effect, as those with a high energy intake are likely to be more physically active and have different lifestyle and dietary behaviours. A prospective study of 33 976 post-menopausal women from Iowa, United States, did not find an association (highest vs lowest tertile of calorie intake, RR=1.20 95% CI=0.67-2.15).[399] Currently the lack of a confirmed biological mechanism and inconsistent epidemiological findings suggest that energy intake is unlikely to be involved in pancreatic cancer aetiology. However, in analyses of dietary factors, it is important to measure energy intake to include multi-variate analyses (see section on energy adjustment page 79), as a crude adjustment for body-size, physical activity and metabolic rate. Adjustment for energy intake therefore gives an assessment of the nutritional density effect of a food, rather than the actual amount.

Dietary fat and fatty acid groups

Fat could act via several mechanisms to induce pancreatic neoplasia. Dietary fat stimulates the release of the hormone cholecystokinin which provokes pancreatic enzyme release and hypertrophy and hyperplasia of acinar cells, causing susceptibility to carcinogens.[400] In rodents fed high-fat diets, compared to rats fed a low fat diet, there was a higher incidence of pancreatic cancer.[401] Individual fatty acids can also exert specific effect that could also influence pancreatic carcinogenesis with evidence that saturated fatty acids induce insulin resistance,[402] through altered insulin secretion and decreased insulin sensitivity.[173-176] Insulin resistance may be a precursor to pancreatic cancer,[326] with insulin a possible carcinogen.

Several epidemiological studies have investigated the effect of dietary fats with a summary of results in Table 25. The US National Institute of Health - American Association of Retired Persons (NIH-AARP) is the largest cohort study undertaken, of 308 736 men and 216 737 women, which identified 865 cases of incident pancreatic cancer in men and 472 in women after 6.3 years follow-up.[403] An increased risk of disease was found in those with the highest intake of total fat (highest vs lowest quintile HR=1.23 95% CI=1.03-1.46), saturated fat (HR=1.36, CI=1.14-1.62) and monounsaturated fat (HR=1.22 CI=1.02-1.46). The strongest association was reported for saturated fat derived from animal sources (HR=1.43, 95% CI=1.20-1.70) with no effects for mono or polyunsaturated fatty acids. Adjustment for occupational physical activity made little difference to the results. Saturated fat intake is correlated with meat intake which includes heterocyclic amines and polyaromatic hydrocarbons found in cooked meats that could have carcinogenic effects and act as confounders.[398] The Multi-Ethnic Cohort (MEC) Study found no association with intakes of total fat and different types of fats derived from all food types.[404] However, fat intake derived solely from meat was associated with an increase in pancreatic cancer (highest quintile vs lowest quintile of energy from fat derived from red meat and processed meat, HR 1.44, 95% CI=1.18 to 1.76). The Netherlands Cohort Study (NCS), with 350 cases diagnosed after 13.3 yrs of follow-up, did not find any association with total fat intake or fatty acid groups,[405] and neither did the the US Nurses' Health Study which identified 178 cases of disease after 18 years of follow-up.[406] All the above cohort studies used an FFQ to record dietary intake, which had been validated against an internal measure (such as 24-hour recall) rather than an external measure such as urinary biomarkers or weighed records. The results from the NIH-AARP, ATBC and MEC studies suggests that fat derived from meat or animal products is more likely to have a positive association with pancreatic cancer risk. Further studies are required using accurate dietary assessment measures such as food diaries which have been validated against external measures.

Table 25. Cohort studies investigating dietary fats and pancreatic cancer.

Study	**Country**	**Number of participants**	**Sex**	**Age**	**Total fat** (p for trend)	**Saturated** (p for trend)	**Monounsat** (p for trend)	**Polyunsat**	***Trans***	**n-6**	**n-3** (p for trend)
ATBC[398]	Finland	27 111	Male	50-69	+ (0.07)	+ (0.02)	0	0	ND	0	0
MEC[404]	US	215 000	Both	45-75	0	0	ND	ND	ND	ND	ND
NHS[406]	US	88 794	Women	30-55	0	0	0	0	0	0*	ND
NIH-AARP[403]	US	>500 000	Both	50-71	+ (0.03)	+ (<0.001)	+ (0.05)	0	0	0	+ (0.01)
NLCS[405]	Netherlands	120 853	Both	55-69	0	0	0	0	0	ND	0[#]

ATBC=Alpha-Tocopherol, Beta-Carotene Cancer prevention study; MEC=Multiethnic Cohort Study;

NHS=Nurses' Health Study; NIH-AARP=National Institutes of Health (NIH)-American Association of Retired Persons (AARP) Diet and Health Study

NLCS=Netherlands Cohort Study.

0 no association; + positive association; ND no data

*data only from linoleic acid, [#]data from individual n-3 fatty acids

Trans fatty acids

The *trans* fatty acid, elaidic acid, has been shown to increase insulin resistance[407] and cholesteryl ester transfer protein (CETP) activity, which causes dyslipidaemia by raising VLDL and lowering HDL cholesterol.[408] Elevated levels of *trans* fatty acids have been associated with both breast and prostate cancer. The three cohort studies (NIH-AARP, NHS and NLCS) that investigated *trans* fats and pancreatic cancer have failed to find an association with pancreatic cancer,[403, 406] which was also the outcome in a Canadian case-control studies.[409] Despite a plausible mechanism for *trans* fatty acids increasing the risk of pancreatic cancer, epidemiological results have not found any associations for trans fatty acids, although studies using more precise methods of measuring their intake could be required if they only exert a small effect size.

N-6 polyunsaturated fatty acids

There is an established relationship between inflammation and the development of pancreatic cancer,[410] highlighted by the 5-fold increased risk of cancer in patients with pancreatitis.[312] N-6 fatty acids promote the production of inflammatory cytokines which, in turn, can stimulate oncogenic pathways such as cell proliferation, apoptosis, and angiogenesis which favour tumour growth.[411] The n-6 polyunsaturated fatty acid (PUFA), arachidonic acid, is found in the diet or is formed from the conversion of the n-6 PUFA, linoleic acid. Arachidonic acid is incorporated into the phospholipid epithelial membrane and once metabolised produces the pro-inflammatory eicosanoids, including prostaglandin E2 (PGE2), leukotriene B4 and thromboxane A2. Experiments in mice injected with human pancreatic cancer cells have demonstrated a tumour stimulating effect of a diet rich in arachidonic acid, with these effects mediated by COX-2 generated prostaglandin E2 (PGE2).[412] In mice fed the n-3 fatty acid, eicosapentaenoic acid, there was the opposite effect, with increased generation of prostaglandin E3, decreased production of PGE2 and reduced growth of pancreatic cancer. These findings suggest that the dietary intake of n-3 and n-6 fatty acids can alter the n3/n-6 ratio, and hence prostaglandin production, which can impact on pancreatic cancer growth. However, n-6 fatty acids can reduce insulin resistance [174] which could reduce the risk of developing pancreatic cancer and hence the role of dietary n-6 fatty acids in this disease needs to be clarified in further work.

Previous cohort studies have investigated the effects of dietary n-6 fatty acids. The Finnish ATBC cohort study and the US Nurses' Health Study assessed dietary n-6 PUFA intake and found no associations after adjusting for co-variates.[398, 406] In the NIH-AARP study, no association was found for total n-6 intake, or linoleic acid intake, although the intake of arachidonic acid was positively associated with pancreatic cancer, with the highest quintile of intake HR=1.33 (95% CI=1.12-1.58, p for trend=0.002).[403] No effect was found for the n-6 PUFAs, linoleic acid or arachidonic acid, in two large case-control studies, one from San-Francisco[413] and one from Canada.[409] More data from further epidemiological studies is required using detailed assessments of n-6 PUFAs to clarify if they have an effect on the risk of pancreatic cancer.

N-3 polyunsaturated fatty acids

N-3 fatty acids all have their first carbon-carbon double bond at the third carbon site from the terminal methyl end of the carbon chain. The three main n-3 fatty acids in human nutrition are α–linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Humans have very little ability to synthesise EPA and DHA so concentrations in the tissues are derived mostly from the diet,[414] particularly fish oils. Marine phytoplankton and zoo plankton readily elongate and desaturate α–linolenic acid to produce abundant EPA and DHA which leads to the incorporation of n-3 fatty acids into the marine food chain.

Laboratory results have demonstrated n-3 fatty acids prevent the proliferation of mammary, prostate, colon, and pancreatic tumours.[415-416] The n-3 fatty acids EPA and DHA have inhibitory effects on the growth of human pancreatic cancer cell lines *in vitro*.[412, 414, 417] In mice models of pancreatic cancer, a feed rich in n-3 PUFA's lead to a reduced incidence, frequency, and proliferative index of pancreatic cancer cells.[418] In vitro, the effect of the omega-3 fatty acid, docosahexaenoic acid (DHA), on two pancreatic cancer cell lines was assessed, which was associated with a dose-dependent decrease in proliferation, through G1/G0 cell cycle arrest and induction of apoptosis.[418] How n-3 fatty acids achieve these reductions of tumour incidence and proliferation in experimental models is not certain although several mechanism have been proposed including alteration of the eicosanoid profile (consisting of prostaglandins, thromboxanes and leukotrienes), reduction of oncogenic mutations and induction of apoptosis.[419]

The fatty acid profile of the phospholipid epithelial membrane determines the fatty acid used as a substrate in the production of eicosanoids. Arachidonic is

the substrate predominantly although if EPA and DHA are present they will be incorporated into the phospholipid membrane at the expense of arachidonic acid.[420] An example of the alteration in the eicosanoid profile due to the available substrate is demonstrated in the the leukotriene family. If 5-lipoxygenase metabolises arachidonic acid the leukotrienes are derived (4-series leukotrienes) have stronger inflammatory effects compared to those derived from EPA and DHA (5-series leukotrienes).

Many of these results have often been achieved at supra-physiological doses of n-3 PUFAs, with accompanying alterations in the general composition of the diet, particularly by lowering the n6/n3 fatty acid ratio, so benefits could be due to a reduced n-6 contribution to the diet rather than an the effects of n-3s *per se*.[421] N-3 fatty acids may prevent the acquisition of genetic mutations. One example of the protective effect of n-3 fatty acids has been described in K-ras genes, which are involved in the initiation of carcinogenesis and are DNA targets for chemical carcinogens. K-ras mutations are an early and fundamental event in the pathogenesis of most exocrine PC[422] and are the most frequent oncogenic alterations in human cancer, and a prime example of activation by point mutation. Ras proteins are vital for cell functions including the regulation of growth, differentiation and apoptosis with K-ras point mutations found in 75-90% of pancreatic cancers.[296, 422] In a study of pancreatic cancer cases, those with K-ras mutations (78%) were compared to those without K-ras mutations (22%) and a food frequency questionnaire used to assess differences in n-3 intake over the preceding year. The highest tertile of dietary n-3 PUFA intake was associated with a reduced rate of K-ras mutation (OR=0.19, 95% CI=0.05-0.81) with a significant trend across tertiles (p=0.024).[422] Thus the prevention this and potentially other oncogenic mutations in the pathway to pancreatic carcinogenesis could account for a protective effect of n-3 PUFAs against the development of cancers.

The Finnish ATBC, US NHS and Netherlands Cohort Study investigated the effects of n-3 fatty acids and fish intake on pancreatic cancer but none found any associations.[398, 405-406] The NIH-AARP study reported an increased risk of pancreatic cancer for total n-3 intake (highest quintile vs lowest HR=1.21 95% CI=1.02-1.44).[403] Hence the experimental data, suggesting that n-3 fatty acids may prevent pancreatic cancer development, is not currently supported by the epidemiological evidence, although further studies are required since the experimental work provides substantial plausible protective biological mechanisms.

Oleic Acid

Oleic acid is an n-9 monounsaturated fatty acid found in animal and vegetable oils, especially olive oil, as well as in rapeseed oil, avocado and nuts. This nutrient occurs naturally in greater quantities than any other fatty acid. Oleic acid is a major component of the Mediterranean diet, which in a meta-analysis was associated with both a reduced all cause mortality as well as mortality from cancer (RR=0.94, 95% CI=0.92-0.96).[423] The mechanisms for its protective effect are yet to be clearly defined, although oleic acid down regulates the oncogenic promoter region of "Her-2/neu" which leads to breast, ovarian and gastric tumours.[424]

Two cohort studies have evaluated oleic acid and the risk of pancreatic cancer, with both the Nurses' Health Study and the NIH-AARP study reporting no differences between the highest and lowest quintile of intake.[403, 406] In a Canadian case-control study of 462 cases of pancreatic cancer and 4721 controls, oleic acid was associated with a reduced risk of pancreatic cancer (highest vs lowest intake OR=0.75, 95% CI=0.55-1.02).[409] However, the same study found several fatty acid groups were associated with a decreased risk, including saturated and MUFAs, which could represent a correlated effect of nutrients in this study. A case-control study from San Francisco reported oleic acid was associated with an increased risk of pancreatic cancer (highest vs. lowest quartile OR=1.4, 95% CI=1.1-1.9, p-trend=0.008). The inconsistencies of these findings suggest that oleic acid may not be involved in the aetiology of pancreatic cancer although further work to clarify the association is required.

Meat and heterocyclic amines

Meat may increase the risk of pancreatic cancer, especially red meat, after it has been cooked as this produces heterocyclic amines (HCAs) and N-nitroso compounds, both of which promote carcinogenesis. HCAs are formed during the high-temperature cooking of meat from reactions involving creatine or creatinine, amino acids, and sugar,[425] with the amount of HCAs produced dependent on both cooking times and the temperature. Evidence from both animal and human studies suggest that HCAs play a role in the pathogenesis of some cancers including pancreatic cancer.[426] HCAs are highly mutagenic toward mammalian cells, and in dietary animal studies, they cause cancers in many organs. When these tumours have been examined, gene alterations have been found in several signalling

systems (*Apc, beta-catenin, Ras*) by the bonding of HCA adducts to DNA.[427] Proportionally more HCAs are produced from red than white meat.

Epidemiological studies have investigated meat and cooking methods in relation to the risk of developing pancreatic cancer. In the Swedish cohort studies, an increased risk was reported in those with the highest consumption of red meat (HR=1.73, 95% CI=0.99-2.98) with the greatest intake of poultry associated with a reduced risk (HR=0.44, 95% CI=0.20-0.97).[428] The NIH-AARP, a cohort study study of over half-a-million people, evaluated the risk of HCA exposure using a meat-specific questionnaire.[429] A meat mutagen and mutagenic activity index was derived which found that men in the highest vs lowest quintile had more than a 2-fold increased risk of developing pancreatic cancer (HR=2.32 95% CI=1.52-3.52, trend across quintiles p=0.001), but no association was found in women. The intake of total red meat and meat cooked at high temperatures were all positively associated with pancreatic cancer among men (fifth versus first quintile: HR=1.41, 95% CI=1.08-1.83, p-trend=0.001; HR=1.42, 95% CI=1.05-1.91, p-trend=0.01; and HR, 1.52, 95% CI, 1.12-2.06, p-trend=0.005, respectively) but again, no associations were found in women. Meat and heterocyclic amines need to be investigated in greater detail to clarify their precise effect using information derived from food diaries.

Antioxidants

There are plausible biological mechanisms for how dietary antioxidants may inhibit carcinogenesis including that in the pancreas. Antioxidants including vitamin C and E, selenium and zinc, stimulate immune function [430-431] and prevent oxidative DNA damage which precedes carcinogenesis [432]. Free radicals can be produced from oxidative damage to cell membranes which has a carcinogenic effect. Ras genes are involved in the initiation of carcinogenesis and are DNA targets for chemical carcinogens. Somatic (acquired) mutations in the K-ras oncogene are an early and fundamental event in the pathogenesis of most exocrine PC.[422] They are the most frequent oncogene alterations in human cancer, and a prime example of activation by point mutation. Ras proteins are vital for cell functions including the regulation of growth, differentiation and apoptosis. K-ras point mutations are found in 75-90% of pancreatic cancers.[296, 422] In a study of pancreatic cancer cases with K-ras mutations (78%) and without K-ras mutations (22%), those with the highest tertile of dietary vitamin E intake had a reduced rate

of K-ras mutation (OR=0.24 95% CI=0.06-0.98, p for trend=0.036) and for vitamin C OR=0.57 (95% CI=0.14-2.38, p for trend=0.28), although other antioxidants were not assessed.[422]

Antioxidants may exert their biological effect through inhibiting inflammation which is a recognised risk factor for the development of several cancers. Chronic inflammation may play a role in pancreatic carcinogenesis,[433] with both hereditary and non-genetic pancreatitis significant risk factors for developing cancer by factors of 53 and 17 respectively.[313, 434] Chronic pancreatitis is associated with the generation of reactive oxygen species. When antioxidant enzyme levels are assessed in pancreatic tissue, there is a gradual decrease in antioxidant enzyme expression in pancreatic cells from normal to chronic pancreatitic to pancreatic cancer cells.[435] In placebo controlled trials in patients with chronic pancreatitis, antioxidants reduced levels of pain and markers of oxidative stress.[436-438]

The experimental data on the effects of antioxidants is supported by limited epidemiological work in pancreatic cancer, with only two prospective cohort studies investigating these micronutrients. The Finnish ATBC study of 27 111 male smokers, reported no associations for the dietary intakes of vitamins C and E and selenium,[398] but that higher serum levels of vitamin E were associated with nearly a halving of risk (highest compared with lowest quintile HR=0.52 95% CI=0.34-0.80, p for trend 0.03). The only other prospective study, which assessed antioxidants, was of 13 979 residents in a retirement community that identified 65 incident cases of pancreatic cancer after 9 years of follow-up.[439] Higher intakes of vegetables, fruits, dietary beta-carotene, and vitamin C were each associated with a reduced risk, although none of these associations were statistically significant. Intakes of vitamin E, selenium and zinc were not assessed. Cohort studies assessing the intake of fruit and vegetables, which are rich in antioxidants, have largely failed to find an association with pancreatic cancer.[398, 440] However, associations of smaller magnitude could be undetected because of measurement error in the dietary assessment methods. The latter is particularly applicable to data collected using FFQs and 24-hour recall. A Swedish cohort study, reported a statistically significant inverse association with the intake of cruciferous vegetables (>=1 serving/week vs never consumption: HR=0.62, 95% CI=0.39-0.99).[441] This protective effect may be due to the high content of glucosinolates, which following degradation into isothiocyanates,[442] inhibit both pancreatic carcinogenesis in animal models and the growth of human pancreatic cancer cell lines.[443-444] A meta-analysis of citrus fruit consumption reported a high intake was associated with a

modest reduction in risk of pancreatic cancer (OR=0.83; 95% CI, 0.70-0.98),[445] and this effect could in part be due to the high vitamin C content found in citrus fruit or other residual confounders.

Randomised controlled trials (RCTs) provide the strongest evidence, and these have been conducted to assess potential health benefits of antioxidants in the prevention of other chronic conditions, particularly cancers and cardiovascular disease. One RCT has specifically assessed antioxidant supplementation and pancreatic cancer, the Finnish ATBC study, which randomised 29 133 male smokers to either alpha-tocopherol (AT; 50 mg/day), beta-carotene (BC; 20 mg/day), both AT and BC, and placebo daily for 5-8 years. No statistically significant differences were found between the treatment groups for pancreatic cancer incidence (AT vs placebo, RR1.34 95% CI=0.88-2.05) or mortality (RR=1.11 95% CI=0.72-1,72).[446] The study population of just male smokers was an appropriate cohort to assess the potential benefits of vitamin E supplementation, as this group has a higher incidence of disease than non-smokers and females. However, the results do not establish whether vitamin E may be of benefit in the latter groups. A Cochrane review which combined 6 RCTs which used other health end-points as the primary outcome, did not find any effect from antioxidant supplementation against pancreatic cancer (RR 1.16, 95% CI 0.90-1.50).[447] To clarify the inconsistencies in the literature of the effects of antioxidants, more cohort studies are required using data derived for the first time from food diaries.

Sugar

The increased risk of pancreatic cancer associated with obesity and type 2 diabetes is probably partly due to raised insulin levels. Cohort studies have reported elevated baseline levels of fasting serum glucose and fasting insulin concentrations associated with a doubling the risk of pancreatic cancer.[325-326] Excess dietary sugar which stimulates insulin release may therefore increase the risk. There is supportive evidence for a role of sugar from both experimental and biomarker data, although results from epidemiological studies on total sugar intake are inconsistent. An investigation of 77 797 Swedish men and women reported that those with the highest quarter dietary of sugar intake had nearly a doubling of risk pancreatic cancer (RR=1.95 95% CI=1.10-3.46).[448] Soft drinks consumption has been studied, as they are a major source of added sugar intake in the Western diet, with around 10 grams of added sugar per 100mls. In the US, the consumption of

≥3 sugar-sweetened soft drinks a week was associated with a 57% (RR=1.57, 95% CI=1.02-2.41) increased risk of developing pancreatic cancer in a cohort of 88 794 women, although no association was found in 49 364 American men.[449] A similar investigation of 60 524 men and women in Singapore reported those consuming ≥2 soft drinks a week had an 87% (HR=1.87, 95% CI=1.10-3.15) increased risk of developing pancreatic cancer compared to those who drank <1 soft drink a month.[450] However, not all investigations have linked sugar consumption to an increased risk of pancreatic cancer. A cohort investigation of 487 922 American men and women, with 1 258 incident cases, found no effect of total added sugar or sugar-sweetened foods and beverages.[451] The data was derived from food frequency questionnaires which have inaccuracies for measuring diet. Several other cohort studies using similar methods also found no effect of sugar or sucrose intake.[452-455] The discrepancies in the sugar data may be due to error in the methods used for recording diet and in the definitions used for sugar. The term "sugar" is most commonly used when referring to "sucrose". However, sugars can be either single sugar molecules (monosaccharides) of glucose, galactose or fructose, or two sugar molecules (disaccharides) of sucrose (glucose + fructose), lactose (glucose + galactose) and maltose (glucose + glucose). Added sugar in the diet is usually in the form of sucrose although particularly in North America, it may be high-fructose corn syrup (a glucose-fructose syrup). Clarification on the role of sugar is required from further cohort studies using accurate measures of dietary assessment, although most current evidence suggests that sugar and sucrose in particular may increase the risk of developing disease, but that the source of sugar is important.

5. Summary of introduction

To date, the recognised risk factors identified for pancreatic cancer are genetic syndromes (i.e. hereditary pancreatitis, Peutz-Jeghers), a family history of pancreatic cancer, previous pancreatitis, type 2 diabetes, tobacco smoking and obesity. Factors with substantial, but not clear evidence of an effect include physical activity, Helicobacter *Pylori* infection, and the use of aspirin and metformin. Dietary nutrients which may be involved in the aetiology of disease are fatty acids, antioxidants and sugar. The lack of defined risk factors for pancreatic cancer could be due to previous work having methodological weaknesses in the study design and imprecise methods to measure exposure risk. This prospective cohort study, of over 25 000 participants, aims to address these limitations, by comparing baseline characteristics and dietary intake in those who develop disease, to those who do not. The investigation minimises measurement error by using a physical activity validated against physiological measures of cardio-respiratory fitness, and also a unique method of recording dietary intake, namely 7-day food diaries, which produces a more accurate measure of nutrient intake compared to other methods.[47] Hence the study design may allow the identification of new risk factors, in particular those with small effect sizes.

Methods

Preface to methods

EPIC-Norfolk was used as the study population which is previously described in chapter 1 (p22-26) as well as the methods used to measure demography (p27), anthropometry (p27), physical activity (p27-28), dietary assessments (p30-33), supplement use (p34), alcohol use (p34) and blood samples (p34).Methods specifically pertaining to the investigation of pancreatic cancer are discussed here.

1. Case ascertainment

The cohort was monitored after recruitment to identify those participants who developed incident pancreatic cancer up to June 2010, i.e. a maximum follow-up time of 17 years after recruitment. Participants with pancreatic cancer were identified by matching the EPIC-Norfolk database with the Norfolk Health Authority records of hospital admissions, the Eastern Cancer Registry and Information Centre (ECRIC) and death certificate records. The International Classification of Diseases-10 (ICD-10) code used was C-25 (malignant neoplasm of the pancreas) and its subdivisions (Table 26).[252] The notes of all potential cases were reviewed by a single medical gastroenterologist (Paul Banim) to verify the diagnoses and obtain clinical diagnostic and staging information. Cases were only included if the diagnosis was compatible with the clinical features of pancreatic exocrine cancer and confirmed either by radiological, endoscopic, surgical or histological investigation. The Eastern Cancer Registry accessed microfilmed data and records on patients diagnosed outside the geographic area to aid case-ascertainment. Cases were excluded if there was diagnostic uncertainty, the diagnosis was endocrine pancreatic cancer, participants had pancreatic cancer prior to enrolment, and if the diagnosis was made within 6 months of entering the study. The later ensured that the dietary data was truly prospective before the development of symptoms.

Table 26. The ICD-10 codes of pancreatic cancer used to identify potential cases of pancreatic cancer in EPIC-Norfolk participants.

C25	Malignant neoplasm of pancreas
C25.0	Malignant neoplasm of head of pancreas
C25.1	Malignant neoplasm of body of pancreas
C25.2	Malignant neoplasm of tail of pancreas
C25.3	Malignant neoplasm of pancreatic duct
C25.4	Malignant neoplasm of endocrine pancreas
C25.7	Malignant neoplasm of other parts of pancreas
C25.8	Malignant neoplasm of overlapping sites of pancreas
C25.9	Malignant neoplasm of pancreas

Statistical analyses

The statistical analysis was performed using the computer program STATA Version 10 (Stata, College Station, Texas, USA). In the analysis, baseline characteristics were compared between participants with and without incident pancreatic cancer using a t-test for normally distributed continuous variables, a Mann-Whitney U test for non-parametric continuous variables and a chi-squared test for categorical ones. Known risk factors for pancreatic cancer and nutritional study exposures studied were defined and classified into categories as shown in Table 27. Cox proportional regression models estimated the hazard ratios (with 95% confidence intervals) of developing incident pancreatic exocrine cancer according to each category of exposure, using the lowest level of exposure as the baseline value, with further analyses made of the trends across categories. All analyses were adjusted for the potential covariates of age at recruitment, gender, cigarette smoking, type 2 diabetes and body mass index (BMI). In the dietary analysis further adjustment was made for the average daily total energy intake (kcal) derived from the 7-DFD and in the antioxidants further adjustment was made for supplements containing the respective antioxidant.

The primary analysis, for each variable, was performed after 10 years of follow-up and the secondary analysis after 17 years, which was the maximum length of follow-up. Ten years was considered the time during which a single measure of dietary intake from the physical activity questionnaire or 7-DFD may be representative of that measured at recruitment. This technique would reduce regression dilution bias caused by a proportion of the cohort altering their behaviour during follow-up which introduces measurement error. The secondary outcome was the risk of pancreatic cancer after the full follow up period of 17 years after recruitment.

Physical activity analysis

A cohort analysis using Cox regression was made of physical activity which was categorised using the four level physical activity index (Table 1). Since the physical activity index score was predominantly derived from the level of occupational physical activity, a separate analysis was made excluding all participants aged 65 years (the state retirement age) and over at enrolment, as those not working were less likely to have their level of physical activity correctly classified which would have introduced measurement error.

Alcohol analysis

Alcohol intake was estimated from the food frequency questionnaire with data available on the whole cohort of 25 639 participants. Alcohol was analysed as a categorical variable as defined in Table 27.

Dietary analysis

For the dietary variables a case-cohort analysis was performed using a representative subset of 3 970 randomly selected participants who did not develop pancreatic cancer. This analysis was required as most but not all of the completed diaries had been coded. Each nutrient was divided into fifths of intake across the distribution of the whole cohort. To evaluate for possible nutrient threshold effects, the lowest fifth of intake was compared to a summation of the four higher fifths.

Table 27. Characteristics and exposures with units and cut-points used in analysis

Characteristic	**Units**	**Cut-points**
Age at recruitment	Years	Continuous variable
Smoking status	--	Never smoked Previous smoker Current smoker
Diabetes	--	Reported at baseline Not reported at baseline
Body mass index	kilograms/metre^2	<25 (normal) 25-<30 (overweight) 30-<35 (obese class I) >35 (obese class II & III)
Alcohol intake	units per week (1 UK unit = 7.9 grams or 10 mls)	Zero >0-<7 7-<14 14-<21 ≥21
Physical activity	Derived from physical activity index (table 2)	Inactive Moderately inactive Moderately active Active
Dietary nutrients	Average daily intake	Fifths

Results

1. Case ascertainment

From the cohort of 25 639 participants (54.7% women) who attended the baseline health check, 111 cases of potential incident pancreatic cancer were identified who had their notes reviewed by a gastroenterologist specialist. Of these, 53 participants (41.5% women) had confirmed incident exocrine pancreatic cancer after 10 years follow-up, with 93 cases (52.7% women) 17 years follow-up. The remaining 18 cases were excluded from analysis with the reasons for exclusion listed in Table 28. Comparing the clinical diagnoses of pancreatic cancer from review of the notes and those at Eastern Cancer Registry and Information Centre (ECRIC) it was possible to calculate the specificity of the cancer registry process if review of the clinical notes was deemed to be the gold standard. A maximum of 9 out of the total 93 cases had not had time to be registered at ECRIC and they were excluded from the analysis. Three patients had not been registered at ECRIC despite reasonable clinical evidence on note review of pancreatic cancer, giving a sensitivity of 96.4% (81/84 x 100).

Clinical features of participants developing pancreatic cancer

The 53 cases diagnosed after 10 years follow-up had a mean age at diagnosis of 69.7 years (SD=8.6 years) (Table 29). The stage of disease at diagnosis was mostly either distant metastatic disease (43.4%, American Joint Committee on Cancer, AJCC stage 4 disease) or locally advanced disease (18.9%, AJCC stage 3)(Table 29). Only 15.1% had cancer localised to the pancreas (stage 0, 1A or 1B) with 9.4% classified as having locally invasive disease (stage 2A or 2B)(Table 29). Histological confirmation of adenocarcinoma was made in 37.8% patients. In those without histology the diagnosis was made using at least two imaging modalities from either: USS, CT, ERCP, MRI. Patients were treated with either: surgery (13.5%), chemotherapy (38.5%) or with palliative measures (48.1%). The median survival of all patients was 4 months (range 0.25 to 25 months) with a mean survival of 6.8 months (SD=6.4 months). In the 93 cases diagnosed after 17 years follow-up, the mean age at diagnosis was 72.3 years (SD=8.9 years) with similar clinical features to those diagnosed after 10 years (Table 29).

Table 28. The reasons for exclusion following review of cases notes.

Reason for exclusion	Number of cases
No record of pancreatic cancer in hospital records or at ECRIC*	3
Prevalent case of pancreatic cancer	4
Neuroendocrine pancreatic cancer	3
Cholangiocarcinoma	2
Ampullary carcinoma	2
Pancreatitis	1
Mesothelioma	1
Carcinomatosis of uncertain origin	1
Diagnosis uncertain	1
Total	18

*ECRIC=Eastern Cancer Registry and Information Centre

Table 29. Clinical features of all cases of pancreatic cancer identified.

Variable	Cases 10 yrs follow-up	Cases 17 yrs follow-up
Total number of cases	53	93
Age at diagnosis (years, mean (SD))	69.7 (8.6)	72.3 (8.9)
AJCC stage of disease, n (%)		
Localised within pancreas (stages 0, 1A & 1B)	8 (15.1)	14 (15.1)
Locally invasive (stages 2A & 2B)	5 (9.4)	9 (9.7)
Locally advanced (stage 3)	10 (18.9)	20 (21.5)
Distant metastases (stage 4)	23 (43.4)	42 (45.2)
No staging data available	7 (13.2)	8 (8.6)
Investigations, n (%)		
One modality	1 (1.1)	1 (1.1)
Two modalities	19 (35.9)	38 (40.8)
Three modalities	13 (24.5)	22 (23.7)
Histology available	20 (37.8)	32 (34.3)
Treatment, n (%)		
Surgical	7 (13.5)	8 (8.6)
Oncological	20 (38.5)	32 (34.4)
Palliative	25 (48.1)	51 (54.8)
Missing data	1 (1.9)	2 (2.2)
Survival following diagnosis		
Available data, n (%)	53 (100)	83 (89.2)
Mean, months (SD)	6.8 (6.4)	5.9 (6.1)
Median, months (range)	4.0 (0.25-25)	4.0 (0.25-25)

AJCC=American Joint Committee on Cancer

2. Physical activity and alcohol intake

Comparison of baseline characteristics used in the physical activity and alcohol analysis

From the cohort of 25 639 participants who attended the baseline health check and completed the physical activity questionnaire, 53 people (41.5% women) developed incident exocrine pancreatic cancer after 10 years follow-up, with 93 cases identified after 17 years (Table 30). Comparison of those with, and without incident disease showed that after both 10 years, and 17 years of follow-up, cases were older at recruitment than those without disease ($p<0.001$). After 10 years of follow-up, cases were more likely to be male, although there were no gender differences after 17 years. There were no statistical differences between cases and non-cases in the average or categories of body mass index (BMI), cigarette smoking or diabetes at baseline (Table 30). After 10 years the age and sex adjusted hazard ratio, compared to BMI <25, for a BMI 25-<30 HR=0.95 (95% CI=0.53-1.69), BMI 30-<35 HR=0.40 (95% CI=0.12-1.35) and BMI >35 HR=1.31 (95% CI=0.31-5.61). The age and sex adjusted hazard ratio, compared to never smoker, for a previous smoker HR=0.61 (95% CI=0.21-1.75) and for a current smoker HR=0.83 (95% CI=0.28-2.44). The age and sex adjusted hazard ratio for the presence of diabetes compared to no diabetes, HR=0.86 (0.21-3.54).

Table 30. Baseline characteristics of the whole cohort (used in the physical activity and alcohol analysis).

	Non-incident disease	Cases 10 yrs follow-up	Cases 17 yrs follow-up
Number of participants	25 546	53	93
Age at recruitment (years, mean (SD))	59.2 (9.3)	63.7 (8.6)[†]	63.7 (8.1)[†]
Age at diagnosis (years, mean (SD))	-	69.4 (8.7)	72.3 (8.9)
Interval to diagnosis (years,mean (SD))	-	5.6 (2.6)	8.6 (4.0)
Gender			
Male, n (%)	11 563 (45.3)	31 (58.5)[#]	44 (47.3)
Female, n (%)	13 983 (54.7)	22 (41.5)	49 (52.7)
Body mass index (mean (SD)kg/m^2)	26.3 (3.9)	26.3 (3.6)	25.8 (3.5)
Cigarette smoking status			
Current smoker, n (%)	2 975 (11.8)	4 (7.6)	9 (9.7)
Former smoker, n (%)	10 721 (42.3)	30 (56.6)	41 (44.1)
Never smoked, n (%)	11 631 (45.9)	19 (35.9)	43 (46.2)
Presence of diabetes			
Present	85 (3.3)	2 (3.8)	4 (4.3)
not present	24 695 (96.7)	51 (96.2)	89 (95.7)

SD=Standard deviation, kg=Kilograms, m^2=metres squared, n=number

[†]$p<0.001$ using unpaired t-test

[#]$p=0.053$ using Pearson's chi-square test

Physical activity results

In the whole cohort after 10 years follow-up, the incidence of disease in the most active category compared to the least active was lower (0.13 cases per 1 000 person-years vs 0.24 cases per 1 000 person-years), although after adjustment for co-variates the hazard ratio was non-significant (HR=0.69, 95% CI=0.28-1.79, p=0.45), with no trend across categories (HR=0.98, 95% CI=0.75-1.27, p=0.88) (Table 31). After 17 years follow-up, the multivariate hazard ratio for the most active vs inactive category was 1.05 (95% CI=0.55-1.99, p=0.88), with the trend across categories HR=1.04 (95% CI=0.85-1.27, p=0.68)(Table 31).

Table 31. Physical activity and the risk of developing pancreatic cancer.

	Categories of physical activity			
	Inactive	Moderately inactive	Moderately active	Active
Number of participants	7 863	7 351	5 776	4 648
% of whole cohort	30.7	28.7	22.5	18.1
10 years follow-up				
Number of P-Y	78 581	73 458	57 688	46 458
Number of cases	19	13	15	6
Cases per 1000 P-Y	0.24	0.18	0.26	0.13
HR (95% CI)[1]	1.00	0.91 (0.45-1.85)	1.41 (0.71-2.84)	0.72 (0.28-1.84)
HR (95% CI)[2]	1.00	0.88 (0.44-1.85)	1.37 (0.70-2.81)	0.69 (0.28-1.79)
17 years follow-up				
Number of P-Y	99 240	93 149	73 246	59 016
Number of cases	32	23	23	15
Cases per 1000 P-Y	0.32	0.25	0.31	0.25
HR (95% CI)[1]	1.00	0.91 (0.53-1.56)	1.22 (0.70-2.14)	1.10 (0.58-2.07)
HR (95% CI)[2]	1.00	0.88 (0.53-1.56)	1.17 (0.67-2.05)	1.05 (0.55-1.99)

HR=hazard ratio, CI=confidence interval, P-Y=person-years.

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking, diabetes and BMI categories.

When the cohort was restricted to those aged less than 65 years at recruitment, physical activity was associated with a reduced risk of pancreatic cancer. Participants <65years are more likely to be employed, with occupational physical activity in the physical activity questionnaire the main determinant of the physical activity index. After 10 years of follow-up, the most active category compared to the least active, had a multi-variate hazard ratio=0.11 (95% CI=0.01-0.88), with a borderline statistically significant trend across categories (HR=0.72, 95% CI=0.49-1.05, p=0.092) (Table 32). After 17 years of follow-up the results were attenuated (most vs least active HR=0.47, 95% CI=0.19-1.14). In summary, increased physical activity was associated with a reduced risk of pancreatic cancer in those aged less than 65 years at recruitment, although no effect was found in the analysis of participants of all ages.

Table 32. Physical activity and the risk of developing pancreatic cancer if aged <65 years at recruitment.

	Categories of physical activity				
	Inactive	Moderately inactive	Moderately active	Active	
Number of participants	4 098	5 098	4 514	3 826	
% of whole cohort	23.4	29.1	25.7	21.8	
10 years follow-up					
Number of P-Y	40 953	50 965	45 098	38 363	
Number of cases	10	5	10	1	
Cases per 1000 P-Y	0.24	0.10	0.22	0.03	
HR (95% CI)[1]	1.00	0.44 (0.15-1.34)	0.99 (0.41-2.38)	0.11 (0.01-0.90)	*
HR (95% CI)[2]	1.00	0.43 (0.15-1.27)	0.96 (0.39-2.33)	0.11 (0.01-0.88)	*
17 years follow-up					
Number of P-Y	51 356	64 211	57 069	48 431	
Number of cases	17	10	14	7	
Cases per 1000 P-Y	0.32	0.16	0.25	0.14	
HR (95% CI)[1]	1.00	0.50 (0.23-1.10)	0.75 (0.36-1.54)	0.50 (0.20-1.20)	
HR (95% CI)[2]	1.00	0.48 (0.22-1.06)	0.71 (0.34-1.48)	0.47 (0.19-1.14)	

HR=hazard ratio, CI=confidence interval, P-Y=person-years.

* p for trend<0.10

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes and BMI category.

Alcohol results

In the whole cohort after 10 years follow-up, the incidence of pancreatic cancer did not significantly change with increased alcohol intake. The highest category of alcohol intake (>21 units per week) compared to those with zero intake had a hazard ratio=0.43 (95% CI=0.12-1.59)(Table 33). After 17 years follow-up, the multivariate hazard ratio for the highest alcohol intake vs zero intake was 0.71 (95% CI=0.27-1.82). However, those with an intake of >0 to <7 units per week had a significantly reduced risk of developing disease compared to those with zero intake of alcohol (HR=0.51, 95% CI=0.30-0.86)(Table 33).

Table 33. Alcohol intake and the risk of developing pancreatic cancer.

	Categories of alcohol intake				
	0	> 0 to < 7	7 to <14	14 to <21	≥ 21
Number of participants	3 638	13 675	4 525	1 927	1 874
% of whole cohort	14.2	53.3	17.7	7.5	7.3
10 years follow-up					
Number of P-Y	36 351	136 668	45 216	19 232	18 729
Number of cases	12	21	9	8	3
Cases per 1000 P-Y	0.33	0.15	0.20	0.42	0.16
HR (95% CI)[1]	1.00	0.52 (0.25-1.06)	0.64 (0.26-1.54)	1.30 (0.52-3.28)	0.47 (0.13-1.74)
HR (95% CI)[2]	1.00	0.49 (0.24-1.00)	0.57 (0.24-1.39)	1.17 (0.46-2.97)	0.43 (0.12-1.59)
17 years follow-up					
Number of P-Y	46 459	173 747	56 894	24 119	23 443
Number of cases	22	35	15	10	6
Cases per 1000 P-Y	0.47	0.22	0.26	0.50	0.26
HR (95% CI)[1]	1.00	0.51 (0.30-0.88)	0.68 (0.35-1.33)	1.32 (0.64-2.72)	0.69 (0.27-1.78)
HR (95% CI)[2]	1.00	0.51 (0.30-0.86)	0.66 (0.33-1.30)	1.29 (0.62-2.86)	0.71 (0.27-1.82)

HR=hazard ratio, CI=confidence interval, P-Y=person-years.

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking, diabetes and BMI categories.

3. Dietary outcomes using 7-day food diaries

Comparison of baseline characteristics used in the dietary analysis

In the 23 658 participants (92.3% of those attending the health check) who completed the 7-day food diary (7DFD) 51 participants were diagnosed with pancreatic cancer (43% women) after 10 years of follow-up, which increased to 88 cases (54% women) after 17 years. Not all food diaries from the whole cohort are currently coded, so a random sample of 3 970 participants had their diaries coded and were used as the comparison population. The baseline characteristics were compared between participants with and without incident disease and are listed in Table 34. Cases were older at recruitment and after 10 years follow-up were more likely to be male. There were no statistical differences in the averages or proportions of body mass index, cigarette smoking and diabetes.

Table 34. Baseline characteristics of the cohort (used in dietary analysis).

	Non-incident disease	Cases 10 yrs follow-up	Cases 17 yrs follow-up
Number of participants	3 970	51	88
Age at recruitment (years, mean (SD))	59.3 (9.4)	64.1 (8.3)[†]	64.2 (7.8)[†]
Age at diagnosis (years, mean (SD))		69.7 (8.6)	72.6 (8.8)
Interval to diagnosis (years,mean (SD))		5.6 (2.6)	8.4 (3.9)
Gender			
Male (%)	1 740 (43.8)	29 (56.9)	40 (45.5)
Female (%)	2 230 (56.2)	22 (43.1)	48 (54.5)
Body mass index (mean (SD) kg/m2)	26.3 (3.9)	26.3 (3.7)	25.7 (3.5)
Cigarette smoking status			
Current smoker (%)	451 (11.5)	4 (8.0)	8 (9.1)
Former smoker (%)	1 670 (42.4)	28 (56.0)	40 (45.5)
Never smoked (%)	1 818 (46.1)	18 (36.0)	40 (45.5)
Presence of diabetes			
present n (%)	121 (3.0)	2 (3.9)	4 (4.5)
not present	3 849 (97.0)	49 (96.1)	84 (95.5)

[†]$p<0.001$ using unpaired t-test

[#]$p=0.062$ using Pearson's chi-square test

Energy and macronutrients

In the primary outcome after 10 years of follow-up, each higher quintile of total energy intake was associated with reduced hazard ratios of developing pancreatic cancer, although none reached statistical significance (highest vs lowest quintile, HR=0.46 95% CI=0.17-1.23, p=0.12) with a HR for the trend across categories of 0.87 (95% CI=0.69-1.10, p=0.24) (Table 35). To assess whether participants may have pre-clinical symptoms causing a reduced energy intake a sensitivity analysis was performed, excluding those diagnosed with pancreatic cancer <2 years after enrolment, with similar results (highest vs the lowest quintile HR=0.41, 95% CI=0.14-1.18, p=0.099) and the trend across quintiles HR=0.84 (95% CI=0.66-1.07, p=0.16). After 17 years of follow-up, energy intake was not associated with pancreatic cancer risk, with the trend across categories HR=0.93 (95% CI=0.78-1.12, p=0.45).

After 10 years of follow-up, all quintiles of increased total fat and protein intake had negative associations compared to the lowest, although again, none were statistically significant (Table 35), with the trends across categories showing no associations (total fat, trend HR=1.00, 95% CI=0.69-1.44, total protein trend HR=0.94, 95% CI=0.70-1.25). Total carbohydrate intake had no relationship with the risk of developing pancreatic cancer (trend across quintiles, HR=1.03, 95% CI=0.72-1.46). After 17 years follow-up, no associations were found between macronutrients and pancreatic cancer (Table 35).

Table 35. Total energy and macronutrient intake and the risk of developing pancreatic cancer.

	Quintile				
	1	2	3	4	5
Number of participants	812	812	811	812	811
Total energy intake					
Cut points (kcal/day)	322 to <1496	1496 to <1765	1765 to <2027	2027 to <2341	2341 to <6050
10 years of follow-up					
Cases	14	8	7	13	9
HR (95% CI)[1]	1.00	0.52 (0.22-1.27)	0.42 (0.17-1.07)	0.72 (0.31-1.67)	0.49 (0.18-1.28)
HR (95% CI)[2]	1.00	0.52 (0.22-1.28)	0.40 (0.15-1.01)	0.67 (0.29-1.57)	0.46 (0.17-1.23)
17 years of follow-up					
Cases	22	17	15	22	12
HR (95% CI)[1]	1.00	0.78 (0.41-1.48)	0.70 (0.36-1.37)	1.09 (0.57-2.10)	0.64 (0.28-1.42)
HR (95% CI)[2]	1.00	0.76 (0.41-1.48)	0.66 (0.35-1.36)	1.00 (0.52-1.94)	0.59 (0.26-1.33)
Total fat					
Cut points (grams/day)	10.7 to <53.5	53.5 to <65.8	65.8 to <77.7	77.7 to <93.5	93.5 to <339.9
10 years of follow-up					
Cases	16	6	8	9	12
HR (95% CI)[1]	1.00	0.35 (0.14-0.89)	0.43 (0.18-1.02)	0.48 (0.20-1.12)	0.63 (0.27-1.45)
HR (95% CI)[2]	1.00	0.39 (0.13-1.13)	0.48 (0.15-1.57)	0.55 (0.14-2.13)	0.91 (0.21-3.96)
17 years of follow-up					
Cases	27	12	19	15	15
HR (95% CI)[1]	1.00	0.44 (0.22-0.87)	0.71 (0.39-1.29)	0.58 (0.30-1.12)	0.63 (0.31-1.26)
HR (95% CI)[2]	1.00	0.39 (0.18-0.86)	0.53 (0.24-1.27)	0.39 (0.15-1.05)	0.49 (0.16-1.48)
Total carbohydrate					
Cut points (grams/day)	10.7 to <53.5	53.5 to <65.8	65.8 to <77.7	77.7 to <93.5	93.5 to <339.9
10 years of follow-up					
Cases	13	7	10	13	8
HR (95% CI)[1]	1.00	0.53 (0.21-1.34)	0.68 (0.29-1.57)	0.85 (0.38-1.89)	0.51 (0.19-1.32)
HR (95% CI)[2]	1.00	0.77 (0.28-2.20)	1.20 (0.38-4.10)	1.44 (0.41-5.54)	0.85 (0.20-4.06)
17 years of follow-up					
Cases	20	17	14	23	14
HR (95% CI)[1]	1.00	0.88 (0.46-1.69)	0.71 (0.35-1.41)	1.25 (0.67-2.36)	0.85 (0.40-1.81)
HR (95% CI)[2]	1.00	1.02 (0.50-2.21)	0.92 (0.38-2.39)	1.64 (0.67-4.65)	1.33 (0.46-4.58)
Total protein					
Cut points (grams/day)	23.5 to <57.9	57.9 to <67.2	67.2 to <75.6	75.6 to <86.4	86.4 to <175.3
10 years of follow-up					
Cases	13	9	8	12	9
HR (95% CI)[1]	1.00	0.65 (0.28-1.52)	0.52 (0.21-1.28)	0.72 (0.31-1.67)	0.55 (0.21-1.40)
HR (95% CI)[2]	1.00	0.78 (0.32-1.93)	0.67 (0.23-1.83)	0.91 (0.31-2.52)	0.71 (0.20-2.30)
17 years of follow-up					
Cases	18	18	17	21	14
HR (95% CI)[1]	1.00	1.01 (0.52-1.94)	0.93 (0.47-1.83)	1.23 (0.63-2.39)	0.91 (0.42-1.97)
HR (95% CI)[2]	1.00	1.15 (0.58-2.28)	1.11 (0.49-2.27)	1.50 (0.64-3.15)	1.25 (0.44-2.94)

HR=hazard ratio, CI=confidence interval.

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Polyunsaturated fatty acids

Total PUFAs had negative associations with pancreatic cancer for all higher quintiles of intake compared to the lowest, after both 10 and 17 years follow-up, although none were statistically significant with no effect for the trend across quintiles (at 10 years, trend for total PUFA, HR=0.98 95 % CI=0.92-1.05, p=0.35). N-6 PUFAs had no patterns of association with the risk of pancreatic cancer (trend HR=1.03, 95% CI=0.80-1.33, p=0.81)(Table 36). However, total n-3 intake had statistically significant negative associations with pancreatic cancer after 10 years of follow-up in the age and sex adjusted analysis (highest vs lowest quintile HR=0.25 95% 0.09-0.94, p=0.041) with the multivariate analysis of borderline statistical significance (HR=0.30 95% CI=0.07-1.21, p=0.092) There was no trend across quintiles (HR=0.84 95% CI=0.67-1.07, p=0.16) (Table 36). Total n-3 fatty acid intake analysed as a continuous variable per gram/day had a multivariate HR=0.42 (95% CI=0.22-0.82, p=0.011). After 17 years of follow-up, the results for total n-3 intake were attenuated, with inverse non-significant associations for individual quintiles and trend across categories. Total n-3 intake as a continuous variable was of borderline statistical significance (HR=0.67, 95% CI=0.43-1.05, p=0.078).

Individual n-3 polyunsaturated fatty acids

An analysis was performed to verify which individual n-3 fatty acids may account for the inverse associations reported for total n-3 intake. After 10 years of follow-up, docosahexaenoic acid (DHA) intake was negatively associated with pancreatic cancer for each higher quintile of intake, with the highest vs lowest quintile associated with a 60% protective effect (HR=0.40 95% CI=0.15-1.08, p=0.070) and a statistically significant multivariate trend across quintiles (HR=0.80, 95% CI=0.65-0.98, p=0.031). After 17 years of follow-up, DHA had negative associations for the highest two quintiles of intake but these were not statistically significant (trend across quintiles HR=0.92, 95% CI=0.79-1.07, p=0.28) (Table 37). For eicosapentaenoic acid (EPA) the three higher quintiles of intake were associated with a non-significant decreased risk after 10 years of follow-up, with the age and sex adjusted trend across categories of borderline statistical significance (HR=0.84, 95% CI=0.69-1.02, p=0.084). After 17 years, individual categories and trends of EPA had no relationship with disease (Table 37). The essential n-3 fatty acid, alpha-linolenic acid, had no relationship with the risk of developing pancreatic cancer (table 7), after either follow-up period.

Table 36. Total polyunsaturated fatty acid (PUFA), n-3 & n-6 PUFA intake and the risk of developing pancreatic cancer.

	Quintile					
	1	2	3	4	5	
Number of participants	812	812	811	812	811	
Total polyunsaturated fat						
Cut points (grams/day)	1.89 to <9.5	9.5 to <12.0	12.0 to <14.5	14.5 to <18.0	18.0 to <84.6	
10 years of follow-up						
Cases	15	8	8	9	11	
HR (95% CI)[1]	1.00	0.49 (0.21-1.16)	0.50 (0.21-1.19)	0.54 (0.23-1.27)	0.69 (0.30-1.61)	
HR (95% CI)[2]	1.00	0.58 (0.23-1.42)	0.62 (0.23-1.67)	0.69 (0.25-1.94)	0.91 (0.31-2.67)	
17 years of follow-up						
Cases	27	15	13	17	16	
HR (95% CI)[1]	1.00	0.57 (0.30-1.07)	0.52 (0.26-1.02)	0.70 (0.37-1.33)	0.76 (0.39-1.48)	
HR (95% CI)[2]	1.00	0.55 (0.28-1.07)	0. 50 (0.24-1.07)	0.68 (0.32-1.46)	0.77 (0.34-1.79)	
n-3 PUFA						
Cut points (grams/day)	0.25 to <1.04	1.04 to <1.31	1.31 to <1.58	1.58 to <1.97	1.97 to <6.58	
10 years of follow-up						
Cases	10	12	15	11	3	
HR (95% CI)[1]	1.00	1.10 (0.47-2.56)	1.38 (0.61-3.10)	0.97 (0.40-2.34)	0.25 (0.09-0.94)	*
HR (95% CI)[2]	1.00	1.29 (0.54-3.12)	1.71 (0.70-4.11)	1.16 (0.44-3.04)	0.30 (0.07-1.21)	
17 years of follow-up						
Cases	21	20	19	17	11	
HR (95% CI)[1]	1.00	0.95 (0.51-1.75)	0.94 (0.50-1.77)	0.87 (0.45-1.68)	0.56 (0.26-1.19)	
HR (95% CI)[2]	1.00	0.94 (0.50-1.82)	0.96 (0.49-1.91)	0.87 (0.42-1.78)	0.57 (0.25-1.30)	
n-6 PUFA						
Cut points (grams/day)	1.3 to <7.8	7.8 to <9.9	9.9 to <12.3	12.3 to <15.6	15.6 to <79.8	
10 years of follow-up						
Cases	15	8	7	9	12	
HR (95% CI)[1]	1.00	0.52 (0.22-1.22)	0.44 (0.18-1.08)	0.58 (0.25-1.36)	0.79 (0.35-1.79)	
HR (95% CI)[2]	1.00	0.58 (0.24-1.44)	0.54 (0.20-1.46)	0.75 (0.28-2.04)	1.04 (0.38-2.87)	
17 years of follow-up						
Cases	28	14	12	17	17	
HR (95% CI)[1]	1.00	0.52 (0.27-0.99)	0.46 (0.23-0.91)	0.71 (0.38-1.33)	0.78 (0.41-1.50)	
HR (95% CI)[2]	1.00	0.51 (0.26-1.00)	0.45 (0.21-0.95)	0.70 (0.34-1.44)	0.81 (0.37-1.77)	

HR=hazard ratio, CI=confidence interval, * borderline statistical significant trend across quintiles (p=0.072)

*denotes p for trend<0.05

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Table 37. Individual n-3 fatty acids and the risk of developing pancreatic cancer.

	Quintile					
	1	2	3	4	5	
Number of participants	812	812	811	812	811	
Alpha Linolenic acid (C18:3n3c)						
Cut points (grams/day)	0.25 to <1.04	1.04 to <1.31	1.31 to <1.58	1.58 to <1.97	1.97 to <6.58	
10 years of follow-up						
Cases	9	13	13	12	4	
HR (95% CI)[1]	1.00	1.39 (0.59-3.26)	1.30 (0.55-3.10)	1.25 (0.52-3.06)	0.40 (0.12-1.36)	
HR (95% CI)[2]	1.00	1.81 (0.74-4.43)	1.76 (0.67-4.61)	1.66 (0.60-4.60)	0.51 (0.13-1.98)	
17 years of follow-up						
Cases	24	18	16	20	10	
HR (95% CI)[1]	1.00	0.78 (0.42-1.43)	0.71 (0.37-1.35)	0.95 (0.51-1.74)	0.49 (0.23-1.07)	
HR (95% CI)[2]	1.00	0.78 (0.42-1.52)	0.69 (0.34-1.43)	0.91 (0.45-1.88)	0.49 (0.20-1.19)	
Eicosapentaenoic acid (C20:5n3c)						
Cut points (grams/day)	0 to <0.02	0.02 to <0.04	0.04 to <0.07	0.07 to <0.16	0.16 to <1.72	
10 years of follow-up						
Cases	10	14	10	11	6	
HR (95% CI)[1]	1.00	1.24 (0.55-2.79)	0.84 (0.35-2.02)	0.88 (0.37-2.08)	0.45 (0.16-1.26)	*
HR (95% CI)[2]	1.00	1.31 (0.57-2.97)	0.90 (0.37-2.19)	0.95 (0.40-2.28)	0.50 (0.17-1.39)	
17 years of follow-up						
Cases	15	22	17	20	14	
HR (95% CI)[1]	1.00	1.31 (0.68-2.52)	1.00 (0.50-2.00)	1.10 (0.56-2.15)	0.75 (0.36-1.56)	
HR (95% CI)[2]	1.00	1.34 (0.68-2.55)	1.00 (0.50-2.05)	1.12 (0.57-2.21)	0.78 (0.36-1.60)	
Docosahexaenoic acid (C22:6n3c)						
Cut points (grams/day)	0 to <0.02	0.02 to <0.05	0.04 to <0.10	0.10 to <0.25	0.25 to <1.98	
10 years of follow-up						
Cases	12	14	10	9	6	
HR (95% CI)[1]	1.00	0.99 (0.45-2.13)	0.69 (0.30-1.60)	0.59 (0.25-1.40)	0.39 (0.15-1.04)	**
HR (95% CI)[2]	1.00	0.98 (0.46-2.16)	0.70 (0.30-1.66)	0.60 (0.25-1.43)	0.40 (0.15-1.08)	**
17 years of follow-up						
Cases	16	20	22	14	16	
HR (95% CI)[1]	1.00	1.08 (0.56-2.09)	1.14 (0.60-2.18)	0.70 (0.34-1.43)	0.82 (0.41-1.64)	
HR (95% CI)[2]	1.00	1.06 (0.55-2.06)	1.12 (0.59-2.15)	0.69 (0.33-1.42)	0.81 (0.40-1.63)	

HR=hazard ratio, CI=confidence interval, *borderline significant trend across quintile (p=0.084), **significant trend where p<0.05

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Monounsaturated fatty acids

Total monounsaturated fatty acid (MUFA) intake was negatively associated with pancreatic cancer for all higher quintiles, compared to the lowest, after both 10 and 17 years follow-up, although none were statistically significant (Table 38). There was no effect for the trend across quintiles (after 10 years follow-up, trend for total MUFA intake, HR=0.97, 95% CI=0.92-1.03). After 17 years, total MUFA intake had a borderline protective effect when the highest quintile was compared to the lowest (HR=0.38 95% CI=0.13-1.14, p=0.085). As well as the effect of total MUFA intake, individual MUFAs were assessed, namely oleic acid, palmitoleic acid and vaccenic acid. After 10 years of follow-up, oleic acid had negative associations for all higher quintiles of intake although these were not statistically significant (Table 38), and no effect for the trend across quintiles (HR=0.83, 95% CI=0.60-1.15, p=0.26). However after 17 years of follow-up, there was a significant reduced risk of pancreatic cancer for the highest quintile of intake (highest vs lowest HR=0.29, 95% CI=0.11-0.84, p=0.022), with a significant trend across quintiles (HR=0.73 95% CI=0.57-0.93, p=0.013)(Table 38). The monounsaturated fatty acid, palmitoleic acid, had positive associations with pancreatic cancer after 10 years (highest vs lowest HR=2.64, 95% CI=0.84-8.30, p=0.097), although none after 17 years follow-up. Vaccenic acid was not associated with pancreatic cancer in any of the analyses.

Table 38. Total and individual monounsaturated fatty acid (MUFA) intake and the risk of developing pancreatic cancer.

	Quintile					
	1	2	3	4	5	
Number of participants	812	812	811	812	811	
Total monunsaturated fat						
Cut points (grams/day)	3.2 to <18.3	18.3 to <22.7	22.7 to <26.8	26.8 to <32.8	32.8 to <103.8	
10 years of follow-up						
Cases	15	9	6	13	8	
HR (95% CI)[1]	1.00	0.54 (0.23-1.24)	0.35 (0.14-0.92)	0.70 (0.32-1.55)	0.44 (0.17-1.11)	
HR (95% CI)[2]	1.00	0.61 (0.23-1.57)	0.38 (0.11-1.32)	0.67 (0.21-2.32)	0.45 (0.10-1.80)	
17 years of follow-up						
Cases	26	16	15	19	12	
HR (95% CI)[1]	1.00	0.60 (0.32-1.11)	0.60 (0.31-1.14)	0.74 (0.39-1.37)	0.52 (0.24-1.08)	
HR (95% CI)[2]	1.00	0.53 (0.29-1.09)	0.46 (0.19-1.07)	0.51 (0.20-1.27)	0.38 (0.12-1.08)	
Palmitoliec Acid (C16:1n7c)						
Cut points (grams/day)	0.11 to 0.64	0.64 to <0.81	0.81 to <0.98	0.98 to <1.23	1.23 to <3.39	
10 years of follow-up						
Cases	8	12	9	8	14	
HR (95% CI)[1]	1.00	1.32 (0.53-0.25)	0.98 (0.37-2.59)	0.84 (0.31-2.29)	1.40 (0.55-3.55)	
HR (95% CI)[2]	1.00	1.73 (0.67-4.40)	1.46 (0.2-4.16)	1.41 (0.45-4.38)	2.64 (0.83-8.31)	
17 years of follow-up						
Cases	15	21	16	18	18	
HR (95% CI)[1]	1.00	1.03 (0.67-2.53)	1.03 (0.51-2.11)	1.17 (0.58-2.36)	1.17 (0.57-2.44)	
HR (95% CI)[2]	1.00	1.41 (0.71-2.82)	1.20 (0.55-2.59)	1.42 (0.64-3.15)	1.55 (0.65-3.73)	
Vaccenic Acid (C18:1n7c)						
Cut points (grams/day)	0 .03 to <1.20	1.20 to <1.57	1.56 to <1.97	1.97 to <2.51	2.51 to <10.68	
10 years of follow-up						
Cases	11	11	6	10	13	
HR (95% CI)[1]	1.00	0.88 (0.38-2.04)	0.46 (0.17-1.28)	0.75 (0.31-1.83)	0.99 (0.41-2.37)	
HR (95% CI)[2]	1.00	1.10 (0.46-2.65)	0.65 (0.21-1.96)	1.08 (0.37-3.13)	1.62 (0.53-4.93)	
17 years of follow-up						
Cases	20	20	10	19	19	
HR (95% CI)[1]	1.00	0.95 (0.51-1.76)	0.49 (0.22-1.04)	0.96 (0.50-1.83)	1.03 (0.52-2.03)	
HR (95% CI)[2]	1.00	0.64 (0.31-1.35)	1.11 (0.55-2.22)	1.04 (0.48-2.28)	1.29 (0.55-3.00)	
Oleic acid (C18:1n9c)						
Cut points (grams/day)	2.5 to <13.2	13.2 to <16.4	16.4 to <19.4	19.4 to <23.7	23.7 to <78.4	
10 years of follow-up						
Cases	14	13	4	12	8	
HR (95% CI)[1]	1.00	0.83 (0.39-1.78)	0.24 (0.08-1.24)	0.72 (0.32-1.63)	0.48 (0.17-1.24)	
HR (95% CI)[2]	1.00	0.92 (0.38-2.21)	0.25 (0.06-0.95)	0.66 (0.20-2.12)	0.46 (0.11-1.80)	
17 years of follow-up						
Cases	27	22	11	16	12	
HR (95% CI)[1]	1.00	0.81 (0.45-1.43)	0.41 (0.20-0.83)	0.63 (0.33 (1.21)	0.51 (0.24-1.06)	**
HR (95% CI)[2]	1.00	0.64 (0.33-1.25)	0.27 (0.11-0.65)	0.35 (0.14-0.85)	0.29 (0.11-0.83)	**

HR=hazard ratio, CI=confidence interval, ** significant trend across quintiles where p<0.05

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Saturated fatty acids and *trans*-fatty acids

There were no associations with the dietary intake of total saturated fats, total *trans* fatty acids, as well as the individual saturated fatty acids of myristic acid, palmitic acid and stearic acid and the risk of developing pancreatic cancer after both periods of follow-up (Table 39 and Table 40).

Table 39. Total and individual saturated fatty acid intake and the risk of developing pancreatic cancer.

	Quintile				
	1	2	3	4	5
Number of participants	812	812	812	812	812
Total saturated fat intake					
Cut points (grams/day)	3.9 to <19.2	19.2 to <24.1	24.1 to <29.4	29.4 to <36.2	36.2 to <125.6
10 years of follow-up					
Cases	12	8	8	12	11
HR (95% CI)[1]	1.00	0.61 (0.24-1.50)	0.61 (0.24-1.50)	0.89 (0.38-2.04)	0.79 (0.33-1.90)
HR (95% CI)[2]	1.00	0.83 (0.33-2.25)	0.98 (0.34-2.99)	1.51 (0.50-4.82)	1.58 (0.44-5.66)
17 years of follow-up					
Cases	22	16	17	17	16
HR (95% CI)[1]	1.00	0.70 (0.37-1.34)	0.78 (0.41-1.48)	0.82 (0.43-1.58)	0.79 (0.40-1.58)
HR (95% CI)[2]	1.00	0.53 (0.29-1.09)	0.46 (0.19-1.07)	0.51 (0.20-1.27)	0.38 (0.12-1.08)
Myristic acid (C14:0)					
Cut points (grams/day)	0.16 to <1.69	1.69 to <2.27	2.27 to <2.89	2.89 to <3.81	3.81 to <13.05
10 years of follow-up					
Cases	9	11	9	9	13
HR (95% CI)[1]	1.00	1.15 (0.48-2.78)	0.95 (0.37-2.39)	0.93 (0.36-2.36)	1.25 (0.52-3.00)
HR (95% CI)[2]	1.00	1.47 (0.59-3.65)	1.31 (0.49-3.55)	1.41 (0.50-4.04)	2.00 (0.69-5.74)
17 years of follow-up					
Cases	17	18	17	20	16
HR (95% CI)[1]	1.00	1.00 (0.51-1.94)	0.99 (0.50-1.94)	1.22 (0.63-2.36)	0.94 (0.47-1.89)
HR (95% CI)[2]	1.00	1.08 (0.55-2.17)	1.07 (0.53-2.25)	1.34 (0.67-2.93)	1.07 (0.49-2.52)
Palmitic acid (C16:0)					
Cut points (grams/day)	2.16 to <9.89	9.89 to <12.46	12.46 to <14.96	14.96 to <18.43	18.43 to <64.24
10 years of follow-up					
Cases	14	6	9	10	12
HR (95% CI)[1]	1.00	0.38 (0.14-0.99)	0.56 (0,23-1.31)	0.61 (0.25-1.42)	0.73 (0.31-1.69)
HR (95% CI)[2]	1.00	0.48 (0.18-1.40)	0.85 (0.29-2.51)	0.98 (0.29-3.30)	1.42 (0.37-5.25)
17 years of follow-up					
Cases	25	13	17	16	17
HR (95% CI)[1]	1.00	0.48 (0.24-0.94)	0.67 (0.36-1.24)	0.67 (0.35-1.29)	0.75 (0.38-1.47)
HR (95% CI)[2]	1.00	0.48 (0.23-1.01)	0.64 (0.29-1.41)	0.62 (0.25-1.52)	0.81 (0.29-2.12)
Stearic acid (C18:0)					
Cut points (grams/day)	0.71 to <4.29	4.29 to <5.46	5.46 to <6.63	6.63 to <8.21	8.21 to <29.52
10 years of follow-up					
Cases	9	14	5	13	10
HR (95% CI)[1]	1.00	1.49 (0.64-3.46)	0.49 (0.16-1.48)	1.29 (0.53-3.11)	1.01 (0.39-2.65)
HR (95% CI)[2]	1.00	2.02 (0.85-5.14)	0.79 (0.23-2.77)	2.23 (0.72-7.23)	2.01 (0.54-7.45)
17 years of follow-up					
Cases	21	20	15	17	15
HR (95% CI)[1]	1.00	0.97 (0.52-1.80)	0.70 (0.36-1.38)	0.87 (0.45-1.67)	0.82 (0.40-1.68)
HR (95% CI)[2]	1.00	1.00 (0.52-1.99)	0.71 (0.33-1.59)	0.85 (0.36-2.00)	0.91 (0.33-2.26)

HR=hazard ratio, CI=confidence interval [1]Adjusted for sex and age

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Table 40. Total *trans* fatty acid intake and the risk of developing pancreatic cancer.

	Quintile				
	1	2	3	4	5
Number of participants	812	812	811	812	811
Cut points (grams/day)	0.1 to <1.7	1.7 to <2.4	2.4 to <3.0	3.0 to <3.8	3.8 to <16.0
10 years of follow-up					
Cases	12	6	10	12	11
HR (95% CI)[1]	1.00	0.47 (0.18-1.25)	0.75 (0.32-1.77)	0.89 (0.39-2.03)	0.79 (0.33-1.98)
HR (95% CI)[2]	1.00	0.59 (0.22-1.64)	1.11 (0.44-2.97)	1.40 (0.52-3.84)	1.43 (0.46-4.42)
17 years of follow-up					
Cases	22	15	15	21	15
HR (95% CI)[1]	1.00	0.68 (0.35-1.32)	0.67 (0.35-1.30)	0.99 (0.54-1.82)	0.71 (0.35-1.41)
HR (95% CI)[2]	1.00	0.71 (0.35-1.40)	0.70 (0.33-1.45)	1.04 (0.50-2.17)	0.79 (0.34-1.87)

HR=hazard ratio, CI=confidence interval

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Meat and fish food groups

Red meat is a rich source of n-6 PUFAs, while fish, in particular fatty fish, has a high content of n-3 PUFAs. White, red and processed meats had no associations with the development of pancreatic cancer for individual categories or trends (Table 41). Fatty fish was not divided into fifths, due to the large number who ate none, and instead was divided into four categories of intake. After 10 years follow-up, but not 17 years, fatty fish intake was inversely associated with the risk of pancreatic cancer in the three higher quartiles of intake, although none reached statistical significance (trend HR=0.89, 95% CI=0.75-1.05, p=0.17) (Table 42). White fish intake had no association with the development of pancreatic cancer (trend across categories HR=0.94 95% CI=0.79-1.13, p=0.53) (Table 42).

Table 41. Meat groups and the risk of developing pancreatic cancer.

	Quintile				
	1	**2**	**3**	**4**	**5**
White meat intake					
Number of participants	905	684	802	789	790
Cut points (grams/day)	0 to <0.1	0.15 to <14.4	14.5 to <25.3	25.4 to <41.4	41.5 to <347.8
10 years of follow-up					
Cases	10	12	5	17	7
HR (95% CI)[1]	1.00	1.59 (0.69-3.68)	0.57 (0.19-1.66)	1.87 (0.85-4.08)	0.83 (0.32-2.20)
HR (95% CI)[2]	1.00	1.60 (0.69-3.72)	0.59 (0.20-1.74)	1.92 (0.88-4.20)	0.86 (0.33-2.27)
17 years of follow-up					
Cases	12	23	11	27	15
HR (95% CI)[1]	1.00	2.45 (1.22-4.93)	1.03 (0.45-2.34)	2.54 (1.28-5.03)	1.56 (0.73-3.34)
HR (95% CI)[2]	1.00	2.43 (1.20-4.89)	1.04 (0.46-2.37)	2.54 (1.29-5.03)	1.59 (0.74-3.40)
Red meat intake					
Number of participants	812	812	811	812	811
Cut points (grams/day)	0 to <8.6	8.7 to <23.0	23.1 to <36.9	37.0 to <55.7	55.7 to 349.3
10 years of follow-up					
Cases	12	8	6	15	10
HR (95% CI)[1]	1.00	0.61 (0.25-1.50)	0.46 (0.17-1.23)	1.10 (0.51-2.36)	0.71 (0.30-1.66)
HR (95% CI)[2]	1.00	0.63 (0.26-1.55)	0.48 (0.18-1.29)	1.14 (0.53-2.46)	0.76 (0.32-1.80)
17 years of follow-up					
Cases	20	17	12	26	13
HR (95% CI)[1]	1.00	0.79 (0.41-1.51)	0.54 (0.26-1.10)	1.14 (0.63-2.04)	0.59 (0.29-1.19)
HR (95% CI)[2]	1.00	0.79 (0.41-1.52)	0.54 (0.26-1.11)	1.13 (0.62-2.03)	0.59 (0.29-1.21)
Processed meat intake					
Number of participants	800	790	793	796	791
Cut points (grams/day)	0 to <5.43	5.46 to <14.2	14.3 to <23.1	23.2 to <36.1	36.2 to <192.0
10 years of follow-up					
Cases	8	13	10	8	12
HR (95% CI)[1]	1.00	1.46 (0.61-3.53)	1.07 (0.42-2.73)	0.84 (0.31-2.23)	1.22 (0.49-3.03)
HR (95% CI)[2]	1.00	1.51 (0.62-3.67)	1.12 (0.44-2.88)	0.89 (0.33-2.39)	1.30 (0.52-3.26)
17 years of follow-up					
Cases	13	21	18	17	19
HR (95% CI)[1]	1.00	1.43 (0.71-2.86)	1.22 (0.60-2.50)	1.18 (0.57-2.44)	1.39 (0.67-2.85)
HR (95% CI)[2]	1.00	1.44 (0.72-2.90)	1.24 (0.60-2.54)	1.21 (0.58-2.50)	1.41 (0.69-2.92)

HR=hazard ratio, CI=confidence interval

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Table 42. Fish intake and the risk of developing pancreatic cancer.

	Quintile				
	1	**2**	**3**	**4**	**5**
White fish intake					
Number of participants	1 504	101	871	730	801
Cut points (grams/day)	0	0.2-<6.4	6.5-<16.3	16.4-<26.5	26.5-250.0
10 years of follow-up					
Cases	19	0	14	8	10
HR (95% CI)[1]	1.00	Not available	1.10 (0.55-2.20)	0.73 (0.32-1.67)	0.79 (0.37-1.71)
HR (95% CI)[2]	1.00	Not available	1.12 (0.56-2.26)	0.76 (0.33-1.74)	0.80 (0.37-1.72)
17 years of follow-up					
Cases	30	2	22	18	16
HR (95% CI)[1]	1.00	0.96 (0.23-4.02)	1.06 (0.61-1.84)	1.05 (0.58-1.89)	0.79 (0.43-1.45)
HR (95% CI)[2]	1.00	0.95 (0.23-4.00)	1.07 (0.61-1.86)	1.08 (0.60-1.95)	0.79 (0.43-1.45)
Fatty fish intake					
Number of participants	1 988	Not available	448	811	811
Cut points (grams/day)	0	Not available	0.01-8.57	8.6-22.6	22.7-259.2
10 years of follow-up					
Cases	30	Not available	6	6	9
HR (95% CI)[1]	1.00	Not available	0.89 (0.37-2.14)	0.48 (0.20-1.16)	0.70 (0.33-1.48)
HR (95% CI)[2]	1.00	Not available	0.90 (0.38-2.22)	0.50 (0.21-1.20)	0.73 (0.34-1.54)
17 years of follow-up					
Cases	44	Not available	13	14	17
HR (95% CI)[1]	1.00	Not available	1.29 (0.70-2.40)	0.76 (0.42-1.39)	0.93 (0.53-1.62)
HR (95% CI)[2]	1.00	Not available	1.30 (0.70-2.41)	0.75 (0.41-1.37)	0.94 (0.54-1.65)

HR=hazard ratio, CI=confidence interval

[1] Adjusted for age and sex.

[2] Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

Dietary antioxidants

There were inverse associations for the dietary intakes of vitamin C, vitamin E and selenium for all the higher quintiles of intake (HRs ranging from 0.47 to 0.84) at 10 and 17 years of follow-up, although no individual one reached statistical significance. Dietary zinc had no association with the risk of developing pancreatic cancer in any analysis (Table 43). Adjusting for the use of supplements, containing the same antioxidant, produced similar results. The inverse association with disease was of greatest magnitude for vitamin E, and in the primary analysis after 10 years of follow-up, the age and sex adjusted trend across quintiles was of borderline statistical significance (HR=0.84, 95% CI=0.68-1.03, p=0.086), with the multivariate result of the same effect size (HR=0.84 95% CI=0.67-1.07, p=0.16) (Table 43). As the risk reduction across each quintile was similar for vitamin C, vitamin E and selenium, the threshold effect for each antioxidant was calculated. The lowest quintile of intake was compared against a summation of the four higher ones. After 10 years of follow-up, the threshold effect for vitamin E in multivariate analysis was 0.53 (95% CI=0.27-1.04, p=0.065) (Table 44). If adjustment was made for the use of supplements containing vitamin E, the HR=0.48 (95%=0.26-0.88, p=0.018), with a similar result after 17 years (Table 44). Dietary vitamin C, adjusted for vitamin C supplement use had a threshold effect after 10 years of follow-up (HR=0.58, 95% CI=0.32-1.08, p=0.087) and after 17 years (HR=0.61, 95% CI=0.38-0.99, p=0.045) (Table 44). The threshold effect for selenium after 10 years was statistically significant after adjusted for selenium supplement use (HR=0.53, 95% CI=0.29-0.99, p=0.048), although not after 17 years (HR=0.69, 95% CI=0.42-1.13, p=0.14). In a post hoc analysis, those in the lowest quintiles for all intakes of vitamin C, vitamin E and selenium intakes were compared to those with higher one. The multivariate HR for developing pancreatic cancer in those with the lowest intakes of all three antioxidants was 5.26 (95%=2.04-14.3, p=0.001), with adjustment for antioxidant supplementation not altering the effect size (Table 45). After 17 years, those with the lowest intake of all three antioxidants the HR=3.70 (95% CI=1.59-8.33, p=0.002).

Serum vitamin C levels were measured at baseline in 22 474 (87.7%) of the initial 25 639 participants who attended the initial health check in EPIC-Norfolk. After 10 and 17 years follow-up, all increasing quintiles of serum vitamin C were inversely associated with the risk of pancreatic cancer (Table 46). After 10 years, 44 incident cases of pancreatic cancer were diagnosed in those with baseline serum vitamin C levels, with a statistically significant reduced risk of developing

pancreatic cancer in the highest vs the lowest quintile (multivariate HR=0.16 (95% CI=0.04-0.73, p=0.018) with a trend across quintiles HR=0.73 (95% CI=0.57-0.92, p=0.008) (Table 46). After 10 years, serum vitamin C as a continuous variable (per micro mol/litre) had a multi-variate inverse association (HR=0.98, 95% CI=0.97-1.00, p=0.026). After 17 years of follow-up, 78 participants who had undergone baseline measurement of serum vitamin C were diagnosed with pancreatic cancer. In this group all higher quintiles were negatively associated, with the highest vs the lowest quintile of serum vitamin C multivariate HR=0.48 (95% CI=0.21-1.11, p=0.085), with a non-significant negative trend across quintiles (HR=0.88 95% CI=0.74-1.04, p=0.12).

Table 43. Quintiles of dietary antioxidant intake and the risk of pancreatic cancer.

	Quintile				
	1	**2**	**3**	**4**	**5**
Number of participants	812	812	811	812	811
Vitamin C					
Cut points (mg/day)	0 to <46.9	47.0 to <65.3	65.4 to <89.1	89.2 to <123.1	123.2 to <654.8
10 years of follow-up					
Cases	16	8	9	7	11
HR (95% CI)[1]	1.00	0.50 (0.21-1.18)	0.57 (0.25-1.29)	0.44 (0.18-1.08)	0.71 (0.33-1.52)
HR (95% CI)[2]	1.00	0.51 (0.21-1.21)	0.61 (0.26-1.39)	0.47 (0.19-1.17)	0.76 (0.34-1.70)
HR (95% CI)[3]	1.00	0.51 (0.22-1.21)	0.61 (0.27-1.40)	0.48 (0.19-1.18)	0.77 (0.34-1.72)
17 years of follow-up					
Cases	24	15	16	13	20
HR (95% CI)[1]	1.00	0.62 (0.32-1.17)	0.66 (0.35-1.25)	0.53 (0.27-1.04)	0.85 (0.47-1.54)
HR (95% CI)[2]	1.00	0.61 (0.32-1.17)	0.65 (0.34-1.24)	0.53 (0.26-1.05)	0.85 (0.45-1.57)
HR (95% CI)[3]	1.00	0.61 (0.32-1.17)	0.64 (0.34-1.22)	0.51 (0.25-1.02)	0.81 (0.44-.152)
Vitamin E					
Cut points (mg/day)	0.9 to <6.8	6.8 to <8.5	8.5 to <10.4	10.4 to <13.1	13.1 to <75.0
10 years of follow-up					
Cases	17	9	8	9	8
HR (95% CI)[1]	1.00	0.53 (0.24-1.19)	0.46 (0.20-1.08)	0.53 (0.23-1.21)	0.47 (0.20-1.11)*
HR (95% CI)[2]	1.00	0.56 (0.24-1.30)	0.48 (0.19-1.17)	0.58 (0.23-1.44)	0.47 (0.17-1.27)
HR (95% CI)[3]	1.00	0.56 (0.24-1.31)	0.48 (0.19-1.18)	0.58 (0.23-1.45)	0.47 (0.17-1.28)
17 years of follow-up					
Cases	27	15	18	12	16
HR (95% CI)[1]	1.00	0.59 (0.31-1.11)	0.72 (0.39-1.31)	0.52 (0.26-1.04)	0.73 (0.39-1.39)
HR (95% CI)[2]	1.00	0.58 (0.30-1.10)	0.68 (0.36-1.29)	0.50 (0.23-1.06)	0.70 (0.33-1.46)
HR (95% CI)[3]	1.00	0.58 (0.32-1.11)	0.68 (0.36-1.30)	0.51 (0.24-1.07)	0.70 (0.34-1.48)
Selenium					
Cut points (µg/day)	7.9 to <40.8	40.8 to <51.4	51.4 to <62.1	62.1 to <76.7	76.7 to <275.5
10 years of follow-up					
Cases	15	8	9	9	10
HR (95% CI)[1]	1.00	0.51 (0.22-1.21)	0.53 (0.23-1.21)	0.51 (0.22-1.20)	0.53 (0.23-1.24)
HR (95% CI)[2]	1.00	0.55 (0.23-1.32)	0.59 (0.25-1.39)	0.59 (0.24-1.43)	0.62 (0.25-1.56)
HR (95% CI)[3]	1.00	0.55 (0.23-1.32)	0.58 (0.24-1.39)	0.59 (0.24-1.43)	0.63 (0.25-1.57)
17 years of follow-up					
Cases	23	15	15	19	16
HR (95% CI)[1]	1.00	0.63 (0.33-1.22)	0.62 (0.32-1.20)	0.79 (0.42-1.47)	0.69 (0.35-1.35)
HR (95% CI)[2]	1.00	0.64 (0.33-1.24)	0.65 (0.33-1.27)	0.84 (0.43-1.61)	0.75 (0.36-1.54)
HR (95% CI)[3]	1.00	0.64 (0.33-1.25)	0.64 (0.33-1.27)	0.84 (0.43-1.61)	0.75 (0.36-1.55)
Zinc					
Cut points (mg/day)	0.8 to <6.3	6.3 to <7.5	7.5 to <8.7	8.7 to <10.3	10.3 to <24.5
10 years of follow-up					
Cases	12	7	12	10	10
HR (95% CI)[1]	1.00	0.59 (0.23-1.51)	0.90 (0.40-2.02)	0.70 (0.29-1.68)	0.69 (0.28-1.68)
HR (95% CI)[2]	1.00	0.72 (0.27-1.90)	1.17 (0.47-2.92)	0.92 (0.33-2.56)	0.90 (0.31-2.67)
HR (95% CI)[3]	1.00	0.71 (0.27-1.88)	1.17 (0.47-2.91)	0.92 (0.33-2.57)	0.88 (0.30-2.61)
17 years of follow-up					
Cases	20	15	22	16	15
HR (95% CI)[1]	1.00	0.79 (0.40-1.54)	1.11 (0.60-2.05)	0.79 (0.40-1.56)	0.78 (0.39-1.60)
HR (95% CI)[2]	1.00	0.84 (0.42-1.69)	1.18 (0.59-2.34)	0.82 (0.38-1.77)	0.85 (0.37-1.97)
HR (95% CI)[3]	1.00	0.83 (0.41-1.68)	1.17 (0.59-2.32)	0.81 (0.37-1.76)	0.84 (0.36-1.95)

HR=hazard ratio, CI=confidence interval, * borderline significant trend across quintiles (p=0.086).

[1] Adjusted for age and sex. [2]Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake

[3]Same as model 2 with the addition of the respective antioxidant supplement e.g, vit C adjusted for vitamin C supplementation.

Table 44. The threshold effect of dietary antioxidants and the risk of pancreatic cancer.

	Threshold effect of Q1 vs Q2-5		
	Q1	Q 2-5	p value
Number of participants	812	3 246	
Vitamin C			
Cut points (mg/day)	0 to 46.95	46.95 to 654.8	
10 years of follow-up			
Cases	16	35	
HR (95% CI)[1]	1.00	0.55 (0.31-1.00)	0.051
HR (95% CI)[2]	1.00	0.58 (0.31-1.07)	0.084
HR (95% CI)[3]	1.00	0.58 (0.32-1.08)	0.087
17 years of follow-up			
Cases	24	64	
HR (95% CI)[1]	1.00	0.66 (0.42-1.06)	0.088
HR (95% CI)[2]	1.00	0.65 (0.40-1.06)	0.085
HR (95% CI)[3]	1.00	0.61 (0.38-0.99)	0.045
Vitamin E			
Cut points (mg/day)	0.87 to 6.76	6.77 to 74.9	
10 years of follow-up			
Cases	17	34	
HR (95% CI)[1]	1.00	0.50 (0.28-0.91)	0.023
HR (95% CI)[2]	1.00	0.53 (0.27-1.04)	0.065
HR (95% CI)[3]	1.00	0.48 (0.26-0.88)	0.018
17 years of follow-up			
Cases	27	61	
HR (95% CI)[1]	1.00	0.64 (0.40-1.01)	0.058
HR (95% CI)[2]	1.00	0.61 (0.36-1.02)	0.062
HR (95% CI)[3]	1.00	0.61 (0.38-0.98)	0.040
Selenium			
Cut points (µg/day)	7.90 to 40.82	40.83 to 275.5	
10 years of follow-up			
Cases	15	36	
HR (95% CI)[1]	1.00	0.52 (0.28-0.97)	0.040
HR (95% CI)[2]	1.00	0.58 (0.30-1.13)	0.11
HR (95% CI)[3]	1.00	0.53 (0.29-0.99)	0.048
17 years of follow-up			
Cases	23	65	
HR (95% CI)[1]	1.00	0.68 (0.42-1.11)	0.12
HR (95% CI)[2]	1.00	0.70 (0.42-1.18)	0.18
HR (95% CI)[3]	1.00	0.69 (0.42-1.13)	0.14
Zinc			
Cut points (mg/day)	0.75 to 6.33	6.34 to 24.51	
10 years of follow-up			
Cases	12	39	
HR (95% CI)[1]	1.00	0.72 (0.37-1.41)	0.34
HR (95% CI)[2]	1.00	0.90 (0.41-1.97)	0.79
HR (95% CI)[3]	1.00	0.90 (0.41-1.97)	0.79
17 years of follow-up			
Cases	20	68	
HR (95% CI)[1]	1.00	0.88 (0.52-1.47)	0.62
HR (95% CI)[2]	1.00	0.93 (0.51-1.68)	0.81
HR (95% CI)[3]	1.00	0.97 (0.31-3.09)	0.96

HR=hazard ratio, CI=confidence interval, µg=microgram, mg=milligram

[1]The model adjusted for age and sex.

[2]The mulitvariate model adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy.

[3] Same as model 2 with the addition of the respective antioxidant supplement e.g. vit C adjusted for vitamin C supplementation.

Table 45. The lowest quintile of intake for all of vitamin C, vitamin E and selenium and the risk of pancreatic cancer.

	Q1 for all of vit C, E & selenium	Q2-5 for any of vit C, E or selenium	p value
Number of participants	98	3 960	
10 years of follow-up			
Cases	6	45	
HR (95% CI)[1]	1.00	0.17 (0.07-0.41)	<0.001
HR (95% CI)[2]	1.00	0.19 (0.07-0.48)	0.001
HR (95% CI)[3]	1.00	0.19 (0.07-0.49)	0.001
17 years of follow-up			
Cases	7	81	
HR (95% CI)[1]	1.00	0.29 (0.13-0.63)	0.002
HR (95% CI)[2]	1.00	0.27 (0.12-0.63)	0.002
HR (95% CI)[3]	1.00	0.28 (0.12-0.64)	0.003

HR=hazard ratio, CI=confidence interval, Q1=lowest quintile of intake.

[1]Adjusted for age and sex.

[2]Adjusted for age, sex, smoking status, diabetes, BMI category and quintile of energy intake.

[3] Same as model 2 with the addition of vit C, vit E & selenium antioxidant supplementation.

Table 46. Serum vitamin C and the risk of developing pancreatic cancer.

	Quintile				
	1	**2**	**3**	**4**	**5**
Number of participants	4691	4362	4459	4689	4273
% of original cohort	18.3	17.0	17.4	18.3	16.7
Cut points (µmol/litre)	3.0 to 37.0	37.4 to 49.0	49.5 to 58.0	58.7 to 69.0	69.1 to 242.0
10 years follow-up					
Number of P-Y	46 854	43 566	44 573	46 861	42 723
Number of cases (n=44)	15	12	7	8	2
Cases per 1000 P-Y	0.32	0.28	0.16	0.17	0.05
HR (95% CI)[1]	1.00	0.93 (0.43-1.99)	0.55 (0.22-1.37)	0.62 (0.26-1.49)	0.17 (0.04-0.77)**
HR (95% CI)[2]	1.00	0.91 (0.42-1.96)	0.52 (0.21-1.31)	0.59 (0.24-1.44)	0.16 (0.04-0.73)**
17 years follow-up					
Number of P-Y	59 693	55 234	56 417	59 194	53 717
Number of cases (n=78)	20	17	15	17	9
Cases per 1000 P-Y	0.34	0.31	0.27	0.29	0.17
HR (95% CI)[1]	1.00	0.97 (0.51-1.86)	0.85 (0.43-1.67)	0.91 (0.47 1.77)	0.51 (0.23-1.15)
HR (95% CI)[2]	1.00	0.96 (0.50-1.84)	0.83 (0.42-1.65)	0.88 (0.45-1.72)	0.49 (0.21-1.10)

HR=hazard ratio, CI=confidence ratio, P-Y=person-years, **p for trend <0.05

[1] Adjusted for age & sex.

[2] Adjusted for age, sex, smoking status, diabetes and BMI category.

4. Summary of results

The primary analysis was the risk of developing pancreatic cancer after 10 years follow-up, adjusted for the co-variates of age, sex, cigarette smoking, diabetes and body mass index category, plus total energy intake for dietary nutrients. The main findings were statistically significant inverse associations for following; first, increased physical activity in participants younger than 65 years; second, increased dietary DHA intake; third, a threshold effect for dietary vitamin E and selenium; fourth, increased serum vitamin C levels. Borderline statistically significant negative associations were found for both total n-3 fatty acid intake and the threshold effect of vitamin C. No other associations were reported with either macro-nutrient or food groups during the 10 year follow-up period. The secondary outcomes, analysed after 17 years of follow-up, reported a statistically significant negative association with oleic acid intake and a threshold effect for vitamin C and vitamin E.

Discussion

The main findings in this study of pancreatic cancer aetiology were for the primary outcomes, after 10 years of follow-up, statistically significant inverse associations for increased dietary DHA intake and a threshold protective effect for vitamin E and selenium with increased physical activity protective in participants aged less than 65 years. Borderline statistically significant negative associations were found for total n-3 fatty acid intake and a threshold effect of vitamin C which was supported by the serum vitamin C data that had a statistically significant negative association with the risk of developing disease. No other statistically significant associations were reported during the 10 year follow-up period. The secondary outcomes, analysed after 17 years of follow-up, found a statistically significant negative association with oleic acid intake and a threshold effect for vitamin C and vitamin E. A borderline result was found for physical activity in those aged less than 65 years with the remaining results non-significant. The effect sizes were large with at least a 50% reduction is risk for the highest category of most risk factors. This work is the first to investigate the aetiology of pancreatic cancer using physical activity questionnaires that have been validated against physiological measures and food diaries in a prospective cohort study. The findings support measuring these variables in future aetiological studies of pancreatic cancer. Factors affecting the interpretation of results will now be discussed.

1. Lifestyle risk factors; physical activity and alcohol use

Physical activity

After 10 years of follow-up, physical activity was associated with a reduced risk of pancreatic cancer in those aged less the 65 years at enrolment, although no effects were found in the whole cohort. The study benefitted from the use of a physical activity questionnaire that had been previously validated against physiological measures of cardio-respiratory fitness, giving a novel attribute to the study design. Measurement tools with a higher degree of accuracy allow the detection of smaller magnitudes of effect. The *a priori* hypothesis was that physical activity could reduce the risk of pancreatic cancer by firstly, improving glucose tolerance and insulin sensitivity, and secondly, reducing serum insulin levels.[376-377] Raised insulin levels are associated with an increased risk of pancreatic cancer [278] by stimulating the proliferation of pancreatic cancer cells.[316-317] Exercise also increases the rate of fat oxidation which enables metabolism of a high fat diet,[408] contrasting with diabetes and obesity which are both risk factors for pancreatic cancer, that decrease the capacity to oxidise fatty acids, allowing some fatty acids to induce insulin resistance.[456]

The findings in this study reflect some, but not all previous epidemiological work. Several investigations did not report an effect of physical activity,[289] [380] including the largest prospective cohort study in 1.3 million UK women undergoing breast cancer screening between the years 1996-2001.[457] After a maximum of 11 years follow-up, 1 710 women died from pancreatic cancer. Physical activity was assessed in a questionnaire that categorised participants by the number of occasions they exercised each week. The findings were adjusted for smoking, BMI and height, with no effect reported for frequency of physical activity (p for trend=0.6).[457] However, the physical activity questionnaire had not been previously validated and mostly assessed leisure time physical activities, which have been shown to be a poor indicator of physiological activity when compared to physiological measures of cardio-respiratory fitness.[458] Physical activity has been investigated in a large number of other epidemiological investigations, leading to two recent meta-analyses, the first from 2008. This meta-analysis concluded there was no evidence of an effect of physical activity derived from leisure activity, although data taken from three cohort studies showed occupational activity was associated with a 25% decreased risk of pancreatic cancer (highest quartile vs lowest RR=0.75; 95% CI=0.58-0.96),[378] consistent with

data from another meta-analysis in 2011.[379] The finding that occupational physical activity exerts a protective effect against the development of pancreatic cancer is consistent with this study's results in those aged below 65 years. The lack of an association of physical activity in the whole cohort, and hence including those over the age of 65 years, is likely to be due to several reasons. Firstly, the physical activity index was derived mostly from occupational physical activity, and those aged 65 years and over will probably have retired, so exercise is likely to be less and potentially have a reduced impact on disease. Furthermore, as participants stopped working and entered retirement, physical activity levels would be expected to change, leading to a regression dilution effect as their activity would be misclassified. Finally, physical activity at work is the most discriminating question to determine the level of total physical activity. If it is no longer applicable to an individual, it becomes very difficult to reliably classify someone's level of physical activity.[458] In those aged over 65 years or not working, there are currently no validated physical activity questionnaires which reliably categorise such people, and hence no effects of physical activity have been demonstrated in this group, in either this study, or previous work. To address the limitations of current work, future studies could include repeated assessments of physical activity. These were obtained in the EPIC-Norfolk participants who completed the physical activity questionnaire after 18 months and 3 years of follow-up. More accurate methods of recording physical activity could also be used, with previous work suggesting a role for heart rate monitoring over several days which improves estimates of physical activity energy expenditure.[459]

The weaknesses of the study were the relatively small number of cases identified, with only 53 cases diagnosed after 10 years of follow-up, which increases the risk of chance findings. However, it was decided to perform the primary analysis after 10 years, rather than 17 years, to reduce the effects of regression dilution bias which could have been considerable. Physical activity remains a difficult exposure to assess reliably and hence reducing the time to analysis improves the accuracy of a single baseline measure. Evidence from this and previous studies suggests occupational physical activity does reduce the risk of pancreatic cancer, but until more reliable methods are found to assess leisure time activity, particularly in those aged over 65 years, it will remain unclear whether this also has an effect on disease risk.

Alcohol intake

Increasing alcohol intake did not significantly alter the risk of pancreatic cancer, although those with a moderate intake (>0 to <7 units per week) did have a reduced risk of disease compared to abstainers of alcohol after 17 years of follow-up. There was no effect of having a high intake of alcohol (>21 units per week). The lack of effect of an increased intake of alcohol is in agreement with previous large cohort studies which have reported similar results.[363-364] This study did not have sufficient numbers to evaluate those with a very high alcohol intake (>35 units/week) which has previously been associated with an increased of disease.[364-365] Hence, the results from this study add to the growing evidence that moderate and high intakes of alcohol do not increase the risk of pancreatic cancer. The finding that moderate intake may be associated with a decreased risk compared to abstainers was replicated by the largest study of moderate alcohol intake undertaken in the Million Women Study. This study, of 1 280 296 middle-aged women in the UK, separated alcohol categories into; non-drinkers, those consuming ≤2 drinks/week, 3-6 drinks/week, 7-14 drinks/week and ≥15 drinks/week. Using those drinking ≤2 drinks/week as the comparison group it reported that those consuming 3-6 drinks per week had a reduced risk of developing disease (RR=0.88 95% CI=0.78-1.00) with a non-statistical increased risk in non-drinkers (RR=1.07 95% CI=0.97-1.20) and those with the highest intake (RR=1.07, 95% CI=0.85-1.35).[460] Hence, the results from this study and previous large cohort studies suggest a possible J-shaped relationship between alcohol intake and pancreatic cancer risk with a moderate intake (>0 to <7 units per week) associated with a decreased risk of disease, although there could be a residual confounder effect of either the lifestyle or dietary patterns.

2. Dietary analysis

Diet is a plausible environmental factor to investigate in the aetiology of pancreatic cancer, with different nutrients having potential causative and protective effects. When assessing the role of diet in disease there are four main limitations to epidemiological studies. First, measurement of diet lacks precision and specificity. Second, nutrient intakes are highly correlated and therefore attribution of causation to one nutrient considered to be acting on its own may be misleading. Thirdly, biological measures of nutrients in tissues may not accurately and reliably reflect dietary intake. Fourth, the physical attributes of a food are not taken into consideration i.e. cooking style and preparation, freshness etc.[260] Each of these limitations should be considered when drawing conclusions from results obtained.

Total energy intake

The results from this study showed negative associations for each higher quintile of total energy intake after 10 and 17 years follow-up, although none reached statistical significance (10 yr F/U, highest vs lowest quintile, HR=0.46 95% CI=0.17-1.23, p=0.12). The study benefitted from the use of a 7-DFD which had been validated in previous studies against 16-day weighed records. Better correlation coefficients for total energy intake were achieved using 7-DFDs when compared to FFQs and 24-hour recalls which were the dietary assessment methods used in previous cohort studies (r=0.59 for 7-DFD vs r=0.52 for FFQ vs r=0.42 for 24-hour recall).[51] To exclude possibility of pre-clinical disease leading to a reduced energy intake, cases diagnosed within 2 years were removed from the analysis, which lead to an accentuation of the effect (10 year F/U, highest vs the lowest quintile HR= 0.41 95% CI=0.14-1.18, p=0.099). There are few biological mechanisms to explain a protective effect of total energy intake, although, increased energy intake may be due to increased physical activity levels.[397]

Only two previous cohort studies have investigated total energy intake and pancreatic cancer. This study's finding of a negative association of energy intake with pancreatic cancer has been replicated in the Finnish ATBC cohort study of 27 111 male smokers with 163 incident cases of pancreatic cancer, which reported the highest vs lowest quintile HR=0.62 (95% CI=0.36-1.07, p for trend=0.05).[398] The other cohort study was the Iowa Women's Health Study, which reported no association (highest vs lowest tertile RR=1.20, 95% CI=0.67-2.15, p for

trend=0.54).[399] Previous cohort studies and results from EPIC-Norfolk, listed in table 5, are not adjusted for physical activity, which is likely to be a confounder for energy intake. However, an analysis was made including physical activity but the results were not significantly altered (adjusting for physical activity, highest vs lowest quintile HR=0.47 95% CI=0.17-1.23; without physical activity HR=0.46 95% CI=0.18-1.25). In future work, energy intake should be adjusted for physical activity to exclude the possibility of total energy intake being a surrogate marker of physical activity and to clarify if total energy intake does have an effect on pancreatic cancer risk.

Dietary fats

This study assessed the effects of total fats, fatty acid classes, fatty acid sub-classes and individual fatty acids. In the primary analysis, after 10 years of follow-up, total fat had negative associations for higher quintiles of intake compared to the lowest, although none were statistically significant. The study benefitted from the use of 7-DFDs which had improved correlation coefficients for fat intake compared to other dietary assessment methods (r=0.63 for 7-DFD vs r=0.55 for FFQ vs r=0.40 for 24-hour recall).[51] Total fat intake has limited plausible biological mechanisms which could alter pancreatic risk. Fat could increase the risk of disease via stimulation of cholecystokinin release which induces hypertrophy of acinar cells,[400] but there are no plausible biological mechanism to account for a protective effect of total fat intake. Previous cohort studies have either found no effect [399, 404-406] or an increased risk of disease with increased total fat intake (Table 25).[398, 403] Therefore, the non-significant negative association found in EPIC-Norfolk, combined with inconsistencies of previous work, suggests that total fat intake is not a risk factor for pancreatic cancer.

Saturated fats

In the primary analysis, no statistically significant associations with pancreatic cancer were found for total saturated fat intake or any of the individual saturated fatty acids. However, all of the highest quintiles had a positive association with disease, with the greatest magnitude found with stearic acid (HR=2.01, 95% CI=0.54-7.45, p for trend=0.36). Saturated fatty acids have biological actions which could account for an increased risk of pancreatic cancer.

Saturated fatty acids induce insulin resistance,[402] by increasing insulin secretion and decreasing insulin sensitivity,[173-176] with insulin resistance and raised insulin levels a possible precursor to pancreatic cancer.[326]

Previous large cohort studies have investigated the association of saturated fatty acids and pancreatic cancer. The US National Institute of Health - American Association of Retired Persons (NIH-AARP) is the largest cohort study undertaken in this area, of 308 736 men and 216 737 women, which identified 865 cases of incident pancreatic cancer in men and 472 in women after 6.3 years follow-up.[403] An increased risk of disease was found in those with the highest intake of saturated fat (HR=1.36, CI=1.14-1.62). The Finish ATBC study found a borderline increased risk with saturated fat intake (HR=1.60, 95% CI=0.96-2.64, p for trend=0.02)[398] The three remaining cohort studies that have published saturated fatty acid data reported no association with pancreatic cancer.[404-406]

The lack of a statistically significant association in EPIC-Norfolk, for saturated fatty acid intake, could be due to a low sample size, with only 51 incident cases for analysis after 10 years. By extending the follow-up period to 17 years, and increasing the number of incident cases to 88, regression dilution bias has a greater effect and no pattern of association was apparent. The NIH-AARP study benefitted from a larger cohort size, with 1 337 cases after only 6.3 years follow-up, and hence their statistically significant findings could be of greater relevance. When interpreting saturated fat results, there is the risk of a residual confounding, as saturated fat intake is correlated with meat intake. Meat has a high concentration of many compounds which could have carcinogenic effects including iron, heterocyclic amines and polyaromatic hydrocarbons which are found in cooked meats.[398] The results from this study did not show a clear association of saturated fatty acid intake and pancreatic cancer, although previous studies have reported an increased risk, indicating that future work is required to clarify the association, using detailed methods of assessing dietary intake of saturated fats, in a cohort large enough to detect potential small effect sizes.

Monounsaturated fatty acids

After 10 years of follow-up, no statistically significant associations were found for total monounsaturated fatty acid (MUFA) intake or any of the individual

MUFAs. After 17 years follow-up, oleic acid had a statistically significant negative association with pancreatic cancer and palmitoleic acid had a positive association, though not statistically significant. Experimental work has been undertaken to clarify if oleic acid has anti-carcinogenic effects. It is predominantly found in vegetable oils, particularly olive oil, which is an integral component of the "Mediterranean diet". Olive oil may have a role in lowering the risk of cancers, with previous epidemiological work suggesting that it is associated with a reduced risk of prevent breast cancer,[461-464] colorectal cancer,[465-466] and gynaecological cancer.[467-468] The benefits of olive oil could either be attributable to the high content of oleic acid, or the antioxidant components of the unsaponifiable fraction. Oleic acid can modify key cancer oncogenes, with experiments showing oleic acid is able to down-regulate the transcription of the key oncogene, Her-2/neu, in breast, ovarian and gastric cancer.[424] The presence of Her-2/neu in pancreatic adenocarcinoma has been assessed in 154 patients, with 32 (21%) showing positivity. Hence, oleic acids ability to down-regulating Her-2/neu, could account for the prevention of some pancreatic cancer cases.[469] Not all experimental work supports a protective effect of oleic acid in cancers. A prospective study from Italy analysed the relationship between erythrocyte membrane fatty acids and postmenopausal breast cancer risk. They found that a higher concentration of oleic acid in the erythrocyte membrane was associated with an increased risk of cancer (highest versus lowest tertile of percentage of total fatty acids, OR=2.79; 95% CI=1.24-6.28).[470]

Previous epidemiological investigations of the effects of total MUFA intake reported a decreased risk of pancreatic cancer in early case-control studies although these findings were not repeated in large cohort control studies. The Finnish ATBC study found no effect[398] and the NIH-AARP study reported an increased risk of pancreatic cancer for the highest quintile of total MUFA intake (HR=1.22, 95% CI=1.02-1.46).[403] Only one previous cohort study and three case-control studies have specifically assessed oleic acid intake and the risk of pancreatic cancer. The NIH-AARP Diet and Health Study reported no effect for oleic acid (highest vs lowest quintile HR=1.16 95% CI=0.97-1.39, p for trend=0.12). An Italian case-control study of 326 pancreatic cancer cases and 652 controls used FFQ data collected by an interviewer during a hospital episode and found no effect of dietary oleic acid.[471] A case-control study from San Francisco of 532 cases and 1701 controls used a FFQ validated against a 7-day food diary, asking participants to report their average intake of foods one year previously.[413] They reported that oleic acid was associated with an increased risk of pancreatic

cancer (highest vs lowest quartile OR=1.4 95% CI=1.1-1.9). A Canadian case-control study of 462 cases and 4721 matched controls used a self-administered FFQ to assess dietary intake 2 years prior to completion and reported that those in the highest vs lowest quartile had a reduced risk of pancreatic cancer, with a multivariate OR=0.75 (95% CI=0.55-1.02, p for trend=0.04).[409] As previously discussed, case-control studies and the use of FFQ data, particularly when validated against 7-DFDs, is an inferior study design to that used in EPIC-Norfolk, and hence the results should be interpreted with caution.

In EPIC-Norfolk, palmitoleic acid had non-statistically significant positive associations with pancreatic cancer for each higher quintile of intake (highest vs lowest, HR=2.64, 95% CI=0.84-8.30). Palmitoleic acid has actions which could modulate the risk of pancreatic cancer. It has been shown to negatively affect cholesterol metabolism and contribute to the metabolic syndrome, which leads to insulin resistance.[472-473] Palmitoleic acid can also alter cell functions, although experimental work is unclear whether this could contribute to carcinogenesis.[474] The only other cohort study to publish data on this fatty acid was the NIH-AARP study, which also found a positive association (highest vs lowest quintile HR=1.34, 95% CI=1.12-1.59, p for trend<0.001). A case-control study from San Francisco reported a positive association (highest vs lowest quartile OR=1.6, 95% CI=1.2-2.1). Although there is limited epidemiological work on palmitoleic acid, the findings are consistent that it may increase the risk of pancreatic cancer.

In summary, total MUFA intake did not alter the risk of pancreatic cancer in EPIC-Norfolk, although oleic acid and palmitoleic acid had possible opposing effects, indicating individual monounsaturated fatty acids may exert biological effects to modify the risk of developing pancreatic cancer. These need to be investigated in future experimental and epidemiological work, with the latter using sufficiently detailed methods of determining the dietary intake of these nutrients.

Polyunsaturated fatty acids

Total PUFA had inverse, but statistically non-significant associations with pancreatic cancer risk. Four cohort studies have published in this area, none of which found effects (Table 25). Individual PUFAs exert a wide range of biological actions, which could hypothetically increase or decrease the risk of pancreatic cancer. N-6 PUFAs are a group of fatty acids with actions which may increase the risk of disease by promoting the production of inflammatory cytokines that

stimulate oncogenic pathways such as cell proliferation and angiogenesis which favour tumour growth.[411] However, n-6 fatty acids can reduce insulin resistance [174] which may reduce the risk of developing pancreatic cancer. In this study, total n-6 fatty acid intake was not associated with pancreatic cancer. This null finding was also replicated in the three cohort studies to publish in this area (table 22), which suggests that n-6 PUFAs are not involved in the aetiology of pancreatic cancer.

The highest quintile of total n-3 PUFA intake had a borderline statistically significant negative association with pancreatic cancer after 10 years of follow-up (HR=0.30, 95% CI=0.07-1.21, p=0.092), with the individual n-3 PUFAs, DHA and EPA, also demonstrating statistically significant or borderline significant negative associations. The tumour suppressive effects of n-3 fatty acids have been demonstrated in laboratory work[415-416] with EPA and DHA exerting inhibitory effects on the growth of human pancreatic cancer cell lines *in vitro*.[412, 414, 417] N-3 fatty acids may prevent somatic mutations in the K-ras genes, which are involved in the initiation of pancreatic carcinogenesis and are targets for chemical carcinogens. This mechanism of preventing one of the most common oncogenic mutations in the pathway to pancreatic carcinogenesis could account for a beneficial effect of n-3 fatty acids.[422] There is also experimental evidence that n-3 fatty acids may slow the improve outcomes in patients with pancreatic cancer although work has yet to be conducted demonstrating a survival benefit.[414]

The inverse association of n-3 PUFA intake with pancreatic cancer in EPIC-Norfolk are not supported by the results of previous cohort studies, with no effect reported in the Finnish ATBC, US Nurses' Health Study and Netherlands Cohort Study.[398, 405-406] The NIH-AARP study reported an increased risk of pancreatic cancer with higher total n-3 intake (highest quintile vs lowest HR=1.21 95% CI=1.02-1.44).[403] In total, the NIH-AARP study investigated three fatty acids classes (saturated, MUFA & PUFA), three fatty acid sub-classes (n-6, n-3 & *trans*) and 12 individual fatty acids (including DHA and EPA), all of which had positive associations with pancreatic cancer. All these groups may increase the risk, although there may have been either a biasing of results or a correlated effect as the dietary assessment method was unable to discriminate the source of dietary fat intake. No previous study has used 7-DFDs that provide the most accurate measure of dietary intake, and allow greater discrimination between nutrients and the assessment of smaller effect sizes.[51]

The benefits seen in this study may not be solely from increased n-3 intake, but also from accompanying alterations in the general composition of the diet, particularly by lowering the n6/n3 fatty acid ratio. The benefits could be due to a

reduced n-6 contribution to the diet, rather than an the effects of n-3s *per se*,[421] although as previously discussed, n-6 PUFA do not appear to have a direct effect on pancreatic cancer risk. The results could also be accounted for by a residual confounding effect, given that EPA and DHA are strongly correlated with fish intake. Furthermore, the substitution of fish in the diet with meat may further confound this effect, as there will be reduced intake of the potential risk factors found in meat such as of iron, heterocyclic amines and polyaromatic hydrocarbons. A limitation of the analysis is that no adjustment has been made for supplementary intake of fish oils, with 24% of participants to known to use cod liver oil supplements in EPIC-Norfolk.[49]

The findings from EPIC-Norfolk supports experimental data that suggests n-3 fatty acids may prevent pancreatic cancer development. There are inconsistencies in epidemiological work, though previous work may have been limited in the methods of determining dietary intake. N-3 PUFAs deserve further investigation as there appears to be emerging biological, epidemiological and intervention work that this group of fatty acids has protective effects against the development and progression of pancreatic cancer.

Dietary antioxidants

This is the first epidemiological study to report the effects of dietary antioxidants and pancreatic cancer using data collected from food diaries. The dietary antioxidants, vitamin C, vitamin E and selenium (but not zinc) had large inverse associations for the development of pancreatic cancer. The lowest quintile of antioxidant intake had at least a 40% greater risk of developing disease. The inverse associations existed in a threshold, rather than a dose-dependent manner. For vitamin C, the diary data was supported by the biomarker results, as increased serum vitamin C levels had a strong dose-dependent inverse association, with those in the highest vs lowest quintile having a 84% reduced risk of pancreatic cancer. For participants consuming the lowest quintiles of dietary intake for all of vitamins C and E and selenium, they had a 5 times greater risk of developing pancreatic cancer.

This epidemiological data supports experimental work for how dietary antioxidants may prevent carcinogenesis by scavenging for free radicals, the latter of which can induce genetic mutations.[475] Antioxidants also have effects stimulating the immune system which can be protect against carcinogenesis.[431]

Established risk factors for pancreatic cancer, such as diabetes and smoking, induce oxidative stress and free radical production which could be prevented by dietary antioxidants.[476] Vitamin E had the largest inverse association with pancreatic cancer. Vitamin E, which is present in vegetable oils, nuts and egg yolk, inactivates free radicals formed from the polyunsaturated fatty acids present in lipid cell membrane.[477] This fat soluble vitamin also prevents the formation of N-nitroso compounds, which are suspected carcinogens for pancreatic cancer.[478-479] Vitamin C is present in fruit, vegetables and milk, and is a water soluble reducing agent that can detoxify hydroxyl and superoxide free radicals. Selenium is found in many different food types, including cereals and meat, with the mineral concentration in foods dependent upon the selenium content of the soil used in production. Selenium is incorporated into selenoproteins, including the enzyme glutathioione peroxidase, which catalyses the removal of hydroperoxides. The antioxidant effects of vitamin C, vitamin E and selenium all prevent oxidative stress[480] which leads to genetic damage and carcinogenesis.[475-476] A second anti-carcinogenic mechanism for antioxidants is their effects on the inflammatory process and suppression of chronic inflammation that may otherwise be involved in cancer development.[433] Both hereditary and non-familial pancreatitis are significant risk factors for pancreatic cancer,[312, 434] with chronic pancreatitis associated with the generation of reactive oxygen species that requires antioxidant enzyme activity to inactivate. Biopsies taken from normal, inflamed and neoplastic pancreatic tissue showed a gradual decrease in antioxidant enzyme expression suggesting a lack of antioxidants may enable the progression to cancer.[435] Antioxidants supplementation in patients with chronic pancreatitis reduces levels of pain and markers of oxidative stress.[436-438] Finally, antioxidants have an effect on genetic mechanisms relevant to pancreatic cancer. Somatic mutations in the K-ras oncogene are an early and fundamental event in the pathogenesis of most exocrine pancreatic cancers. Ras proteins are vital for cell function and regulation of growth, differentiation and apoptosis and K-ras point mutations are found in 75-90% of pancreatic cancers.[296] In a study of 121 pancreatic cancer cases with (78%) and without (22%) K-ras mutations, those in the highest tertile of dietary vitamin E intake had a reduced rate of mutation (OR=0.24 95% CI=0.06-0.98).[422] Although statistically non-significant, an increased intake of vitamin C was also associated with reduced K-ras mutations.[422] These findings suggest that increased dietary antioxidant intake may prevent a key genetic mutation found in most cases of pancreatic cancer.

To confirm the experimental data that dietary antioxidants do have a causal role in preventing pancreatic cancer supportive data epidemiological studies is

required, ideally from prospective cohort investigations. To the best of our knowledge, there are just two cohort investigations which have investigated dietary antioxidants. The first, a prospective study of diet in the elderly reported higher intakes of vegetables, fruits, dietary beta-carotene, and vitamin C were each associated with a reduced risk of pancreatic cancer, although none of these associations were statistically significant.[439] The second cohort investigation was the Finnish ATBC study of 27 111 male smokers. It used FFQs to measure the dietary intake of vitamins C, E and selenium and reported no associations with pancreatic cancer.[481] (5)There are studies that investigated food groups which contain antioxidants, such as citrus fruits which are rich in vitamin C. A meta-analysis of four case-control studies of citrus fruit, reported an increased intake had an inverse association with pancreatic cancer (highest vs lowest quintile of intake OR=0.83; 95% CI=0.70-0.98), although such retrospective studies have methodological biases which limit their validity.[445] A Cochrane review of randomised controlled trials of antioxidant supplements, including vitamins E, C and selenium, either solely or in combinations, did not find any effect of supplementation on the incidence of pancreatic cancer.[447] The current uncertainty in the role of antioxidants is due to the relatively few studies, small numbers in some work, using less accurate measures of diet and unrepresentative populations. However, the data from EPIC-Norfolk, showing inverse associations of several dietary antioxidants in a threshold manner, supports the continued investigation of these micronutrients in the aetiology of pancreatic cancer.

Serum antioxidants and serum vitamin C

Biomarkers can be a more accurate measurement of diet than questionnaire based methods. In this study, serum vitamin C had a strong inverse association with pancreatic cancer. After 10 years of follow-up the highest vs the lowest quintile level had a multivariate HR=0.16 (95% CI=0.04-0.73, p=0.018) with a hazard ratio trend across quintiles of 0.73 (95% CI=0.57-0.92, p=0.008). After 17 years of follow-up the results were attenuated with the highest v lowest quintile HR=0.48 (95% CI=0.21-1.11, p=0.085) and a trend across quintile HR=0.88 (95% CI=0.74-1.04, p=0.12). Serum vitamin C levels are mostly determined by dietary intake but are also influenced by demographic and lifestyle factors. This was demonstrated in a French study of 1 821 women and 1 307 men, that reported serum vitamin C concentrations were higher in women, non-smokers and the non-

obese.[482] Smoking reduces serum vitamin C levels due smoke-related oxidant production,[483] resulting in an increased turnover of this antioxidant. However, in the French study, dietary intake of vitamin C was the strongest determinant of serum levels (Pearson correlation coefficient=0.28, $p<0.0001$). Hence serum vitamin C is likely to be a true reflection of intake, be it from the diet or supplements and in the EPIC-Norfolk analysis, adjustment was made for sex, BMI and smoking which are also known to affect serum vitamin C levels.

Serum vitamin C levels and pancreatic cancer have not been previously investigated, although low baseline serum vitamin C was associated with an increased risk of all cancers in men in the French SU.VI.MAX (SUpplementation en VItamines et Mineraux AntioXydants) study.[484] This was a randomised double-blind, primary prevention trial of low-dose antioxidant supplementation (120mg of vitamin C, 30mg of vitamin E, 6mg of beta-carotene, 100µgrams of selenium & 20mg of zinc) which reported antioxidant supplementation lowered total cancer incidence in men, but not in women.[484] Furthermore, in the same study cohort men had lower baseline serum antioxidants levels than women, and men with low baseline serum antioxidant levels gained the greatest reduction in cancer risk with antioxidant supplementation.[485] These findings support a threshold effect for antioxidants, with low levels of antioxidants positively associated with pancreatic cancer, but increased intakes not leading to a further reduction of risk.

Serum levels of the antioxidants, vitamin E and selenium and the risk of pancreatic cancer have been previously investigated. The Finnish ATBC study, reported raised serum vitamin E levels at baseline were associated with a halving of risk of developing pancreatic cancer for those in the highest compared with the lowest quintile (HR=0.52; 95% CI-0.34-0.80; p for trend=0.03).[481] These results contrast with a nested case-control study from a population of 25 620 men and women from Maryland, USA. They identified 22 cases of pancreatic cancer that were matched with 44 controls and reported the lowest tertile of vitamin E was associated with a lower risk of pancreatic cancer (OR=0.2, 95% CI=0.04-1.17).[486] However, the lowest tertile of serum selenium was associated with a 4 times greater risk (OR=3.9, 95% CI=1.13-13.2). Another study which evaluated baseline serum vitamin E levels was the Finnish Mobile Clinic Health Survey, undertaken in 36 365 men and women which identified 766 cancers after a mean follow-up of 8 years. They reported individuals with a low level of vitamin E had a 1.5-fold (no confidence interval cited) increased risk of cancer compared with those with higher

concentrations. The association was strongest among non-smoking men and women with low levels of serum selenium.[487] Seventeen cases of pancreatic cancer were identified in men, and in those with lower levels (lowest three quintile vs two highest quintiles) of serum vitamin E the relative risk of disease was 4.8 (no confidence interval cited) although no effects were found in women.[486] In summary, there is emerging, but as yet incomplete evidence that dietary antioxidants prevent pancreatic cancer. This includes plausible biological mechanisms, some epidemiological data and a randomised controlled trial data. The findings in EPIC-Norfolk of inverse associations for increased dietary intake of antioxidants and serum levels of vitamin C supports this hypothesis. To confirm causality further large cohort investigations need to report their findings on antioxidants. Randomised controlled trials could also be undertaken which focus on those with a low intake or serum levels of antioxidants, as they are at particular risk of developing pancreatic cancer and evidence suggest they gain most benefit from antioxidant supplementation.

3. Strengths and limitations of the study

Internal validity

Chance

The internal validity of a study is dependent on chance, bias, confounding and measurement error. There are several advantages and limitations to this study which need to be considered when interpreting the findings. A relatively large population cohort was used in EPIC-Norfolk, of over 25 000, but the number of incident cases of pancreatic cancer after 10 years was relatively small, at 53 cases, allowing chance findings to become more likely, although for all the associations found in this study there are plausible biological mechanisms to explain their action, with either supporting animal or human intervention studies. Studies such as the NIH-AARP and EPIC [363, 403] had more than 400 000 participants with over 1300 incident cases of incident pancreatic cancer which reduces the likelihood of chance findings. However, these large cohort studies did not benefit from utilising the more accurate methods of measuring risk exposures used in EPIC-Norfolk, such as the 7-day food diaries, and hence due to measurement error, may be unable to detect small effects derived from exposures.

Selection bias

The prospective design of this study minimises several potential sources of bias. Since dietary data was collected and recorded in real time over a one week period, recall bias due to errors of estimation of food intake are reduced. The prospective study design also reduced selection bias as cases and non-cases are drawn from the same population. If cases and non-cases are compared at the time disease is identified, there is the potential for differential reporting of dietary intakes, which is a limitation of the case-control design. Also, symptoms may alter behaviour in those with pancreatic cancer, in particular they may alter their diet by decreasing foods which precipitate symptoms causing a "protopathic" bias[123] and leading to type 1 error. To minimise bias introduced by a disease altering behaviour prior to its diagnosis, no cases were included if they were diagnosed within 6 months of enrolment into EPIC-Norfolk. Furthermore, a sensitivity analysis was performed for total energy intake, whereby all those diagnosed within 2 years were excluded, which made little difference to results, suggesting that energy intake was not altered in those later diagnosed with pancreatic cancer within two years of enrolment.

Regression dilution bias

All the analyses relied on a single baseline measurement, which after prolonged follow-up, can lead to regression dilution bias due to participants altering their diet. Prospective studies which analyse disease risk from just one initial baseline survey of an exposure, may underestimate the magnitude of risk of disease after longer periods of follow-up. This effect is amplified if the analysis includes many co-variates, all of which become less accurate over time.[257] This random measurement error occurs as some of the population will change their magnitude of exposure to the risk over time. In EPIC-Norfolk, we considered that physical activity and dietary intake were particularly vulnerable to variation over time. Ideally, the analysis should be made after the shortest period possible prior to the development of symptoms to minimise the effects of regression dilution bias. Since pancreatic cancer is relatively uncommon, the primary analysis was performed after ten years to allow the accumulation of a significant number of incident cases, and the secondary analysis after seventeen years of follow-up. In future work in the dietary analysis, we intend include data from diaries completed by participants after 18 months and 5 years of follow-up which will help to minimise regression dilution bias.

Follow-up bias

Follow-up bias could occur if those with a specific characteristic were more likely to move away from the catchment area of the local hospital where incident cases of pancreatic cancer were identified. For example; if more participants with a "high level" of physical activity moved outside the catchment area of the local hospital compared to "inactive" participants, then our study would conclude a higher level of physical is associated with a lower risk of disease. However, this is unlikely to occur on a large scale as the study population had little outward migration.[41] Follow-up bias could also occur due to a limitation in the method of identifying cases of potential pancreatic cancer. However, the methods used were robust using three separate data records, namely the Norfolk Health Authority records of hospital admissions, the Eastern Cancer Registry and Information Centre (ECRIC) and death certificate records. It is unlikely that any significant number of pancreatic cancer cases were not identified, particularly since ECRIC was able to source cancer registry data throughout the UK.

Confounders

In EPIC-Norfolk, multivariate analysis adjustment was made for the known risk factors of age, sex, smoking, diabetes and obesity (measured by body mass index). Pancreatitis is also a known risk factor for disease, but none of the incident cases of pancreatic cancer reported a history of pancreatitis at baseline. No assessment or adjustment was made for presence of the genetic disorders associated with pancreatic cancer (i.e. Peutz-Jeghers, HNPCC) or a family history of pancreatic cancer although these conditions are rare and only make a minimal contribution to the population's risk of developing disease.

External validity

Generalisable and Valid

The results found in EPIC-Norfolk are generalisable to a white UK population of both genders. The age group studied are the most susceptible to pancreatic cancer and the type of disease was representative of that seen clinically, with the staging at diagnosis and survival times equivalent to those reported in previous studies. The findings are valid as they have been demonstrated in a large, normal, diverse UK population rather than in animal models or *in vitro* models of disease. The level of physical activity or doses of nutrients that were associated with an effect were achieved in a population with a normal lifestyle or diet, rather than at exposures levels that are difficult to attain in normal living behaviour.

4. Summary of discussion findings

The lifestyle risk factors for pancreatic cancer are not well established, due to several methodological issues. First, the disease has a relatively low incidence rate, so fewer cases are available for study in cohort studies. Second, the poor survival times of patients with pancreatic cancer causes difficulties in recruiting for case–control studies. Also there is an increased susceptibility of retrospective studies to recall bias, selection bias, and exposure misclassification. Thirdly, although studies of families with high rates of pancreatic cancer have identified several predisposing genetic variants, these variants are rare and contribute little to the overall population burden of pancreatic cancer. Fourth, with no screening tests able to detect pancreatic cancer at an early more treatable stage, risk factor identification may not impact on patient outcomes, in the absence of a clear commitment to risk factor modification or the development of novel screening modalities.[488] This prospective cohort study of pancreatic cancer is the first to use 7-day food diaries to evaluate dietary exposures, which are the most accurate pragmatic dietary assessment method in large scale epidemiological work. Dose-dependent inverse associations were found for total n-3 fatty acids, docosahexaenoic acid and oleic acid with a threshold inverse association found for vitamin C, vitamin E and selenium. The findings for vitamin C were supported by the serum analysis, which also showed an inverse effect. Increased occupational physical activity also lowered the risk of pancreatic cancer in those aged less than 65 years at enrolment. There is experimental data that provides plausible biological mechanisms to explain why these dietary nutrients and physical activity may protect against pancreatic cancer, but currently the evidence from aetiological epidemiological work is both minimal and inconsistent. However, our work on these fatty acids and antioxidants supports measuring these factors in future aetiological work. If causal associations are confirmed in such epidemiological studies, then population-based dietary recommendations may help prevent pancreatic cancer.

CHAPTER THREE

THE ASSESSMENT OF NUTRITION AND DEPRESSION IN PATIENTS RECEIVING CHEMOTHERAPY FOR PANCREATIC CANCER

Abstract

Background

Nutritional depletion and depression are common in patients with pancreatic cancer. There is minimal work evaluating the most appropriate clinical service for identifying these problems to enable appropriate clinical treatment to be instituted. The aim of this clinical survey was to assess the value of a dedicated clinician screening for malnutrition and depression, document their prevalence and initiate relevant treatments through a "pancreatic support service (PASS)". The efficacy of PASS was assessed by measuring survival and clinical outcomes of patients in a retrospective group (RG), before the implementation of PASS, and after, in a prospective group (PG) of patients with pancreatic cancer.

Methods

The RG received one or more doses of palliative chemotherapy for exocrine pancreatic cancer during the year 2009 at the Norfolk and Norwich Hospital. The outcomes were compared with the PG, treated during 2010, who in addition to their standard care were also seen by PASS. The primary outcomes were survival rates and the number of chemotherapy doses received. The secondary outcomes were hospital admissions (number and length) in patients who died within 12 months of diagnosis, weight change, use of oral supplements and pancreatic enzyme replacement therapy (PERT), and whether depression had been screened for and assessed. Furthermore, the PG was screened for psychological symptoms using the hospital anxiety and depression (HAD) scale.

Results

Comparison of the RG (16 patients, 56% female) to the PG (19 patients, 47% female) found no differences in survival rates at 12 months (RG 31% vs 42% PG, p=0.51) or the number of chemotherapy doses administered (RG=9 vs 6 PG, p=0.19). For secondary outcomes, the median number of hospital admissions was higher in the RG vs PG (2 vs 1, p=0.034), with a longer duration of admission in the former (11 days vs 4 days, p=0.017). PERT was prescribed less frequently in the RG (50% vs 79%, p=0.072). The RG had fewer patients with documented evidence of a psychological assessment (44% vs 74%, p=0.072), but a higher proportion were treated for psychological symptoms (5 patients vs 1 patients, p=0.042). The HAD scale identified 43% of patients met the criteria for further evaluation of depression or anxiety. There were no statistically significant differences in the use of oral nutritional supplements, dosing of PERT or weight change. Although the differences were not statistically significant, PG patients lost less weight (RG= -7.2% vs -2.9% in PG, p=0.38).

Conclusion

The introduction of PASS did not increase survival times or the number of doses of chemotherapy received, although was associated with a reduced number and duration of hospital admissions. The reasons for this should be explored in future work as this is a group of patients with an extremely poor prognosis. This preliminary data suggests a significant weight improvement might be achieved which should be assessed in more patients and also if such a service influences quality of life. Screening for anxiety and depression identified over one third of patients had symptoms and emphasises this should be part of routine practice.

Introduction

Pancreatic cancer may present with abdominal pain, jaundice, steatorrhoea, weight loss and depression. The latter two are common and lead to a deterioration in patients' quality of life if not detected early and managed appropriately. Weight loss occurs in up to 90% of patients at diagnosis and involves three mechanisms; namely reduced calorie intake, malabsorption and altered metabolism. In patients undergoing chemotherapy for unresectable pancreatic cancer, weight loss is associated with poor clinical outcomes including a reduced quality of life, increased clinical complications and the early cessation of chemotherapy. The triad of weight loss, reduced oral intake and systemic inflammation characterise the cancer cachexia syndrome with associated poor outcomes. No randomised controlled trials have been conducted assessing nutritional therapies to arrest weight loss, and hence current recommendations by national societies are based on uncontrolled data. Weight may be stabilised with increased calorie and oral supplement drinks, which are associated with improved survival times. Malabsorption due to pancreatic exocrine deficiency can be corrected with pancreatic enzyme replacement therapy (PERT). Reduced endogenous pancreatic enzyme secretion occurs in 80% of patients with pancreatic cancer. Patients who undergo biliary stenting lose less weight if prescribed PERT. Depression is commoner in patients with pancreatic cancer than in other malignancies and is often the first symptom, characterised by a sense of impending doom. The depression is related to both specific biological processes induced by the tumour and the psychological consequences of the diagnosis. Depression has a significant impact upon morbidity by worsening patients' pain, anorexia, anxiety and weight loss. This can be avoided if depression is identified and treated stage at an early stage. In patients receiving chemotherapy for pancreatic cancer, there is minimal data on the frequency of nutritional therapy use or frequency of depressive symptoms. A clinical service to screen for nutritional and depressive symptoms, plus providing appropriate therapy may improve clinical outcomes. This study evaluated such an approach.

Pancreatic cancer is relatively common and one of the most difficult cancers to treat as it usually presents at an advanced stage with few therapies to significantly improve survival. In this context, it is important to assess patients for treatable symptoms that may otherwise impair their quality of life, including weight loss and depression. This review describes pancreatic physiology, the clinical presentation and treatment of pancreatic cancer, specifically weight loss

and depression, assessing their prevalence, aetiological mechanisms and treatment in such patients. The PubMed database was used to search for relevant papers up to July 2011. The papers identified were also used to source references and citations that were not found on the electronic database.

1. Pancreatic physiology

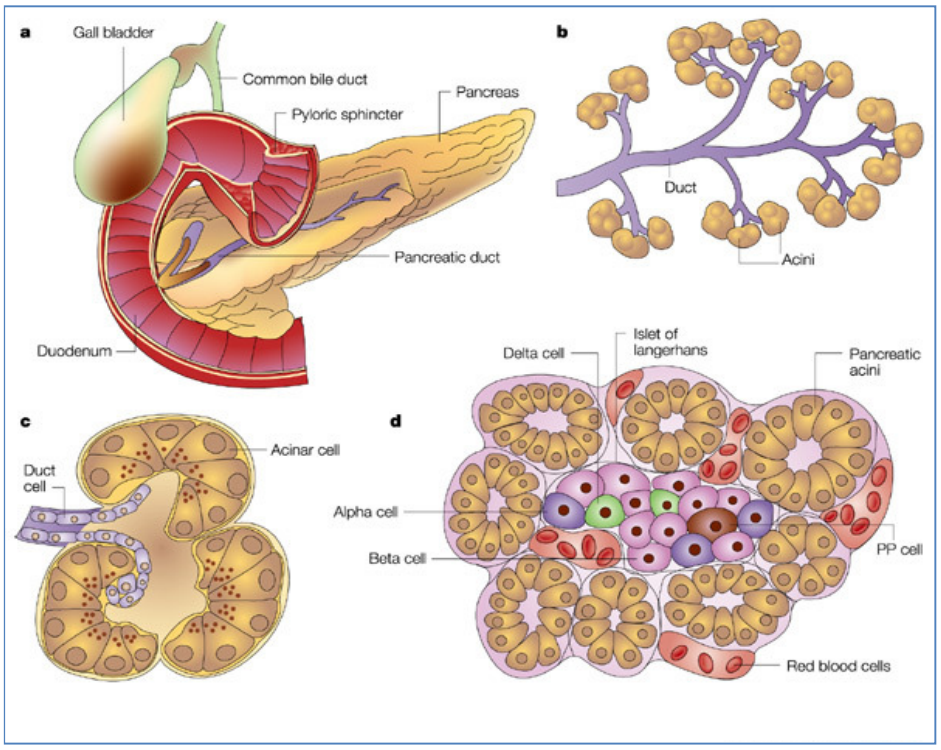
The pancreas is a gland with both endocrine and exocrine functions. The exocrine pancreas secretes pancreatic juice which consists of two components: firstly pancreatic enzymes released from the acinar cells and secondly an aqueous alkaline solution rich in sodium bicarbonate secreted by the ductal cells lining the pancreatic ducts (Figure 1). The acinar cells secrete three different types of pancreatic enzymes to facilitate digestion i) proteolytic enzymes for protein breakdown, such as trypsinogen, ii) pancreatic amylase to digest carbohydrate, and iii) pancreatic lipase to hydrolyse triglycerides. The three major pancreatic proteolytic enzymes are trypsinogen, chymotrypsinogen, and procarboxypetidase. Each is secreted in an inactivated form to prevent damage to the surrounding pancreatic parenchyma and ducts, though upon reaching the duodenum, an enzyme in the duodenal lumen called enterokinase initiate their activation. Pancreatic amylase hydrolyses the alpha-bonds of polysaccharides such as starch and glycogen to produce disaccharides including maltose. Pancreatic lipase hydrolyses dietary triglycerides to monoglycerides and free fatty acids. As the pancreas is the only significant source of lipase production, a deficiency results in fat malabsorption which manifests as steatorrhoea, defined as greater than 7g of fat in a 24 hour stool collection.[489]

The endocrine function of the pancreas is provided by the Islet of Langerhan's which consist of four cell types. These include alpha cells which secrete glucagon in response to low blood glucose and stimulate the conversion of hepatic glycogen to glucose. Beta cells release insulin that allows glucose to taken up by insulin-dependent tissues. Delta cells are located in the pancreas islet cells as well as the stomach and intestine and produce somatostatin, an inhibitory hormone that suppresses the release of many gastric (e.g. gastrin, cholecystokinin and secretin) and pancreatic (e.g. glucagon, insulin) hormones as well as pancreatic exocrine functions. Finally, F cells or PP cells are few in number and release pancreatic polypeptide which aids regulation of the endocrine and exocrine secretary function of the pancreas (Figure 15).

Incidence, epidemiology and histology of pancreatic cancer

These have been previously described on pages 135-138.

Figure 15. The anatomy and histology of the pancreas.

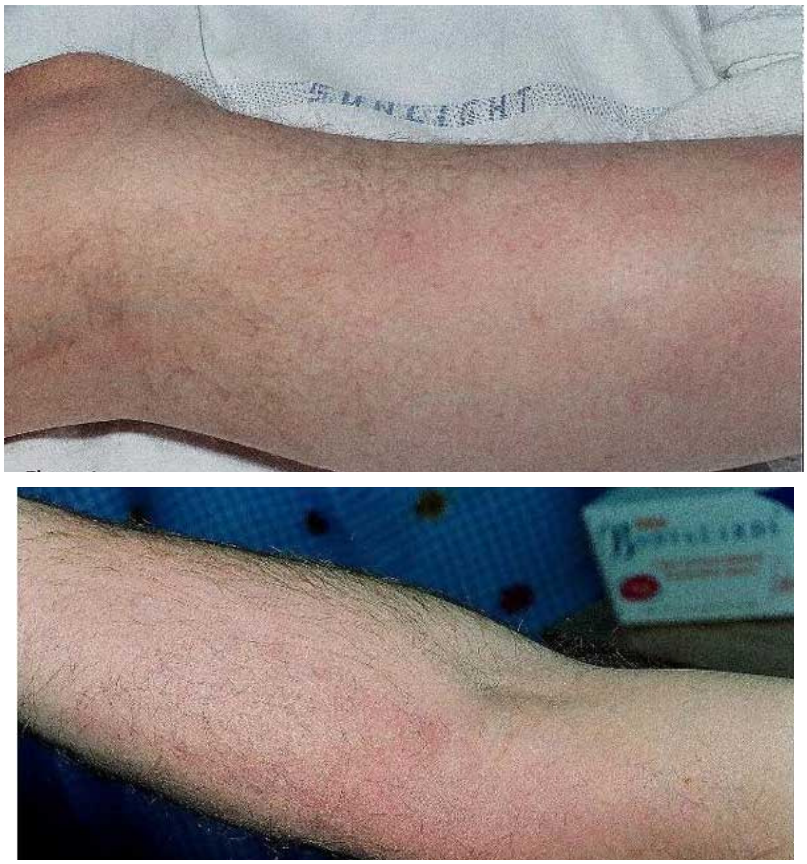


a) The gross anatomy of the pancreas. **b**) The exocrine pancreas. **c**) Histology of a single acinus. **d**) A pancreatic islet surrounded by acini. The acinar cells secrete digestive enzymes into the ducts, supplemented by an alkaline solution from the ductal cells. There are four types of islet cells which form the Islet of Langerhans and secrete hormones into the blood; Alpha cells (secrete glucagon), Beta cells (insulin), Delta cells (somatostatin) and PP cells (pancreatic polypeptide).[490]

Clinical symptoms and signs

Patients with pancreatic cancer can present with upper abdominal and/or back pain and jaundice, although weight loss is the commonest symptom found in approximately 90%.[491-492] Weight loss is often profound around the time of diagnosis, with an average loss of 3 kg per month.[491] Psychological symptoms, and in particular depression, are common. Both weight loss and depression, though frequent, may not initially be recognised as a manifestation of pancreatic cancer as they can be attributed to other illnesses. Since the symptoms of pancreatic cancer are often non-specific, this can delay diagnosis. Other less common presentations include late onset diabetes mellitus in the absence of obesity, deep vein thrombosis and thombophlebitis migrans (Trousseau's syndrome) (Figure 16). The latter is characterised by the development of recurrent (i.e. migratory) superficial thrombophlebitis due to an acquired coagulopathy that is strongly associated with malignancy. The clinical signs of pancreatic cancer are jaundice, hepatomegaly, a palpable gallbladder (Courvoisier's sign), Troisier's sign (Virchow's node), an abdominal mass and ascites.[493]

Figure 16. Thrombophlebitis migrans (Trousseau's syndrome) in a 62 year old patient.



Thrombophlebitis migrans seen in the leg (top picture) and forearm (bottom) in a 62 year old man with metastatic pancreatic cancer, characterised by pain, erythema and swelling of the extremities.

Diagnosis and staging of disease

In patients presenting with jaundice, trans-abdominal ultrasound may identify the presence of an obstructed biliary tree, a pancreatic mass and liver metastases with a diagnostic accuracy of 75%.[494] In anicteric patients or when common bile duct dilatation is confirmed on USS, a contrast-enhanced computerised tomography scan (CT scan) is the most useful diagnostic tool to identify a pancreatic mass lesion with a diagnostic sensitivity of 97% and specificity of 80%.[495] In patients with unexplained weight loss, a CT scan is important to prevent delaying the diagnosis of a pancreatic lesion. Furthermore, a CT scan can stage and predict unresectable lesions in 90% of cases, although it is less accurate in determining a resectable lesion.[495] Positron emission tomography (PET) and PET-CT do not add any extra diagnostic accuracy for determining operability.[285] Small hepatic metastases and peritoneal deposits are likely to remain undetected prior to laparotomy. Endoscopic ultrasonography (EUS) is used to characterise suspected pancreatic tumours and can detect smaller pancreatic mass lesions of only 2-3mm diameter.[496] Magnetic resonance imaging (MRI) gives similar results to a non-enhanced CT scan though can be useful for characterising cystic lesions and for patients allergic to intravenous contrast. Prior to surgical resection, laparoscopy and laparoscopic ultrasound may be offered to aid the accuracy of disease staging, particularly in patients who present a major operative risk.

Tumour markers can aid the diagnosis and management of patients with pancreatic cancer. Serum carbohydrate antigen (CA) 19-9 is clinically most useful for monitoring a patient's response to treatment although it is also the most widely used and validated tumour marker for pancreatic cancer, although it has its limitations in early tumours. The chosen cut-points for serum levels of CA 19-9 determine the accuracy of the test which was demonstrated when comparing levels in patients with and without pancreatic cancer. Using a threshold of 37kU/l, CA 19-9 has a sensitivity of 81% and a specificity of 90% for pancreatic cancer but by increasing the cut off to 100kU/l the specificity improves to 98%, but sensitivity drops to 68%.[497] Several benign diseases elevate CA 19-9 including acute and chronic pancreatitis, liver cirrhosis, cholangitis and obstructive jaundice. Serum CA 19-9 is also elevated in 67% of patients with cholangiocarcinoma, 41% of patients with gastric cancer, 34% with colon cancer and 49% of those with hepatocellular cancer.[498] Serum CA 19-9 should be used with caution in the initial investigation of patients with pancreatic cancer, when a significantly elevated level may increase the suspicion for disease, though a normal value should not prevent

further investigations.[497] Serum CA 19-9 has a role in estimating prognosis following surgical resection and in monitoring patients receiving chemotherapy. Future work aims to establish more accurate markers of pancreatic cancer, which is currently being investigated using genomic analysis to identify proteins that are over-expressed in disease.[295] If autoimmune pancreatitis is in the potential differential diagnosis, immunoglobulin G class subtypes should be measured to assess for elevated IgG4 levels.

Patients with characteristic features of pancreatic cancer do not require a histological specimen prior to operation,[499] although a diagnostic biopsy should be sought in patients with uncharacteristic lesions prior to surgery and in those referred for chemotherapy. Obtaining a histological sample can be difficult due to the anatomical location of the pancreas. If an endoscopic retrograde cholangiopancreatography (ERCP) is performed to gain biliary drainage, then biliary brushings can be taken for cytology. EUS and fine needle aspiration (FNA) can be used to acquire tissue with a diagnostic accuracy of >90% sensitivity and ~100% specificity.[285] FNA is the procedure of choice to gain histology in advanced pancreatic cancer or to diagnose small uncharacterised lesions. If liver metastases are present, percutaneous biopsy is used to obtain a tissue sample under USS or CT guidance. Confirmatory histology is required in planning chemotherapy and characterising prognosis and occasionally alternative diagnoses are made including lymphoma or autoimmune pancreatitis which have different treatments and clinical outcomes.

2. Treatments

Resectable disease

Prior to deciding treatment, accurate staging of the cancer is essential and the management requires a multidisciplinary approach. A tumour is unlikely to be resectable if it is >5cm diameter or if there is involvement of the superior mesenteric artery or celiac axis.[500] Post-operative short and long-term survival results are improved in high-volume surgical centres.[285] For tumours of the pancreatic head, a pylorus preserving pancreaticoduodenectomy is appropriate where a resection is made of the first and second part of the duodenum; the head of the pancreas; the common bile duct; and the gallbladder (Figure 17). The main advantage of this operation is that the pylorus, and thus normal gastric emptying, is preserved. If there is duodenal or gastric involvement then a proximal pancreaticoduodenectomy with antrectomy is required (classical Whipple)

Figure 18). For tumours of the pancreatic body and tail tumours a distal (left) pancreatectomy is performed. In patients found to have unresectable disease at surgery, a surgical bypass (hepaticojejunostomy and/or gastroenterostomy) can be fashioned to prevent future biliary tract or duodenal obstruction.

The survival following surgery can be improved with the use of adjuvant chemotherapy. The results from two large randomised controlled trials reported that adjuvant systemic chemotherapy with either 5-fluorouracil (5-FU) and folic acid (ESPAC-1 trial)[501] or Gemcitabine (CONKO-001 trial)[502] improved median disease-free survival from 6.9 months in the control group to 13.4 months in the gemcitabine arm ($p<0.001$).[502] The ESPAC-3 trial was the largest adjuvant trial ever conducted for pancreatic ductal adenocarcinoma and reported no significant difference in survival between adjuvant 5-FU+folic acid vs Gemcitabine.[503] The ESPAC-4 trial is currently recruiting, and randomizing patients to either adjuvant Gemcitabine or combination Gemcitabine and Capecitabine (GemCap).

Figure 17. Pylorus preserving pancreaticoduodenectomy. (Source *Cancer Help UK http://cancerhelp.cancerresearchuk.org/type/pancreatic-cancer/treatment/surgery/surgery-to-try-to-cure-pancreatic-cancer#whipple*)

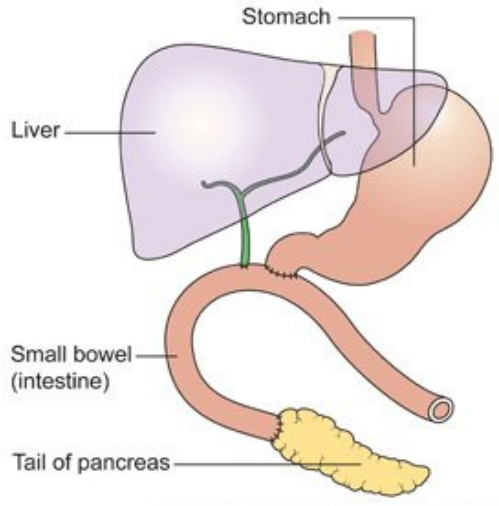
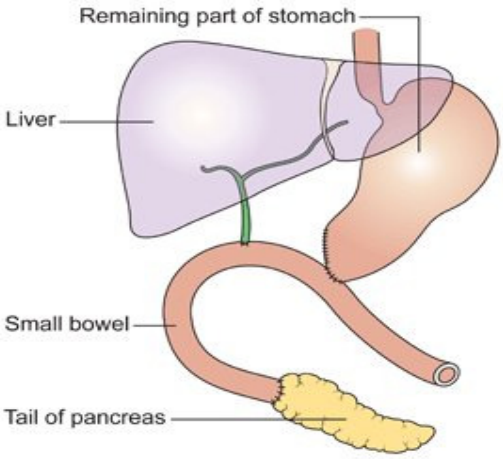


Figure 18. A proximal pancreaticoduodenectomy with antrectomy (classical Whipple). (Source *Cancer Help UK*)



Management of Inoperable disease

Chemotherapy and radiotherapy only make a minor impact on survival and the quality of life in patients with inoperable pancreatic cancer. Gemcitabine is given to those with locally advanced or metastatic pancreatic cancer as randomised controlled clinical trials demonstrated superiority over 5-fluorouracil (5FU), with a small benefit in median survival (5.7 vs 4.4 months, p=0.0025) and an improved 1 year survival (18% vs 2%, p=0.019) with improvement in disease-related symptoms.[504] Despite several trials of gemcitabine combination regimens, only a small survival benefit has been reported by the addition of capecitabine.[505] Similarly, the incorporation of biological agents, such as Erlotinib, has also been disappointing. Erlotinib is a tyrosine kinase inhibitor that acts on the epidermal growth factor receptor, showing a survival improvement from 5.9 to 6.4 months (p=0.025).[506] The recently published FOLFIRINOX study from France, randomised 342 patients with metastatic pancreatic cancer to either gemcitabine or a combination of oxaliplatin, irintecan, leucovorin and fluorouracil (FOLFININOX) and reported the new regime improved median survival from 6.8 months to 11.1 months (p<0.001).[507] FOLFIRINOX was associated with more adverse events although quality of life scores at 6 months were improved. The TeloVac trial is currently recruiting patients with locally advanced and metastatic disease to compare standard chemotherapy with a chemo-immunological agent (GV1001 telomerase vaccine). Telomeres are found at the end of a chromosome and are regions of repetitive DNA which protect the chromosome from deterioration. Cancer cells undergo frequent division leading to shortened telomeres, but if they become too short, the cell may die. Some cancer, including pancreatic, escape this fatal process by up-regulating an enzyme called telomerase, which adds telomeric DNA to critically shortened chromosomes and ensuring continued cell survival. Experimentally, the GV1001 vaccine targets the over-expressed telomerase, enabling the immune response to recognise the enzyme and illicit an immune response against telomerase which prevents enzyme function and facilitates cancer cell death.[508]

For non-metastatic inoperable pancreatic cancer, chemoradiation is the standard treatment in the USA, a policy based upon the results of the Gastro-Intestinal Study Group trial. This reported an improved median survival for the combined-modality therapy group (radiation combined with 5-fluorouracil followed by streptozocin, mitomycin, and 5-fluorouraci) compared with chemotherapy (streptozocin, mitomycin, and 5-fluorouracil alone) of 42 weeks vs 32 weeks survival (p<0.005).[509] However, initial trials of gemcitabine and radiation

were terminated due to excess toxicity, but studies using lower radiotherapy doses demonstrated were better tolerated. Chemoradiation is of particular benefit in patients who after 3 months of induction chemotherapy are without disease progression and have a good performance status. Here the subsequent introduction of chemoradiotherapy improved median overall survival from 11.7 months to 15.0 months (p=0.0009).[510] The use of radiation with either concurrent gemcitabine or capecitabine following 'induction' GemCap chemotherapy is currently being investigated in the SCALOP trial, for inoperable tumours ≤6cm diameter. The rationale is to select patients with chemo-responsive disease from those with a more rapidly progressive clinical course. Significant clinical improvements in long-term survival will probably only be achieved through continuing investigation of the pathophysiological processes of this cancer.

Palliative treatments

In many patients, palliative treatments are the principle focus, with pain the most important symptom to manage. The severity of pain in pancreatic cancer is related to the tumour size, the presence of lymph node metastases and tumour invasion of either the anterior pancreatic capsule, intrapancreatic, or coeliac plexus nerves. Increased pain correlates with a reduced median survival (9 months with severe pain vs 29 months if without pain).[511] Treatment requires the early introduction of high-dose opioid analgesia with both long acting preparations and short acting ones for breakthrough symptoms. Postprandial epigastric pain can be due to pancreatic enzyme insufficiency which may respond to pancreatic enzyme replacement therapy (PERT). Pain control with neurolytic coeliac plexus block should be reserved for patients failing to benefit from opioid analgesia. Previous trial data established that coeliac plexus block improved pain control, though not the patients' quality of life.[512] Patients and their relatives should be reassured that increasing pain will be managed promptly to reduce the associated fear and stress that might otherwise occur.

Obstructive jaundice is a common presenting symptom of pancreatic cancer and usually represents a tumour in the head of the pancreas. A survey of 381 patients with pancreatic cancer reported that 48% of cases presented with jaundice, with most associated with pain (34%) rather than painless jaundice (13%).[513] Pruritis often complicates obstructive jaundice and is due to excess bile acids in the blood and skin [514] or elevated endogenous opioids.[515] Achieving biliary drainage and consequent resolution of the jaundice should improve patients' symptoms. If this cannot be achieved quickly topical treatment with aqueous cream or

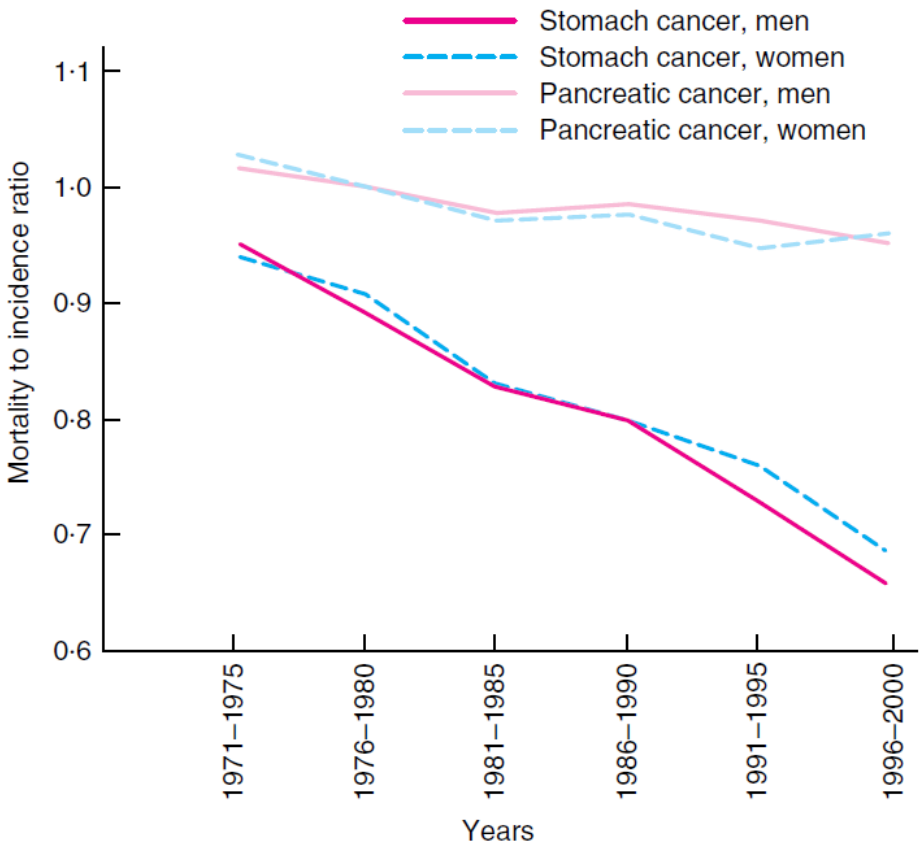
emulsifying ointment should be administered. Medical therapies to be considered are anti-histamines and ondansetron (a 5-HT3 antagonist). If ondansetron is effective, it can be switched to a selective serotonin reuptake inhibitor (such as paroxetine), a cheaper long-term alternative, which also has antagonist effects on the 5-HT3 receptor.[516]

Biliary drainage is usually achieved using endoscopic retrograde cholangiopancreatography (ERCP) with stenting. Plastic stents usually obstruct after 3 months and hence should only be used in patients with a poor prognosis or undergoing surgery. Metal and covered stents, which are considerably more expensive than plastic stents, are used in patients where survival is expected to exceed 3 months.[285] Duodenal obstruction occurs in around 15% of patients and can be treated with an expandable duodenal stent, placed either endoscopically or under radiological guidance. The complications of stents are perforation, bleeding and recurrent obstruction.[517] In patients medically fit for surgery, biliary obstruction can be treated by performing a Roux-en-Y loop hepatojejunostomy, and duodenal obstruction can be managed with a gastrojejunostomy.

Prognosis

Pancreatic cancer has a poor prognosis with only 16% of patients surviving beyond 1 year and only 0.2% - 3% beyond 5 years.[274-275] The late presentation of this aggressive tumour accounts for the low number of patients who are suitable for potentially curative surgery (10-15%).[285] The mortality to incidence ratio for pancreatic cancer did not improve significantly between the years 1971-2000, whilst stomach cancer mortality dropped significantly (Figure 19).[518] Following radical resection, the 5-year survival from pancreatic cancer is only 10% with most patients developing metastatic disease.[519] Of those patients receiving chemotherapy (30-40%) the median survival time is 6-7 months [500, 506] and for patients not fit for chemotherapy (40-50%) it is lower at 3-4 months.[520] These poor survival times for all stages of the disease emphasize the need for early appropriate supportive and palliative therapy in patients to prevent a deteriorating quality of life. Certain histological variants of ductal adenocarcinoma (10% of all exocrine tumours) have a worse prognosis than typical ductal adenocarcinoma. However, other exocrine tumours carry a more favourable prognosis, including mucinous and serous cystadenocarcinoma.

Figure 19. Mortality to incidence ratio for pancreatic and stomach cancer in men and women, 1971-2000 (source; Fitzsimmons *et al, Br J Surg 2007*[518]).



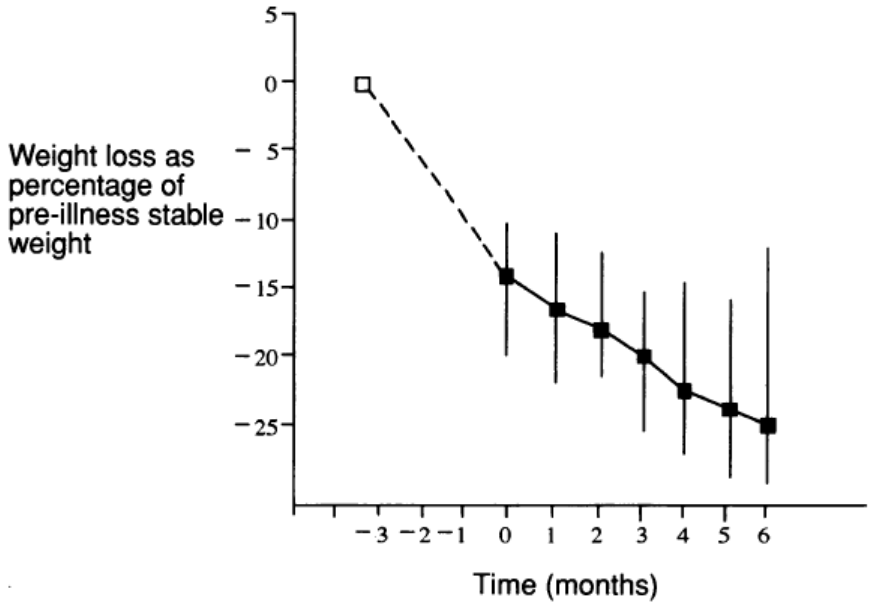
3. Nutritional aspects in patients with pancreatic cancer

Weight loss

Weight loss is a common symptom in patients, but often receives less attention than the consideration of chemotherapy and relief of jaundice. Weight loss is due to a poor nutritional intake, altered metabolism and malabsorption which lead to cachexia and a deteriorating quality of life. A nutritional assessment and provision of dietary supplementation may not always be a clinical priority, although it is beneficial in terms of decreasing morbidity and mortality. Disease progression in pancreatic cancer leads to severe anorexia and weight loss, with up to 90% of patients having significant weight loss at the time of diagnosis.[491-492] Patients have lost an average of 15% of their pre-illness weight at the time of diagnosis and up to 25% 6 months after diagnosis (Figure 20).[491]

Weight loss in pancreatic cancer is not only due to a reduced calorie intake but also alterations in metabolism. Patients are hypermetabolic, with a raised resting energy expenditure (REE) [521-522]. Although the metabolic mechanism of weight loss is not fully understood, it is thought to be induced by pro-inflammatory cytokines (such as interleukin (IL)-1beta, IL-6, IL-8 and tumour necrosis factor-alpha) derived from the tumour.[523] The host's systemic inflammatory response leads to a reprioritisation of protein metabolism with breakdown of this macronutrient.[524] Activation of neuroendocrine stress hormones and tumour specific factors contribute to hypermetabolism. The rise in REE accelerates weight loss and muscle wasting, leading to reduced physical activity.

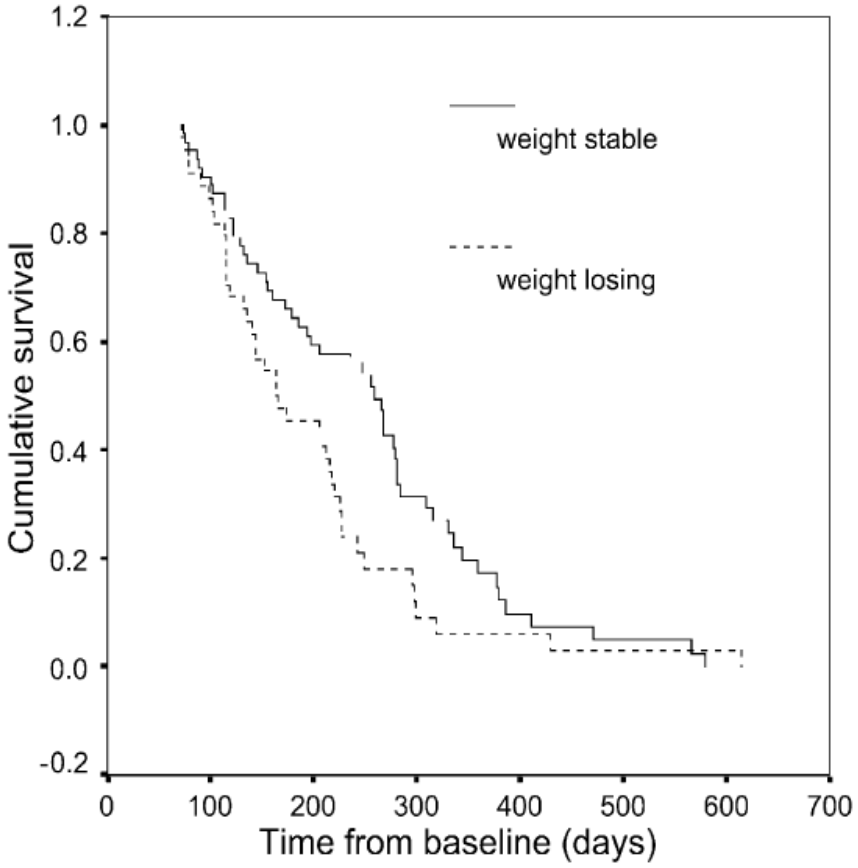
Figure 20. Weight loss as a percentage of pre-illness stable weight in 20 patients with unresectable pancreatic cancer (source; Wigmore SJ *et al Br J of Cancer 1997*[491]).



The percentage weight loss between onset of weight loss and the time of diagnosis (time 0 months) is indicated by the broken line.

The clinical significance of weight loss is its impact on survival, tolerance of chemotherapy and quality of life. In patients with cancer of any type, weight loss is an independent predictor of survival.[525] Achieving weight stabilisation following the diagnosis of pancreatic cancer is associated with an improved median survival which was demonstrated in a study of 109 patients with unresectable pancreatic cancer, who all received oral nutritional supplementation. Eight weeks after diagnosis, patients who were weight stable (<1kg weight loss) had improved survival times compared to those who had lost weight (>1kg) (median survival of 259 days vs 164 days, p=0.019) (Figure 21).[526] Weight stabilisation was also associated with improved quality of life scores (global quality of life scores at 8 weeks of 55 vs 47, p=0.037). To assess the impact of weight loss on chemotherapy dosing, The Royal Marsden Hospital recorded the presence or absence of weight loss in 1 555 patients with advanced oesophageal (179 patients), gastric (433), colorectal (781) or pancreatic (162) cancer.[527] In patients receiving chemotherapy who had weight loss at presentation, the average duration of therapy was 30 days less (120.3 days vs 150.5 days, p=<0.0001). The authors equated this to an average reduction of 18% in chemotherapy received. There were also increased complications in patients with weight loss undergoing chemotherapy with higher rates of stomatitis and plantar-palmar syndrome with 7% of patients with weight loss vs 1% without weight loss experiencing increased grade 3-4 plantar-palmar syndrome (p<0.0001) (Table 47).[527] The plantar-palmar or hand foot syndrome is characterised by red and tender palms and soles of the feet which look and feel like sunburn (Figure 22). Although these results are not solely applicable to patients with pancreatic cancer, they emphasize the potential role weight loss may have influencing survival, treatment schedules and side-effects of treatment.

Figure 21. Comparison of survival from baseline for both weight losing (n=44) and weight stable (n=63) pancreatic cancer patients (source; Davidson W *et al, Clin Nutr 2004*[526]).



Kaplan-Meier log rank statistic 5.53, p=0.019

Figure 22. Plantar-palmar syndrome induced by chemotherapy (source; http://www.flickr.com/photos/sketchesbymez/2681808756/).

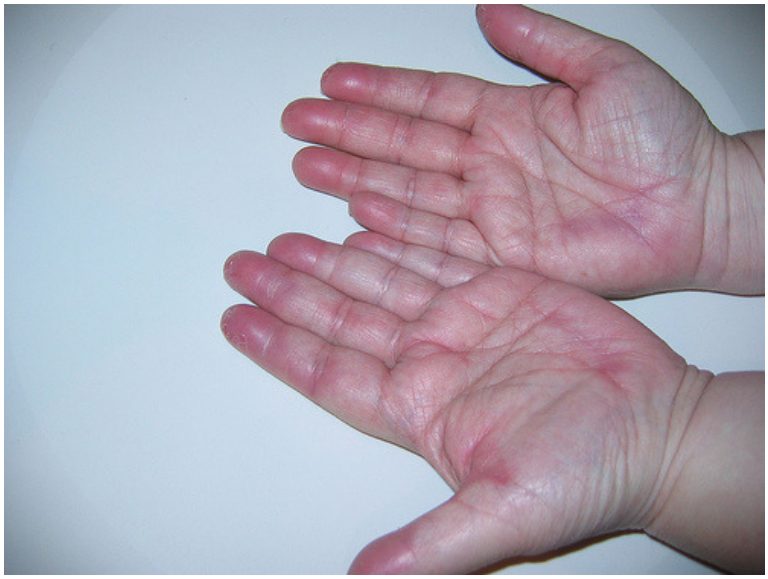


Table 47. Plantar-palmar syndrome induced by chemotherapy and its relationship to weight loss at presentation in different tumour types (source; Andreyev HJ *et al, Eur J Cancer 1998*[527]).

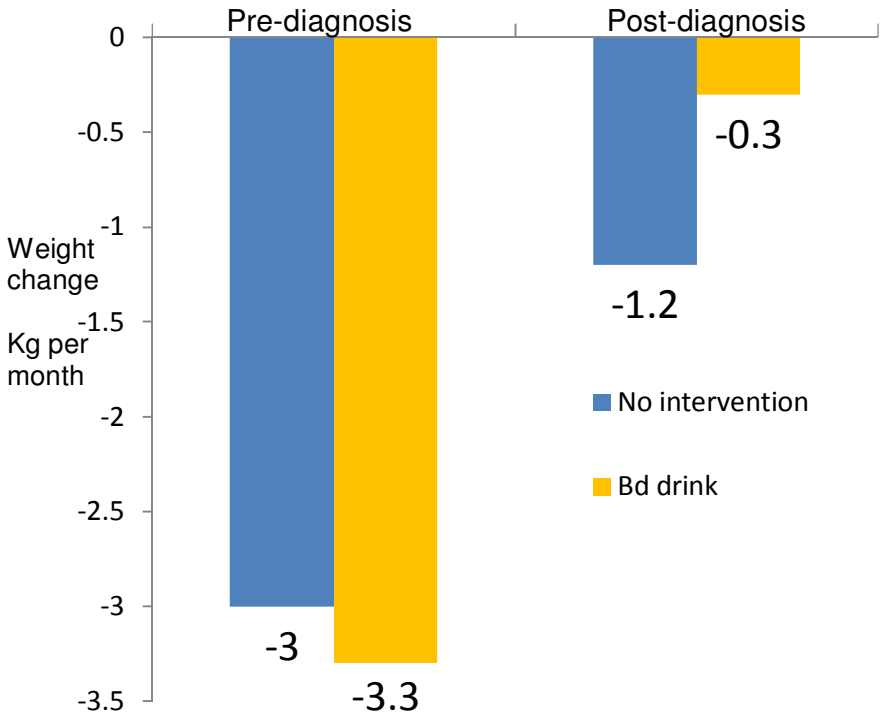
	No weight loss	Weight loss	No weight loss	Weight loss	No weight loss	Weight loss	Any toxicity
Grade of toxicity	0	0	1–2	1–2	3–4	3–4	
Oesophageal	65	65	27	33	5	2	$P=0.82$
Gastric	69	53	26	41	1	3	$P=0.0007$
Pancreatic	71	52	22	39	1	7	$P=0.0001$
Colorectal	52	46	42	45	2	8	$P=0.065$

Oral nutritional supplementation

An adequate calorie intake is required to slow or arrest the weight loss in pancreatic cancer. The British Society of Gastroenterology (BSG)528 and the American Gastroenterogical Association (AGA)529 currently advise that dietary supplementation should be considered in patients with pancreatic cancer, although the specific dosing and timing is not given. The BSG states “attention to dietary intake, and the use of specific nutritional supplements, may improve well being”. No randomised trials have specifically assessed whether this approach is beneficial in patients with pancreatic cancer probably due to logistical and ethical constraints. However, a follow-up study of 200 patients with unresectable pancreatic cancer, who had lost at least 5% of body weight, were all prescribed 2 cans of oral nutritional supplementation per day (one can=237ml, 310kcal, 16g protein, 6g fat) and then randomised patients “to receive” or “not to receive” an additional n-3 polyunsaturated fatty acid supplement of 1.1 grams of eicosapentaenoic acid (EPA) plus antioxidants in each carton. The rate of weight change in both groups prior to diagnosis was -3.3kg/month which slowed to an average of -0.3kg/month in both groups after 8 weeks of oral supplemental feeding.530 This compared to an observational study of 20 patients with unresectable pancreatic cancer who did not receive routine oral supplementary feeds. Here patients lost 3kg/month prior to diagnosis and continued to lose weight at a rate of 1.2kg/month following diagnosis491 suggesting the rate of weight loss post-diagnosis is reduced in patients receiving oral supplemental feeds compared to patients receiving standard care (

Figure 23). Although there are limitations in comparing two different studies, the reduced rate of weight loss those receiving oral supplementary feeds supports their use in attenuating weight loss. In participants randomised to receive EPA and antioxidant supplements, who remained fully compliant, gained lean body mass and recorded an improved quality of life suggesting there may be a role for supplemental n-3 fatty acids in patients. The n-3 fatty acids, eicosapentaenoic acid is derived from fish oils, has been shown experimentally to have anti-inflammatory and anti-tumour properties. The n-3 fatty acids EPA and DHA have inhibitory effects on the growth of human pancreatic cancer cell lines *in vitro,*[412, 414, 417] and induce apoptosis in a dose-dependent manner.[419] N-3 fatty acids inhibit the activation of NF-kB in cancer cells lines. NF-kB is associated with resistance to gemcitabine due to the production of anti-apoptotic proteins.[531] The inhibition of NK-kB facilitates apoptosis and may have the ability to improve gemcitabine sensitivity. Overall, prescribing oral nutritional supplements, based on the limitations of the current evidence available, appears to be appropriate in patients with unresectable pancreatic cancer.

Figure 23. The rate of weight loss in patients with pancreatic cancer, pre and post-diagnosis, receiving either no dietary intervention or a twice daily oral nutritional feed of 237ml (1.5kcal/ml) (sources; Wigmore SJ *et al, Br J Cancer1997* [486] and Fearon KC *et al, Gut 2003*[491, 530]).



Pancreatic enzyme replacement therapy

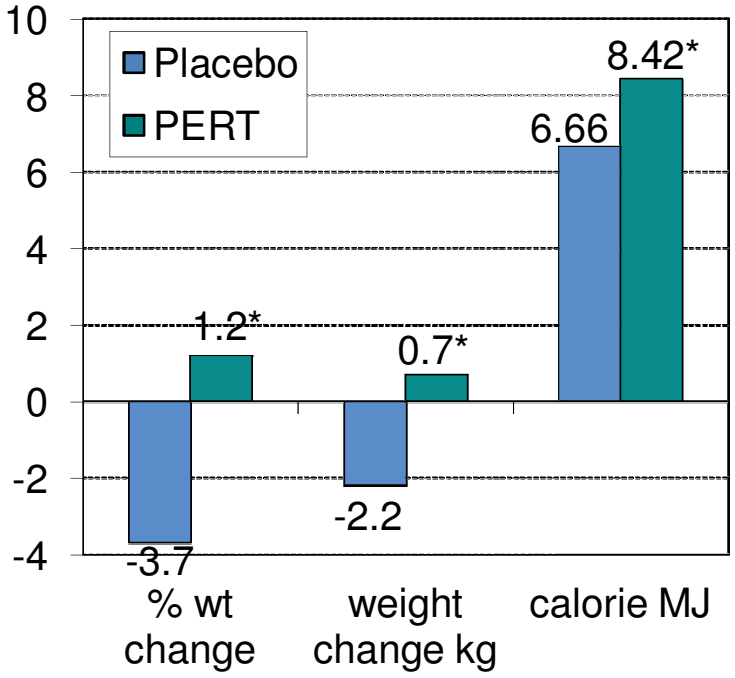
Pancreatic enzyme replacement therapy (PERT) is licensed for use in patients with evidence of pancreatic exocrine deficiency, although this can be difficult to diagnose. Once clinical signs of steatorrhoea and weight loss have developed, approximately 90% of exocrine function has been lost.[532] The gold standard for assessing pancreatic exocrine function is the cholecystokinin (CCK)/secretin pancreatic function test, although this is invasive, time consuming, costly and only available in specialised centres. In-direct (tubeless) tests of pancreatic function, such as faecal elastase-1, are useful in diagnosing severe exocrine deficiency.[533-534] However, these have limited sensitivity and specificity in diagnosing mild to moderate disease. Hence there is no non-invasive test available which reliably diagnoses mild exocrine deficiency. Once clinical symptoms are evident, the patient is likely to have severe exocrine deficiency and have already lost weight.

Patients with pancreatic cancer often have exocrine deficiency, with a study of 25 patients without overt clinical signs of malabsorption, reporting biochemical evidence of exocrine deficiency in 80% of patients.[535] The BSG and AGA both recommend the use of PERT in patients with pancreatic cancer with the BSG stating that "compared with untreated patients, patients with advanced pancreatic cancer who are given pancreatic enzyme supplements enjoy a better quality of life and improved symptoms score". This recommendation was based on two clinical investigations which evaluated the effects of PERT on patients with pancreatic cancer.[536-537] The first assessed the biochemical effects PERT therapy in 12 patients with biopsy proven advanced pancreatic cancer. The study found that patients with significant fat malabsorption (6 of 12 patients with coefficient of absorption <85%) benefitted from the introduction of PERT, with the average coefficients of fat absorption improving by over 20%, although those with mild malabsorption (coefficient of absorption >85%) did not benefit.[536] The second study was a randomised controlled trial of PERT in patients with unresectable pancreatic cancer, who had recently undergone biliary stent insertion to relieve obstructive jaundice. The 21 patients were randomised to receive either PERT (50 000 lipase units with meals, 25 000 lipase units with snacks) or placebo. Those receiving PERT had a significant improvement in the percentage body weight change (placebo= -3.7% vs +1.2%, p=0.02) and calorie intake (placebo=6.66MJ vs 8.42MJ, p=0.04) compared to those in the placebo group (Figure 24).[537] Neither study included patients undergoing chemotherapy, although they may gain

particular benefit by maintaining an anabolic state and a reduction in complications. Some medical oncologists already advocate the routine prescription of PERT to all patients at the time of diagnosis of pancreatic cancer to prevent the development or progression of weight loss.[538]

The current but limited evidence supports the use of pancreatic enzyme replacement and nutritional supplements in patients with pancreatic cancer, although it is unclear which groups would benefit as well as the timing and dosing of prescription. More work is needed to investigate whether the routine nutritional assessment of patients and potential early prescription of PERT leads to an improvement in outcomes. Such investigations should include an assessment of the doses, frequency of prescription and their impact on survival as well as clinical parameters such as weight change and side-effects from chemotherapy.

Figure 24. A randomised controlled trial investigating the effect of pancreatic enzyme replacement therapy on weight change in patients with pancreatic cancer (source; Bruno MJ *et al, GUT 2005*[537]).



*significant difference, p<0.05

4. Depression

Depression is a common symptom in patients with pancreatic cancer, caused by both biological mechanisms and the psychological consequences of the diagnosis. This review will discuss the frequency and impact of depression and whether it can be managed if identified and treated early.

Prevalence

Depression is commoner in patients with pancreatic cancer than other malignancies, with a prevalence of between 33%-50%.[539-540] The relationship with depression was originally described in the 1930's by Yaskin [541] who reported the symptoms of depression, anxiety and feelings of premonition of serious illness as the earliest manifestations of pancreatic cancer. In the 1960's, Fras et al formalised this work by evaluating patients prior to the diagnosis of different cancers.[542] They found that patients diagnosed with pancreatic cancer were more likely to have experienced psychiatric symptoms, especially depression, than patients diagnosed with colon cancer (76% vs 20%, p value not calculated). Furthermore, nearly half experienced psychiatric symptoms as their earliest manifestation of disease. A US study evaluated the prevalence of depression in 130 patients with newly diagnosed pancreatic cancer. Using the Beck depression inventory to screen for depression they reported 38% had a score (≥15) that suggests high levels of depressive symptoms.[543] The largest study to evaluate the presence of depression in gastrointestinal cancer patients was conducted in 262 Chinese inpatients, which reported depression was higher in pancreatic cancer than in other cancers; 78% of pancreatic cancer patients; 60% of hepatocellular carcinoma patients; 36% of gastric patients; 24% gastric patients; 19% of colon patients.[544] However, to assess the presence of depression, they solely used the Hamilton Rating Scale for Depression-24 (HAMD-24) questionnaire which was developed to screen for depression symptoms rather than to diagnose depression.[545] Furthermore, the HAMD-24 creates a score from 24 stem questions which places participants in one of four categories; severe depression (score >35), mild to moderate depression (score >20), suspected depression (score 8-20) and no depression (<8). In the Chinese study, any participant with suspected depression (score>8) was classified as having cancer-related depression when normally a clinical assessment would be required to confirm the diagnosis. The HAMD-24 has four stem questions which assesses symptoms that are common in pancreatic cancer, with two questions

concerning weight loss and one each on fatigue and loss of appetite. Therefore, it is not surprising that the authors reported a high rate of depressive symptoms in such patients. The prevalence of depression in pancreatic cancer has been evaluated in several studies though the diversity of findings can be attributed to the low sample size, the definition depressive symptoms, and varied demographics. [539-540]

Biological mechanism

Pancreatic cancer may lead to depressive symptoms either due to the psychological aspects of the diagnosis, or via biochemical mechanisms including immunological, hormonal, paraneoplastic and biochemical changes.[546] The immunological effects can be mediated by proteins released from tumours stimulating the production of antibodies that block serotonin receptors, which can induce depressive symptoms.[547] Solid tumour malignancies can be associated with serotonin depletion induced via increased urinary excretion of this hormon.[548]. Biochemical changes of cancer including anaemia, hypercalcaemia, and acid-base abnormalities, as well as the production of biogenic amines or neuropeptides can also alter the psychological state.[540]

Diagnosis of depression and screening tools

Although depression in pancreatic cancer is a relatively common, the first challenge is to recognise the condition, which can be complicated in such patients as depressive symptoms, such as anorexia and weight loss, are also symptoms of the cancer. The reliability of any data on the incidence of depression is dependent on the appropriate choice of assessment tool in defining depression, and the varied rates, as discussed in the previous section, are likely to relate to the different methods of diagnosis. The most robust definition of depression is taken from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and requires a structured clinical interview by skilled trained staff.[549] The advantage to structured clinical interviews are the robustness of the definition of depression, although they require considerable resources and therefore are usually used to confirm the diagnosis of depression rather than as a screening tool.[550] Self-report screening tools are useful in routine practice and can identify significant functional impairment during follow up.[551] Therefore, it is recommended that self-reported screening tools are used as a first step to identify depression followed by a more

detailed structured interview, if required, to confirm the diagnosis.[552] One such screening tool is the Hospital Anxiety and Depression Scale (HADS), used to identify patients who may benefit from clinical assessment of their mood and which has been validated in patients with terminal cancer.[553] This focuses on the loss of pleasure response (anhedonia) rather than the somatic symptoms that may be found in both cancer and depression (i.e. anorexia, weight loss). The loss of the pleasure response is considered to be a disturbance of neurotransmitter function and more responsive to antidepressant medication.[554]

Impact

Depression does not affect survival in pancreatic cancer [555] but has a significant impact upon morbidity. There is worsened pain, anorexia and anxiety which compound weight loss with an associated low quality of life scores.[544] Depression in the terminally ill should not be considered normal, as articulated by the quote "when depression is considered a normal phenomenon in cancer patients, its impact on the quality of life is trivialised".[546] The early recognition and treatment of depression can lead to an improvement in function and a sense of well being.[549]

Treatment of depression

Once depression is diagnosed in patients with pancreatic cancer, organic causes of depressive symptoms should be excluded and if found treated appropriately. These include metabolic abnormalities (i.e., hypercalcaemia), anaemia, nutritional deficiencies (i.e., iron or vitamin B12 deficiency) and drug side-effects particularly chemotherapy induced depression.[556] If no reversible causes of depression are found the patient should be considered for counselling and support, which can be accessed via palliative support teams. Pharmacological therapy can also be offered with the choice of medication dependent of the side effect profile, with the literature suggesting tricyclic antidepressants are commonly used,[546] although local expertise has found mirtazapine to be useful as its side-effect profile includes appetite stimulation and weight gain which could have obvious benefits.[557] A systematic review in 2006 found limited evidence to guide the choice management of depression in cancer patients, largely due to a lack of adequately powered studies of pharmacological or psychotherapeutic interventions

targeted at cancer patients with depression.[558] The lack of studies was addressed by a Scottish investigation in 2008, conducted in 200 outpatients diagnosed with both depression and cancer who were randomised to either standard care or to a combination of pharmacological and psychotherapy delivered by a cancer nurse over an average of seven sessions. This reported that the intervention improved depression scores after a period of three months ($p<0.05$) although the findings may not be generalizable to all cancer patients, including pancreatic cancer patients, as those with a prognosis of less than 6months were excluded.[559]

5. Aims of this research

Nutritional depletion and depression are common in patients with pancreatic cancer, although the prevalence in many UK hospitals, including ours has not been previously assessed. Additionally there is little work on the most appropriate clinical service for identifying these factors in clinical practice so that treatment could be instituted. Furthermore, such a service needs to be assessed for its potential effects on survival and clinical outcomes. The aim of this clinical survey was to have a dedicated clinician in place to screen for these conditions, describe their prevalence and initiate any relevant treatments. Preliminary data on its potential value could be assessed by measuring survival and clinical outcomes in patients before and after the commencement of this service.

The primary outcomes chosen to evaluate the effectiveness of PASS were comparison of survival rates as well as the number of chemotherapy doses received since this reflects the physical well being of the patient.[527] The secondary outcomes were hospital admission rates, weight change over time, use of oral supplement and pancreatic enzyme replacement therapy and finally evidence of psychological assessment and treatment. Hospital admission rates are of importance to both the patient and health care provider as reduced hospital admission rates reflect a reduction in complications of disease and lead to reduction in total costs of providing care. Weight change over time was assessed as weight loss is associated with complications and a deteriorating quality of life.[527] The use of oral supplements and pancreatic enzyme supplements were compared to assess if the introduction of PASS lead to an increased use of these therapies. Evidence of psychological assessment and treatment was made to verify whether the routine screening for symptoms lead to an increase in treatment. Identification of any benefits would suggest that a pancreatic support service (PASS) should be assessed in larger studies and considered in the routine management of patients with pancreatic cancer.

Methodology

1. Overview of the study design

This study compared patients receiving one or more doses of palliative chemotherapy for suspected exocrine pancreatic cancer in 2009 against patients treated in 2010, who in addition to their standard care, were also seen by PASS. Patient baseline data was collected on age at diagnosis, initial staging of pancreatic cancer and the type of chemotherapy treatment given. The data collected for the primary outcomes were survival rates and the number of chemotherapy doses received. The secondary outcomes were the number of hospital admissions in patients who died within 12 months, weight change, nutritional and depression assessment and treatment. Data was collected in the prospective survey on depression screening scores and the frequency of depression treatment.

Study protocol

Patients receiving chemotherapy for pancreatic exocrine cancer at the Norfolk & Norwich University Hospital NHS Trust during 2009 had data collected retrospectively concerning their management and clinical outcomes. These outcomes were then compared against those collected prospectively in patients treated in 2010, who in addition to their standard care, were also seen a PAncreatic Support Service (PASS). The clinician in PASS assessed nutritional status, medical and psychological symptoms and implemented appropriate treatments if required. The ideal study design to evaluate the effect of such a new clinical service would be a randomised controlled trial. However, randomising patients to receive or not receive this clinical support service was deemed to be unethical as the management was already stipulated by national societies of gastroenterology in the UK and US.[528-529] Consequently, in a pragmatic approach, we surveyed and compared outcomes in patients treated prior to and after the implementation of PASS. Demonstrating a beneficial outcome of PASS would support its wider use.

This cross-sectional survey documented the nutritional status and screen for depression and in the comparative analysis provide a crude assessment of the value of PASS. Patients treated with chemotherapy were chosen to assess the efficacy of PASS as they could be reviewed during their chemotherapy appointments and hence not require additional hospital attendances. Also, as they

were fit enough to undergo chemotherapy, they were likely to have reasonable survival times where a potential benefit of PASS may be demonstrated. Those receiving palliative measures were judged to have very short survival times where nutritional and psychological intervention may be inappropriate. Patients undergoing chemotherapy were either treated with gemcitabine monotherapy, gemcitabine and capecitabine, or entered into the Telovac trial. This randomised patients to either

a) gemcitabine & capecitabine over a total of 8 weeks;

b) gemcitabine and capecitabine together, over a period of 8 weeks and then a course of Telovac injections;

c) Gemcitabine and capecitabine (as A & B) but start Telovac in first week.

All patients receiving chemotherapy were aiming to receive at least eight doses of gemcitabine, regardless of which treatment group they were in, with those on gemcitabine monotherapy expected to receive three weekly doses of gemcitabine (one cycle of treatment), a week off, and then repeated three weekly doses for six cycles. However, it was expected that most patients would stop therapy before this although occasionally some patients are considered for longer courses. There were no age limitations for patients in the survey although patients undergoing adjuvant chemotherapy were not included in the study and as well as those with pancreatic endocrine cancer.

Retrospective survey

The retrospective survey was of patients diagnosed with in-operable pancreatic cancer who received chemotherapy at The Norfolk & Norwich University Hospital NHS Trust during the year 2009. Potential patients were identified from the pancreatic multi-disciplinary meeting records of that year. The oncology computer database and medical records of each patient who had received chemotherapy in 2009 were reviewed using a data collection sheet (appendix 1).

Prospective survey

All new patients with pancreatic cancer receiving palliative chemotherapy during the year 2010 were identified at the pancreatic multi-disciplinary meeting. The PASS reviews were undertaken by either a pancreatic cancer nurse specialist or specialist registrar in gastroenterology, Dr Paul Banim. The initial PASS review

aimed to be completed during the first or second visit for chemotherapy, with subsequent monitoring at 4 weekly intervals during attendance for chemotherapy. At the initial meeting, details of the PASS service were discussed with the patient, who was also give an information leaflet on PASS (appendix 2). At each review an assessment of physical symptoms was performed (appendix 3). If new clinical problems or diagnoses were identified, appropriate management plans were instigated. More specifically, to assess a patient's nutritional status, they were weighed and questioned on appetite, dietary intake and malabsorption symptoms. Following the protocol listed below, if nutritional deficiencies were identified, advice was given, referral to a dietician was made and when appropriate, oral nutritional supplementation or pancreatic enzyme replacement therapy prescribed. An additional leaflet was given to patients commenced on PERT with advice on dosing (appendix 4). To assess for psychological symptoms the patients were given a HAD Scale (appendix 5) to complete in their own time at home and return at their next hospital visit.

The protocol followed at each PASS review was as follows;

i. A record was made of medical symptoms including weight, abdominal pain, steatorrhoea, nausea, vomiting and jaundice. If symptoms were present, appropriate therapy was implemented via the general practitioner or secondary care specialist.

ii. PERT was considered if was history of weight loss of >2% body weight or 1kg. Creon 25 000 units to 40 000 units was prescribed, two with each meal and one with snacks. The dose was increased further if clinically indicated.

iii. If there was ongoing weight loss or poor appetite, nutritional advice was given and oral nutritional supplementation and dietetic review considered. The oral nutritional supplement supplied was Ensure Plus twice daily with meals. This is a 220ml milk or yogurt style drink which has a high nutritional content containing protein 6.3g, fat 4.9g, carbohydrate 20.2g, vitamins, minerals and trace elements. The energy value is 1 390KJ/cartoon (330 kcal = 1.5kcal/ml) and patients will have a choice of 16 flavours.

iv. Assessment of mood was made using the Hospital Anxiety and Depression Scale (HADS). Patients were scored out of 21 for each of anxiety and depression. A score in either category of 0 to 7 was considered normal, a score of 8 to 10 suggestive of disease and a score of 11 or more indicated a

probable mood disorder.[554] Those scoring 8 or more were advised to see their GP to discuss implementation of treatment. Patients scoring ≥ 25/42 in total or ≥ 14/21 for either depression or anxiety were referred to palliative care for further assessment.

v. At follow-up reviews compliance was checked with nutritional therapy and PERT.

Patients continued to undergo PASS review until chemotherapy was stopped (and hence routine hospital attendance ceased). Information on survival and hospital admission was obtained from hospital records and computer databases.

2. Analysis

The primary and secondary outcomes were compared between the retrospective and prospective groups using t-tests for normally distributed continuous variables, Mann-Whitney U tests for non-parametrically distributed continuous variables and chi-squared tests for categorical variables. The precise definitions of the outcomes to be assessed were as follow;

Primary outcomes

1) Patient survival rates at

a) 6 months

I b) 12 months

Survival was defined as that from the 1st investigation suggesting pancreatic cancer until death. Data was also available on average survival times, although the follow-up times for each group were different and hence cannot be compared.

2) Number of chemotherapy doses administered

Defined as the number of gemcitabine doses received by the patient.

Secondary outcomes

3) Hospital admissions. To allow a direct comparison between the two groups, the numbers of hospital admissions were assessed in those whose survival time was less than 12 months.

a) median number of admissions following 1st dose of chemotherapy

b) median number of hospital inpatient days

4) Weight change during follow-up

a) weight (kilograms) change per week

b) percent weight change per week

Weight change per week was defined as weight recorded at the booking clinic subtracted by the last recorded weight for each patient and then divided by the number of weeks between the two.

5) Use of oral supplements and pancreatic enzyme replacement therapy

a) Comparison of documented use of any oral supplements used

b) Comparison of documented use of PERT being used

c) Comparison of the total daily dose of PERT

6) Depression screening and treatment

a) Comparison of documented evidence of a psychological assessment being made.

b) Comparison of the proportion of patients receiving treatment for a psychological disorder.

Non-comparative data

Non-comparative data was available for the use of the HAD scale.

7) HAD Scale

Description of patient scores to detect depression

Approval of the study

The study was designed by members of the pancreatic multi-disciplinary team. This included medical, surgical, oncological, palliative care consultants as well as dieticians and cancer specialist nurses. All parties were in agreement with the final study design. An ethical submission was not made as the assessments and intervention were those recommended by national societies of gastroenterology.

Results

The number of patients with pancreatic cancer who received chemotherapy during 2009 was 16 (9 females) and in 2010 was 19 (9 females), with no statistical differences between the age, gender and clinical characteristics of the groups (Table 48). In the retrospective group, the median interval between first the investigation and initial chemotherapy dose 1.9 months (range 0.9 to 12.7 months) with one case of suspected pancreatic tumour taking nearly a year to confirm diagnosis. In the prospective group, the interval was 1.8 months (range 1.2 to 4.2 months). The retrospective group tended to have more advanced disease (56% with distant metastases vs 37% in the prospective group) and a higher proportion entered into the Telovac trial (50% vs 32%), although, both the differences were not statistically significant. In the prospective group, 16 (84%) of the 19 patients underwent the first PASS review, with 11 (57%) patients seen at the second review, 6 (32%) at the third, 4 (21%) at the fourth and 2 (11%) at the fifth PASS review.

Descriptive data, which cannot be compared due to differing lengths of follow-up, shows that all retrospective patients had died after a within a follow-up period of 25 months with a mean survival time (from first investigation to death) of 10.1 months (SD=5.1 months) and a median survival of 10.5 months (2.4 to 23.3 months). In the prospective group, on October 2nd 2011, 2 of the 19 patients remained alive, after 15 and 13 months follow-up. The median survival the group was 9.5 months (range 2.5 to unknown). Of the 17 who had died, the mean survival was 9.4 months, although inclusion of the two patients still alive improves it to 10.0 months.

Table 48. Comparison of the baseline characteristics between the retrospective and prospective groups.

Descriptor	Retrospective Year 2009	Prospective Year 2010
Number of participants	16	19
Age at 1st investigation (years, mean (SD))	65.7 (11.3)	68.9 (6.9)
Interval between 1st investigation and 1st chemotherapy dose, (months)		
mean (SD)	2.8 (2.9)	2.1 (0.9)
median (range)	1.9 (0.9-12.7)	1.8 (1.2-4.2)
Gender, n (%)		
Male	7 (44)	10 (53)
Female	9 (56)	9 (47)
Stage of disease , n (%)		
Locally invasive	0 (0)	3 (16)
Locally advanced	7 (44)	9 (47)
Distant metastases	9 (56)	7 (37)
Oncological treatment, n (%)		
Gemcitabine	8 (50)	12 (63)
Gemitabine and Capecitabine	0 (0)	1 (5)
Telovac trial	8 (50)	6 (32)

3. Primary outcomes

Survival rate and number of chemotherapy doses

There were no differences in the survival rates after 6 months (retrospective group (RG) 75% vs 68% prospective group (PG), p=0.67) and 12 months (RG 31% vs 42% PG, p=0.51) (Table 49). The median number of chemotherapy doses administered was 9 RG (range 2 to 24 doses) vs 6 PG (1 to 18 doses) (p=0.19).

4. Secondary outcomes

Hospital admissions

Hospital admissions were compared in all those who survived less than 12 months. The median number of admissions was higher in the RG vs PG (2, (range of 0 to 5) vs 1 (0 to 4), p=0.034) and they also had a longer median duration of admission (11 days, (0 to 75) vs 4 days (0 to 11), p=0.017) (Table 50).These results were skewed in the retrospective group by one patient requiring a prolonged period of hospital stay during the terminal stages of disease and accrued a 75 day inpatient stay.

Table 49. Primary outcomes; survival rate and chemotherapy doses.

Descriptor	Retrospective (n=16)	Prospective (n=19)
Survival after 1st investigation		
Alive after 6 months, n (%)	12 (75%)	13 (68%)
Alive after 12 months, n (%)	5 (31%)	8 (42%)
Chemotherapy doses		
Doses given, median, n (range)	9 (2-24)	6 (1-18)

Table 50. Comparison of hospital admissions in those surviving less than 12 months.

Descriptor	Retrospective (n=11)	Prospective (n=11)
Hospital admissions, n, median (range)	2 (0-5)	1 (0-4)*
Duration of admissions, days, median (range)	11 (0-75)	4 (0-11)**

*p=0.034, **p=0.017

Weight change during follow-up

At the initial booking visit, patients in the retrospective group were slightly heavier than those in the prospective group at the initial booking visit (mean = 68.9 kg vs 63.8 kg, p=0.29) (Table 51). There were 11 (69%) patients with follow-up weights in the retrospective group and 15 (79%) patients in the prospective group, and these were used to calculate the total weight change and rate of weight change in each patient. None of the data related to changes in weight between groups were statistically significant. The median total weight change in the RG= -1.15kg (-9.3 to 16.8 kg) vs -1.5 kg (-9.6 to 13.0 kg) in PG (p=0.98). The median weight change per week in RG= -0.18 kg (-0.56 kg/wk to 0.33 kg/wk) vs -0.05 kg/wk (-1.0 kg/wk to 0.73 kg/wk) in PG (p=0.74). The median total percentage weight change in the RG= -7.2% (-23.1% to 11.4%) vs -2.9% (-10.3 to 16.6) in PG (p=0.38). The median weekly change in the RG= -0.42% (-0.77% to 0.45%) vs 0.03% (-1.15% to 1.68%) in the PG (p=0.32).

Use of oral nutritional supplements and pancreatic enzyme replacement

In the retrospective group, 5 (31%) patients were documented in the clinical notes to have used oral nutritional supplementation, e.g. *Ensure plus,* compared to 11 (58%) (p=0.29) in the prospective group (Table 52). PERT was prescribed less frequently in the retrospective group (50% vs 79%, p=0.072), although there were no differences between groups for the total mean daily dose (RG=95 000 units vs PG=88 700, p=0.73).

Table 51. Comparison of weight change between groups.

Descriptor	Retrospective (n=16)	Prospective (n=19)
Booking weight, kg, mean (SD)	68.9 (14.5)	63.8 (13.1)
Follow-up weight available, n (%)	11 (69)	14 (74)
Total weight change, kg, median	-1.15 (-9.3 to 16.8)	-1.5 (-9.6 to 13.0)
Weight change/week, kg, median	-0.18 (-0.56 to 0.33)	-0.05 (-1.0 to 0.73)
Total % weight change (range)	-7.2 (-23.1 to 11.4)	-2.9 (-10.3 to 16.6)
Total % weight change/week (range)	-0.42 (-0.77 to 0.45)	-0.03 (-1.15 to 1.68)

kg=kilograms

Table 52. The comparison of oral nutritional supplements and pancreatic enzyme replacement therapy (PERT) use between groups.

Descriptor	Retrospective (n=16)	Prospective (n=19)
Use of oral supplements, n (%)	5 (31)	11 (58)
Use of PERT, n (%)	8 (50)	15 (79)*
PERT dose, units/day, mean (SD)	95 000 (36 645)	88 667 (42 655)

*p=0.072

Depression screening

In the retrospective group, fewer patients had documented evidence of a psychological assessment (44% vs 74%, p=0.072), although a higher proportion were treated for psychological symptoms (5 patients vs 1 patients, p=0.042) (Table 53). The hospital anxiety and depression scores were completed by 15 of the 19 (79%) patients in the prospective group. Here a total of 6 patients (43% of those returning the questionnaire) had scores indicating a possible or probable anxiety (3 patients) or depressive disorder (5 patients), with two patients scoring highly in both the categories. Following the second PASS review, seven patients returned a completed questionnaire (two failed to return) with one patient scoring in the "probable" anxiety and depression category and two in the "possible" depression category.

Table 53. Comparison of evidence of depression screening and treatment between groups as well as outcome of hospital anxiety and depression (HAD) scores in the prospective group.

Descriptor	Retrospective (n=16)	Prospective (n=19)
Patients with documented evidence of psychological assessment, n (%)	7 (44)	15 (79)*
Patients treated for depression, n (%)	5 (31)	1 (5)**
1st HAD score (completed, n=14)		
Anxiety, n (% of completed)		
normal = score ≤7,	N/A	11 (79)
possible = 8 to 10		3 (21)
probable = ≥11		0
Depression, n (% of completed)		
normal = score ≤7	N/A	9 (64)
possible = 8 to 10		3 (21)
probable =≥11		2 (14)
2st HAD score (completed=7)		
Anxiety, n (% of completed)		
normal	N/A	6 (86)
possible		0 (0)
probable		1 (14)
Depression		
normal	N/A	4 (57)
possible		2 (29)
probable		1 (14)

*p=0.032, **p=0.042

5. Summary

This survey of patients with pancreatic cancer receiving chemotherapy, found that after the implementation of PASS, a higher proportion of patients were prescribed PERT (p=0.072), underwent psychological assessment (p=0.032), and in those surviving less than 12 months, hospital admission were fewer (p=0.034) and shorter (p=0.017). However, there were no differences in the primary outcome measures of survival rates at 6 months and 12 month or in the number of chemotherapy doses administered. In the secondary outcomes, despite the retrospective group undergoing psychological assessment less frequently (44% vs 79%, p=0.032), there was a significantly higher proportion of patients receiving treatment (31% retrospective group vs 5% prospective, p=0.042). Using the self-administered HAD Scale highlighted that 43% of patients warranted further evaluation for psychological symptoms. There were no statistically significant differences in the use of oral supplements, dosing of PERT or weight change. Although the differences were not statistically significant, patients in the prospective group did loose less weight and larger numbers may be needed to evaluate if there is a real effect.

Discussion

This study reported that over one third of patients with pancreatic cancer, when screened for psychological symptoms, had possible clinical anxiety or depression. They continued to lose weight after diagnosis, although there is some suggestive evidence this may be reduced by pancreatic enzyme replacement therapy. The results justify national guidelines which advise screening for depression and weight loss. The implementation of PASS, a new clinical support service, was associated with some improved measures of clinical care, e.g. reduction in the number and duration of hospital admission. However, it did not significantly influence the primary outcome measures, namely rates of survival and number of chemotherapy doses. The findings of this study should be interpreted with caution due to the limitations of the study design, particularly the small number of patients enrolled and the inability to directly compare patient groups and hence the study should be regarded as a feasibility study, furthermore no assessment was made of the cost effectiveness, although if these deficiencies are rectified in future work, the benefits of a clinical service such as PASS could justify its broader use. The outcomes and limitations of this study as well as areas of future work are now discussed in greater detail.

Implementing PASS did not change patient survival rates, with survival after 12 months in the retrospective group (RG) 31%, vs 42% (p=0.51) in the prospective group (PG). The median survival time after the first investigation in the retrospective group was 10.5 months and in the prospective one 9.5 months, with no differences in median survival times after the 1st dose of chemotherapy. These figures are similar to those in the original trial of gemcitabine monotherapy, which reported an overall median survival of 5.0 to 7.2 months.[560] With extended follow-up of the prospective group, where 2 of the 19 patients remain alive after 15 months follow-up, it is possible that the mean survival time will improve. An improvement in survival times could occur in patients who are reviewed by PASS due to the increased prescription of PERT preventing malnutrition and the early identification of medical complications of disease. However, PASS may not be responsible for prolonging survival, with evaluation of the seven patients who survived over 12 months, revealing only two patients were seen on three or more occasions by PASS, with two patients never actually reviewed by PASS as their chemotherapy was stopped after a single dose. The lack of any benefit in survival implies focusing on nutritional aspects is not sufficient to help mitigate the effects

of the cancer. Although survival time was unaffected, future work should look at other areas such as quality of life.

The other primary outcome was the number of doses of gemcitabine administered, which did not differ between groups. The clinical aim is give patients to 18 doses of gemcitabine in 6 cycles over 6 months, although most stopped chemotherapy early. We chose to evaluate the number of chemotherapy doses given as weight loss is predictive of shorter chemotherapy courses and increased side-effects.[527] Less chemotherapy doses often indicates poor survival time, although three patients in the prospective group received 4 or less doses of chemotherapy and were still alive after one year of follow-up. Again, the lack of effect of PASS suggests evaluating aspects of care, such as nutrition, is unable to affect the clinical status in the patient given chemotherapy.

A secondary outcome that was measured was weight loss following diagnosis, which was less than expected in both the groups. A probable explanation for this was identified during the clinical review the prospective group, where three patients developed ascites or peripheral oedema which was accompanied by marked weight gain. In future work a reliable assessment of nutritional status would need to be used as patients with pancreatic cancer are likely to develop fluid retention due the associated catabolism and low serum albumin. There are alternative and more reliable assessments of nutritional status. These include mid-arm muscle circumference measurements that estimate changes in muscle volume[561] and laboratory assessments which assess body composition change. These include bioimpedance analysis, which estimates body fluid volumes, although it has not been validated in those who retain fluid secondary to a disease state. Dual-energy X-ray absorptiometry (DEXA), which can be used to measure total body composition and fat content is another potential method. We hypothesised that the assessment of nutritional status would maintain patients' weight due to the early prescription of PERT and oral nutritional supplements. Although the changes in percent of body weight per week between the two groups were not statistically significant (RG= -0.42 vs -0.03 PG, p=0.32) the differences would support further investigation in a larger study. These should include alternative assessments of nutritional status to more reliably clarify if these interventions are associated with improved outcomes.

In the PG, 80% of patients screened for ongoing weight loss or symptoms of malabsorption may require PERT, compared to 50% in the RG (p=0.072). The higher prescription rates in the PG may have occurred due to the existence of PASS, or bias, if patients in the retrospective group were more likely to be

misclassified as non-users. This bias could occur if general practitioners (GPs) prescribed PERT but was not documented in hospital records. To prevent the delay in the use of PERT all patients with pancreatic cancer should be prescribed these agents at the time of diagnosis[538] at an appropriate dose of PERT of at least 40 000-50 000 units with each meal and 10 000-25 000 units with each to avoid sub-therapeutic dosing.[562] In summary, the results suggest that PERT was not prescribed as frequently in the retrospective group, although it is recommended in national guidelines. In future work, the impact of early PERT prescription needs to assess quality of life in addition to clinical outcomes.

A further secondary outcome we assessed was depression, which has a high incidence in patients with pancreatic cancer. We screened for this in the prospective group using the HAD Scale which identified 43% of patients with a potential mood disorder. Ideally these patients would have undergone a structured interview with a specialist in psychiatric health to determine the presence or absence of a mood disorder. The patient with the highest HADS score, of 21 points, had already been identified with a mood disorder prior to PASS assessment. They were already under review by the palliative care team and being treated with counselling and medications (mirtazapine and nozinan). Another patient who scored 21 points declined the offer for further assessment, although his GP was notified. The completion of the HAD scale was initially undertaken by the PASS clinician. However, after interviewing a patient with known depression with his wife, it became apparent that his responses were unreliable in the structured interview setting. Subsequently, patients were asked to complete the questionnaire at home and return it at their subsequent hospital attendances to hopefully achieve more representative answers. The manner of delivering the HAD Scale needs to be more formally assessed in future work. Furthermore, as depression is common in pancreatic cancer, there needs to be the appropriate psychological services available to manage the mood disorders diagnosed.

Surveying anti-depressive use, found that despite lower rates of screening for depressive symptoms, the retrospective group had a higher rate of prescriptions. However, the timing of introduction of these medications was not verified, and hence it is uncertain whether these medications had been introduced following the diagnosis of cancer, or if the patients were on long-term prescriptions. To improve future work, the timing and indication for the anti-depressive medication should be determined at the time of note review.

The study's intention had been for all patients receiving chemotherapy to be reviewed by PASS every four weeks. This was rarely achieved primarily due to

a lack of availability of a PASS member during the patients' visit for chemotherapy. Other PASS reviews did not occur due to altered chemotherapy appointment times, if review was deemed clinically inappropriate or if the patient was too unwell. Future work in this area would need a more robust method to ensure all patients are reviewed in a timely fashion, which ultimately would require more than one PASS reviewer.

There were several limitations, including a small sample size with heterogeneous groups and several extraneous variables which could affect outcomes. These variables included patients receiving different chemotherapy regimes, were treated by different clinical teams and during the development of the study protocol awareness of PERT and the prevalence of depression in pancreatic cancer may have altered clinicians' practice. Furthermore, although all patients with pancreatic exocrine cancer were included, some had less aggressive histological types of carcinoma such as mucinous adenocarcinoma. Bias could have occurred due to the methods used to collect data in the two groups. In the retrospective group, patients receiving PERT, oral supplements or depression screening may not have been identified if the data was not recorded in the medical notes or if the information was missed upon review of the notes. This misclassification was less likely to occur in the group where the data was collected prospectively. These limitations could be rectified in future work including the assessment of quality of life in patients reviewed in PASS. This was considered during the development of the study protocol and the intention was to measure the quality of life using the ETORC-30 in patients at 3 months, however, relatively few patients reached this stage. To clarify whether PASS leads to an alteration in the quality of life of patients, patients could undergo QOL screening routinely and it could repeated again after the reintroduction of PASS. The cost effectiveness of PASS was not assessed in this study which should be addressed in future work. In this work the time required in supplying the clinical PASS was not measured and was given without cost. Future work should evaluate the time and cost of supplying and managing the PASS service as well as additional costs from the increased use of therapies. However, these may be offset by a reduction in clinical complications and hospital admissions, which would need to be accounted, as well as ultimately improving the care and quality of life of patients with pancreatic cancer.

In conclusion, this survey identified several aspects about the clinical management and delivery of care for patients with pancreatic cancer;

- Screening for anxiety and depression identified symptoms in over one third of patients. Emphasis should be placed in the clinical management of screening for this condition.
- There was no impact on the primary outcomes of survival and doses of chemotherapy with PASS. Future work should assess if such a service could affect quality of life outcomes.
- PASS was associated with a reduced the number and duration of hospital admissions and the reasons for this should be explored. Such a benefit would be of importance to the patient.
- Weight loss is common in patients with pancreatic cancer. PASS did appear to improve this parameter although the differences were not statistically significant. Future larger studies are required to see assess in a significant improvement can be achieved. The PASS service did lead to a greater number of PERT prescriptions.
- The work undertaken so far will help provide clinicians with standards against which to audit the care of patients with pancreatic cancer.

Appendices

Appendix 1. The EPIC physical activity questionnaire from which the 4 level physical activity index was derived.

1. We would like to know the type and amount of physical activity involved in your work. Please tick what best corresponds to your present activities from the four possibilities

- Sedentary occupation _____
  You spend most of your time sitting (such as in an office)

- or Standing occupation
  You spend most of your time standing or walking. However, your work does not require intense physical effort (e.g. shop assistant, hairdresser, guard, etc.)

- or Physical work
  This involves some physical effort including handling of heavy objects and use of tools (e.g.plumber, cleaner, nurse, sports instructor, electrician, carpenter, etc.)

- or Heavy manual work
  This involves very vigorous physical activity including handling of very heavy objects (e.g. docker, miner, bricklayer, construction worker,etc.)

2. In a typical week during the past 12 months, how many hours did you spend on each of the following activities? (Put '0' if none)

- Walking, including walking to work, shopping and leisure
  _____ hours per week

- Cycling, including cycling to work and during leisure time
  _____hours per week

- Gardening
  _____ hours per week

- Housework such as cleaning, washing, cooking, childcare
  _____hours per week

- Do-it-yourself
  _____hours per week

- Other physical exercise such as keep fit, aerobics, swimming, jogging
  _____ hours per week

3. In a typical week during the past year did you practise any of these activities vigorously enough to cause sweating or a faster heartbeat?

Yes ____ No ____Don't know ____

- If yes, for how many hours per week in total did you practise such vigorous physical activity? (Put '0' if none)
  _____hours per week

4. In a typical day during the past 12 months, how many floors of stairs did you climb up? (Put '0' if none)

_____floors per day.

*Pa*ncreatic *S*upport *S*ervice

PASS

A support service for patients receiving chemotherapy for pancreatic cancer

Norfolk and Norwich University Hospital

What is *PASS*?

The *PA*ncreatic *S*upport *S*ervice, *PASS*, is for patients with pancreatic cancer who are having chemotherapy. If you are agreeable, we aim to meet you regularly (usually every 4 weeks) during your attendance for chemotherapy. We will focus on maintaining your weight and controlling symptoms**.** *PASS* is run by a doctor and nurse.

About your pancreas.

Your pancreas is a gland located high in your abdomen. The pancreas produces a liquid containing enzymes. Enzymes are proteins which break down food into small fragments which are then absorbed into your body.

What is pancreatic cancer?

In pancreatic cancer there is a growth within the pancreas reducing the amount of these enzymes. This means food is not well absorbed and you can lose weight.

Treatments for cancer of the pancreas.

Treatments are used to slow the growth of the tumour and to improve your symptoms. These include chemotherapy, offered by your oncology doctors, as well as nutritional treatments, medicines and other methods to control your symptoms.

What may *PASS* do for me?

We aim to meet you during your planned clinic or chemotherapy appointments at the hospital. Our aims are to:

1. Help to explain your diagnosis and answer questions you may have.
2. Monitor your weight.
3. If you lose weight offer capsules containing pancreatic enzymes and nutritional drinks to help reverse this.
4. Assess any symptoms you may have and how you are feeling. Further treatments will be suggested to you if required.

What are nutritional drinks?

If you lose weight we will offer you high energy drinks to prevent further weight loss. These contain all the major food groups, minerals and vitamins. These should be taken between your normal meals. Maintaining your weight will help you feel better and stay healthier.

What are pancreatic enzyme capsules?

These capsules replace the loss of your own pancreas enzymes and help digest your food. Using these capsules can help prevent pain and weight loss. The capsules are taken with meals and snacks.

Follow-up

PASS aims to meet you every 4 weeks to help identify any new symptoms. We will also ask how the illness is affecting your quality of life and mood to see if there is anything further we can help with. We will review you during your planned attendance for treatment or clinics to prevent an extra visit for you.

Summary

PASS aims to help you and your family with problems that pancreatic cancer causes. We aim to identify and manage these issues quickly. The doctors in the *PASS* clinic work closely with your other specialists and your GP is kept informed. All recommendations are supported by national guidelines from the British Society of Gastroenterology. *PASS* is optional and we completely understand if you prefer not to use it. Please give us any feed back on *PASS*.

Contacts

If you have any questions please contact either;

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or Maria Cremin, Cancer Nurse Specialist on telephone 01603 288844, email Maria.Cremin@nnuh.nhs.uk.

Appendix 2. The data record sheet used in the retrospective survey.

Demographic data Study number_____ DOB_____

Baseline data

Date 1st investigation suggesting pancr cancer _____ Age at diagn _____

Male ☐ Female ☐

Staging 1) within pancreas 2) local spread 3) distal spread

Chemo received 1) Gem_____ 2) Gem & Cap _____

3a) Telovac trial A - gemcitabine & capecitabine over a total of 8 weeks.

3b) B gemcitabine and capecitabine together, over a period of 8 weeks (the same as A). Then a course of GV1001 vaccine injections.

3c) Gemcitabine and capecitabine (as A & B) & start GV1001 vaccine in first week.

Outcome data Date of death = _____

1) Survival time post diagnosis, (months) _____

Outcome 1a) alive six months ☐ 1b) alive one year ☐

2) Total hospital readmission rate and duration

Number _____ and total duration days _____

No exceptions. Time parameter – from 1st chemo dose until death or over one year period.

3) Chemotherapy tolerance;

a) Number of cycles received ☐

4) Weight change over time Height BMI at chemo booking

	Kg	date
Weight at diagnosis		
Weight booking 4 chemo		
Last recorded weight		

5) Use of oral nutritional supplements

Any supplements recorded on computer?

Any supplements recorded in notes?

Date started?

6) Use of Pancreatic Enzyme Replacement Therapy

Any PERT recorded on computer?

Any PERT recorded in notes? total daily dose

Date started?

7) **Psychological health;**

Any assessment of psychological health recorded? On computer? In notes?

Provision of treatment

Prescribed pharmacological tx?

Referral for further assessment?

Additional notes.

Appendix 3. The patient history and data collection sheet used during each PASS review.

PASS Name _____Age ____ Date of birth_____ date of review _____

Hospital no._____ Tel number _____ Chemo cycle

Weight (kg) date Height BMI

Booking visit pre chemo wt _____ TNM staging _____

Diagnoses

DH

allergies

Eats pork?

Social history

1) **Symptoms** – score 0=not present, 1=mild, 2=moderate, 3=severe

Anorexia	Nausea	Vomiting
Abdo pain	weight loss	Bowels freq (x/day)
Loose stool	Fatigue	Jaundice

2) **Nutrition**

Supplemental drink Y / N date started dose

Compliance 1) nil or poor 2) partial 3) complete

PERT Y / N date started dose

Compliance 1) nil or poor 2) partial 3) complete

3) **HADS** score _____ 4) Complications , hospital admissions, chemo SEs?

5) Notes /outcome

Guidance on Supplements

1) Lost weight or poor appetite, give nutritional advice (including diet sheet).

2) Consider nutritional supplementation if despite advice struggling to maintain good oral intake.

3) If patient is having difficulties with food intake despite advice consider dietetic review.

PERT If history of weight loss or recorded weight loss >2% or 1kg start PERT. See PERT prescribing sheet.

Appendix 4. The information leaflet given to patients started on pancreatic enzyme replacement.

Guidance for patients on Creon

Your pancreas is a gland located high in your abdomen that produces a liquid containing enzymes. Enzymes are proteins which break down food into small fragments which are then absorbed into your body. In pancreatic cancer there is often a reduction in these enzymes – this causes food to be poorly digested and can lead to diarrhoea and weight loss.

Creon helps to replace the loss of your normal pancreatic enzymes.

Creon capsule sizes available are Creon 10 000, 25 000 & 40 000 units

The capsule can be taken whole or opened and mixed with food i.e. jam or honey.
The capsule should not be chewed or crushed.
The dosing can be split – i.e. capsules can be taken before and after a meal.

No Creon is required when eating foods low in fat ie fruit, fizzy drinks, boiled sweets

Guide for dosing of Creon

Meal	grams of fat	Dose of Creon (lipase units)
ie fruit and non-milk drinks	< 2g fat	0
Small snack/biscuit	3-5g	10,000
Snack, milk based ensure	5 -10g	20,000 – 30,000
Main meal	10 – 20g	40,000 – 50,000

Appendix 5. The Hospital Anxiety and Depression Scale given to patients to complete.

H.A.D. SCALE

NAME: ……..……. DATE:

Doctors are aware that emotions play an important part in most illnesses. This questionnaire is designed to help your doctor to know how you feel. Read each item and place a firm tick in the box opposite the reply, which comes closest to how you have been feeling in the past week. Don't take too long over your replies; your immediate reaction to each item will probably be more accurate than a long thought-out response.

Tick only one box in each section

I feel tense or "wound up"

Most of the time	
A lot of the time	
Occasionally	
Not at all	

I feel as if I am slowed down:

Nearly all the time	
Very often	
Sometimes	
Not at all	

I still enjoy the things I used to enjoy

Definitely as much	
Not quite so much	
Only a little	
Hardly at all	

I get a sort of frightened feeling like "butterflies" in the stomach:

Not at all	
Occasionally	
Quite often	
Very often	

I get a sort of frightened feeling as if something awful is about to happen

Very definitely and quite badly	
Yes, but not too badly	
A little, but it doesn't worry me	
Not at all	

I have lost interest in my appearance:

Definitely	
I don't take so much care as I should	
I may not take quite as much care	
I take just as much care as ever	

I can laugh and see the funny side of things:

As much as I always could	
Not quite so much now	
Definitely not so much now	
Not at all	

I feel restless as if I have to be on the move:

Very much indeed	
Quite a lot	
Not very much	
Not at all	

Worrying thoughts go through my mind:

A great deal of the time	
A lot of the time	
From time to time but not too often	
Only occasionally	

I look forward with enjoyment to things

As much as I ever did	
Rather less than I used to	
Definitely less than I used to	
Hardly at all	

I feel cheerful:

Not at all	
Not often	
Sometimes	
Most of the time	

I get sudden feelings of panic:

Very often indeed	
Quite often	
Not very often	
Not at all	

I can sit at ease and feel relaxed:

Definitely	
Usually	
Not often	
Not at all	

I can enjoy a good book or radio/TV programme:

Often	
Sometimes	
Not often	
Very seldom	

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Presentations of abstracts at national and international meetings

2011

Pancreatic Society of Great Britain and Ireland, Dublin, December 2011.

Oral presentation

"Dietary n-3 fatty acids in the aetiology of pancreatic cancer. Data from a UK prospective cohort study using 7-day food diaries."

Digestive Diseases Week, Chicago, May 2011.

Three posters of distinction

"Dietary deficiencies of iron and niacin in the aetiology of symptomatic gallstones – Data from a UK prospective cohort study using 7-day food diaries."

"Caffeinated coffee is associated with a reduction of symptomatic gallstones in men"

"The role of dietary cholesterol and transfatty acids in the aetiology of gallstones."

Two poster presentations

"Physical activity and the risk of developing pancreatic cancer"

"Do oleic acid and n-3 fatty acids prevent pancreatic cancer?"

British Society of Gastroenterology, Birmingham, March 2011.

Oral presentation

"Dietary deficiencies of iron and niacin in the aetiology of symptomatic gallstones – Data from a UK prospective cohort study using 7-day food diaries."

Four poster presentations

"Physical activity and the risk of developing pancreatic cancer"

"Do oleic acid and n-3 fatty acids prevent pancreatic cancer?"

"Caffeinated coffee is associated with a reduction of symptomatic gallstones in men"

"The role of dietary cholesterol and transfatty acids in the aetiology of gallstones."

2010

Digestive Diseases Week, New Orleans, May 2010.

Two oral presentations

"Antioxidants in the aetiology of pancreatic cancer"

"Sucrose in the aetiology of pancreatic cancer"

Poster of distinction

"Lipids in the aetiology of gallstones"

British Society of Gastroenterology, Liverpool, March 2010.

Oral presentation

"Lipids in the aetiology of gallstones."

Two poster presentations

"Antioxidants in the aetiology of pancreatic cancer"

"Sucrose in the aetiology of pancreatic cancer"

2009

Digestive Diseases Week, Chicago, May 2009.

Two oral presentations

"Alcohol intake and development of gallstones: an inverse association – a UK prospective cohort study"

"BMI and waist circumference and the risk of developing gallstone disease"

British Society of Gastroenterology, Glasgow, March 2009.

Plenary poster

"Alcohol intake and development of gallstones: an inverse association – a UK prospective cohort study."

2008

Digestive Diseases Week, San Diego, May 2008.

Oral presentation

"Physical Activity and Reduced Risk of Symptomatic Gallstones - A Prospective Cohort Study in Norfolk"

British Society of Gastroenterology, Birmingham 2008.

Poster presentation

"Physical Activity and Reduced Risk of Symptomatic Gallstones - A Prospective Cohort Study in Norfolk."

Peer reviewed publications relating to this thesis