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Conference on 'Nutrition and health: cell to community'

Symposium 1: Nutrition and epigenetics
Recent advances in understanding the role of diet and obesity in the development of colorectal cancer

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Colorectal cancer (CRC) is a major cause of premature death in the UK and many developed countries. However, the risk of developing CRC is well recognised to be associated not only with diet but also with obesity and lack of exercise. While epidemiological evidence shows an association with factors such as high red meat intake and low intake of vegetables, fibre and fish, the mechanisms underlying these effects are only now being elucidated. CRC develops over many years and is typically characterised by an accumulation of mutations, which may arise as a consequence of inherited polymorphisms in key genes, but more commonly as a result of spontaneously arising mutations affecting genes controlling cell proliferation, differentiation, apoptosis and DNA repair. Epigenetic changes are observed throughout the progress from normal morphology through formation of adenoma, and the subsequent development of carcinoma. The reasons why this accumulation of loss of homoeostatic controls arises are unclear but chronic inflammation has been proposed to play a central role. Obesity is associated with increased plasma levels of chemokines and adipokines characteristic of chronic systemic inflammation, and dietary factors such as fish oils and phytochemicals have been shown to have anti-inflammatory properties as well as modulating established risk factors such as apoptosis and cell proliferation. There is also some evidence that diet can modify epigenetic changes. This paper briefly reviews the current state of knowledge in relation to CRC development and considers evidence for potential mechanisms by which diet may modify risk.

Colorectal cancer: Obesity: Inflammation: Diet: DNA methylation

In the UK, mortality rates from cancer and CVD are currently very similar and account for the vast majority of deaths. In the USA, cancer is now a greater cause of death before the age of 85 years than CVD[1]. Colorectal cancer (CRC) remains a significant cause of death in the UK; in 2007 it was the second most common cancer to be diagnosed in women (thirty-seven cases per 100 000 population) and the third most common cancer in men (fifty-five cases per 100 000) and overall the third most common cause of cancer death (Office for National Statistics, http://www.statistics.gov.uk). This is associated with an increase in incidence but improved survival (Fig. 1). These values are similar in the USA, Australia, New Zealand and most of Europe, whereas in some developing countries age-adjusted figures suggest the incidence may be as low as one-fiftieth of that in developed countries[2]. Furthermore, populations moving from low-incidence areas to high-incidence areas take on the disease profile of the new country within one generation[3], and the incidence in Japanese men, who have in recent years taken on a westernised lifestyle, has increased to levels above those observed in the UK and USA[4]. Such changes might be repeated across much of the developing world, as lifestyles become more akin to those now present in developed countries.

Although there are some well-recognised genetic causes of CRC, the vast majority of cases are considered to be sporadic, which, together with the evidence from migrating

Abbreviations: APC, adenomatous polyposis coli; CC, colon cancer; CGI, CpG island; CRC, colorectal cancer; RR, relative risk; WCRF, World Cancer Research Fund.

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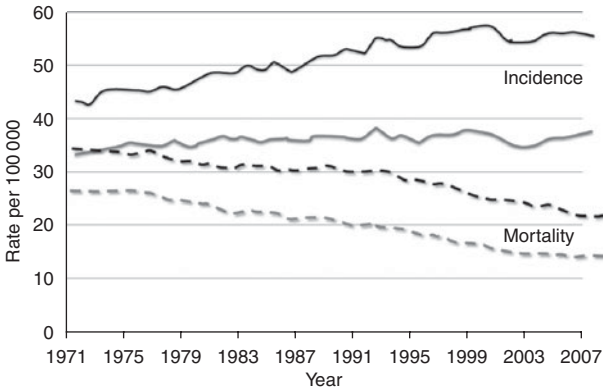

Fig. 1. Colorectal cancer incidence and mortality rates in England between 1971 and 2007 in men (black) and women (grey). Adapted from data published online by the UK Office for National Statistics.

Table 1. Relative risk (RR) of diagnosis of colorectal cancer in relation to dietary and lifestyle factors that were reported to be significant in the WCRF/AICR 2007 report[9]. Analysis is based on comparison between highest- *v.* lowest-exposure categories

	Number of cohorts	RR	95% CI	*P* =
Waist circumference	6	1·79	1·47, 2·18	<0·001
BMI	27	1·34	1·25, 1·44	<0·001
Ethanol	9	1·56	1·27, 1·92	<0·001
Red meat*	12	1·35	1·19, 1·53	<0·001
Processed meat	13	1·3	1·14, 1·47	<0·001
Physical activity	8	0·75	0·61, 0·91	0·031
Fish	13	0·88	0·78, 1·10	0·046
Milk and dairy products	15	0·89	0·80, 1·00	0·044
Fibre	13	0·9	0·81, 0·99	0·04

*This value has subsequently been reported to be incorrect on the associated website; the value should be 1·26 with an estimated 95% CI of 1·1, 1·4.

populations, suggests a strong effect of environment on the risk of CRC. The important role of diet in disease aetiology and risk of sporadic colon cancer (CC) has been recognised for many years[5–8]. In general, epidemiological studies suggest that the effects of most environmental factors are greater in the colon than in the rectum. However, data relating to CRC are available for more dietary and lifestyle factors than those for CC, and so for comparative purposes, it is these values from the World Cancer Research Fund (WCRF) systematic literature review that are summarised in Table 1[9]. The food groups for which there is currently most evidence to support a role in CRC risk are meat, dairy products, fish, vegetables and fibre. Additionally, the importance of obesity as a major risk factor has been recognised for over 15 years[10] with evidence from subsequent studies being very consistent, such that quantitative analysis of studies to date shows a highly significant effect ($P<0.001$)[11]. In this paper, we will review in outline our current knowledge of the mechanisms underlying tumour development and recent evidence supporting the possible effects of diet on CRC risk. This review will focus on those food groups where there is reasonable evidence to suggest an effect on tumorigenesis and consider potential mechanisms of action of associated dietary components.

Mechanisms of colorectal carcinogenesis

Apart from the relatively small proportion of cases associated with known mutations either in the adenomatous polyposis coli (APC) gene, referred to as familial adenomatous polyposis, or DNA mismatch repair genes, known as HNPCC (hereditary non-polyposis CRC), CRC is mostly a disease of old age. The sequential progression from healthy epithelium to metastatic tumour described by Vogelstein[12] is a widely accepted model of tumorigenesis, particularly in relation to CRC. In this model, CRC is considered to develop from normal mucosa through the premalignant adenoma by the step-wise accumulation of mutations. The lining of the colon consists of about $1{\cdot}5 \times 10^7$ invaginations or crypts, each containing at least four stem cells that divide about once daily for a lifetime[13]. Each time a cell divides the copied DNA must be checked for errors and either repaired or the cell removed to avoid an accumulation of errors. However, with over 10^{12} divisions in a lifetime, it is likely that editing errors will accumulate[13]. It is therefore proposed that mutations may arise spontaneously, without specific exposure to any environmental mutagens, but purely as a result of chance[14]. Once key genes such as those involved in DNA repair, apoptosis and control of cell division are affected, the mutation rate is likely to increase. Therefore, any factor that might distort normal homoeostatic controls such that cell proliferation rates increase or normal asymmetric division is disturbed is likely to increase risk. If cells containing mutations in, for example, tumour suppressor genes are not removed by apoptosis, the mutation will remain within the stem cell population of that crypt and may replicate at a disproportionate rate. For example, the best-recognised tumour suppressor gene associated with apoptosis is p53, which is a late-stage mutation in 75% of carcinomas[15]. APC is seen as a 'gatekeeper' gene in this process that functions in suppressing β-catenin signalling and to modify cell adhesion[16].

All CRC display either microsatellite instability or chromosome instability. Microsatellite instability occurs in 15% of CC and results from inactivation of the DNA mismatch repair system. Microsatellite instability promotes tumorigenesis through generating mutations in target genes that possess coding microsatellite repeats, such as β-catenin, transforming growth factor-β receptor II and the pro-apoptotic protein BAX (B-cell lymphocytic-leukaemia proto-oncogene-2 associated X-protein)[17–18]. Chromosome instability is found in the majority of CC and leads to a different pattern of gene alterations that contribute to tumour formation. Genes involved in chromosome instability include those coding for APC, K-ras (involved in controlling cell division), SMAD4 (regulator of gene transcription) and p53[19]. A more recent development in our understanding of CRC development has been the increasing recognition of the role of epigenetics, as reviewed recently by Duthie[20]. The modification of expression of genes regulating mitosis, apoptosis and DNA repair as a result of aberrant methylation of the promoter

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regions[21–24] is particularly important. Such changes appear to be strongly age related, an effect that is exacerbated in inflammatory bowel disease[23,25–26]. Furthermore, a specific CpG island (CGI) phenotype has been described, which has been suggested to have a different aetiology to chromosome instability, but is strongly associated with microsatellite instability[27].

The driving forces for tumour progression are complex, and include not only ageing but potentially also inflammation[28] and environmental mutagens[29], as well as underlying genetic predisposition. However, there is increasing evidence of interactions between lifestyle factors and genetic polymorphisms[30].

Mechanisms by which diet may impact on tumour progression

Prevention or slowing of CRC development may be possible, despite the inevitabilities of ageing and underlying genetic causes, by reducing epithelial cell proliferation, supporting the apoptotic removal of damaged cells and minimising inflammation and exposure to mutagens. Certain dietary factors have been shown to modify mitosis and apoptosis both in animal models and human intervention studies, although in the latter case it is hard to link these effects with reduced tumour development due to the long timescale of disease development. While there has been a considerable focus over many decades on the mutation profiles and modified signalling pathways associated with cancers and adenomas, the events leading to the formation of adenomas have received relatively little attention; yet prevention of initial perturbations to the system should probably be the desired target for lifestyle modifications. Removal of adenomas during colonoscopy reduces risk of CRC and thus it is predicted that lifestyle factors that reduce the numbers of such pre-neoplastic lesions should be protective[31]. However, chemopreventive strategies have tended to focus on inhibition of adenoma recurrence, rather than prevention of initiation. This is mainly because it is more feasible to offer medication to a targeted group of 'at-risk' patients, rather than to large populations of healthy people who are unlikely to develop cancer over a limited period of intervention. Even intervention studies in patients considered to be at risk of developing CRC are difficult, if the aim is to measure actual cancer occurrence rather than adenoma recurrence, because tumour development may take decades, while most dietary interventions, especially those using foods rather than supplements, are generally only feasible for a limited period. Therefore, much of our knowledge about changes in the gut mucosa prior to formation of pre-neoplastic lesions must be based on animal studies, while an understanding of the effects of environmental factors in human subjects must be gleaned from observational studies.

Recent research has generally focused on assessing whether dietary factors can modify cell signalling mechanisms known to be involved in CRC. An in-depth and comprehensive appraisal of the role of diet in chemoprevention is provided by Knasmuller *et al.*[32]. In considering studies concerned with hypothetical mechanisms, we must always bear in mind the issue of the doses used in such studies, and the likelihood of cells being exposed to such concentrations outside the experimental situation. In relation to the colon, we must consider both luminal and systemic access, including rate of metabolism and excretion, and for dietary factors that escape absorption in the small intestine, their possible interactions with the colonic microflora[33]. The Wnt signalling pathway involving APC and β-catenin and the modification of a myriad of signalling pathways affecting apoptosis, mitosis and metastasis[34–35,36], all likely to modify CRC risk, is of particular interest[37,38]. Second, modification of responses to growth factors such as epidermal growth factor, vascular endothelial growth factor[39] and insulin-like growth factor[40] is potentially important. These factors modify cell cycle control through mitogen-activated protein kinase and phosphoinositide 3-kinase via RAS signalling. Phosphoinositide 3-kinase catalyses the production of phosphatidylinositol (3,4,5)-triphosphate; (a lipid second messenger) involved in cell survival, that is blocked by phosphatase and tensin homologue[41–42]. All of these, and related factors, are currently the subject of intensive research as potential chemotherapeutic targets, but have in many cases also been shown to be altered either directly or indirectly by dietary factors, albeit in many studies at supra-physiological doses. For example, epidermal growth factor receptor antagonism increases survival of $APC^{min/+}$ mice[43] and a wide variety of studies have shown a role for the insulin-like growth factor family in disease aetiology, including modified signalling of phosphoinositide 3-kinase and extracellular-signal-regulated kinase 1[44–45]. Insulin-like growth factor 2 is the most overexpressed gene in CRC, and expression is normally under epigenetic regulation, which may be lost during tumour development[46]. Additionally dietary factors that could prevent the deregulation of transforming growth factor-β and p53 signalling found in many cases of sporadic CRC[47] would not only impact on apoptosis and DNA repair but would also have a much wider role in modifying p53 mediated stress responses and homoeostasis[48]. More recently, the Ephrin family have been implicated in tumour development[49] and reduced expression due to promoter methylation of the ephrin receptor EphB has been shown to be common in microsatellite stable tumours[50].

Obesity and physical activity

In the 2007 WCRF report, obesity, lack of exercise and high meat consumption were considered the only convincing environmental factors to affect CRC risk[9]. These data suggest that people in the highest category of BMI have a highly significantly increased incidence of CRC ($P<0.001$); similarly, the relative risk (RR) for waist:hip ratio is 1·82 (95% CI 1·17, 2·82). In contrast, people in employment involving high levels of physical activity have an RR of CRC of 0·59 (95% CI 0·49, 0·73). The European Prospective Investigation in Cancer study provides particularly useful data, due to its size and range of diets consumed across Europe. The data provided by the European Prospective Investigation in Cancer in relation to obesity and exercise were consistent with the overall

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meta-analysis undertaken by WCRF, and of particular note was the analysis of the interactions between these two factors. In this study, the RR of CC associated with being both lean and active was 0·38 (95% CI 0·21, 0·68) compared to men who had a BMI of over 30 and were inactive[51].

The mechanisms by which obesity drives CRC progression are not fully elucidated; however, obesity is associated with increased levels of circulating inflammatory mediators such as TNF-α, IL-6 and C-reactive protein, which may in turn lead to insulin resistance and raised levels of insulin and insulin-like growth factor 1 and reduced levels of the insulin-like growth factor-binding proteins 1 and 2[52,53]. Increased levels of these growth factors have been shown to increase cell proliferation and suppress apoptosis *in vitro*. In addition to the effects on classical markers of inflammation and insulin resistance, adiposity is also associated with increased levels of the 'adipokines' leptin and resistin and reduced levels of adiponectin. It is therefore proposed that CRC risk is increased in the overweight and obese due to a sub-clinical chronic inflammatory systemic milieu that impacts on mucosal inflammation, increasing NF-κB expression and subsequently levels of inducible nitric oxide synthase and Cox-2[52,54]. However, there is evidence that excessive intake of food in a meal may in itself be pro-inflammatory even in the absence of obesity[55] and thus the link between obesity and CRC may at least in part be related to higher food intake. In particular, high levels of circulating NEFA, as are found following a meal as well as in obesity, lead to increases in systemic inflammation[56]. Furthermore, the reported benefits of exercise are most apparent in those who have a relatively low food intake[51]. Thus, it is difficult to separate the impact of total energy intake and obesity as both are associated with increased systemic inflammation.

It is currently an open question as to whether obesity could modify DNA methylation. The chronic inflammation found in inflammatory bowel disease has been shown to be associated with increased CpGI methylation of the promoters for several genes including oestrogen receptor α, myogenic differentiation (controls removal of cells from cell cycle) and p16[23] and therefore there is reason to postulate that obesity-related inflammation might drive a similar process. Additionally, we have provisional data suggesting that methylation of the estrogen receptor promoter in the colon of mice genetically disposed towards obesity-related diseases (the ApoE Leiden mouse model[57]) that have been given a high-fat diet is higher than in those on a control diet or in wild-type mice (Go Elliott, NJ Belshaw, R Kleeman and EK Lund, unpublished results). However, Slatterly and co-workers report that obese subjects have a twofold higher incidence of CpGI phenotype low tumours and no difference in CpGI phenotype high tumours[58]. Similarly, Shima and co-workers found no effect of BMI on promoter methylation of any of the sixteen CpGI phenotype specific and non-specific genes analysed in samples from two human cohort studies although the expected association between high BMI and increased risk was reported for these cohorts[59].

The observation that exercise reduces cancer risk, independently of any potential impact on BMI, raises interesting questions in relation to the chronic inflammation hypothesis. The literature with respect to the effects of exercise on systemic inflammation is mixed and the effect may depend on the model used and the level of exercise. However, in human subjects, moderate exercise has been reported to reduce markers of inflammation in the circulation, while at the same time increasing neutrophil activity [60,61], although results from other studies are contradictory[62].

Meat and fat intake

Most recent assessments of the impact of total fat intake on CRC risk suggest that there is no detectable effect once other confounding factors have been taken into account, in particular body weight[63,64]. However, the epidemiological evidence that meat consumption is potentially harmful was considered convincing by the WCRF group of experts. This effect is particularly associated with red and processed meat consumption; the RR for CC, for highest *v.* lowest consumers, was 1·30 (95% CI 1·08, 1·56) for red meat and 1·36 (95% CI 1·19, 1·55) for processed meat. A more recent meta-analysis casts doubt on these results, concluding that the increased risk associated with red meat consumption was difficult to separate from factors such as obesity, inactivity and a low intake of fruit, vegetables and fibre[65]. Furthermore, recent cohort studies have come to opposing conclusions such that in 2010 two studies found no association of CRC with red meat intake[66,67] while one[68] reported a positive association. It is interesting to note that Spencer *et al.*[67] commented on the relatively low meat intake of this English population. This lack of effect is consistent with the observation in an earlier study from the same group that there was no difference in risk between vegetarians and meat consumers in the UK[69]. The inconsistencies probably arise due to differences in the range of meat intakes in different studies, and it may be that a statistically significant effect can only be found when populations of high meat eaters are included. In addition, the evidence for a pro-carcinogenic effect of processed meat has also been questioned[70]. Furthermore, the impact of red meat consumption on CRC risk has been shown in a number of studies to be modified by polymorphisms in the genes associated with heterocyclic amines activation[71,72], carcinogen metabolising enzymes and xenobiotic transporters[73,74] and PPARγ, which is associated with energy homoeostasis[75]. Such studies may provide insight into inconsistencies between observational studies, not solely in relation to meat consumption but also with regard to other potential risk factors.

A range of mechanisms has been suggested for the pro-carcinogenic effects of meat. Perhaps the most firmly established is the hypothesis that heterocyclic amines in meat act as carcinogens; evidence to support this concept continues to be published[76–78]. However, there is a paradox in the fact that chicken generally contains higher levels of heterocyclic amines than red meat but its consumption is not associated with increased risk of CRC[79]. An alternative hypothesis associated with the haem content of red meat can provide three possible mechanisms. First, haem is a source of Fe, much of which is not absorbed in

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the small intestine, and which can drive the production of free radicals and increase oxidative stress in the colonic lumen[80,81]. However, free radicals are short lived and therefore have to be generated close to, or within tissue to have any effect. Furthermore, the evidence from animal studies that unabsorbed Fe present at physiological levels is a major problem is not convincing[82]. A second potential mechanism focuses on the endogenous formation of nitroso compounds, as consumption of red or processed meat, but not white meat or fish, causes a dose-dependent increase in fecal apparent total N-nitroso compounds and the formation of nitroso-compound-specific DNA adducts[83]. However, Joosen and co-workers found no difference in apparent total N-nitroso compounds formation in response to processed meat consumption as compared to red meat, although mucosal DNA oxidative damage was higher in response to processed meat[84]. The nitroso compound hypothesis provides an attractive explanation for the possible pro-carcinogenic effects of red meat but does not fully explain why processed meat would be more harmful than red meat. However, the effect of processed meat on DNA oxidative damage implies that diet-derived anti-oxidants might be able to counteract this effect[84]. Third, the haem moiety, which has a porphyrin ring structure, has been shown in animal studies to increase tissue damage[85], an effect that is suggested to be caused by erosion of the protective mucous layer and damage to surface epithelium[86] that provides a possible link with the hypothesis that chronic inflammation of the mucosa increases risk and that removal of the mucous layer affects the response of the mucosa to the normal resident microbiota[87]. This inflammatory effect can be counteracted by simultaneous feeding of the plant porphyrin structure chlorophyll[88] that may provide an explanation for any possible protective effect of leafy vegetable consumption. In this respect, it is of interest to note that the impact of high meat consumption on CRC risk in the European Prospective Investigation in Cancer cohort is apparently minimal in those consuming diets high in fibre and fish[89].

Milk and dairy products

Consumption of milk and dairy products provide a significant level of protection against CRC. This is most likely to be associated with the Ca content of these foods. Higher serum levels of both Ca and vitamin D are associated with reduced CRC risk[90–93], although it is not necessarily the case that they act together, as is found in relation to bone health. The WCRF 2007 report[9] described a significantly reduced CC risk associated with Ca intake (RR = 0·95; 95% CI 0·95, 0·98) but not for CRC. A more recent meta-analysis similarly found no significant effect of Ca intake on CRC risk[92]. High serum levels of both are associated with higher levels of apoptosis, although the effect of vitamin D is most pronounced in disease-free patients while Ca is protective in adenoma patients[94]. Ca has been proposed to act through modification of cell signalling or in saponification of bile acids in the colon. Vitamin D is a nuclear receptor ligand that will modify apoptosis and mitosis through the RAS-mitogen-activated protein kinase and phosphoinositide 3-kinase pathways[92,95–97], and because of this may well interact with nutrients that affect overlapping pathways. However, the effect is likely to be complex as indicated by the observation that CRC is associated with overexpression of the vitamin D receptor[98].

Fish consumption

The protective effects of fish consumption on gastrointestinal disease have been discussed in detail in number of recent reviews[53,99–101]. A recent meta-analysis of fish consumption and CRC risk by Geelen and co-workers has suggested a borderline protective effect of fish[100], with RR highest *v.* lowest = 0·88 (95% CI 0·78, 1·00). This result is similar to that reported in the WCRF systematic literature review, but the paper also reported that in studies in which the difference in intake was more than seven times per month, the effect was more significant (RR = 0·78; 95% CI 0·66, 0·92) suggesting that protective effects are most apparent once fish consumption approaches two portions per week. Health benefits associated with fish consumption are generally linked to the *n*-3 long-chain PUFA content. Unfortunately it is extremely difficult to undertake a meaningful analysis of observational data with respect to *n*-3 PUFA intake, not only because of the poor FFQ data which often fail to identify the type of fish consumed, but even if fish type is roughly identified, the PUFA contents of fish and other foodstuffs are not well characterised in food tables. Furthermore, meta analyses often do not distinguish between fish and vegetable derived *n*-3 PUFA, and yet animal and *in vitro* studies suggest the latter have much lower bioactivity in relation to cancer end-points[102]. There are, however, a number of credible mechanisms whereby fish oils might reduce cancer risk, and animal studies provide convincing evidence of effect, albeit at higher concentrations than are achieved in most western diets.

We and others have suggested that the multiple double bonds found in fish oils increase oxidative stress in pre-neoplastic cells and thus increase the production of reactive oxygen species pushing them towards apoptosis and removal from the system before becoming established[103,104]. An alternative area of interest focuses on the anti-inflammatory nature of fish oils. Both EPA (C20:5) and DHA (C22:6) are substrates for a wide range of anti-inflammatory or inflammation resolving lipid signalling molecules, including resolvins and protectins[105], as well as competing with the *n*-6 PUFA arachidonic acid as a substrate for PG H synthase 1 and 2 (Cox-1 and Cox-2), and thereby increasing the production of less inflammatory prostanoids such as PGE_3 rather than PGE_2[106]. These molecules, including both the original PUFA and their oxidative products the eicosanoids and docosanoids, act as ligands for a number of nuclear receptors, most notably the PPAR with target genes involved in control of cell cycle, apoptosis and inflammation[107]. In this context, it is interesting to note that the effects of fish consumption are modified by polymorphisms in related genes[108]. However, it is also recognised that PUFA are involved in G-protein coupled receptor signalling at the cell surface of adipocytes and macrophages as part of an anti-inflammatory

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response[109]. It is very clear that lipid signalling is as yet a poorly explored area, with new insights into potential mechanisms being published on a regular basis. For example, we have recently shown that EPA can modulate the recently recognised ephrin receptor pathway, providing another route by which fish oil could protect against CRC[110].

Although EPA and DHA are the most well-recognised bioactive compounds in seafood, fish is also known to contain high levels of very bioavailable organic selenium[111] and has particularly high levels of the non-essential amino acid taurine[112]. Selenium intake is recognised to be potentially important in relation to CRC prevention although the epidemiology described in the 2007 WCRF report is based on case–control studies rather than cohort studies. However, intervention studies support a protective effect of selenium in relation to CRC[113,114] and this effect may be associated with epigenetic modifications involving histone acetylation, global methylation and demethylation of promoters[115]. The population of the UK has traditionally obtained most selenium from cereals, but with the introduction of bread flours from Europe and a reduced intake of bread, fish has become a more important source of this element (relative concentrations of selenium in the UK are now reported to be 6 μg/100 g white bread, while tuna contains 57 μg/100 g and cod 33 μg/100 g[116]). Selenium deficiency is associated with low levels of key selenoproteins, which have both anti-oxidant and anti-inflammatory properties, and it may be that supra-physiological levels have benefits associated with other anti-carcinogenic effects such as induction of apoptosis, DNA repair and control of angiogenesis[117]. Although oil-rich fish is considered an excellent source of vitamin D, in reality the levels are low and would require significant daily consumption of, for example, herring to counteract the effects of low UV exposure[93,118]. Furthermore, a recent systematic review has cautioned against excessive vitamin D supplementation[95] and it may be that appropriate exposure to sunlight is to be preferred.

Fruit and vegetable consumption

The WCRF 2007 report suggests that there is no evidence from observational studies for any protective effect of fruits on CRC risk[9] but the evidence in relation to non-starchy vegetables (Table 1) does suggest a possible protective effect; RR for CC, for highest *v*. lowest consumers, is 0·86 (95% CI 0·75, 0·99). There are many factors associated with consumption of vegetables that may be protective. Vegetables are a source of a range of dietary fibres, if defined broadly, including resistant starch in, for example, processed peas, oligosaccharides such as inulin found in chicory, Jerusalem artichoke and garlic, and pectin from plant cell walls in all vegetables including legumes and root vegetables. Meta-analysis of cohort studies confirm a protective effect of fibre (Table 1) in which there appears to be a clear dose–response relationship such that for every 10 g fibre consumed per day there is a 10% decrease in risk[9]. No similar dose response was found for non-starchy vegetables. The potential mechanisms by which fibre may modulate risk have been a topic of research for many decades[119]. The initial concept that fibre increased fecal bulk and diluted toxins, although potentially still valid, has been largely superseded by a focus on the production of SCFA, in particular butyrate as a result of the fermentation of soluble fibre[38]. Butyrate is the main metabolic fuel for colonocytes and so supports cell proliferation and also the maintenance of tissue integrity. In cell culture, butyrate induces apoptosis and similar effects have been seen using colonic lavage[120]. Furthermore, dietary supplementation with resistant starch, from which butyrate is produced on fermentation in the colon, can protect against CRC in animal models, an effect associated with increased levels of plasma butyrate, However, the balance between butyrate-supporting cell growth and tissue integrity and the levels required to induce apoptosis *in vivo* are not well defined[38].

Vegetables are also a source of a number of vitamins in particular vitamin C, carotenoids, vitamin E and folic acid. The impact of vitamin intake on CRC risk has been previously reviewed by Johnson[121]. These vitamins are well absorbed and therefore if they have any effect it would be expected to be through modification of the systemic environment rather than any luminal affect. Vitamins C and E and A are considered to be anti-oxidants but the evidence for such specific effects in human subjects is mixed, mainly because of the difficulty in identifying which dietary component is having an effect in observation studies, and due to the difficulties associated with undertaking intervention trials in healthy groups at an appropriate point for disease prevention and over a sufficiently long timescale. Similarly, there are remarkably few publications suggesting any protective effect of these compounds in animal models. Furthermore, two intervention studies on lung cancer in smokers[122–123] had to be stopped because of increased incidence of disease. Interestingly, it has been reported that elevated plasma vitamin C levels are associated with reduced apoptosis in colonic crypts from adenoma patients, an effect predicted to increase tumour promotion[103]. Folic acid status has been shown to be important in development of CRC and potentially linked to one carbon metabolism and DNA methylation, but the story is complex[124]. High intake of folate has been reported in a number of observational studies to be protective against the development of CRC[125], but observations on populations exposed to increased levels of folic acid following food fortification have been interpreted to show an increase in disease progression post initiation[126–127]. Furthermore, studies that have considered plasma folate levels have been inconsistent; most recently, analysis of data from the European Prospective Investigation in Cancer cohort showed no link with CRC and no effect of methylenetetrahydrofolate reductase polymorphisms.

Consumption of vegetables leads to the intake of a wide range of phytochemicals with known biological functions that are not actually considered as essential nutrients. Two large groups of such phytochemicals are the phenolic compounds and the glucosinolates. While glucosinolates are found only in brassica vegetables such as broccoli and cabbage and closely related plants in the *Brassicaceae* (*Cruciferae*) family such as mustard, watercress and

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rocket, phenolics are present in most plants. Within the brassica family of plants, glucosinolates are present at much higher concentrations than phenolics and so are often assumed to be the main bioactive non-nutrient phytochemical. The biological activity of glucosinolates from brassicas in relation to cancer have been extensively reviewed[128,129], and include induction of drug metabolising enzymes and endogenous anti-oxidants, cell cycle arrest and induction of apoptosis[130,131]. There is also evidence that the isothiocyanate metabolites of glucosinolates can have epigenetic effects through changes in histone acetylation and protection against aberrant CGI methylation in a potentially beneficial manner[132–134]. Furthermore, it has recently been reported that one glucosinolate derivative, 3,3′-diindolylmethane, can modify inflammation in an animal model of colitis[135]. The epidemiological evidence for a protective effect of brassicas is weak, but this may be related to similar limitations of such studies to those mentioned above for fish oils, namely the lack of precision of the questions asked in FFQ and the quality of information in food databases. Furthermore, the putative protective effects would appear to be dependent on the genotype of the individual[136].

There is relatively little epidemiological evidence to specifically link polyphenols to CRC prevention, although both soya and tea consumption have been suggested as being protective. A meta-analysis by Yan *et al*. reported a protective effect of dietary soya in women but not in men, which may be linked to the presence of high concentrations of the phytoestrogens genistein and diadzein in soya protein[137,138]. This effect may be similar to the protective effects of hormone replacement therapy. In contrast, a recent Chochrane systematic review investigating consumption of green tea and a range of health outcomes reported conflicting results in relation to CRC[139]. Chemopreventive attributes have been demonstrated, using animal models, for a range of foods high in particular polyphenols such as resveratrol from grapes or epigallocatechin gallate from tea[140], and a number of mechanisms proposed including induction of apoptosis, suppression of inflammation and reduced cell proliferation[141]. In addition, epigallocatechin gallate and curcumin have been shown to modify CGI methylation in a range of cell lines including colorectal cells[142–144]. However, isoflavones not only act as oestrogen receptor ligands but have been shown by us to modify oestrogen receptor expression in the colon[137]. Moreover, they probably change the expression of many genes, as a result of being ligands for nuclear receptor transcription factors in a manner that still requires clarification[145].

Cereals

The WCRF review suggests that there is no good evidence that the consumption of grain is specifically protective in relation to CRC[9] although, as mentioned earlier, fibre is considered protective and cereals, in particular whole grains, are an excellent source of fibre. Cereals may also be a source of phytoestrogens, lignins and micronutrients, particularly selenium as discussed earlier. Since the publication of the WCRF report three cohort studies have specifically reported on cereal grain intake. Schatzkin *et al*. reported a modest protective effect in the NIH-AARP study[146], while Nomura and colleagues found no effect in a multi-ethnic study also from the USA[147]. Furthermore Egeberg and colleagues investigating a European population found a modest protective effect of whole grain consumption in men but not in women[148]. A more recent meta-analysis of cohort studies[149] has reported a modest protective effect but overall the evidence is rather inconsistent.

Summary

The most compelling evidence that food intake may impact on the risk of CRC does not relate to any single nutrient or food, but suggests instead the importance of an excessive energy intake compared to energy expenditure. The underlying mechanisms associated with excess food intake and obesity are likely to be similar to those better recognised in relation to metabolic syndrome, namely hyperinsulinaemia, chronic inflammation leading to increased cell proliferation, reduced DNA repair and apoptosis, and perhaps increased promoter methylation of related tumour suppressor genes. Very high intakes of meat are also associated with increased risk, but studies suggest that such levels may be rarely reached in UK populations. Epidemiological evidence supporting the effect of specific protective factors is generally less convincing, but consumption of some vegetables, dairy products, fibre and perhaps fish all look to be potentially protective. Proposed mechanisms of action generally focus on similar themes of immunomodulation, decreased cell proliferation and increased apoptosis, with a more recent emphasis on cell signalling, gene expression and epigenetic modification of these parameters. The current evidence therefore suggests that a significant proportion of cases of CRC could be prevented by maintaining BMI within the recommended range, exercising, being exposed to sunlight at levels consistent with the avoidance of sunburn, and consuming a diet rich in fibre, vegetables and perhaps dairy products and fish. However, an understanding of the underlying mechanisms of action at the cellular level still requires intensive research.

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