Obesity and Colorectal Cancer Risk: Impact of the Gut Microbiota and Weight-Loss Diets

Harry J. Flint* and R. John Wallace

Gut Health Division, Rowett Institute of Nutrition and Health, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK

Abstract: The link between obesity and colorectal cancer risk in man is well established. This review investigates the role that the intestinal microbial population plays in this link and the impact of weight-loss diets on colorectal cancer risk. Changes in the composition of the intestinal bacterial community have been implicated in contributing to obesity. The robustness of these claims is analysed here, along with the role of bacterial metabolism in colon cancer risk. Weight-loss diets, low in carbohydrate and high in protein and fat, present an additional hazard to individuals struggling with obesity. Intestinal bacteria ferment carbohydrates to products that are generally regarded as being beneficial to health and protective against cancer. Some commensal species also appear to suppress inflammation. On the other hand, when carbohydrate limits the growth of intestinal bacteria, protein is broken down and the amino acids released are fermented to products that are inflammatory and possibly carcinogenic. We advocate the inclusion of non-digestible but fermentable carbohydrate in weight-loss diets to avoid these problems. High-fat diets enhance the escape of fats to reach the intestine, the implications of which are not fully understood. Even more fat reaches the intestine when dietary lipase inhibitors or fat-absorbing non-digestible dietary additives are used. Consequences for gut health of the increased fat concentration in the intestine seem to vary between individuals, the possible reasons for which are discussed here.

Keywords: Gut microbiota, weight loss diets, colorectal cancer, gut metabolism, diet composition, obesity, gut bacteria.

1. INTRODUCTION

The evidence that obesity is a pre-disposing factor in the aetiology of cancer is becoming overwhelming [1,2]. Gastrointestinal cancers feature significantly in the associated risk. This review investigates the role that the intestinal microbiota has in relation to obesity and colorectal cancer, and how the microbiota may be an intermediary in linking the two. Is the increased risk of colorectal cancer because obese people consume more hazardous foods? Or because more food bypasses the small intestine, then forming carcinogenic or inflammatory products? If the latter, what is the role of the intestinal microbiota? Weight-loss diets are vital to restore obese individuals to a healthy weight, but do they by themselves introduce a hazard to gut health, and how may this be avoided? These questions will be addressed from the standpoint of gut microbiology.

The human intestine is colonised throughout by communities of resident micro-organisms. The large intestine in particular harbours vast numbers of micro-organisms (>10^{11}/g contents) that gain energy largely from incompletely digested dietary components arriving from the small intestine. The full range of interactions between these complex communities and the host is still being uncovered, but gut micro-organisms are known to influence many aspects of nutrition, health and gut development. The impact of gut pathogens is obvious and well known, but it is increasingly recognised that resident commensal microorganisms play an important role in health and normal gut function. Anaerobic fermentation of available substrates in the large intestine yields organic acids that are taken up by the colonic mucosa and are utilised as energy sources by the host, contributing an estimated 10% of total energy from the diet [3]. Overall, microbial activity produces a vast array of metabolites that include vitamins, antioxidants, anti-inflammatories, toxins, carcinogens, promoters of apoptosis, receptor ligands, hormone analogues, regulators of gene expression and signalling molecules [4]. Furthermore, interactions between microbial cell components and the innate and adaptive immune system add a further layer of complexity with the potential to influence inflammation locally in the gut, as well as systemically.

The potential contribution of gut micro-organisms to the development of obesity and diabetes has been suggested by a number of recent papers [5-7] but although new information is emerging rapidly, the overall picture is far from clear. Here we discuss recent work relating to the role of colonic bacteria and their metabolites in obesity and obesity-related diseases such as diabetes and colorectal cancer.

2. CONTRIBUTION FROM MICROBIAL FERMENTATION OF DIETARY CARBOHYDRATES IN THE COLON TO ENERGY RETRIEVAL FROM THE DIET

'Non-digestible' or 'low-digestible' carbohydrates that survive to the large intestine include non-starch polysaccharides present in plant cell walls, resistant starch, inulin, and a range of oligosaccharides, sugars and sugar alcohols [8]. The host derives energy from these dietary components indirectly through absorption of the short chain fatty acid products of anaerobic microbial fermentation. Roberfroid [9] has suggested a calorific value of 1.5 kcal/g for non-digestible inulin and oligofructose, compared with 3.9 kcal/g for fructose directly absorbed in the small intestine. While the theoretical

*Address correspondence to this author at the Gut Health Divison, Rowett Institute of Nutrition and Health, Buckburn, Aberdeen, UK; Fax: +44 (0) 1224 716687; Tel: +44 (0)1224 716651; E-mail: h.flint@rri.sari.ac.uk

1876-8237/10 2010 Bentham Open

energy supply to the host from SCFA is 70% of that from directly absorbed sugar, other factors including bacterial growth requirements reduce the energy recovered [9]. Replacement of readily digested carbohydrate by an equivalent amount of non-digestible carbohydrate in the diet therefore reduces the energy supplied to the host. As there is evidence that non-digestible carbohydrates contribute to satiety, they can therefore be helpful in achieving a calorie-controlled diet [10-12].

The three major SCFA products of bacterial fermentation in the colon have different fates and effects upon the host. Butyrate is the main energy source for the colonic epithelium, and is considered to have a protective role against colitis and colorectal cancer [13-15]. Propionate is gluconeogenic and has been associated with reducing cholesterol [16] whereas acetate contributes to lipogenesis. SCFA also interact with gut receptors, and potentially influence a wide range of functions including gut motility and inflammation [17,18]. Changes in the production rates of the major SCFA resulting from changes in diet composition therefore have the potential to profoundly influence host physiology [19-21].

While the great majority of cultured colonic bacteria can utilise soluble carbohydrates for growth, a more limited selection appear able to degrade polysaccharides present in insoluble food particles [22,23]. Leitch *et al.* [24] found relatives *Eubacterium rectale*, *Ruminococcus bromii* and *Bifidobacterium adolescentis* to be the main colonisers of resistant starch particles, and these same species were detected using stable isotope probing with labelled starch by Kovatcheva-Datchary *et al.* [25]. Many other species ferment smaller soluble carbohydrates that are derived from the diet or are released by primary polysaccharide degraders [26-29]. Interestingly, a higher proportion of the gram-negative *Bacteroidetes* 16S rRNA sequences were recently shown to be present in the liquid phase compared with insoluble fibre particles in human stool [30]. This suggests that this group mainly utilises soluble carbohydrates, which seems consistent with what we know about the organisation of its carbohydrate-utilising enzyme systems [27]. In contrast, members of the low % G+C gram-positive *Firmicutes* phylum, in particular a group of *Ruminococcus*-related organisms, showed a preferential association with the particulate phase [30]. There is evidence therefore that the major phylogenetic groups of colonic bacteria differ in their substrate preferences for soluble and insoluble dietary carbohydrates [30].

3. EVIDENCE FOR ALTERED GUT MICROBIOTA COMPOSITION IN OBESITY

The predominant groups of bacteria found in the large intestine and in faecal samples are obligate anaerobes belonging to the phyla *Bacteroidetes* and *Firmicutes* [31-33] with *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* also present. Butyrate-producing species belong to the *Firmicutes* [34,35] while *Bacteroidetes* are likely to contribute greatly to propionate production via succinate [19].

Ley *et al.* [36] reported that the fecal microbiota of genetically obese (*ob/ob*) mice showed higher % *Firmicutes* and lower % *Bacteroidetes* when compared with lean controls. The explanation for this might lie with altered gut transit, gut environment and substrate supply resulting from higher feed intakes in the obese animals. Gut pH for example is known to correlate with transit [37-39] and can affect the composition of the gut microbiota [40,41]. Alternatively, these changes might be the consequence of differences in host physiology, e.g. interactions with the immune system, associated with the obese state.

These studies also pose the question whether the composition of the gut microbiota plays a role in obesity, either by changing the recovery of energy from the diet or by altering host physiology. Turnbaugh *et al.* [6] inoculated germ free mice with fecal microbiota from either *ob/ob* or lean animals. Animals receiving microbiota from *ob/ob* animals showed greater fat deposition over a two week period than those receiving lean microbiota. Although feed intakes differed slightly, the difference was not statistically significant [6]. It was therefore proposed that the *ob/ob* derived microbiota were more effective in releasing energy from dietary residue and in driving lipogenesis than the lean-derived microbiota. Microbial fermentative activity is also reported to influence the production of fasting induced adipose factor, which may also account for changes in lipogenesis [42]. Based on these small animal studies, therefore, the obese state is proposed to influence gut microbiota composition, while the gut microbiota composition is also proposed to be a factor contributing to the obese state.

In a study on twelve obese human subjects, Ley *et al.* [5] suggested that such effects might be important in human obesity. Crucially it was claimed that the % of total bacteria represented by *Bacteroidetes* in fecal samples from obese subjects was far lower (at around 2% of total bacteria) than in lean controls (at around 25% of total bacteria). Furthermore low carbohydrate or low fat diets resulting in weight loss appeared to result in a gradual recovery of the % *Bacteroidetes* over a 52 week period. These findings have not been corroborated, however, in studies published subsequently. Duncan *et al.* [43, 44] found no significant BMI-related changes in % *Bacteroidetes* and *Firmicutes* in fecal samples among 47 subjects whose BMI ranged from 19 to 43. These studies employed both FISH microscopy and real-time PCR to examine the microbiota composition of freshly recovered fecal samples without storage. Detection of the *Bacteroides*-related bacteria by Duncan *et al.* [43, 44] by FISH microscopy relied on the Bac303 probe that recognises the great majority, although not 100%, of representatives of the *Bacteroidetes* phylum [45]. Subsequent studies using 16S rRNA sequence analysis have also failed to detect a lower % *Bacteroidetes* in obese compared with non-obese subjects [46, 47]. Indeed a recent study by Schwiertz *et al.* [48] that examined 98 individuals detected the same numbers of *Bacteroides*, and the same total bacterial cell counts, by FISH microscopy in faecal samples from obese and lean volunteers. Based on median proportions, however, the *Firmicutes* to *Bacteroidetes* ratio was somewhat higher in lean (BMI<25) compared to overweight (BMI>25<30) or obese (BMI>30) subjects [48] which is the opposite of the difference reported by Ley *et al.* [5]. Interestingly these changes in bacterial phyla correlated with higher total SCFA, and a higher proportion of propionate, in faecal samples from the obese compared with the lean group [48]. Zhang *et al.* [46] noted that their three obese subjects harboured

methanogenic archaea, whereas their three lean subjects did not. Since methane-excretors represent around 50% of the human population [49], much larger subject numbers are clearly required to assess whether there is any statistically significant association between obesity and the possession of methanogens. Indeed Schwiertz *et al.* [48] reported a significantly lower incidence of carriage, and also lower populations, of methanogens belonging to the genus *Methanobrevibacter* in obese compared with lean individuals.

It is not clear at present whether these contrasting findings from different studies on the faecal microbiota of obese subjects reflect technical differences in storage procedures or in methods of microbial community analysis, or differences in diet composition or subject groups. We can conclude, however, that dramatic shifts in microbial community composition are not an invariable consequence of the obese state in humans. The more subtle changes suggested by the work of Schwiertz *et al.* [48] seem most likely to be related to differences in dietary intake between lean and obese individuals. The influence of weight loss diets on the colonic microbiota has been clearly demonstrated [43, 44] and is considered below.

4. CONSEQUENCES OF REDUCED CARBOHYDRATE WEIGHT-LOSS DIETS FOR GUT MICROBIOTA COMPOSITION AND METABOLISM

Diets that are low or very low in carbohydrates, but relatively high in protein, offer an important approach for achieving weight loss in overweight individuals. Johnstone *et al.* [50] designed medium- and low-carbohydrate, high protein weight loss diets, producing non-ketogenic and ketogenic conditions respectively, and showed that both diets resulted in significant weight loss when provided *ad libitum* to male volunteers over four week periods. The low carbohydrate diet however entailed the almost complete elimination of starch, and a fourfold reduction in fibre (non-starch polysaccharides). This diet had a major effect on fermentative activity in the large intestine, reducing total fecal short chain fatty acids two-fold and butyrate four-fold [43] (Fig. (**1**)). Analysis of faecal bacterial populations showed that a major group of butyrate producing bacteria, those related to *Eubacterium rectale* and *Roseburia spp.*, became reduced fourfold in the low carbohydrate diet, correlating with the reduction in butyrate. Since the study involved a cross-over design it was possible to show that the bacterial changes were related to the dietary shift rather than to weight loss [44]. Another recent study involving 91 overweight or obese volunteers also reported significant reductions in faecal SCFA, and especially butyrate, on low carbohydrate weight loss diets [51]. The disproportionate reduction in faecal butyrate concentration with reduced carbohydrate intake in these studies implies reduced butyrate production rates in the colon. Since there is persuasive evidence that butyrate plays a role in protection against colorectal cancer [14,15] adoption of reduced carbohydrate diets in the long term may increase the risk of colorectal cancer. The potential additional consequences from increased protein and fat intake in such weight loss diets for colorectal cancer risk will be considered further below

Ley *et al.* [5] reported longer term changes (over 52 weeks) in the faecal bacterial community of obese subjects during weight loss resulting from low fat or low carbohydrate diets. This study reported a progressive increase in % *Bacteroidetes* on weight loss diets that was apparently related to weight loss. As noted earlier, however, the initial

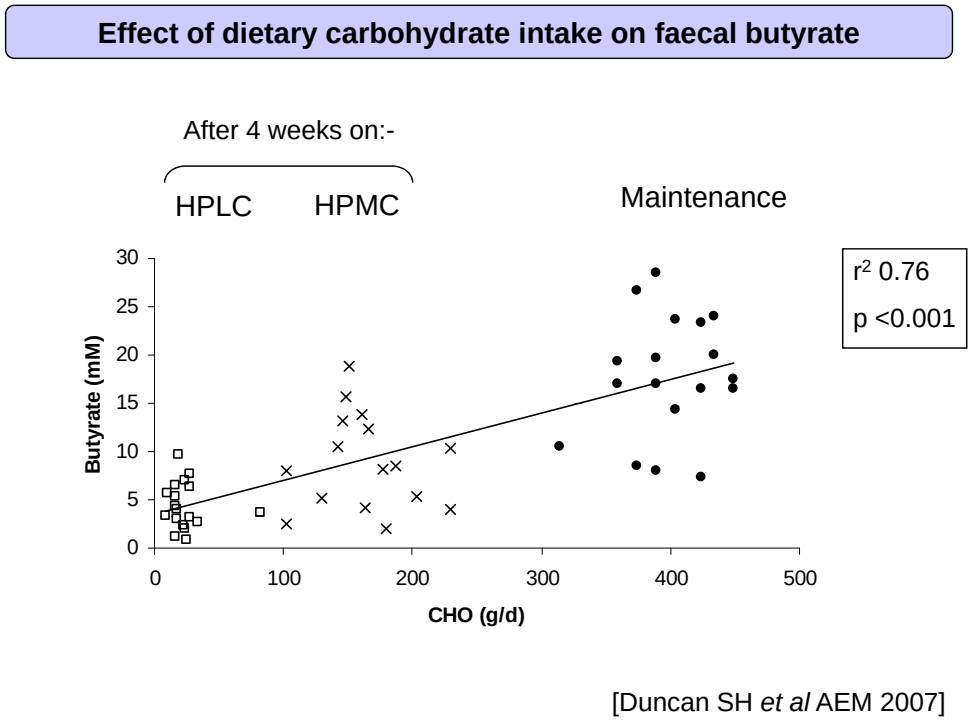

Fig. (1). Effect of reduced carbohydrate intake (M = weight maintenance diet, HPMC = medium carbohydrate weight loss diet, HPLC = low carbohydrate weight loss diet) upon faecal butyrate concentration in obese male volunteers.

values reported for % *Bacteroidetes* in the twelve obese subjects in this study were far lower than have been reported elsewhere.

5. IMPACT OF HIGH PROTEIN INTAKES UPON GUT METABOLISM

One of the commonest dietary strategies to help the obese to lose weight is to restrict the intake of carbohydrate. The foods consumed thus become high in protein and fat. The protein component is believed to have important effects on satiety [50]. A side effect of this type of diet may be that it is harmful to gut health, a risk that will be discussed in this and the next section. The consumption of high-protein diets is often associated with the aetiology of colonic cancers. The consumption of meat is usually cited as a cause of this correlation, partly due to the formation of toxic products in the stomach and intestine. These products are formed mostly by the action of intestinal bacteria. The way that meat is cooked is an important factor: cooking itself can induce the formation of carcinogenic compounds, but also over-cooking can lead to the passage of more protein to the large intestine, where it is broken down by bacterial proteases. Numerous products of the subsequent metabolism of amino acids by intestinal bacteria have been suggested to be toxic. The bacteria responsible for protein metabolism are considered to be *Bacteroides* and related genera. Here we review the evidence for these assertions, and attempt to identify areas of knowledge that are weak and need to be improved in order to understand how high protein intake affects gut health, including interactions with other constituents of the diet.

The epidemiological evidence linking high protein consumption, particularly meat, is much less clear than is sometimes implied. COMA's [52] report concluded, regarding the relation between colorectal cancer and meat consumption: "There is inconsistent evidence from cohort studies and weakly consistent evidence from case-control studies of an effect of total meat consumption on risk of colorectal cancer. There is moderately consistent evidence from cohort studies of a positive association between the consumption of red or processed meat and the risk of colorectal cancer with the higher scoring studies tending to find a significant effect of increased risk although the strength of the association is small." The World Cancer Research Fund/American Institute for Cancer Research [53] made a similar report: "The epidemiological evidence for an association of protein with colorectal cancer is inconsistent." Significantly, they added: "The data are not fully separable from data on calories and fat; no judgement is possible." Other confounding factors, including physical activity [54], consumption of food and vegetables – with their intrinsic beneficial effects – are closely linked as lifestyle parameters. Hill [55] concluded that the evidence was so weak that meat consumption should be positively encouraged for the other nutritional benefits that they provide. A more recent review [56] even concluded that the high-protein, high-fat Atkins diet might actually help reduce the risk of colorectal cancer. The overall picture can be seen in a study of half a million people which concluded that there was indeed an increased mortality associated with red and processed meat consumption, partly due to cancer [57].

There is, on the other hand, abundant evidence that would support the view that the cooking, digestion and metabolism of protein, particularly in the form of red meat, leads to the formation of potentially mutagenic or genotoxic compounds in the gastrointestinal tract. The method of cooking meat may be a contributory factor. Charring of meat during cooking, e.g. by barbecue, forms heterocyclic aromatic amines (HAA), including 2-amino-1-methyl phenylimidazo (4,5-*b*)pyridine, 2-amino-2-amino-3-methylimidazo [4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline (MeIQx). HAA are highly mutagenic and are considered to be initiators of the carcinogenesis sequence. Activation occurs by hepatic *N*-oxidation, then *O*-acetylation may occur in other tissues [58], yielding more potent carcinogens. Norat and Riboli [59] conducted a survey of available epidemiological evidence from 32 case-control and 13 cohort studies: while the confounding factors were bewilderingly complex, their conclusion was that the consumption of meat led to a modest increase in risk of colorectal cancer; the relation between methods of cooking and colorectal cancer incidence were not consistent and the evidence was not conclusive. The review of epidemiological evidence by Cross and Sinha [60] provided a comprehensive analysis of cooking, red meat and colon cancer; their conclusions were slightly stronger in favour of a link. Most recently, Ferrucci *et al.* [61] found that, in asymptomatic women undergoing colonoscopy, colorectal adenomas were associated with high intake of red meat, pan-fried meat, and the heterocyclic amine, MeIQx. The intestinal microbiota appears to be important in modulating the effects of HAA [62]. DNA damage caused by IQ in germ-free rats was 3-5 times less than in conventional animals [63]. HAA are converted by human intestinal microbiota to direct-acting genotoxins [64-66] and detoxification products of HAA formed in the liver are reactivated in the gut by bacterial enzymes [67,68]. In contrast to this apparent activation by bacteria, fermented dairy products [69] and lactic acid bacteria [70] have apparent antimutagenic properties that may be protective against HAA toxicity. The mechanism was suggested to be one whereby the bacteria bound the toxic HAA, thus preventing their absorption from the gut [69]. The reader is directed to Cross and Sinha [60] for a more complete review of HAA and colon cancer.

A simpler effect of over-cooking might be that over-cooking, particularly ground or minced meat (usually red meat), causes protein to resist digestion, leading to the passage of more protein to the colon and its subsequent digestion by intestinal bacteria. There, the release of high amounts of amino acids may lead to the production of potentially hazardous products, as described below.

The other class of compounds derived from meat/protein consumption and metabolism that appear in the gastrointestinal tract and that have been studied in most detail is *N*-nitroso compounds (NOC). The general formula for NOC and some examples are given in Fig. (**2**). NOC are DNA-alkylating agents that are mutagenic and potentially carcinogenic [71]. NOC occur in some foods, such as cured meats, sausages and smoked fish. Knekt *et al.* [72] found that consumption of these foods gave a two-fold increase in colorectal cancer risk. The genotoxicity of faecal water and its NOC content both increase markedly with diets high in

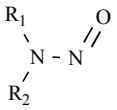

Fig. (2). The general formula of *N*-nitroso compounds. R_1 and R_2 can be the same (CH_3 as in *N*-nitrosodimethylamine) or different, and can be alkyl, aliphatic or other organic derivatives. R_1 and R_2 can combine in a ring system, as in *N*-nitrosoproline. See Mirvish [175].

protein and low in carbohydrate [73,74]. Most problems associated with NOC arise from their endogenous formation in the digestive tract. NOC are formed both in the stomach and in the large intestine. In the acid conditions of the stomach, nitrite, which is derived from dietary nitrate, reacts with secondary amines to form NOC. Red meat rather than white meat stimulates endogenous intestinal *N*-nitrosation [75,76]. The haem present in red meats enhances the acid-catalysed reaction in the stomach. Haem becomes nitrosylated and then becomes a nitrosating agent, reacting with a variety of diet-derived constitutents to form NOC and others such as nitrosothiols. Haem alone, supplied as an 8 mg dietary supplement, caused an increase in faecal NOC in man [77]. Thus, the formation of *N*-nitroso compounds seems to begin with a chemical reaction. Subsequent bacterial activity in the colon appears to modify the nitroso compounds of gastric origin. It is believed that the mixture of amines found in gut contents, which include polyamines, histamine, piperidine, tyramine and 2-phenylethylamine [78,79], may be precursors to carcinogenic NOC [80]. As with HAA, lactic acid bacteria may be beneficial in protecting against the genotoxic effects of NOC [81]. Some results are confusing, however. Massey *et al.* [82] found that germ-free rats did not produce NOC in their faeces, implying that the intestinal microbiota are essential for NOC to appear in the colon and faeces. On the other hand, the composition of NOC in ileal contents from ileostomy patients did not appear to be different from that of faeces [83], implying perhaps a minor role for bacterial metabolism in NOC formation and any associated mutagenic or genotoxic hazard. Plant porphyrins inhibit the nitrosation of haem [84]. Since porphyrins are abundant in plants, in chlorophyll, for example, and are structural analogues of haem, one of the beneficial effects of consuming fruit and vegetables may be to lower the formation of NOC and nitro compounds in general in the stomach.

In spite of the attention given to NOC, and previous claims about relations between NOC and genotoxicity and/or cancer [85], in a study of 11 human volunteers, Cross *et al.* [86] failed to find a correlation between total faecal water *N*-nitroso compounds and genotoxicity as measured by the comet assay.

Other compounds derived from protein breakdown have been implicated in colonic cancer [80]. Ammonia results from the deamination of amino acids. Faecal ammonia concentrations increased in response to increased meat in the diet [77,87]. Ammonia has been shown, perhaps surprisingly, to be damaging to intestinal cells at concentrations of 5-10 mM [88], which is similar to colonic concentrations. Hydrogen sulfide is produced by intestinal bacteria from dietary sulfate [89] and also the metabolism of the sulfur-containing amino acids, cysteine and methionine, in the colon [90]. Dietary protein from meat is an important substrate for sulfide generation by bacteria in the human large intestine [91]. Concentrations are relatively low, at 0.3 to 3.4 mM, in faeces [91-93]. Sulfide is an irritant and has been shown to interfere with tissue metabolism in a manner that would make the cells more vulnerable to carcinogenesis. Moore *et al.* [92] found inhibition of butyrate oxidation caused by sulfide, and Christl *et al.* [93] showed that low concentrations of sulfide significantly increased cell proliferation rates and other changes normally seen in ulcerative colitis. A direct effect of sulfide on carcinogenesis seemed improbable until it was demonstrated by Attene-Ramos *et al.* [94] that sulfide concentrations as low as 1 mM caused DNA damage in an *in vitro* system. The damage was only evident when cellular DNA repair mechanisms had been inactivated by hydroxyurea and 1-β-arabinofuranosylcytosine. The chemical nature of the damage indicated a free-radical mediated effect. Thus, sulfide must be considered as a potential genotoxin in the colon. Aromatic amino acids form various phenolic compounds as the result of intestinal metabolism. These include *p*-cresol, phenylpropionate, phenol, indole and phenylacetate [95]. The role of these compounds in carcinogenesis is for the most part unclear. Phenol reacts with nitrite to form the mutagen, diazoquinone [96]. Indole and other tryptophan metabolites have been linked to cancer [97], though the mechanism seems to be as a promoter rather than as a direct carcinogen [98].

The microbiology associated with the formation of hazardous, perhaps carcinogenic, products from protein in the gut is rather out-of-date. Moore and Moore [99] used traditional anaerobic culture techniques to show that high numbers of *Bacteroides* spp. were associated with patients at risk for colon cancer. *Bacteroides vulgatus* was most strongly associated with high-risk groups of polyp patients and Japanese Hawaiians. Although *B. vulgatus* did not appear to be proteolytic from Tannock's [100] survey of mouse isolates, nor did *B. fragilis*, the proteolytic properties of the latter species have subsequently been documented in some detail [101,102]. Amine production appeared from most-probable-number estimates to be a property of the great majority of the intestinal microbiota [103], but the bacteria were not identified. Many pure cultures were aminogenic, but no representatives of *Roseburia* or *Faecalibacterium* – species that we now know to be among the predominant ones in the 'core' bacterial community of the colon [104] – were tested. Amino acid fermenters were enumerated in a comprehensive study by Smith and Macfarlane [105]. Numbers were high, with most isolates being *Firmicutes*. However, the methodology would not have resulted in the cultivation of the non-saccharolytic 'hyper-ammonia-producing' bacteria that have been found to be so important in ammonia production in the rumen [106,107]. Other key microorganisms that participate in key aspects of intestinal metabolism include sulfate-reducing bacteria (SRB) and methanogens. Although an inverse relation between methanogenesis and sulfate reduction, due to a competition for hydrogen, was reported in some studies [108,109], Florin [110] reported compelling evidence to the contrary. SRB are

ubiquitous in the bowel [111], which is not consistent with the ideas of Gibson *et al.* [108,109]. The relations between diet, different human subjects and their intestinal bacterial community studies should now be probed using modern sequencing-based methods, which have been used successfully to characterise the gut microbiota in other areas of health [26]. For example, *Firmicutes* are under-represented in cultural isolations, probably because they are more difficult to grow than some other species; molecular microbial ecology eliminates that cultural bias.

Although diet has a major part to play, protein will always be present in the gut, whether from endogenous secretions of from intestinal bacterial themselves. Many important issues remain with protein nutrition, both fundamentally and in relation to gut health and obesity:

- Protein passing to the intestine threatens gut health. The method of cooking protein affects its digestibility. Thus, the method of food preparation must be crucial in weight-loss diets.
- The type of protein is important too. Red meat is a high-protein food with many nutritional virtues, but its haem content causes concern. It is clear that plant porphyrins inhibit the gastric reactions that convert haem to hazardous N-nitroso compounds. Should high-protein weight-loss diets rich in animal protein routinely be amended by the addition of chlorophyll-rich vegetables?
- The intestinal microbiota from vegetarians was more protective against the toxic effects of IQ than that of meat eaters [112]. Are there key protective effects of the microbiota that we can enhance using appropriate dietary ingredients?
- Obesity tends to increase with age. The genotoxicity of faecal waters increases with age [86]. Are the two linked? We also know that age has a major influence on the predominant bacteria present in the colon [113], but how are these changes linked to genotoxicity?
- Faecal water genotoxicity was positively associated with faecal nitrogen concentration [114]. As most faecal nitrogen is of bacterial origin, is bacterial load important, and why?
- Fecapentaenes are mutagenic products of intestinal bacterial metabolism [115,116]. The origin of their precursor is uncertain, but it seems that vegetarians excrete more fecapentaenes than omnivores [117]. A survey of 718 faecal samples indicated that 50% of samples that were mutagenic contained higher concentrations of fecapentaenes [118]. In view of their possible importance, it is very odd that no research has been carried out on fecapentaenes in recent years.

The solution to many of the problems associated with protein breakdown products in the colon is for the intestinal bacteria to assimilate these products during growth. If fermentable carbohydrate is available, bacteria will assimilate ammonia and amino acids rather than ferment amino acids to generate ATP. Thus, non-digestible but fermentable carbohydrate not only provides beneficial effects to the colon in the form of butyric acid, it removes potentially hazardous protein breakdown products too.

6. IMPACT OF HIGH FAT INTAKES UPON GUT METABOLISM

The average Western adult consumes about 100 g of triacylglycerols (TAG) and 4–8 g of phospholipid each day [119]. The efficiency of absorption in the small intestine is generally high (76–99%, depending on the TAG ingested [120]. Dietary medium-chain fatty acids are absorbed completely from the upper GI tract and do not appear in significant quantities in the large intestine [121]. Unsaturated FAs are much more readily absorbed than saturated fatty acids [122]. In addition, the specific distribution of fatty acids as glycerol esters in TAG influences their absorption [123]. Therefore, the nature of the lipid entering the small intestine, and its absorption through the stomach wall, directly affects the amount and type of lipids entering the large intestine.

Lipid passage to the large intestine is increased under conditions where there is a lack of lipase, or lipase is inhibited, or bile salts are absent. Under these conditions, the flow of lipids to the colon can be significant, the effects of which seem to vary from individual to individual. Flows of up to 134 g were observed in individuals suffering from cystic fibrosis, or a pancreatic deficiency, or those who had undergone GI resectioning [124,125]. Orlistat, an anti-obesity drug that acts by inhibiting GI lipases, decreased fat absorption by approximately 30% and led to increased faecal fat [126]. Other treatments for obesity include the absorption of fats onto matrixes that decrease absorption, such as chitosan [127]. The obesity epidemic will inevitably lead to more use of these and similar treatments, and the consequent effects on gut metabolism and health. Common side-effects of increased lipid entering the colon include bloating, GI discomfort, faecal incontinence and urgency, increased steatorrhoea, oily spotting and increased defecation [128], and losses of fat-soluble vitamins [129]. The increased flow of dietary lipids entering the large intestine may also lead to an increase in faecal bile acid content, with its own health implications [130]. Fatty acids released by host and bacterial lipases may also have a detergent effect that damages the mucosa, predisposing to tumor development [131].

Large differences seem to occur naturally between individuals in the flow of lipid to the intestine, for which there is presently no explanation. DAG concentrations in ten faecal samples from healthy donors examined by Morotomi *et al.* [132] indicated that the amount of faecal DAG varied considerably (>27-fold) among the individuals, but that there was little variation (<4-fold) in samples taken from one donor over a period of 115 days. The study of Vulevic *et al.* [133] using batch culture fermentations (at pH 6.8, 7.5 and 8.5) to monitor DAG production by faecal bacteria also demonstrated inter- and intraindividual variation in DAG production. One might speculate that several factors will influence lipid flux, including the dietary lipids consumed, the lipid-metabolising activity of the GI microbiota and the activity of bile acids production. The first step in the metabolism of TAG and its constituent fatty acids, glycerol and glycerides is lipolysis. In spite of its importance to gut health, there seems to have been no systematic study of lipolysis in the mixed intestinal microbiota or of lipase activity in individual bacterial species from the human intestine. This lack of knowledge about intestinal bacterial

lipase seems to be an important deficiency in our understanding of the human colon, especially with fat passage to the intestine likely to be of increasing importance in coming years as we combat the global obesity problem.

The composition of fatty acids in human faeces differs greatly from the fatty acid composition of foods [134]. Partly, this may be due to differential absorption of fatty acids from the GI tract, and partly it is due to the metabolic activity of bacteria in the intestine. There are two main routes for fatty acid metabolism in the intestine – hydration, leading to the formation of hydroxy fatty acids (HFA) from monounsaturated fatty acids, and reduction (known as biohydrogenation) that leads ultimately to the formation of saturated fatty acids such as stearate and palmitate. The first evidence was that some HFA were present in faeces but not in the diet [134]. This work showed that hydroxystearic acid (HAS) was produced in significant amounts only from oleic and linoleic acids when incubated with human faeces. Thus, HSA were formed by hydration of the Δ^9 double bond in unsaturated FAs rather than by the oxidation of the saturated acid. Thomas [135] showed that many anaerobic bacteria, including some colonic species, carried out hydration of oleic acid to HSA. *Clostridium perfringens* was the most active species. Pearson [136] incubated 228 strains of intestinal bacteria from five genera with oleic acid, and found that 103 strains formed HSA. Thus, HSA formation from unsaturated fatty acids is a widespread function among intestinal bacteria. Other HFA may be formed as intermediates in the metabolism of PUFA. Devillard *et al.* [137] found that some *Roseburia* strains formed a hydroxy fatty acid, identified as a 10-hydroxy,*cis*-12-18:1. Strains of *Lactobacillus*, *Lactococcus*, *Eubacterium*, *Propionibacterium*, *Bifidobacterium* and *Faecalibacterium* produced the same HFA, although to a lesser extent than most *Roseburia* strains. The 10-hydroxy,*cis*-12-18:1 was converted by the mixed intestinal flora transiently to *cis*-9,*trans*-11-18:2 then to *trans*-11-18:1 [137].

The other main route of fatty acid metabolism, biohydrogenation, as had been found in the rumen [138] and in mixed intestinal bacteria from rats [139], was not confirmed in the human intestine until the work of Howard and Henderson [140]. The biohydrogenation of linoleic acid (LA, *cis*-9,*cis*-12-18:2), occurs mainly by conversion to the conjugated dienoic acid, rumenic acid (RA, *cis*-9,*trans*-11-18:2; a conjugated linoleic acid or CLA), which is then hydrogenated to vaccenic acid (VA, *trans*-11-18:1), then to stearic acid (18:0) (Fig. (**3**)). α-Linolenic acid (LNA, *cis*-9,*cis*-12,*cis*-15-18:3) is also metabolised rapidly by the faecal microbiota, a similar metabolic route forming a mixture of 18:3 and 18:2 isomers [140]. *Butyrivibrio fibrisolvens*, *Roseburia inulinivorans* and *Roseburia hominis* produced VA rapidly from LA, presumably via RA. The bacteria responsible for the conversion of vaccenic acid to stearic acid in the human colon are unknown. Identification of these bacteria may be difficult. Experience with ruminal bacteria [141,142] demonstrated that the bacteria responsible, *Butyrivibrio proteoclasticus*, are extremely sensitive to the toxic effects of unsaturated fatty acids. Growth of the bacteria was necessary for stearate formation to occur, but, as LA was toxic at concentrations as low as 5 μg/ml, growth was inhibited by the substrate. qPCR based on 16S rRNA gene sequences indicated that *B. proteoclasticus* was present only at very low numbers in human faeces (S. Muetzel, unpublished results), indicating that, as with the earlier steps in the pathway, the species responsible for stearate formation in the two gut ecosystems might be different.

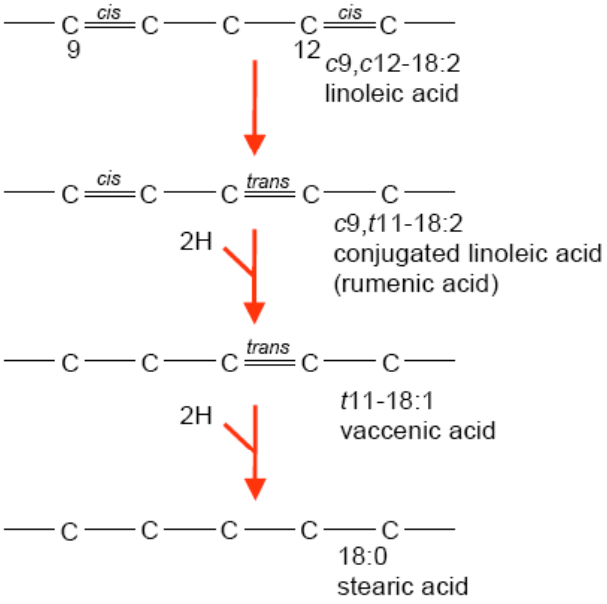

Fig. (3). Metabolic pathway of the conversion of linoleic acid to stearic acid by colonic bacteria

HSA concentrations in faeces increase as a consequence of various clinical conditions [143]. Patients with ileal disease, ileal resections or small intestinal bacterial colonisation all had more than 5% HSA in their faeces. Whether HSA has any implications for health other than a consequence of disease or abnormality is less clear. HSA is chemically similar to ricinoleic acid (12-hydroxy-*cis*-9-octadecenoic acid), the major FA in castor oil, a known cathartic. The presence of HSA in human feces led to the suggestion that it contributes to the diarrhoea frequently associated with steatorrhoea [134]. Wiggins *et al.* [143] demonstrated that, in general, the percentage of HSA in faeces increased as the faecal fat output rose. In individuals without steatorrhoea and excreting 20 g fat/day, less than 5% of the faecal fat comprised HSA, while in individuals with steatorrhoea (and, consequently, excreting more fat), between 6 and 23% of the faecal fat comprised HSA. However, no correlation was found between HSA levels and steatorrhoea in the majority of cases.

In addition to converting VA to RA, Δ^9-desaturase in host tissues converts stearic acid to oleic acid (*cis*-9-18:1). Oleic and stearic acids are known to decrease plasma cholesterol concentrations [144], so biohydrogenating C-18 PUFA and MUFA might be considered in some ways beneficial to health. However, it is the MUFA and PUFA themselves that many view to be more beneficial to health.

RA and VA are considered to have possibly potent effects on human health. *In vitro* and *in vivo* animal studies have suggested that RA has anti-carcinogenic, anti-atherosclerotic and immune-modulating effects, as well as favourable influences on body composition, blood lipids, liver metabolism and insulin sensitivity [145-147]. As yet, there is insufficient evidence to evaluate the impact of RA in humans [148]. VA may arguably be considered to be

functionally equivalent to RA. VA is converted to RA via the host's Δ^9-desaturase, an enzyme present in the intestine and liver [149-151]. VA suppresses the growth and affect cellular responses of human mammary and colon cancer cell lines through its conversion to RA by Δ^9-desaturase in these cells [152]. Therefore, increasing RA and VA supply may have potential benefits on health.

One possible delivery mechanism to increase RA and VA availability to the host is to utilise the biohydrogenating ability of intestinal bacteria. RA formed in the intestine might be absorbed and contribute to systemic RA. However, experiments with germ-free rats inoculated with a human faecal microbiota and fed a diet enriched with sunflower-seed oil indicated that no benefit accrued in terms of tissue concentrations of CLA [153]. Kamlage *et al.* [154] found that glucose inhibited CLA formation by mixed faecal microorganisms, and speculated that this may be the reason for the earlier result. It is also possible that CLA was not absorbed from the intestine. Nevertheless, even if CLA absorption from the intestine is minimal, there may be *in situ* benefits from intestinal CLA production. In mouse models of inflammatory bowel disease, CLA were shown to exhibit anti-inflammatory properties via endoplasmic and nuclear mechanisms [155,156]. Further studies demonstrated that RA exerted anti-carcinogenic activity in the rat colon [157] and exhibited anti-proliferative properties on the growth of human colon cancer cells *in vitro* [158]. Therefore, mechanisms by which RA might be delivered to and formed in the intestine have important implications for long-term human gut health.

In an effort to increase the amount of RA available to humans, probiotic bacteria have been suggested as a possible method for increasing CLA in the human intestine [159]. The reasoning in this approach is that ingested bacteria could use dietary LA to produce CLA. *Lactobacillus*, *Propionibacterium* and *Bifidobacterium* species are known to be involved in the formation of CLA from LA [136]. In the study of Coakley *et al.* [159], strains of *Bifidobacterium breve* and *Bifidobacterium lactis* were identified that were able to convert LA to CLA highly efficiently. Bifidobacteria have long been used as probiotics in human foods and they have been shown to elicit specific health benefits upon the host [160,161]. Therefore, the identification of probiotic bifidobacteria with the ability to synthesise RA may offer novel opportunities in the rational design of improved health-promoting functional foods [159].

There seems little doubt that the consumption of diets high in fat is a risk factor for colonic cancer [130]. While in some studies [74] it is not possible to separate the effects of fat and high protein, often meat, there is good reason to suppose that a high-fat diet in general predisposes to colonic disease. The effects may be direct, as with the examples already cited, but they may be indirect, via the passage of bile acids to the colon. Faecal bile acid concentrations are higher in populations with a high incidence of colon cancer [162-164]. Bile acids are converted to secondary bile acids by the colonic microbiota. Secondary bile acids are carcinogenic [130,165]. Thus, if high dietary fat leads to the escape of sequestered lipids to the intestine, the associated bile acids may be released by bacterial bile salt hydrolases and form their toxic products [166]. Faecal water from people receiving a high-fat diet [167] contained much higher concentrations of secondary bile acids than samples from individuals on a normal diet [130,168].

Thus, the high fat component of common weight-loss diets could be hazardous for gut health, but the effects would be expected to vary according to the chemistry of the lipids. Vegetable oils with a high linoleic acid content, such as sunflower oil, could be anti-inflammatory if delivered in the correct amounts.

7. INFLUENCE OF GUT MICROBES UPON THE DEVELOPMENT OF DIABETES

Recent studies using mice have suggested the hypothesis that circulating lipopolysaccharide (LPS) originating from Gram-negative gut bacteria may promote inflammation and the development of diabetes [169]. It is further proposed that high fat diets cause an increase in circulating LPS either because of increased gut permeability, or increased transport of LPS across the gut wall, in the presence of dietary fat [169-171]. Supplementation of a high fat diet that contained almost no fermentable carbohydrate with fructo-oligosaccharides reversed some of these effects at the same time as increasing the population of bifidobacteria detected in faeces [172]. Although it is suggested that this effect of FOS might be mediated through an influence of bifidobac-teria on intestinal permeability, this group accounted for only a small % of total bacteria. Thus it is not ruled out that other bacterial groups, or indeed an overall stimulation of microbial fermentation, might account for the beneficial effects of FOS in this case. There is also evidence from animal models for complex involvement of the gut microbiota in type 1 diabetes [173]. Human studies have indicated that diets supplemented with resistant starch may help to reduce insulin resistance, possibly as a result of the increased supply of fermentation acids [174].

If high fat diets do indeed promote LPS-driven inflammation, this could be an important factor in explaining the increased cancer risk that is associated with obesity, given the linkage between inflammation and cancer. On this hypothesis it would be the dietary intake associated with the obese state, rather than obesity itself, that increases cancer risk.

CONCLUSIONS

The involvement of the gut microbiota as a possible causative agent in human obesity and diabetes remains unclear despite some very interesting, high profile papers over the last few years. A number of new mechanisms and hypotheses have been suggested, however, that will continue to drive investigations forward in this area. The WCRF [54] report recognises obesity as a significant risk factor in colorectal cancer. The mechanistic reasons for this remain unclear. Either the obese state itself increases the risk of cancer initiation and growth, or the habitual dietary intake of obese individuals increases the risk of colorectal cancer. In the latter case, particularly, the gut microbiota may play a contributory role via the metabolic transformation of high intakes of dietary fat and protein to produce toxins and carcinogens, or as agents of inflammation. This applies even

if the species composition of the colonic microbiota is substantially unaltered in the obese state; increased microbial loads and metabolic outputs in the colon resulting from high dietary intakes, together with effects of dietary components (e.g. fats) on gut permeability, have the potential for considerable physiological impact on the obese host.

Studies conducted recently with obese human volunteers on high protein, reduced carbohydrate weight loss diets show that they result in multiple changes in large intestinal metabolism that would be predicted to increase the risk of colorectal cancer in the long term. Since obesity is considered to be an important factor in increasing colorectal cancer risk, as discussed above, it might be argued that the weight loss achieved through such short term diet change justifies the potential negative aspects of the diet. It appears desirable however to achieve weight loss and weight control regimes that do not compromise gut health, and this goal should be achievable at least partially by the judicious design of weight-loss diets, recognising the impact of different constituents of food on gut microbiology. The types of dietary carbohydrate need to be considered carefully so as to ensure that sufficient non-digestible but fermentable carbohydrate reaches the colon, and these should be a substantial component of weight-control diets. Any increase in dietary protein and fat intake needs to involve careful choice of ingredients and cooking methods, and meals with high meat content should be balanced with green vegetables.

ACKNOWLEDGEMENTS

The Rowett Institute of Nutrition and Health receives support from the Scottish Government Rural Environment Research and Analysis Directorate.

REFERENCES

[1] Vainio H, Bianchini F. IARC handbooks of cancer prevention. Volume 6: Weight control and physical activity. Lyon, France: IARC Press 2002.

[2] Polednak AP. Trends in incidence rates for obesity-associated cancers in the U.S. Cancer Detect Prev 2003; 27: 415-21.

[3] McNeil NI. The contribution of the large intestine to energy supplies in man. Am J Clin Nutr 1984; 39: 338-42.

[4] Holmes E, Wilson ID, Nicholson JK. Metabolic phenotyping in health and disease. Cell 2008; 134: 714-7.

[5] Ley RE, Turnbaugh PJ, Klein, S, *et al.* Microbial Ecol– human gut microbes associated with obesity. Nature 2006; 444: 1022-3.

[6] Turnbaugh PJ, Ley RE, Mahowald MA, *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444: 1027-31.

[7] Cani PD, Amar J, Iglesias MA, *et al.* Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56: 1761-72.

[8] Macfarlane GT, Gibson GR. Carbohydrate fermentation, energy transduction and gas metabolism in the human large intestine. In: Mackie RI, White BA, Eds. Gastrointestinal Microbiology. UK: Chapman and Hall London 1997; vol 1: pp. 269-318.

[9] Roberfroid MB. Calorific value of inulin and oligofructose. J Nutr 1999; 129: 1436S-7S.

[10] Nilsson AC, Ostman EM, Holst JJ, Bjorck IME. Including indigestible carbohydrates in the evening meal of healthy subjects improves glucose tolerance, lowers inflammatory markers, and increases satiety after a subsequent standardized breakfast. J Nutr 2008; 138: 732-9.

[11] Parnell JA, Weimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. Am J Clin Nutr 2009; 89: 1-9.

[12] Willis HJ, Eldridge AL, Beiseigel J, Thomas W, Slavin JL. Greater satiety response with resistant starch and corn bran in human subjects. Nutr Res 2009; 29: 100-5.

[13] Pryde SE, Duncan SH, Stewart CS, Hold GL, Flint HJ. The microbiology of butyrate formation in the human colon. FEMS Microbiol Lett 2002; 217: 133-9.

[14] Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer R-J. Review article: the role of butyrate on colonic function. Alimen Pharmacol Ther 2008; 27: 104-19.

[15] McIntyre AP, Gibson P, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a gut model. Gut 1993; 34: 386-91.

[16] Venter CS, Vorster HH, Cummings JH. Effects of dietary propionate on carbohydrate and lipid metabolism in man. Am J Clin Nutr 1989; 85: 543-9.

[17] Brown AJ, Goldsworthy SM, Barnes AA, *et al.* The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem 2003; 278: 11312-9.

[18] Tazoe H, Otomob Y, Kaji I, *et al.* Roles of short chain fatty acid receptors, GPR41 and GPR43 on colonic functions. J Physiol Pharmacol 2008; 59: 251-62.

[19] Louis P, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. J Appl Microbiol 2007; 102: 1197-208.

[20] Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and non-starch polysaccharides. Physiol Rev 2001; 81: 1031-64.

[21] Cummings JH, Macfarlane GT. The control and consequences of bacterial fermentation in the human colon. J Appl Bacteriol 1991; 70: 443-59.

[22] Robert C, Bernalier-Donadille A. The cellulolytic microflora of the human colon : evidence of microcrystalline cellulose-degrading bacteria in methane-excreting subjects. FEMS Microbiol Ecol 2003; 46: 81-9.

[23] Macfarlane GT, Englyst HN. Starch utilization by the human large intestinal microflora. J Appl Bacteriol 1986; 60: 195-201.

[24] Leitch ECM, Walker AW, Duncan SH, Holtrop G, Flint HJ. Selective colonization of insoluble substrates by human colonic bacteria. Environ Microbiol 2007; 72: 667-9.

[25] Kovatcheva-Datchary P, Egert M, Maathuis A, *et al.* Linking phylogenetic identities of bacteria to starch fermentation in an *in vitro* model of the large intestine by RNA-based stable isotope probing. Environ Microbiol 2009; 11: 914-26.

[26] Flint HJ, Duncan SH, Scott KP, Louis P. Interactions and competition within the microbial community of the human colon: links between diet and health. Environ Microbiol 2007; 9: 1101-11.

[27] Flint HJ, Bayer EA, Rincon MT, Lamed R, White BA. Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. Nat Rev Microbiol 2008 6: 121-31.

[28] Falony G, Viachou A, Verbrugge K, de Vuyst L. Cross-feeding between Bifidobacterium longum BB536 and acetate converting, butyrate-producing colon bacteria during growth on oligofructose. Appl Environ Microbiol 2006; 72: 7835-41.

[29] Belenguer A, Duncan SH, Calder AG, *et al.* Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. Appl Environ Microbiol 2006; 72: 3593-9.

[30] Walker AW, Duncan SH, Harmsen HJM, *et al.* The species composition of the human intestinal microbiota differs between particle- associated and liquid phase communities. Environ Microbiol 2008; 10: 3275-83.

[31] Eckburg PB, Bernstein CN, Purdom E, *et al.* Diversity of the human intestinal microbial flora. Science 2005; 308: 1635-8.

[32] Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ. Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiol Ecol 2002; 39: 33-39.

[33] Suau A, Bonnet R, Sutren M, *et al.* Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl Environ Microbiol 1999; 24: 4799-807.

[34] Barcenilla A, Pryde SE, Martin JC, *et al.* Phylogenetic relationships of butyrate-producing bacteria from the human gut. Appl Environ Microbiol 2000; 66: 1654-61.

[35] Louis P, Flint HJ. Diversity, metabolism and Microbial Ecol of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 2009; 294: 1-8.

[36] Ley RE, Backhed F, Turnbuagh P, *et al.* Obesity alters gut microbial ecology. Proc Natl Acad Sci USA 2005; 102: 11070-5.

[37] Lewis SJ, Heaton, KW. Increasing butyrate concentration in the distal colon by accelerating intestinal transit. Gut 1997; 41: 245-25.

[38] Stephen AM, Wiggins HS, Cummings JH. Effect of changing transit time on colonic microbial metabolism in man. Gut 1987; 28: 601-9.

[39] El Oufir L, Flourié B, Bruley Des Varannes S, *et al.* Relations between transit time, fermentation products, and hydrogen consuming flora in healthy humans. Gut 1996; 38: 870-7.

[40] Walker AW, Duncan SH, Leitch ECM, Child MW, Flint HJ. pH and peptide supply can radically alter bacterial populations and short chain fatty acid ratios within microbial communities from the human colon. Appl Environ Microbiol 2005; 71: 3692-700.

[41] Duncan SH, Louis P, Thomson J, Flint HJ. The role of pH in determining the species composition of the human colonic microbiota. Environ Microbiol 2009; 11: 2112-22

[42] Backhed F, Ding H, Wang T, *et al.* The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004; 101: 15718-23.

[43] Duncan SH, Belenguer A, Holtrop G, *et al.* Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. Appl Environ Microbiol 2007; 73: 1073-8.

[44] Duncan SH, Lobley GE, Holtrop G, *et al.* Human colonic microbiota associated with diet, obesity and weight loss. Intl J Obes 2008; 32: 1720-4.

[45] Manz W, Amann R, Ludwig W, *et al.* Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum Cytophaga-Flavobacter-Bacteroides in the natural environment. Microbiology 1996; 142: 1097-106.

[46] Zhang H, DiBaise JK, Zuccolo A, *et al.* Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA 2008; 106: 2365-70.

[47] Turnbaugh PJ, Hamaday M, Yatsunenko T, *et al.* A core microbiome in obese and lean twins. Nature 2009; 457: 480-4.

[48] Schwiertz A, Tars D, Schafer K, *et al.* Microbiota and SCFA in lean and overweight healthy subjects. Obesity 2010; 18(1): 190-5.

[49] Florin THF, Zhu G, Kirk KM, Martin NG. Shared and unique environmental factors determine the ecology of methanogens in human and rats. Am J Gastroenterol 2000; 95: 2872-9.

[50] Johnstone AM, Horgan GW, Murison SD, Bremner DM, Lobley GE. Effects of high-protein ketogenic diet on hunger, appetite, and weight loss in obese men feeding ad libitum. Am J Clin Nutr 2008; 87: 44-55.

[51] Brinkworth GD, Noakes M, Clifton PM, Bird AR. Comparative effects of very low carbohydrate, high fat and high carbohydrate, low-fat weight loss diets on bowel habit and faecal short chain fatty acids and bacterial populations. Br J Nutr 2009; 101: 1493-502.

[52] COMA (Committee on Medical Aspects of Food and Nutrition Policy). Nutritional aspects of the development of cancer: report of the working group on diet and cancer. London: The Stationery Office 1980.

[53] World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC, USA 1997.

[54] World Cancer Research Fund/American Institute for Cancer Research. Food, nutrition, physical activity, and the prevention of cancer. Washington, DC, USA 2007.

[55] Hill M. Meat, cancer and dietary advice to the public. Eur J Clin Nutr 2002; 56: S36-S41.

[56] Fleming ME, Sales KM, Winslet MC. Diet and colorectal cancer: implications for the obese and devotees of the Atkins diet. Colorectal Dis 2005; 7: 128-32.

[57] Sinha R, Cross AJ, Graubard BI, Leitzmann MF, Schatzkin A. Meat intake and mortality. A prospective study of over half a million people. Arch Int Med 2009; 169: 562-71.

[58] Minchin RF, Reeves PT, Teitel CH, *et al.* N-and O-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in COS-1 cells. Biochem Biophys Commun 1992; 185: 839-44.

[59] Norat T, Riboli E. Meat consumption and colorectal cancer: A review of epidemiologic evidence. Nutr Rev 2001; 59: 37-47.

[60] Cross AJ, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. Environ Mol Mut 2004; 44: 44-55.

[61] Ferrucci LM, Sinha R, Graubard BI, *et al.* Dietary meat intake in relation to colorectal adenoma in asymptomatic women. Am J Gastroenterol 2009; 104: 1231-40.

[62] Knasmuller S, Steinkellner H, Hirschl AM, Rabot S, Nobis EC, Kassie F. Impact of bacteria in dairy products and of the intestinal microflora on the genotoxic and carcinogenic effects of heterocyclic aromatic amines. Mut Res-Fund Mol Mech Mut 2001; 480: 129-38.

[63] Kassie F, Rabot S, Kundi M, Chabicovsky M, Qin HM, Knasmuller S. Intestinal microflora plays a crucial role in the genotoxicity of the cooked food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline (IQ). Carcinogenesis 2001; 22: 1721-5.

[64] Bashir M, Kingston DGI, Carman RJ, van Tassel RL, Wilkins TD. Anaerobic metabolism of 2-aminoimidazo-3-methyl-3H [4,5-f]quinoline (IQ) by human fecal flora. Mutat Res 1987; 190: 187-90.

[65] Carman RJ, van Tassel RL, Kingston DGI, Bashir M, Wilkins TD. Conversion of IQ, a dietary pyrolysis carcinogen to a direct acting mutagen by normal intestinal bacteria of humans. Mutat Res 1988; 206: 335-42.

[66] Rumney CR, Rowland IR, O'Neil K. Conversion of IQ to 7OHIQ by gut microflora. Nutr Cancer 1993; 191: 67-76.

[67] Aeschbacher HU, Turesky RJ. Mammalian cell mutagenicity and metabolism of heterocyclic amines. Mutat Res 1991; 259: 235-50.

[68] Hambly RJ, Rumney CJ, Fletcher ME, Rijken PJ, Rowland IR. Effect of high and low risk diets on gut microflora associated biomarkers of colon cancer in human flora associated rats. Nutr Cancer 1997; 27: 250-5.

[69] Tavan E, Cayuela C, Antoine JM, Trugnan G, Chaugier C, Cassand P. Effects of dairy products on heterocyclic aromatic amine-induced rat colon carcinogenesis. Carcinogenesis 2002; 23: 477-83.

[70] Tavan E, Cayuela C, Antoine JM, Cassand P. Antimutagenic activities of various lactic acid bacteria against food mutagens: heterocyclic amines. J Dairy Res 2002; 69: 335-41.

[71] Saffhill R, Margison GP, O'Connor PJ. Mechanisms of carcinogenesis induced by alkylating agents. Biochim Biophys Acta 1985; 823: 111-45.

[72] Knekt P, Jarvinen R, Dich J, Hakulinen T. Risk of colorectal and other gastro-intestinal cancers after exposure to nitrate, nitrite and N-nitroso compounds: A follow-up study. Int J Cancer 1999; 80: 852-6.

[73] Silvester KR, Bingham SA, Pollock JRA, Cummings JH, O'Neill IK. Effect of meat and resistant starch on fecal excretion of apparent N-nitroso compounds and ammonia from the human large bowel. Nutr Cancer - Int J 1997; 29: 13-23.

[74] Rieger MA, Parlesak A, Pool-Zobel BL, Rechkemmer G, Bode C. A diet high in fat and meat but low in dietary fibre increases the genotoxic potential of 'faecal water'. Carcinogenesis 1999; 20: 2311-6.

[75] Hughes R, Cross AJ, Pollock JRA, Bingham S. Dose-dependent effect of dietary meat on endogenous colonic N-nitrosation. Carcinogenesis 2001; 22: 199-202.

[76] Bingham SA, Hughes R, Cross AJ. Effect of white versus red meat on endogenous N-nitrosation in the human colon and further evidence of a dose response. J Nutr 2002; 132: 3522S-5S.

[77] Cross AJ, Pollock JRA, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. Cancer Res 2003; 63: 2358-60.

[78] Drasar BS, Hill MJ. Human intestinal flora. London: Academic Press 1974.

[79] Murray KE, Adams RF, Earl JW, Shaw KJ. Studies of the free faecal amines of infants with gastroenteritis and of healthy infants. Gut 1986; 27: 1173-80.

[80] Hughes R, Magee EA, Bingham S. Protein degradation in the large intestine: relevance to colorectal cancer. Curr Issue Intest Microbiol 2000; 1: 51-8.

[81] Pool-Zobel BL, Neudecker C, Domizlaff I, *et al.* Lactobacillus- and Bifidobacterium-mediated antigenotoxicity in the colon of rats. Nutr Cancer 1996; 26: 365-80.

[82] Massey RC, Key PE, Mallett AK, Rowland IR. An investigation of the endogenous formation of apparent total N-nitroso compounds in conventional microflora and germ-free rats. Food Chem Toxicol 1988; 26: 595-600.

[83] Lunn JC, Kuhnle G, Mai V, *et al.* The effect of haem in red and processed meat on the endogenous formation of N-nitroso compounds in the upper gastrointestinal tract. Carcinogenesis 2007; 28: 685-90.

[84] de Vogel J, Jonker-Termont DSML, van Lieshout EMM, Katan MB, van der Meer R. Green vegetables, red meat and colon cancer: chlorophyll prevents the cytotoxic and hyperproliferative effects of haem in rat colon. Carcinogenesis 2005; 26: 387-93.

[85] Bingham SA, Pignatelli B, Pollock JRA, *et al.* Does increased endogenous formation of N-nitroso compounds in the human colon explain the association between red meat and colon cancer? Carcinogenesis 1996; 17: 515-23.

[86] Cross AJ, Greetham HL, Pollock JR, Rowland IR, Bingham SA. Variability in fecal water genotoxicity, determined using the Comet assay, is independent of endogenous N-nitroso compound formation attributed to red meat consumption. Environ Mol Mut 2006; 47: 179-84.

[87] Cummings JH, Hill MJ, Bone ES, Branch WJ, Jenkins DJA. Effect of meat protein and dietary fiber on colonic function and metabolism. 2. Bacterial metabolites in feces and urine. Am J Clin Nutr 1979; 32: 2094-101.

[88] Visek WJ. Diet and cell growth modulation by ammonia. Am J Clin Nutr 1978; 31: S216-S20.

[89] Beerens H, Romond C. Sulfate-reducing anaerobic bacteria in human feces. Am J Clin Nutr 1977; 30: 1770-6.

[90] Macfarlane GT, Macfarlane S, Gibson GR. Synthesis and release of proteases by Bacteroides fragilis. Curr Microbiol 1992; 24: 55-9.

[91] Magee EA, Richardson CJ, Hughes R, Cummings JH. Contribution of dietary protein to sulfide production in the large intestine: an *in vitro* and a controlled feeding study in humans. Am J Clin Nutr 2000; 72: 1488-94.

[92] Moore JWE, Millard S, Babidge W, Rowland R, Roediger WEW. Hydrogen sulphide produces diminished fatty acid oxidation in the rat colon *in vivo*: Implications for ulcerative colitis. Aust NZ J Surg 1997; 67: 245-9.

[93] Christl SU, Eisner H-D, Dusel G, Kasper H, Scheppach W. Antagonistic effects of sulfide and butyrate on proliferation of colonic mucosa; a potential role for these agents in the pathogenesis of Ulcerative Colitis. Digest Dis Sci 1996; 41: 2477-81.

[94] Attene-Ramos MS, Wagner ED, Gaskins HR, Plewa MJ. Hydrogen sulfide induces direct radical-associated DNA damage. Mol Cancer Res 2007; 5: 455-9.

[95] Smith EA, Macfarlane GT. Formation of phenolic and indolic compounds by anaerobic bacteria in the human large intestine. Microbial Ecol 1997; 33: 180-88.

[96] Kikugawa K, Kato T. Formation of a mutagenic diazoquinone by interaction of phenol with nitrite. Food Chem Toxicol 1988; 26: 209-14.

[97] Bryan GT. Role of urinary tryptophan metabolites in etiology of bladder cancer. Am J Clin Nutr 1971; 24: 841-7.

[98] Dunning WF, Curtis MR, Maun ME. The effect of added dietary tryptophane on the occurrence of 2-acetylaminofluorene-induced liver and bladder cancer in rats. Cancer Res 1950; 10: 454-9.

[99] Moore WEC, Moore LH. Intestinal floras of populations that have a high risk of colon cancer. Appl Environ Microbiol 1995; 61: 3202-7.

[100] Tannock GW. Characteristics of Bacteroides isolates from cecum of conventional mice. Appl Environ Microbiol 1977; 33: 745-50.

[101] Gibson SAW, Macfarlane GT. Characterization of proteases formed by Bacteroides fragilis. J Gen Microbiol 1988; 134: 2231-40.

[102] Macfarlane GT, Gibson GR, Cummings JH. Comparison of fermentation reactions in different regions of the human colon. J Appl Bacteriol 1992; 72: 57-64.

[103] Smith EA, Macfarlane GT. Studies on amine production in the human colon: Enumeration of amine forming bacteria and physiological effects of carbohydrate and pH. Anaerobe 1996; 2: 285-97.

[104] Tap J, Mondot S, Levenez F, *et al.* Towards the healthy human intestinal microbiota phylogenetic core? Microbial Ecol 2009; 57: 580-1.

[105] Smith EA, Macfarlane GT. Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. FEMS Microbiol Ecol 1998; 25: 355-68.

[106] Russell JB, Onodera R, Hino T. Ruminal protein fermentation: new perspectives on previous contradictions. In: Tsuda T, Sasaki Y, Kawashima R, Eds. Physiological aspects of digestion and metabolism in ruminants. USA: Academic Press 1991; pp. 681-697.

[107] Eschenlauer SC, McKain N, Walker ND, McEwan NR, Newbold CJ, Wallace RJ. Ammonia production by ruminal microorganisms and enumeration, isolation, and characterization of bacteria capable of growth on peptides and amino acids from the sheep rumen. Appl Environ Microbiol 2002; 68: 4925-31.

[108] Gibson GR, Cummings JH, Macfarlane GT. Competition for hydrogen between sulphate-reducing bacteria and methanogenic bacteria from the human large intestine. J Appl Bacteriol 1998; 65: 241-7.

[109] Gibson GR, Macfarlane GT, Cummings JH. Occurrence of sulphate-reducing bacteria in human faeces and the relationship of dissimilatory sulphate reduction to methanogenesis in the large gut. J Appl Bacteriol 1988; 65: 103-11.

[110] Florin THJ. Hydrogen sulfide and total acid-volatile sulfide in feces, determined with a direct spectrophotometric method. Clin Chim Acta 1991; 196: 127-34.

[111] Fite A, Macfarlane GT, Cummings JH, *et al.* Identification and quantitation of mucosal and faecal desulfovibrios using real time polymerase chain reaction. Gut 2004; 53: 523-9.

[112] Kassie F, Lhoste EF, Bruneau A, *et al.* Effect of intestinal microfloras from vegetarians and meat eaters on the genotoxicity of 2-amino-3-methylimidazo[4,5-f]quinoline, a carcinogenic heterocyclic amine. J Chromat B-Anal Technol Biomed Life Sci 2004; 802: 211-5.

[113] Saunier K, Dore J. Gastrointestinal tract and the elderly: functional foods, gut microflora and healthy ageing. Digest Liv Dis 2002; 34: S19-S24.

[114] Hughes R, Pollock JRA, Bingham S. Effect of vegetables, tea, and soy on endogenous N-nitrosation, fecal ammonia, and fecal water genotoxicity during a high red meat diet in humans. Nutr Cancer – Int J 2002; 42: 70-7.

[115] Van Tassell RL, MacDonald DK, Wilkins TD. Production of a fecal mutagen by Bacteroides spp. Infect Immun 1982; 37: 975-80.

[116] Van Tassell RL, Kingston DGI, Wilkins TD. Metabolism of dietary genotoxins by the human colonic microflora - the fecapentaenes and heterocyclic amines. Mut Res 1990; 238: 209-21.

[117] de Kok TM, Vanfaassen CMA, Bauschgoldbohm RA, Tenhoor F, Kleinjans JCS. Fecapentaene excretion and fecal mutagenicity in relation to nutrient intake and fecal parameters in humans on omnivorous and vegetarian diets. Cancer Lett 1992; 62: 11-21.

[118] Schiffman MH, Van Tassell RL, Andrews AW, *et al.* Fecapentaene concentration and mutagenicity in 718 North American stool samples. Mut Res 1989; 222: 351-7.

[119] Thomson ABR, De Pover A, Keelan M, Jarocka-Cyrta E, Clandinin MT. Inhibition of lipid absorption as an approach to the treatment of obesity. Meth Enzymol 1997; 286: 3-44.

[120] Mu H, Posgaard T. The metabolism of structured triacylglycerols. Prog Lipid Res 2005; 44: 430-48.

[121] Newmark H, Lupton JR. Determinants and consequences of colonic luminal pH: implications for colon cancer. Nutr Cancer 1990; 14: 161-73.

[122] Hashim SA, Babayan VK. Studies in man of partially absorbed dietary fats. Am J Clin Nutr 1978; 31: S273-S6.

[123] Bracco U. Effect of triglyceride structure on fat absorption. Am J Clin Nutr 1994; 60: 1002S-9S.

[124] Wiggins HS, Howell KE, Kellock TD, Stalder J. The origin of faecal fat. Gut 1969; 10: 400-3.

[125] Juste C. Dietary fatty acids, intestinal microbiota and cancer. Bull Cancer 2005; 92: 708-21.

[126] Zhi JU, Moore R, Kanitra L. The effect of short-term (21-day) orlistat treatment on the physiologic balance of six selected macrominerals and microminerals in obese adolescents. J Am Coll Nutr 2003; 22: 357-62.

[127] Pittler MH, Ernst E. Complementary therapies for reducing body weight: a systematic review. Int J Obes 2005; 29: 1030-8.

[128] Chanoine JP, Hampl S, Jensen C, Boldrin M, Hauptman J. Effect of orlistat on weight and body composition in obese adolescents - A randomized controlled trial. J Am Med Assoc 2005; 293: 2873-83.

[129] Rössner S, Sjostrom L, Noack R, Meinders AE, Noseda G. Weight loss, weight maintenance, and improved cardiovascular risk factors after 2 years treatment with orlistat for obesity. Obes Res 2000; 8: 49-61.

[130] Bernstein H, Bernstein C, Payne CM, Dvorakova K, Garewal H. Bile acids as carcinogens in human gastrointestinal cancers. Mutat Res 2005; 589: 47-65.

[131] Vonk RJ, Kalivianakis M, Minich DM, Bijleveld CMA, Verkade HJ. The metabolic importance of unabsorbed dietary lipids in the colon. Scand J Gastroenterol 1997; 32: 65-7.

[132] Morotomi M, Guillem JG, Lo Gerfo P, Weinstein B. Production of diacylglycerol, an activator of protein kinase C, by human intestinal microflora. Cancer Res 1990; 50: 3595-9.

[133] Vulevic J, McCartney AL, Gee JM, Johnson IT, Gibson GR. Microbial species involved in production of 1,2-sn-diacylglycerol and effects of phosphatidylcholine on human fecal microbiota. Appl Environ Microbiol 2004; 70: 5659-66.

[134] James AT, Webb JPW, Kellock TD. The occurrence of unusual fatty acids in faecal lipids from human beings with normal and abnormal fat absorption. Biochem J 1961; 78: 333-9.

[135] Thomas PJ. Identification of some enteric bacteria which convert oleic acid to hydroxystearic acid *in vitro*. Gastroenterology 1972; 62: 430-5.

[136] Pearson JR. Alteration of dietary fat by human intestinal bacteria. Proc Nutr Soc 1973; 32: 8A-9A.

[137] Devillard E, McIntosh FM, Duncan SM, Wallace RJ. Metabolism of linoleic acid by human gut bacteria: different routes for biosynthesis of conjugated linoleic acid. J Bacteriol 2007; 189: 2566-70.

[138] Polan CE, McNeill JJ, Tove SB. Biohydrogenation of unsaturated fatty acids by rumen bacteria. J Bacteriol 1964; 88: 1056-64.

[139] Eyssen H, Parmentier G. Biohydrogenation of sterols and fatty acids by the intestinal microflora. Am J Clin Nutr 1974; 27: 1329-40.

[140] Howard FAC, Henderson C. Hydrogenation of polyunsaturated fatty acids by human colonic bacteria. Lett Appl Microbiol 1999; 29: 193.

[141] Maia MRG, Chaudhary LC, Figueres L, Wallace RJ. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie van Leeuwenhoek 2007; 91: 303-14.

[142] Paillard D, McKain N, Chaudhary LC, *et al.* Relation between phylogenetic position, lipid metabolism and butyrate production by different Butyrivibrio-like bacteria from the rumen. Antonie van Leeuwenhoek 2007; 91: 417-22.

[143] Wiggins HS, Pearson JR, Walker JG, Russell RI, Kellock TD. Incidence and significance of faecal hydroxystearic acid in alimentary disease. Gut 1974; 15: 614-21.

[144] Bonanome A, Grundy SM. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. N Eng J Med 1998; 318: 1244-8.

[145] Belury MA. Dietary conjugated linoleic acid in health: Physiological effects and mechanisms of action. Ann Rev Nutr 2002; 22: 505-31.

[146] Wahle KWJ, Heys SD, Rotondo D. Conjugated linoleic acids: Are they beneficial or detrimental to health. Prog Lipid Res 2004; 43: 553-7.

[147] Tricon S, Yaqoob P. Conjugated linoleic acid and human health: a critical evaluation of the evidence. Curr Opin Clin Nutr Metab Care 2006; 9: 105-10.

[148] Position statement by the Scientific Advisory Committee on Nutrition. SACN Update on trans fatty acids and health. London: TSO (The Stationery Office) 2007.

[149] Rhee SK, Kayani AJ, Ciszek A, Brenna JT. Desaturation and interconversion of dietary stearic and palmitic acids in human plasma and lipoproteins. Am J Clin Nutr 1997; 65: 451-8.

[150] Turpeinen AM, Mutanen M, Aro A, *et al.* Bioconversion of vaccenic acid to conjugated linoleic acid in humans. Am J Clin Nutr 2002; 76: 504-10.

[151] Mosley EE, McGuire MK, Williams JE, McGuire MA. cis-9,trans-11 conjugated linoleic acid is synthesized from vaccenic acid in lactating women. J Nutr 2006; 136: 2297-301.

[152] Miller A, McGrath E, Stanton C, Devery R. Vaccenic acid (t11-18: 1) is converted to c9,t11-CLA in MCF-7 and SW480 cancer cells. Lipids 2003; 38: 623-32.

[153] Kamlage B, Hartmann L, Gruhl B, Blaut M. Intestinal microorganisms do not supply associated gnotobiotic rats with conjugated linoleic acid. J Nutr 1999; 129: 2212-7.

[154] Kamlage B, Hartmann L, Gruhl B, Blaut M. Linoleic acid conjugation by human intestinal microorganisms is inhibited by glucose and other substrates in vitro and in gnotobiotic rats. J Nutr 2000; 130: 2036-9.

[155] Bassaganya-Riera J, Hontecillus R, Beitz DC. Colonic anti-inflammatory mechanisms of conjugated linoleic acid. Clin Nutr 2002; 21: 451-9.

[156] Bassaganya-Riera J, Reynolds K, Martino-Catt S, *et al.* Activation of PPAR gamma and delta by conjugated linoleic acid mediates protection from experimental inflammatory bowel disease. Gastroenterology 2004; 127: 777-91.

[157] Nichenametla SN, South EH, Exon JH. Interaction of conjugated linoleic acid, sphingomyelin, and butyrate on formation of colonic aberrant crypt foci and immune function in rats. J Toxicol Environ Health 2004; A67: 469-81.

[158] Kemp MQ, Jeffy BD, Romagnolo DF. Conjugated linoleic acid inhibits cell proliferation through a p53-dependent mechanism: Effects on the expression of G1-restriction points in breast and colon cancer cells. J Nutr 2003; 133: 3670-77.

[159] Coakley M, Ross RP, Nordgren M, Fitzgerald G, Devery R, Stanton C. Conjugated linoleic acid biosynthesis by human-derived Bifidobacterium species. J Appl Microbiol 2003; 94: 138-45.

[160] O'Connor EB, Barrett E, Fitzgerald G, Hill C, Stanton C, Ross RP. Production of vitamins, exopolysaccharides and bacteriocins by probiotic bacteria. In: Tamine A, Ed. Probiotic dairy products. Oxford, USA: Blackwell Publishing Ltd. 2005; pp. 167-194.

[161] Khedkar CD, Ouwehand AC. Modifying the gastrointestinal microbiota with probiotics. In: Ouwehand A, Vaughan EE, Eds. Gastrointestinal microbiology. New York: Taylor & Francis Ltd. 2006; pp. 315-33.

[162] Reddy BS, Hedges AR, Laakso K, Wynder EL. Metabolic epidemiology of large bowel cancer - fecal bulk and constituents of high-risk North American and low-risk Finnish population. Cancer 1978; 42: 2832-8.

[163] Hill MJ, Crowther JS, Drasar BS, Hawksworth GS, Aries V, Williams RE. Bacteria and aetiology of cancer of large bowel. Lancet 1971; 1: 95-100.

[164] Hill MJ. Bile, bacteria and bowel cancer. Gut 1983; 24: 871-5.

[165] McGarr SE, Ridlon JM, Hylemon PB. Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature. J Clin Gastroenterol 2005; 39: 98-109.

[166] Ridlon JM, Kang DK, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res 2006; 47: 241-59.

[167] Stadler J, Stern HS, Yeung KAS, *et al.* Effect of high fat consumption on cell proliferation activity of colorectal mucosa and on soluble fecal bile acids. Gut 1988; 29: 1326-31.

[168] de Kok TM, van Maanen JM. Evaluation of fecal mutagenicity and colorectal cancer risk. Mut Res 2000; 463: 53-101.

[169] Cani PD, Amur J, Iglesias MA, *et al.* Metabolic eudotoxomia initiates obesity and insulin resistance. Diabetes 2007; 56: 1761-72.

[170] Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. Curr Pharm Des 2009; 15: 1546-58.

[171] Cani PD, Possemiers S, van de Wiele T, *et al.* Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2 driven improvement in gut permeability. Gut 2009; 58: 1091-103.

[172] Cani PD, Neyrinck M, Fava F, *et al.* Selective increases of bifidobacteria in gut mircoflora improve high-fat-diet-induced

diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007; 50: 2374-83.

[173] Wen L, Ley RE, Yu P, *et al.* Innate immunity and intestinal microbiota in the development of type 1 diabetes. Nature 2008; 455: 1109-10.

[174] Robertson MD, Bickerton AS, Dennis AL, Vidal H, Frayn KN. Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism. Am J Clin Nutr 2005; 82: 559-67.

[175] Mirvish SS. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. Cancer Lett 1995; 93: 17-48.

Received: September 07, 2009 Revised: September 23, 2009 Accepted: September 23, 2009