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Ultra-processed foods and food additives in gut health and disease

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Abstract

Ultra-processed foods (UPF) and food additives have become ubiquitous components of the modern human diet. There is increasing evidence of an association between diets rich in UPF and gut disease, including inflammatory bowel disease, colorectal cancer and irritable bowel syndrome. Food additives are added to many UPF and themselves have been shown to impact gut health. For example, evidence shows effects of some emulsifiers, sweeteners, colours and microparticles/nanoparticles on a range of outcomes including the microbiome, intestinal permeability and intestinal inflammation. Broadly speaking, the evidence for an effect of UPF on gut disease comes from observational epidemiological studies, while in contrast, the evidence for the effect of food additives comes largely from pre-clinical studies conducted *in vitro* or in animal models. Fewer studies have investigated the effect of UPF or food additives on gut health and disease in human intervention studies. Hence, the aim of this article is to critically review the evidence for an impact of UPF and food additives on gut health and gut disease and to discuss the clinical application of these findings in light of the current level of evidence.

Key points

Ultra-processed foods (UPF) are widely consumed in the food chain, and epidemiological studies indicate increased risk of gut diseases including inflammatory bowel disease, colorectal cancer and possibly irritable bowel syndrome.

Food additives commonly added to UPF, including emulsifiers, sweeteners, colours and microparticles/nanoparticles, have been shown in pre-clinical studies to impact the gut, including the microbiome, intestinal permeability and intestinal inflammation.

Few studies have investigated the effect of dietary restriction of UPF or food additives on the risk or management of gut disease, though multi-component diets have shown some initial promise.

[H1] Introduction

Human diet is rapidly evolving, driven by changes in population demographics, urbanisation, employment patterns and enabled by advances in science and technology in both farming and the food industry. Farming practices have changed over centuries from small local provision to machine-facilitated industries growing food at scale and distributing it worldwide. At the same time, food processing and food additives have enabled ready-to-eat foods with attractive appearance and organoleptic properties with long shelf lives and requiring little preparation. All of these have led to a food supply considerably different to that from a century ago[1], resulting in major shifts in dietary exposures to which many have linked the rise in non-communicable disease, including many diseases of the gastrointestinal (GI) tract.

Ultra-processed foods (UPF) and food additives are key features of this change in food supply and have become ubiquitous components of diet, particularly in (although not restricted to) high income countries. There is increasing evidence of an association between diets rich in UPF and gut disease, and also that some food additives, such as emulsifiers, sweeteners, colours and nanoparticles, may alter the intestinal microbiota and permeability in a way that appears linked to the promotion of chronic intestinal inflammation, scientific literature that will be extensively discussed in this critical review.

Evidence for an effect of UPF and food additives on gut health and disease comes from a range of sources including pre-clinical studies *in vitro* and in animal models as well as observational epidemiological studies, with many fewer human intervention trials. Despite this, the public, patients and health professionals have considerable interest and appetite for information and evidence in this area. Hence, the central aim of this article is to provide a critical review of the evidence for an impact of UPF and food additives on gut health (including microbiology, permeability, inflammation) and gut disease and to discuss the clinical application of these findings given the current level of evidence.

In order to achieve this aim, an online literature search was performed using the Medline database for studies investigating mechanisms (e.g. in vitro, animal studies), associations from observational studies (e.g., case-control, cohort) and causal or effectiveness outcomes from intervention studies (e.g. randomised controlled trials) in relation to UPF and food additives in ut health and disease. All studies that addressed the aim of this review were potentially eligible,

and strengths and limitations of study design that impact interpretation of the outcome are discussed.

[H1] Ultra-processed foods

Ultra-processed foods are widely available in the food supply, although their definition is subject to much debate. Historically, terms such as "convenience food", "fast food" or "junk food" have been used despite the negative connotations and lack of robust criteria. At least eight classification systems have been used to categorise foods based upon the level of processing [2], all of which broadly speaking use criteria based upon the extent (i.e. how much the food differs from the unprocessed ingredient), nature (e.g. changing the matrix, use of food additives), location (whether at home- or commercially-produced) and purpose (e.g. for convenience, appearance) of processing[2].

Examples of the different classification systems are shown in **Box 1**. Importantly, there are several anomalies between the systems making comparison between studies that use different UPF classification systems challenging.

NOVA is the most widely used classification system and has been adopted by the Food and Agriculture Organization of the United Nations[3]. Consisting of four categories, NOVA's definition of UPF would include carbonated soft drinks; sweet, fatty or salty packaged snacks; confectionery; biscuits, pastries, and cakes; margarine and other spreads; sweetened breakfast cereals; ready meals; meat, poultry or fish nuggets; sausages, burgers and hot dogs; powdered and packaged soups, noodles and desserts (**Box 1**). Few would dispute these being UPF, but it is important to note that many food items that may be considered part of a healthy diet, including packaged wholemeal bread, some fruit yoghurts, fortified juices and plant-based meat alternatives are also included in the UPF category.

Despite its widespread use, the NOVA classification for UPF is contested. For example, it uses location of processing in its definition, thus two breads made with similar ingredients, recipe and conditions would be classified differently if prepared at home (a processed food) or in a commercial plant (a UPF). NOVA also considers the purpose of processing, for example stating that UPF are made to be branded, attractive and low cost. This is not only challenging to define

but also implies an ideological perspective that packaged, colourful or cheap foods are less healthful than homemade, plain or expensive foods.

UPF are widely consumed in diets although with considerable variation across countries. A systematic review of 99 studies including 1,378,454 participants across 20 countries[6], reported UPF intakes among adults to contribute anything from 10% of energy intake (Italy)[7] to 59.7% (United States)[8]. Time series studies indicate secular trends of increasing UPF consumption, in Canada increasing from 24.4% of total energy in 1938 to 54.9% in 2001[9], in Sweden increasing by 142% between 1960 and 2010[10], and in young people in the USA increasing from 61.4% of total energy in 1999 to 67% in 2018[11].

As well as wide variation in UPF intake across countries and over time, there is wide inter-individual variation in intake. Factors associated with higher UPF intake are reported to include (factors in parentheses show the direction associated with higher intakes) demographic characteristics such as sex (females)[12], age (younger) [6,12,13], income (lower income)[12], educational level (lower educational level) and domiciliary status (living alone)[12] and anthropometric and behavioural characteristics including body mass index (overweight and obesity)[6,12], physical activity (lower physical activity)[12] and eating behaviours (greater screen time during meals)[13].

Higher UPF intakes have been associated with greater energy density, higher intakes of free sugars, fat, saturated fat, together with lower intakes of protein, dietary fibre and numerous micronutrients[14-16]. UPF intakes are also related to dietary pattern, being higher in vegans and vegetarians[17] and lower in those with higher diet quality index[16] and those adhering to national dietary guidelines[18] and Mediterranean diet[13].

There is debate regarding whether observations of association between UPF intake and disease could result at least in part from these demographic, anthropometric, behavioural and dietary variables that are not sufficiently adjusted for in epidemiological analyses. In one study of over 9,000 people in the UK, UPF intake was associated with calculated cardiometabolic risk following multivariable adjustment, however, once a diet quality index was factored into the adjustment model this association did not remain[16]. In contrast, a review of 37 cohort studies comparing UPF intake with a health outcome demonstrated that the majority of identified

associations remained significant following adjustment for either one or more nutrients (e.g. free sugar, fat, saturated fat) or diet quality index or dietary pattern score (e.g. Healthy Eating Index, Mediterranean Diet Score) [19].

All diets are different between individuals, and one person consuming high UPF intake from pastries, cakes, ready-meals and burgers would have a very different nutrient intake and diet quality than somebody with the same UPF intake from wholemeal bread, fruit yoghurts, and fortified breakfast cereal. It has been shown that diets broadly meeting national guidelines for a healthy diet can be designed from UPF although this approach has not been tested in people[20] and epidemiological studies relying on generic FFQs would unlikely be able to differentiate on this issue. Whilst variations exist in the foods contributing to UPF intake in individuals, at the population level the association with poorer nutrient profile and lower diet quality remain[14-16,18]. Therefore, it is crucial that epidemiological studies of UPF are sufficiently adjusted for intake of nutrients or dietary patterns that are relevant to the disease of interest.

[H2] Epidemiological evidence for impact of UPF on gut health and disease

Numerous cohort studies have reported associations between higher intake of UPF and mortality [21,22] and morbidity including greater risk of coronary artery disease[23], cardiovascular disease[24,25], type 2 diabetes[26], cancer[27], with a meta-analysis of observational studies reporting increased risk of overweight, obesity, metabolic syndrome and depression[28]. In one of the few experimental studies, a domiciliary feeding study, a high UPF diet resulted in greater energy consumption and weight gain than an isocaloric unprocessed diet matched for fat, sugar and fibre content[29]. These data suggest that processing *per se*, rather than just differences in energy and nutrient content, may impact ingestive behaviour and healthy-related outcomes. The Scientific Advisory Committee on Nutrition in the United Kingdom published a statement regarding the association of food processing with health outcomes following a search and analysis of 20 systematic reviews of RCTs and cohort studies[30]. The majority of systematic reviews of primary studies showed associations between intakes of UPF and poor health outcomes that the statement described as '*concerning*', however, the inconsistent and sometimes inadequate adjustment for covariables made it unclear whether the associations related to food processing *per se*, or due to nutrient intake profiles associated with high consumption of UPFs (e.g. increased energy density, and high intakes of saturated fat, free sugars and salt) and as such the evidence should be treated with caution.

Importantly, there is accumulating evidence of a role for UPF in increasing the risk of disorders of the GI tract, including inflammatory bowel disease (IBD), functional gastrointestinal disorders (FGID) and several intestinal cancers (**Table 1**).

Inflammatory bowel disease. Five cohort studies have thus far investigated the association between UPF intake and risk of IBD (**Table 1**). Following adjustment for multiple variables, in the three studies reporting data for IBD combined (Crohn's plus UC), the risk of developing IBD in the highest compared with the lowest quantile of UPF intake ranged from HR 1.15[35], RR 1.44[33] to HR 1.92[31] although only the latter was statistically significant In contrast, all four studies analysing the risk of Crohn's disease specifically, reported statistically significant increased hazard ratios (HR) of 1.48[34], 1.61[35], 1.7[32] and 4.9[31], whereas none reported statistically significant associations with ulcerative colitis, with HR of 0.93[34], 1.01[35], 1.2[32] and 1.52[31].

It is important to note that in the French NutriNet-Santé cohort, the very short follow-up period (average 2.3 years) inevitably resulted in a low number of incident cases (75 cases/105,832 in cohort), that may result in a type II error due to inadequate power to detect an association with UPF intake should it exist. In addition, self-reported cases were only confirmed by medical record review in a subsample of 15% (i.e. in only 11 cases)[33].

A meta-analysis of four of these cohort studies has been performed demonstrating in the highest compared with the lowest quantile of UPF intake there was an increased risk of development of Crohn's disease (HR 1.71, 95% CI 1.37–2.14), but not ulcerative colitis (HR 1.17, 95% CI 0.86–1.61)[42].

The association of UPFs with risk of Crohn's disease, but not UC, is interesting but not without precedence. The evidence for other behavioural factors (e.g. smoking)[43] and for dietary treatments of active disease (e.g. exclusive enteral nutrition, EEN)[44] in IBD is discordant between Crohn's disease and UC. Dietary ligands and metabolites have greater impact on the small intestine compared, which might explain why diversion of luminal flow results in lower recurrence of Crohn's disease [45].

Functional gastrointestinal disorders. To date only one study has investigated the association between UPF intake and FGID, a case-control study using data from the NutriNet-Santé cohort in France. Following adjustment for multiple variables, in the highest quartile of UPF intake there was a 25% greater odds of irritable bowel syndrome (IBS) (OR 1.25) and of functional dyspepsia (OR 1.25), but no association with functional constipation or functional diarrhoea[36] (**Table 1**).

Gastrointestinal cancers. Three cohort studies[27,37,41], and at least three case-control studies[38-40] have investigated the association between UPF intake and gastrointestinal cancer, all in relation to adenoma or colorectal cancer (CRC)[27,37-41] with the exception of pancreatic cancer[41] (**Table 1**).

Following multiple adjustments two cohort studies report the highest quintile of UPF intake to be associated with CRC with a HR of 1.23[27] and 1.29 (men only)[37]. In the latter study, cancer location was also relevant in men, being significant for distal colon cancer (HR 1.72) but not for proximal colon cancer or rectal cancer. In contrast, in women there was no association between UPF intake and risk of CRC, nor any specific colorectal location[37]. Two case-control studies reported 30%[38] and 40%[39] greater odds of CRC whilst another reported 75% greater odds of colorectal adenoma in the highest tertile of UPF intake, which was also statistically significant for stage (greater odds for advanced adenoma) and location (greater odds for proximal adenoma)[40]. Data from these observational studies were recently included in a systematic review including 462,292 participants, with the meta-analysis reporting the highest intake of UPF to be associated with CRC with a RR of 1.26 (95% CI 1.14–1.38). This association was significant in subgroup analysis both in cohort studies only (RR = 1.16, 95%CI 1.08–1.25) and in case-control studies only (RR = 1.41; 95% CI 1.22–1.63)[46]. Importantly, people with high UPF intake commonly consume lower intakes of dietary fibre and higher intakes of processed meat[15], both of which are risk factors for CRC[47,48] and is rarely specifically adjusted for in these cohort studies.

In the only study of UPF and pancreatic cancer, in the highest quartile of UPF intake there was a greater risk of pancreatic cancer with HR of 1.49[41].

Overall, these epidemiological studies provide strong and consistent evidence that high intakes of UPF are associated with an increased risk of Crohn's disease and CRC, and evidence from single studies of an association with IBS and pancreatic cancer.

[H2] Challenges of investigating associations between UPF and gut disease

Epidemiological studies have been crucial in uncovering the associations of UPF with gut disease. However, methodological differences between studies may be responsible for the wide variations in risk reported across studies for the same disease, including differences in recording dietary intake, calculation of UPF exposure, populations being observed and the approach to disease ascertainment. In addition, numerous limitations in the conduct and reporting of observational studies, some of which are inherent to all nutritional epidemiology and some that are specific to UPF that impact the interpretation of this evidence.

The majority of studies assessed UPF intake using food frequency questionnaires (FFQ) that assess the frequency (and sometimes portion size) of a discrete list of food items or food groups, and to our knowledge none of the FFQs used were explicitly validated to measure UPF intake. These established generic FFQs are likely insufficiently granular to accurately measure UPF intake (which was not their initial design intention) and therefore require food items to be classified for UPF status *a posteriori*. Some food items on an FFQ are easy to correctly classify, for example, food commodities (e.g. banana, egg) are evidently unprocessed and those containing food additives (e.g. "low calorie sodas", "candy bars") are evidently UPF. In one analysis it was possible for three researchers to independently assign 70.2% of FFQ food items to a NOVA processing category[49]. However, some food items on FFQ are more ambiguous to classify (e.g. "oil and vinegar dressing" which would not be a UPF if home-made but would be a UPF if it was a commercial preparation containing food additives) or alternatively because the food item descriptors cross UPF boundaries (e.g. "Pie, home-baked or ready-made"). In the aforementioned study, investigation of ingredients, discussion with dietitians and consensus meetings resulted in 95.6% of FFQ items being able to be classified[49]. Despite this, in the Nurses Health Studies and the Health Professionals Follow-up Study, nine food items on the FFQ remain challenging to classify and are provisionally classified conservatively as not being UPF, with the recommendation that sensitivity analyses be performed whereby these nine food items are re-classified as UPFs. In the studies cited in this review, re-classifying these nine food items as UPF did not materially alter the findings of disease risk for IBD[50] or CRC[37]. Therefore

with the level of detail and granularity on standard FFQs, it is not possible to classify items as UPF with 100% sensitivity and specificity, and importantly, few studies sufficiently detail how this classification is performed.

Furthermore, long follow-up periods are a strength of cohort studies to accurately capture disease onset, however this relies on using FFQ data collected many years ago, since which time the composition of many foods has changed and the availability of UPF has grown considerably. These secular changes in food composition and availability further complicate accurate classification of FFQ food items into UPF categories over time. In contrast, an advantage of the data from the NutriNet-Santé cohort[33,36] is the use of multiple online 24 h recalls with extensive food lists to enable more accurate classification of foods into UPF categories.

There is variability in how UPF intakes are quantified. For example, some studies calculate 'servings of UPF per day'[31,35] although agreement on serving sizes is not always consistent across studies and across countries. Other studies report the 'weight of UPF consumed', which will inflate the contribution from high volume (e.g. sugar-sweetened soft drinks) compared to low volume (e.g. sugar sweets/candy) UPFs that may otherwise have similar composition and processing. Finally, most studies report 'percentage energy from UPF' which has the advantage of adjusting for those with higher intakes of all foods (including between males and females) but which may under-represent the contribution from UPFs formulated to be low in energy, such as low-calorie soft-drinks with artificial sweeteners (<40 kcal/litre) compared to sugar-sweetened beverages (190-420 kcal/litre), despite identical exposure to UPF.

Nutritional epidemiology traditionally sought to relate exposure to a single nutrient with subsequent health/disease. A single molecule can normally be accurately measured in food and consistently reported across studies to represent a consistent exposure. However, in studies measuring UPF intake, we attempt to assimilate exposure to a large quantity of a food containing one food additive together with exposure to a small quantity of a food that has undergone extensive processing and contains many different classes of food additives. Overall the issue faced by researchers is that it is challenging to measure and report UPF intake when the exposure of interest are a range of different foods and not a single molecule.

Finally, it is crucial when considering applying the evidence from epidemiological studies that the quantities of UPF intake associated with risk are considered. For example, in two studies the highest risk categories consumed were >5 servings[31] and >3.7 servings (energy adjusted)[41] per day which were compared with reference categories consuming <1 servings/d[31] or <0.9 servings (energy adjusted)[41] per day. Therefore in discussing risk reduction, these are major reductions in UPF intake that would require dramatic shifts in dietary behaviour to achieve intakes reflecting the reference category.

[H2] UPF and the gut microbiome

The intestinal microbiota has gained attention for its impact on intestinal and metabolic health. As an example highlighting this concept, patients with IBD harbour compositionally altered microbiota, characterized by the depletion of health-associated members, such as *Faecalibacterium prausnitzii*[51] together with the overgrowth of pathobiont members of the intestinal ecosystem, such as adherent and invasive *Escherichia coli*[52]. Moreover, the observation that microbiota transplantation from patients with IBD into germ-free interleukin 10–deficient mice drives severe colitis compared to transplantation from healthy controls suggests a functional role played by the intestinal microbiota in the promotion of chronic intestinal inflammation observed in IBD[53].

The impact of UPF on gut microbiome composition and metabolism is often cited as a causal mechanism through which increased risk of gut disease is mediated. Despite this assumption, there is limited empirical research specific to the impact of UPF collectively on the human microbiome. Over recent decades, many studies have investigated the effect of so-called "Western diet" characterised by high animal product and low plant food intakes and thus high intakes of energy, fat and sugar and low intakes of fibre on the gut microbiome. However, whilst these tell us of the impact of excess intakes of fats and free sugars and deficiency of fibre, they do not explicitly characterise the effect of exposure to UPFs, which has been investigated in very few studies.

A murine feeding study compared the effect of UPF foods (chow made solely from hamburgers and French fries purchased from a fast-food chain for 6-weeks) supplemented with nothing, calcium or a multivitamin/mineral compared with standard chow on a range of bone markers plus caecal microbiome[54]. There was no difference in α-diversity (Shannon index) between

control and UPF diet, and it was actually higher in both the UPF plus supplement groups compared to control chow. The three UPF groups differed from control in ß-diversity (Bray-Curtis), with *Parasutterella* and *Bifidobacterium* more abundant and *Bacteroidetes* (phylum) and *Roseburia* less abundant in UPF than controls. There was considerable difference in fat content between control (6.2% fat) and UPF diets (38% fat), and the extent to which this was alone responsible for the differences, rather than the UPF nature of the diet, is unclear[54].

An observational study divided adults living in Spain into those habitually consuming <3 servings UPF /d (n=96) and >5 servings UPF/d (n=90), as measured by FFQ and conducted 16S rRNA sequencing on stool samples[55]. In women, there were no differences in any measure of α-diversity based upon UPF intake categories, however, in men the number of OTUs, Shannon index and Chao1 were all significantly lower in those with high UPF intakes. Overall, those consuming higher UPF intakes had greater abundance of *Gemmiger, Granulicatella, Parabacteroides, Shigella, Bifidobacterium* (the latter actually considered to have beneficial impacts on gut health),[56] and lower *Lachnospira* and *Roseburia*, and at the phylum level greater Actinobacteria, than those in the low UPF intake group, with some differences between findings in women and men[55].

A further observational study of 441 adults living in Colombia recorded UPF intake (as percentage energy) from a 24-h recall and microbiome through 16S rRNA gene sequencing[57]. There was no association between UPF intake and diversity (Shannon). However, high UPF intakes were significantly associated with the abundance of 17 species, including both lower and higher abundance of several Oscillospira spp., higher *Bilophia* sp. And lower *Lachnospira* sp. And *Bifidobacteria adolescentis*.

In both observational studies, microbiome analyses were adjusted for age, BMI and other demographic characteristics, but not for diet quality indices such as Mediterranean Diet Score[55] and Healthy Eating Index[57], and therefore an influence of diet quality rather than specifically food processing on microbiome outcomes cannot be excluded. Aside from the studies investigating specific nutrients or food components (e.g. high fat diet or high sugar intake), there is a surprising lack of intervention studies of the impact of UPF on the gut microbiome.

[H1] Food additives

Food additives have been used for many years to enhance the appearance, taste, texture and shelf-life of foods. Food additives are defined as 'substances that are not normally consumed as food itself but are added to food intentionally for a technological purpose'[58]. Food additive intake has risen in recent decades, with the growing demand for convenient products with longer shelf-life[59].

The categories of food additives, and which compounds are in specific categories, varies between legislative bodies. In the European Union, food additives are broadly categorised into colours, sweeteners, and 'additives other than colours and sweeteners'[58], whilst other legislative bodies group food additives into functional classes including colours, sweeteners, emulsifiers, stabilisers, gelling agents and thickeners[60,61]. An observational study of 274 patients with Crohn's disease in Australia, China and Hong Kong has shown that in the previous year and compared with healthy controls, they consumed higher intakes of total food additives, total emulsifiers (as well as polysorbate-80, carboxymethylcellulose (CMC) and carrageenan), total sweeteners (as well as aspartame, sucralose and saccharin), and the nanoparticle titanium dioxide[62].

Any food containing a commercial food additive would be considered a UPF. However, unlike UPF there is considerable mechanistic research on the role of some classes of food additives on gut health and disease, which have implicated them as a potential key contributor to the deleterious impact of modern diet on health [63]. In particular, *in vitro*, animal studies as well as many fewer studies in humans have shown effects of some food additives on the microbiome, mucous, permeability and inflammation in the gut, as summarised in **Figure 1**.

[H1] Food additive emulsifiers

Among the most commonly used food additives are those with emulsifying and thickening properties, which will be referred to as emulsifiers in this review. Emulsifiers are defined as food additives that form or maintain a uniform emulsion of two or more food phases (e.g. oil and water) and are added to UPF to improve organoleptic properties and extend shelf-lives [64]. Numerous emulsifiers are found in UPF [64], with six emulsifiers being amongst the ten most consumed food additives, according to a recent analysis from the French prospective cohort NutriNet-Santé [65]. Intakes have been measured for some emulsifiers[66], including sorbitan esters (mean daily intake 7.14 mg/kg/bw) and sucrose esters and sucroglycerides (15.82 mg/kg/bw).

Although in some instances, subgroups with particularly high intakes may exceed the ADI, e.g. sorbitan esters whereby those in the 97.5th centile of intake consume 383% of ADI and for sucrose esters and sucroglycerides where they consume 150% of ADI. As presented **Table 2**, accumulating experimental evidence of the impact of emulsifiers on intestinal and gut health suggest that these compounds may be implicated in the rapid increase in the incidence of chronic inflammatory diseases in the post–mid-20th century [67-89].

[H2] Food additive emulsifiers – in vitro and animal models

Initial studies demonstrated that emulsifiers carboxymethylcellulose and polysorbate 80 promoted small intestinal bacterial overgrowth[67] and bacterial translocation across in vitro epithelium[68], respectively. In a 2015 study, Chassaing and colleagues demonstrated that dietary emulsifiers are sufficient to detrimentally impact the intestinal microbiota in a way that drives chronic inflammatory diseases[70]. In wild-type mice, bacteria were only rarely observed within 10 μm from the surface epithelium, and the average closest bacteria detected over multiple high-powered fields was about 25 μm[70]. However, in mice fed with dietary emulsifiers carboxymethylcellulose and polysorbate 80, bacteria could be found in direct contact with the epithelium, and the average distance of the closest bacteria decreased to less than 10 μm[70]. In this seminal work, such effects of emulsifier exposure on the microbiota were associated with the development of chronic colitis in genetically susceptible mice, while wild-type mice developed chronic low-grade intestinal inflammation and metabolic dysregulation. These findings were subsequently validated in independent studies using other models and/or other dietary emulsifiers [71,74,76,78-81,83,88].

Importantly, microbiota/epithelium distance inversely correlates with the extent of intestinal inflammation, supporting the central and direct role played by mucus penetrating bacteria in emulsifier-induced promotion of chronic intestinal inflammation [70,93,94]. Follow up studies also demonstrated that carboxymethylcellulose and polysorbate-80 consumption-induced alterations in microbiota to create a favourable niche that led to increased tumour development in mouse models of colorectal cancer[85] as well as alterations in anxiety-like and social behaviours, together with alterations in the expression of neuropeptides implicated in the modulation of feeding [77].

Mechanistically, the effects of emulsifier consumption are eliminated under germ-free condition[70], while transplantation of microbiota from emulsifier-treated mice to wild type germ-free recipient mice was found to be sufficient to transfer some parameters of low-grade inflammation and metabolic syndrome, indicating a central role played by the microbiota in mediating these effects [70]. Moreover, in three follow up studies, the direct impact of dietary emulsifier on the intestinal microbiota were demonstrated, in a host-independent manner, through the use of *in vitro* microbiota systems, supporting the concept that the intestinal microbiota is the major target of emulsifiers [73,82,86], while direct impact on the mucus layer appears limited [75,76]. Importantly, when transferred to germfree recipient animals, emulsifier-treated *in vitro* microbiota are sufficient to induce most of the host and microbial alterations observed in mice directly treated by emulsifiers, further supporting the notion that the microbiota is directly impacted by these commonly used food additives in a manner that subsequently drives intestinal inflammation[73]. Further supporting this concept, independent studies demonstrated a transgenerational effect of emulsifier consumption, where emulsifier-induced alterations in microbiota composition appear sufficient to drive metabolic dysregulation and colitis susceptibility in the offspring, even if they were never directly exposed to emulsifiers [78,90,95].

Interestingly, emulsifier consumption by gnotobiotic mice colonised with a highly-restricted microbiota comprised of only 8 bacteria ('Altered Schaedler Flora', ASF), was not sufficient to induce microbiota encroachment, intestinal inflammation nor altered metabolism [73], suggesting that a complex microbiota, containing specific species, is required for the detrimental effects of emulsifiers. The use of various gnotobiotic approaches has highlighted the microbial requirements for emulsifier (CMC and P80)-induced chronic inflammation and elucidated their mechanism of action.[84]

[H2] Food additive emulsifiers – human clinical studies

Studies in healthy humans include a cross-sectional study using data collected from six 24-h dietary recalls among 588 U.S. men and women over a one year period demonstrating a greater emulsifier intake positively associates with the inflammatory biomarker glycoprotein acetyls (GlycA) [87]. A recent double-blind controlled-feeding study investigated the impact of CMC consumption on the gut microbiota and gut health in healthy human participants [89]. Results obtained from this pilot trial demonstrated that CMC consumption is sufficient to induce post-

prandial abdominal discomfort as well as to detrimentally alter the intestinal microbiota composition and faecal metabolome [89].

Following these studies on dietary emulsifiers, together with the increasing appreciation of the role played by the intestinal microbiota in IBD, diet has become a potential therapeutic target for the management of gastrointestinal inflammation. Studies of emulsifiers specifically in patients with gut disease are currently very limited. For example, a 14-day feasibility study in 20 patients with Crohn's disease confirmed that dietary advice could significantly reduce emulsifier intake which was associated with an improvement in patient reported outcome and IBD control, however, this was uncontrolled and unblinded [81]. A re-supplementation trial of carrageenan in 12 patients with ulcerative colitis demonstrated reduced relapse rates in those on carrageenan restriction[96].

Altogether, these observations demonstrate the need for further studies focusing on the role played by long-term emulsifier exposure in healthy individuals as well as in various diseases characterized by a chronic intestinal inflammation state, including IBD. An adequately powered randomised, placebo-controlled, re-supplementation trial of an emulsifier-restriction is underway[97] and should bring new information on the role played specifically by emulsifiers in IBD management.

[H1] Artificial sweeteners

Artificial sweeteners are food additives that have a higher intensity of sweetness than caloric sweeteners such as sucrose, corn syrups and fruit juice concentrates.[98] Most artificial sweeteners transit through the gastrointestinal tract without being digested by the host, and thus come into direct contact with the microbiota.[99,100] Due to growing levels of obesity and type 2 diabetes, many people have been advised to transition from sugar to artificial sweeteners[101]. Up to 32% of adult Americans consume products containing sweeteners.[102] From studies conducted since 2008, the risk of exceeding the ADI globally is low except in those with specific dietary requirements such as children with specific medical needs or people with diabetes[103]. For example, mean daily intakes have been measured for acesulfame (1.62 mg/kg/bw) and aspartame (2.93 mg/kg/bw), and even in those people with intakes at the 97.5th centile were consuming 61% of ADI (5.48 mg/kg/bw) and 24% of ADI (9.63mg/kg/bw), respectively. However, estimated consumption has been calculated based on toxicology and

carcinogenesis assessments, and thus whether lower intakes may impact the microbiome and gut health is yet to be ascertained[66]. Interestingly, there is a temporal correlation with artificial sweetener consumption and the incidence of IBD, although such ecological comparisons are open to considerable confounding[104].

Numerous *in vitro* and animal studies that have investigated artificial sweeteners and their role in gut health, some of which are summarised below (**Table 3**).

[H2] Artificial sweeteners – in vitro and animal models

[H3] Microbiota diversity and composition

The effect of the artificial sweeteners saccharin, sucralose and aspartame on C57BL/6 mice has been examined[114]. The gut microbiome from mice drinking water supplemented with saccharin (0.1 mg/ml, FDA ADI of 5 mg/kg body weight) clustered separately from control groups but also differed from their starting microbiome composition with more than 40 OTUs with significantly different abundance.

SAMP1/YitFc (SAMP) mice which are a model of spontaneous Crohn's-like ileitis were exposed to three levels of sucralose: low dose (1.08 mg/mL) a high dose (3.5 mg/mL, FDA acceptable daily intake) and mega-dose (35 mg/mL, 10x FDA acceptable daily intake). Six weeks of exposure to sucralose did not worsen ileitis severity, but caused alterations in faecal microbiota in both SAMP mice and control mice strain AKR/J. Additionally, in the SAMP mice only, there was a significant increase of Proteobacteria, myeloperoxidase activity and larger clusters of bacteria within the villi, suggesting that sucralose may affect individuals with a genetic susceptibility to Crohn's disease[116].

In a Sprague-Dawley rat model (used to study obesity), 8 weeks exposure to aspartame (5-7 mg/kg/day, equivalent to 2-3 cans of artificially-sweetened soft drinks, where the acceptable daily intake is 40-50 mg/kg/day) resulted in an increase in Firmicutes:Bacteroidetes ratio, Enterobacteriaceae, Roseburia ssp. And *Clostridium leptum*[109]. Unfortunately, baseline microbiota was not analysed prior to aspartame administration, and thus these changes may be related to underlying differences between obese and normal rats and differences in energy consumption thus illustrating the importance of controlling for confounders. However, not all studies have shown microbiome changes that may promote inflammation. A murine model

(C57BL/6) given 0.72 mg/ml of sucralose (which after allometric scaling and adjustment for increased murine metabolic rate is equivalent to the EFSA ADI for sucralose of 15 mg/kg/bw) showed no consistent changes in gut microbiota. Additionally, there was no evidence of changes in caecal length or weight and no signs of watery stools, indicating that sucralose intake did not lead to colitis [122].

These models, along with those described in **Table 3** highlight the varying impact of artificial sweeteners on the gut microbiome but demonstrate the difficulty interpreting these studies with differing models and methodologies.

[H3] Bacterial translocation, gene regulation and bacterial cell-to-cell communication

Male C57BL/6J mice fed sucralose (0.1 mg/ml equivalent to the FDA acceptable daily intake of 5 mg/kg/d in humans) for 6 months had altered gut bacteria composition (14 genera, including those associated with inflammation such as *Ruminococcaceae Ruminococcus*). Additionally, genes related to LPS, flagella protein and fimbriae synthesis increased significantly as well as bacterial toxin genes, such as toxic shock syndrome toxin-1[120]. In another study, also in C57BL/6J mice, supplementation with saccharin for 6 months (0.3mg/ml, FDA ADI for humans) resulted in upregulation of several bacterial genes including those involved with LPS, flagella, fimbriae and bacterial toxins, again demonstrating that artificial sweeteners can impact bacterial penetrability and gene regulation[111].

It has also been postulated that artificial sweeteners may have an effect through quorum sensing, which is a sophisticated network of cell-to-cell communication that enables bacteria to interact and adjust gene expression based on their population density. Most gram-negative bacteria use N-acyl homoserine lactone (AHL) mediated quorum systems. Aspartame, saccharin and sucralose disrupt the AHL-mediated communication systems which could affect protein binding within the gut microbial community[127]. This is of interest in IBD, as people with IBD have lower abundance of the AHL signalling molecule (3-oxo-C12:2-HSL) compared to healthy controls, thus implicating this mechanism in disease pathogenesis[128].

[H3] Intestinal permeability, inflammation, colitis and carcinogenesis

In the azoxymethane/DSS (AOM/DSS) model of colitis-associated CRC, C57BL/6 mice supplemented with 1.5 mg/ml sucralose in drinking water for 6 weeks developed higher

numbers and larger cancers, as well as more severe weight loss, more blood in stools, reduced colonic length, and a higher mortality compared to the AOM/DSS group alone. The addition of sucralose to the AOM/DSS model led to increases in mucosal 20ublic20ng, claudin-1, claudin-4 (indicating gut barrier dysfunction), TNF-α and IL-6, and lower levels of IL-10 and TNF-receptor associated factor-6 (TRAF-6) when compared to the AOM/DSS only group[118]. Sucralose and aspartame influence tight junction proteins claudin-3 and claudin-15 in Caco-2 monolayers, implicating artificial sweeteners in disruption of gut permeability. [124] Similar effects were seen with sucralose (1.5mg/ml) in a DSS-induced colitis model in C57BL/6 mice[119]. However, in a different model of T-cell induced colitis, immunodeficient CD45.2*Tcra*$^{-/}$ mice given congenic CD45.1 naïve CD4^{+} T cells that received sucralose (0.72mg/ml) had a reduced number of donor CD45.1^{+}CD4^{+} T cells, and at day 21 showed reduced numbers of IFN-γ–producing CD4^{+} T cells suggesting that sucralose mitigates T-cell mediated responses. However, this only considers specific inflammatory mechanisms of one cell type in a complex system[122].

Acesulfame-K (150 mg/kg/day) caused histological damage, greater gut permeability and elevated levels of IFN-γ, IL-1β and TNF–α in C57BL/6J mice. There was also higher expression of MAdCAM-1, reduced α-diversity and significant changes in many genera compared to controls. However, the dose used was markedly higher than that consumed by humans (FDA acceptable daily intake 15mg/kg/bw/day, making extrapolation difficult. Interestingly, when microbiota was transferred from mice exposed to acesulfame-K to non-exposed mice, the above changes did not reoccur, which suggests that unlike some of the findings for emulsifiers, the effects seen in this model are not microbiota driven[105].

The *in vivo* experiments described differ in their methodology, sweeteners and doses. Critically, these effects are reviewed over a relatively short time span, whereas any potential effects of artificial sweeteners in humans would follow chronic exposure and are intertwined with the effects of other dietary components on gut homeostasis that may compound one another. Within sweeteners themselves, it must be noted that some contain fillers such as maltodextrin, which may themselves interact and influence the microbiome[129]. The range of artificial sweeteners may exert effects through different mechanisms, and it is important to be specific about which sweeteners result in which effects. Sweetener and control groups may also differ in energy intake and macronutrient composition, that may be partly responsible for the

observed changes in microbiota[130]. Thus, mouse models are useful for mechanistic insights, but will never fully recreate the complex genetic and environmental factors surrounding humans [131,132].

[H2] Artificial sweeteners – human studies

Several small studies of the effect of sweeteners on the human gut have been performed (**Table 4**). One observational study of 31 humans measured dietary intake using a 4-day food diary to record habitual sweetener intake and compared gut microbiome measured from a faecal sample on the fifth day. Microbiota composition did not differ between consumers and non-consumers of sweeteners, but bacterial diversity evaluated by UniFrac analysis was different between consumers and non-consumers of both acesulfame K and aspartame. However, background diet was not controlled for, and dose-response relationships were not examined as the groups were simply dichotomised into consumers or non-consumers. This analysis may therefore miss important associations in those with highest sweetener intake[136]. An intervention study of seven healthy adults (non-habitual users of artificial sweeteners) who were supplemented with saccharin (5 mg/kg, the FDA acceptable daily intake) for 7 days reported that those who developed poorer glycaemic responses (responders, n=4) were found to have different microbiome clustering to non-responders (n=3)[114]. Transfer of day 7 stool from post-sweetener exposed responders to germ-free mice, resulted in significant glucose intolerance compared to germ-free mice who received day 1 stool from the same responders prior to sweetener exposure. This study did not analyse whether there were any deleterious effects on intestinal permeability, inflammation or carcinogenesis but this does illustrate that sweeteners may influence the microbiome that may in turn lead to the manifestation of disease, and importantly illustrates the inter-individual variation in responses by individuals, which perhaps is influenced by other factors including host genetics as well as other environmental exposures.

In contrast, other trials have shown no changes in gut microbiota after artificial sweetener consumption. Thus, a randomized placebo-controlled trial of saccharin in 54 healthy volunteers, reported that those given the maximum acceptable daily intake of saccharin did not show a change in microbial diversity or composition. Additionally, a double-blind randomized crossover trial of aspartame and sucralose in healthy volunteers demonstrated that neither sweetener induced a change in microbial diversity, composition or metabolite production (such

as SCFAs). However, these studies were carried out over short intervention periods and have differing methodologies. [134 135 137].

One randomised controlled trial has compared an artificial sweetener-containing diet (50-100 mg/d of 80% sucralose and 20% aspartame, acesulfame K and saccharin) to an artificial-sweetener-restricted diet (<10 mg/d) in healthy volunteers half of whom experienced gastrointestinal symptoms at baseline[138]. After 5 weeks, the incidence of diarrhoea, postprandial discomfort, constipation and burning increased in the sweetener-containing group; whereas abdominal pain, postprandial discomfort, burning, early satiety and epigastric pain decreased in the sweetener-restricted diet group. No microbial analysis was performed in this study.

Finally, the International Agency for Research on Cancer (IARC) recently reclassified aspartame as "possibly carcinogenic to humans" with reference to "limited evidence" for increased risk of hepatocellular carcinoma and "inadequate evidence" for other types of cancer [140]. This decision was based upon three large cohort studies that used consumption of artificially sweetened beverages as a proxy for aspartame intake and found positive associations with artificially sweetened beverage consumption and hepatocellular carcinoma risk [141-143]. Contrastingly, a recent prospective cohort study in post-menopausal women (aged 50-79 years) demonstrated that sugar-sweetened beverages were associated with chronic liver disease and liver cancer, whereas artificially sweetened beverages did not show the same association. Unfortunately, researchers were not able to extract data for individual artificial sweeteners [144].

The studies thus far have demonstrated that some artificial sweeteners may promote some changes in microbiota and inflammation, but the data for humans is far from consistent and most studies were conducted in healthy volunteers. This underscores the need for adequately powered RCTs coupled with mechanistic studies to definitively determine whether aspartame and other sweeteners are pro-inflammatory or indeed carcinogenic and whether their exclusion can manage some gut diseases.

[H1] Food colours

Food colours are additives that are added to foods to make up for colour losses (e.g. due to exposure to light, air, moisture, variations in temperature), to enhance naturally occurring

colours or to add colour to foods that would otherwise be colourless or coloured differently [145]. Food colours have no nutritional value.[146] Intake of food colours has been examined in the United States, and current levels of consumption are reportedly within safety limits even in high consumers[147]. Despite this, there is limited data on the effect of food colours on gut health. One study investigated two common food colours red-40 (E129, acceptable daily intake 7 mg/kg/d) and yellow-6 (E110, acceptable daily intake 4 mg/kg/d). Red-40 is an organic compound that contains the functional azo group (-N=N-)[148] and is metabolised by AZO-reduction in the gastrointestinal tract, releasing two metabolites, 1-amino-2naphthol-6-suphonate sodium salt (ANSA-Na) and cresidine-4-sulphonate sodium salt (CSA-Na). Yellow-6 also yields ANSA-Na when metabolised and has been shown to induce colitis in a R23FR mouse model (mice that conditionally overexpress IL-23R in CX3CR1+ myeloid cells)[147].

Although red-40 did not induce colitis in control mice, in R23FR mice, red-40 induced colitis when given after the induction of IL-23, suggesting colitis is only triggered in the presence of IL-23. Yellow-6 also promoted colitis in R23FR mice. These findings were microbiota dependent as they did not occur in germ-free mice. It seems that the colitogenic properties of red-40 are activated after being metabolised by commensal bacteria[149,150], as colitis was not observed in germ-free mice exposed to red-40, independent of changes in microbiota diversity or I abundance. The mechanism for this seems to be mediated by CD4+ cells and is dependent on IFN-γ but not TNF-α, IL-22, IL-17a or IL-17f as only IFN-γ blockade decreased colitis severity. Given the role of IFN-γ in IBD, it would be pertinent to know whether these deleterious changes occur in people with IBD and whether any impacts extend to non-immune mediated gut disease.

Translating these findings to humans is once again difficult. First, the colours examined in these pre-clinical models are not the most widely used food colours that humans are exposed to through diet[65]. Second, the interaction of colours with other foods and food matrices may also impact their effects on the gut.

[H1] Microparticles / nanoparticles

Dietary microparticles are defined as inorganic bacterial sized particles (0.1-1 mm) often used as food additives to influence the colour, consistency or appearance. They are also used in tooth paste and as a carrier or coating in many pharmaceuticals and are highly stable and resistant

to degradation. The most commonly used microparticles are inorganic compounds of titanium dioxide (TiO_2, E171), aluminium silicate (AlSi, E559) and silicon dioxide (SiO_2, E551). Titanium dioxide has been used as a whitening / brightening agent, a clouding agent in non-dairy creamers, a flour bleaching agent and to separate layers of different colours in sweets, whereas aluminium silicates are used as anti-caking agents. There is likely significant contamination of microparticle food additives with nanoparticles (<100 nm) which can penetrate cell membranes although are unable to penetrate the intact intestinal mucus layer. In 2022, the European Union banned the use of TiO_2 as a food additive although its use is still permitted in medicinal products[151] although this continues to be used in other countries including in the United Kingdom, likely leading to considerable confusion for consumers.

The daily intake of dietary microparticles varies between populations and dietary patterns with estimates for silicates of 35 mg/d[152] and $Ti0_2$ ranging from 2.5-469 mg/d in adults and up to 556 mg/d in children[151,153].

[H2] Microparticles / nanoparticles – in vitro and animal models

$Ti0_2$ is absorbed by intestinal epithelial cells and macrophages triggering the release of proinflammatory cytokines[154]. $Ti0_2$ accumulates in immune cells within Peyer's patches in exposed rats[155]. In murine models $Ti0_2$ ingestion exacerbated induced colitis via activation of the inflammasome[154]. Long term $Ti0_2$ exposure is associated with release of reactive oxygen species, altered gene transcription impacting the transcriptome and both dysplasia and colorectal cancer in rodent colitis models[156,157]. Similar findings have been reported for dietary aluminium intake, which also impairs intestinal barrier function[158].

[H2] Microparticles/nanoparticles – human studies

In healthy subjects, $Ti0_2$ is trapped within the lumen by the intestinal mucous layer[159]. However, microparticles have been detected within phagocytes in intestinal lymphoid aggregates in patients with IBD[160]. In addition, serum titanium levels are elevated in patients with active ulcerative colitis compared with controls[154].

The role of microparticles in driving intestinal inflammation in Crohn's disease has been assessed in two dietary intervention studies[161,162]. An initial pilot RCT in 20 patients with active Crohn's disease reported a significant reduction in disease activity in those on a low

752 microparticle diet ($Ti0_2$/AlSi) compared with control with seven patients in the intervention
753 group achieving clinical remission[162]. However, a subsequent 16 week randomised controlled
754 study in 83 patients with active Crohn's disease reported no difference in clinical response or
755 remission rates between the low and normal dietary microparticle groups[161]. One key
756 difference between these trials is that the intervention in the pilot study restricted all processed
757 food whereas the larger multicentre trial restricted only food containing microparticles.
758 Therefore, it is possible that the restriction of food additives other than microparticles was
759 responsible for the preliminary benefit seen in the pilot study.
760
761 Despite the findings in the larger RCT that there is no evidence that microparticles exacerbate
762 Crohn's disease, the EU has recently banned the use of TiO2 in food sources.
763
764 **[H1] Evidence for dietary restriction of UPF and food additives in clinical trials**
765 The concept of restricting dietary intake of UPF and food additives as a therapy for GI disease
766 largely focussed on the IBD population and arose from the epidemiological studies and animal
767 models highlighted previously in this review. However, one study has also investigated the
768 impact of artificial sweetener restriction on functional gastrointestinal symptoms in healthy
769 volunteers (see section on "Artificial sweeteners – human studies").
770
771 Trials that have investigated this in some way include (i) focussed interventions designed to
772 restrict only UPF or a specific food additive (these are discussed earlier in the relevant sections
773 on emulsifiers and sweeteners); (ii) diets that intentionally restrict UPF or food additives in
774 addition to other dietary components; and (iii) diets that will likely reduce intakes as part of
775 wider dietary intervention not specifically targeting UPF or food additives (**Table 5**).
776
777 Interpretation of the impact of dietary interventions requires careful analysis of the population
778 included, the nature, delivery and blinding of the intervention and any control, as well as the
779 outcome studied. For example, many patients with IBD have functional gastrointestinal
780 symptoms in the absence of active intestinal inflammation[170]. Whilst modifying dietary intake
781 may have a marked impact on such symptoms, this will not necessarily correlate with
782 improvement of underlying inflammation. Thus, although a low FODMAP diet may improve
783 functional symptoms in quiescent IBD it does not impact underlying disease activity[165].
784

Clinical trials of diets that that intentionally restrict UPF or food additives in addition to other dietary components have recently been published. The Crohn's disease exclusion diet (CDED) is a whole food diet designed to reduce exposure to components hypothesised to negatively impact the microbiome, intestinal permeability and the mucosal immune system and is combined with partial enteral nutrition. The diet mandates daily consumption of specific foods such as chicken and eggs alongside an allowed list of fruit, vegetables, and simple/complex carbohydrate but excludes dairy, gluten, all food additives (including emulsifiers and artificial sweeteners) and all "processed foods". A recent 6 week randomised controlled induction trial in children with active Crohn's disease demonstrated that the CDED with PEN was significantly more tolerable than exclusive enteral nutrition, which is a current standard of care for this patient group[163]. There was no difference in symptom based and objective assessment of efficacy between the two approaches. Most management approaches use CDED alongside partial enteral nutrition, as described above, and it is important to note that enteral formulas themselves are UPF and many contain food additives including emulsifiers[171]. In the only trial where CDED was used alone, it was shown to be as effective as CDED plus partial enteral nutrition in a small RCT of adults with active Crohn's disease, although there was no control group in this comparison[164] (**Table 5**).

Additional multicomponent dietary interventions likely to restrict UPF and food additive intake that have undergone assessment of clinical efficacy in RCTs in Crohn's disease include the specific carbohydrate diet, Mediterranean diet, low meat diet and Crohn's disease anti-inflammatory diet (**Table 5**). Two ongoing studies of the CD-TREAT diet plan are in progress (one uncontrolled study in active Crohn's disease, one randomised trial comparing CD treat with standard diet after EEN)[172,173]. CD-TREAT is a prescriptive, personalized diet that aims to recreate the impact of EEN on the gut microbiome and metabolome by the exclusion of certain dietary components (e.g., gluten, lactose, and alcohol) and matching of others (macronutrients, vitamins, minerals, and fibre) using ordinary food. Careful analysis of the impact of these interventions on UPF and food additive intake in addition will be required to assess whether any observed benefit can be ascribed to their restriction.

Implications for policy, food industry, clinical practice, and research

The increased availability and consumption of UPF, including those containing food additives, alongside the findings of the evidence in this review have numerous implications for policy, food industry, clinical practice and research.

In terms of policy, many national dietary recommendations refer in broad terms to food processing, however, thus far only seven countries explicitly recommend reducing intakes of UPF (Belgium, Brazil, Ecuador, Israel, Maldives, Peru, Uruguay) and five countries explicitly recommend consuming more 'unprocessed' or 'minimally processed' foods (Brazil, Brunei Darussalam, Kenya, Malta, New Zealand)[174]. In the UK, the Scientific Advisory Committee on Nutrition reported that existing dietary recommendations to reduce saturated fat, free sugars, and salt were already relevant to UPF, however, there remained issues regarding whether the evidence for the associations of UPF intake with health outcomes were independent of the poor nutritional profile of such diets as well as the limited information on the impact of UPF, and their reduction, on population subgroups (e.g. socio-economic status, older people)[30].

Some countries have introduced fiscal policies, such as taxation, in relation to specific food groups (e.g. sugar-sweetened drinks) or for foods where specific nutrient profiles are breached (e.g. where free-sugar content is above specified limits). Although some of these policies make explicit mention of targeting UPF, the criteria for fiscal policy intervention often relate to the products' nutritional profile rather than degree of processing[175]. Labelling of foods as being UPF is currently not mandated, although a recent RCT in 21,159 people in France showed that a front of pack label indicating whether the product was a UPF (black border on nutrient score), resulted in 174-fold greater odds of correctly identifying almost all UPFs[176]. Mandatory labelling of food additives on ingredients lists is a requirement, but the existence of hundreds of different food additives and the lack of consensus on labelling approaches (e.g. chemical names vs E numbers) can make these challenging for consumers to identify.

In terms of food additives, food policy in relation to the use, and the quantity, is regionally determined. For example, the decision to ban nanoparticle TiO_2 in the EU was based upon evidence of potential for genotoxicity (e.g. DNA strand breaks, chromosomal damage), immunotoxicity and neurotoxicity. The method through which food additive safety is determined relates to strict experimental evidence of carcinogenicity, toxicity, and mortality in animals, whereas evidence for alterations to microbiome are rarely included.

Given the high intakes of UPF in many high-income countries (exceeding 50% of total energy in some)[6], reducing UPF and food additive exposure would require extensive behaviour change by the public and widespread product reformulation by food industry. Optimal reformulation of UPF would require improved understanding of which processes or components are responsible for the potential harmful health effect in order that these specifically can be altered, removed or replaced[177]. Importantly, some of the important functions of foods additives (e.g. microbiological safety, long shelf life), would still need to be addressed in reformulated products.

There are also clinical implications to any approach that requires avoidance or reduction in intake of UPF and food additives. In view of the extremely limited evidence from RCTs of the impact of UPF and food additives in gut disease, in particular on clinical endpoints, we submit that it is too early to recommend that patients should follow a diet that restricts these foods. It is important that clinicians understand that the overwhelming majority of evidence for UPFs is from epidemiology that investigates the risk of developing disease in the general population, rather than their use in disease management. If RCTs are able to prove causality and the effectiveness of UPF and food additive restriction, then health professionals will require a good understanding of what UPF are, which is currently not well understood even by food and nutrition professionals (nutritionists, food technologists, dietitians and doctors)[178]. Currently, the public also have a relatively poor understanding of what foods are UPF[179], and the optimal methods of educating them on this are unknown. Finally, the impact of UPF and food additive restriction on nutrient intake is an important clinical consideration, as this would require a dramatic dietary change for some patients, and an impact on nutritional status in vulnerable patients should be avoided.

There are numerous implications for research on UPF and food additives. Studies are urgently required to investigate the effect of UPF on gut health and disease, similar in design to the only feeding study thus far comparing high UPF diet with isocaloric low UPF diet[29], although adequately powered studies with adequate duration in free-living patient populations may be more practical, economically viable and externally valid to clinical practice than domiciliary feeding studies. The evidence to date relates mostly to disease risk, and RCTs investigating reducing UPF intake on disease prevention are warranted but would need to be very large and

would be financially costly. Trials of UPF and food additive restriction in disease management are required including in the treatment and maintenance of IBD. Studies are required that investigate whether the presence of food processing and food additives in UPF *per se*, as opposed to their nutrient profile, are responsible for the reported health risks. For example, RCTs are required comparing two high UPF diets comprising foods with poorer nutrient profile (e.g. cakes, pastries, ready meals) and improved nutrient profile (e.g. wholemeal bread, fruit yoghurts, fortified breakfast cereals) to investigate whether processing and food additives offsets the benefits of a beneficial nutrient profile.

Robustly designed RCTs of UPFs and food additives have challenges that are specific to dietary intervention studies[180]. Dietary collinearity means that reducing intake of one component may unintentionally influence intake of nutrients (e.g. reducing sweeteners may increase free-sugar intake, reducing emulsifiers may reduce fat intake) as well as other food additives from the same class (e.g. reducing carboxymethylcellulose may reduce global emulsifier intake[181]) or different class (e.g. reducing emulsifiers may reduce stabiliser intake[182]) due to frequent co-occurrence that may confound the findings. Control groups are notoriously challenging in dietary intervention trials and the choice of standard or alternative diets can confound blinding whilst placebo diets are intensive to design and deliver[183].

Identifying the culprits for any effect of UPF on health is required, in order that interventions, policy and reformulation can target the source of potential harm. For example, *in vitro* studies show that not all emulsifiers impact the microbiome[86], and may not all be considered potentially deleterious to the gut health and disease. Additionally, the two emulsifiers with most extensive evidence of effects on gut health in animal models (**Table 2**), carboxymethylcellulose and polysorbate-80, are only present in 179 and eight foods respectively, in the UK [181]. Finally, although currently the major culprit is thought to be food additives, contamination from packing materials may also be implicated. For example, perfluoroalkyl and polyfluoroalkyl substances are commonly used in food packaging and can migrate into food [184] and have been shown to impact the gut microbiome, barrier function and inflammation in animal models[185]. As such these other potential mechanisms of UPF impact on health should also be investigated.

Conclusion

Data have accumulated over the last decade to suggest a central role played by the diet in general, and UPF intake in particular, in gut health in general, and in the pathogenesis of gastrointestinal diseases in particular. While many suspects have been identified, food additives largely used by the food industry seem to be at play in detrimentally impacting the intestinal environment. Such advances were made possible thanks to rapid developments toward the understanding of the intestinal microbiota, but significant additional efforts now appear needed to transition from animal-based observation to the clinical settings. Moreover, such investigation of dietary components in gastrointestinal disorders will need to take great consideration of the multi-factorial aspect of these diseases. While numerous challenges appear in the path of this field of research, ambitious RCTs are underway and should soon bring significant new understanding of what patients with some gastrointestinal disorders should, and should not, eat. Moreover, accumulating knowledge on the diet-microbiome-intestine trialogue should provide innovative approaches for the prevention of these chronic and debilitating disorders.

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Competing interests

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1480 **Table 1 Summary of epidemiological studies of UPF and risk of gut disease**

Reference	Study design and population	Numbers in cohort (or number of controls)	Follow-up	Dietary assessment method and UPF classification	Disease diagnosis method	Risk reporting	Disease	Incident cases	Risk of gut disease (unadjusted or minimally adjusted)	Risk of gut disease (adjusted)	Variables adjusted for
Inflammatory bowel disease											
Narula et al, 2021[31]	Cohort study (PURE) 21 countries, 59.2% female 50.2 y (SD 9.7 y)	116,037	Median 9.7 y (IQR 8.9-11.2)	FFQ (country specific, 1 year recall) Researcher-defined UPF	Self-report, followed by medical record confirmation in 20% of positive cases	HR (95% CI) ≥5 serves/d Reference <1 serves/d	IBD	467	3.18 (2.49 to 4.07) P<0.001	1.92 (1.28 to 2.90) P=0.004	Age, sex, geographical region, education, alcohol intake, smoking status, location, BMI, energy intake, Alternate Health Eating Index (AHEI)
							Crohn's	90	5.84 (3.57 to 9.54) P<0.001	4.90 (1.78 to 13.45) 0.008	
							UC	377	2.63 (1.97 to 3.51) <0.001	1.52 (0.96 to 2.41) 0.06	
Lo et al, 2022[32]	Cohort study (Nurses' Health Study I and II; Health Professionals Follow-up Study) USA 83.0% female 44.7-45.7 y mean	245,112	5,468,444 person years (mean 22.3 y)	FFQ (1 year recall) NOVA	Self-report, followed by medical record confirmation in all positive cases	HR (95% CI) Q4 (median 46.4% energy from UPF) Reference Q1 (median 21.0% energy from UPF)	Crohn's	369	1.75 (1.29 to 2.35) P=0.0001	1.70 (1.23 to 2.35) P=0.0008	Age, cohort, questionnaire cycle, race/ethnicity, family history of IBD, smoking status, BMI, physical activity, energy intake, AHEI, regular NSAID use, oral contraceptives; menopausal hormone therapy.
							UC	488	1.25 (0.97 to 1.62) P=0.11	1.20 (0.91 to 1.58) P=0.25	

Vasseur et al, 2021[33]	Cohort study (NutriNet-Santé) France 78.0% female 43.3 y (SD 14.7)	105,832	238,924 person years (mean 2.3 y , SD 2.2 y)	≥3 x online 24h recall NOVA	Self-report, followed by medical record confirmation in 15% of cases	RR (95% CI) Q3 (>19.1% food weight as UPF) Reference Q1 (<12.4% food weight as UPF)	IBD	75 (27 Crohn's, 48 UC)	1.81 (1.05 to 3.12), P=0.03	1.44 (0.70 to 2.94) P=0.30	Age, sex, income, education, marital status, residence, BMI, physical activity, smoking status, hormonal contraception, number of 24h recalls, energy intake, "healthy" dietary pattern
Meyer et al, 2022[34]	Cohort study (EPIC) 8 European countries 68.6% female 51.7 y (SD 10.1)	413,590	4,920,526 person years (mean 13.2 y)	EPIC FFQ (1 year recall) NOVA	Self-report, followed by medical record confirmation in all cases	HR (95% CI) Q4 (mean 50.6% energy from UPF) Reference Q1 (mean 13.3% energy from UPF)	Crohn's	179	NR	1.48 (0.79 to 2.76)	Age, sex, centre, education, smoking status, BMI, physical activity, energy intake, alcohol consumption
							UC	431	NR	0.93 (0.61 to 1.43)	
Chen et al, 2022[35]	Cohort study (UK Biobank) United Kingdom 54.8% female 56.2 y (SD 7.9)	185,849	16,247 person years (mean 9.8 years, IQR 9.5-10.8)	≥1 x online 24h recall NOVA	Hospital or primary care record review	HR (95% CI) Q5 (per serving, energy kJ from UPF, % energy from UPF) Reference Q1	IBD	841	Per serving 1.34 (1.07 to 1.67), P=0.001 Energy from UPF 1.20 (0.97, 1.49), P=0.010 % energy from UPF	Per serving 1.16 (0.91, 1.48), P=0.091 Energy from UPF 1.18 (0.95, 1.46), P=0.017 % energy from UPF 1.15 (0.93, 1.42), P=0.097	Age, age-squared, sex, ethnicity, deprivation, smoking status, drinking status, education, physical activity, BMI, IBD genetic risk, and total energy (for 'per serving' only).

									1.21 (0.98, 1.50), P=0.016		
							Crohn's	251	Per serving 1.52 (1.02 to 2.27), P=0.001 Energy from UPF 1.49 (1.01 to 2.20), P=0.007 % energy from UPF 2.09 (1.39 to 3.16), P<0.001	Per serving 1.61 (1.03 to 2.51), P=0.002 Energy from UPF 1.46 (0.98 to 2.16), P=0.011) % energy from UPF 2.00 (1.32 to 3.03), P=0.001	
							UC	590	Per serving 1.27 (0.97 to 1.65), P=0.070 Energy from UPF 1.10 (0.85 to 1.42), P=0.174 % energy from UPF 0.97 (0.75 to 1.25), P=0.581	Per serving 1.01 (0.75 to 1.35), P=0.956 Energy from UPF 1.08 (0.83 to 1.39), P=0.235 % energy from UPF 0.91 (0.70 to 1.18), P=0.948	

Functional gastrointestinal disorders											
Schnabel et al, 2018[36]	Case-control study (NutriNet-Santé) France 76.4% female 50.4 y (SD 14.0)	33,343	NR	≥3 x online 24h recall NOVA	Rome III questionnaire (self-report)	OR (95% CI) Q4 (>20.6% food weight as UPF) Reference Q1 (<9.7% food weight as UPF)	IBS	3516	1.21 (1.09 to 1.34), P<0.0001	1.25 (1.12 to 1.39), P<0.0001	Sex, age, income, education, marital status, residence, BMI, physical activity, smoking, energy intake, season of food records, time between food and FGIDs questionnaire, Adherence to national diet recommendation score
							Functional constipation	1785	1.02 (0.89 to 1.16), P=0.91	0.98 (0.85 to 1.12) P=0.66	
							Functional diarrhoea	368	1.02 (0.77 to 1.36) P=0.77	0.92 (0.69 to 1.24) P=0.70	
							Functional dyspepsia	1303	1.32 (1.12 to 1.55) P=0.0002	1.25 (1.05 to 1.47) P=0.004	
Gastrointestinal cancer											
Fiolet et al, 2018[27]	Cohort study (NutriNet-Santé) France 78.3% female 42.8 y (SD 14.8)	104,980	426, 362 person years (median 5 y)	≥2 x online 24h recall NOVA	Self-report, followed by medical record confirmation in >90% of positive cases	HR (95% CI) Q4 (>23.2% of food weight as UPF) Reference to Q1 (<11.8% of food weight as UPF)	Colorectal cancer	153	1.49 (0.92 to 2.43) P=0.1	1.23 (1.08 to 1.40) P=0.07	Age, sex, energy intake, number of dietary records, smoking, education, physical activity, height, BMI, alcohol intake, family history; intakes of lipids, sodium, carbohydrates, 'Western' dietary pattern.
Wang et al, 2022[37]	Cohort study (Health Professionals Follow-up Study, Nurses'	Men 46,341 Women 159,907	24-28 years	FFQ (1 year recall) NOVA	Self-report, followed by medical record confirmation in all positive cases where possible	HR (95% CI) Q5 (Men ≥7.9, Women ≥7.2 energy-adjusted	Colorectal cancer	Men 1,294 Women 1,922	Men 1.24 (1.04 to 1.47) P=0.04 Women 1.08 (0.94 to 1.24) P=0.08	Men 1.29 (1.08 to 1.53) P=0.01 Women 1.04 (0.90 to 1.20) P=0.29	Age, year of questionnaire, race, family history of cancer, endoscopy history, alcohol intake, physical activity, smoking

	Health Study I and II) USA Men 53.5-54.9 y mean Women 52.0-53.0 y mean					servings /d of UPF) Reference Q1 (Men ≤4.1, Women ≤4.0 energy-adjusted servings /d of UPF)	Proximal colon cancer	Men 443 Women 797	Men 1.32 (0.97 to 1.79) P=0.22 Women 1.16 (0.93 to 1.45) P=0.11	Men 1.34 (0.98 to 1.82) P=0.20 Women 1.11 (0.89 to 1.39) P=0.24	status, smoking pack years, energy intake, aspirin use, menopausal status, postmenopausal hormone use
							Distal colon cancer	Men 368 Women 455	Men 1.62 (1.18 to 2.23) P=0.002 Women 1.10 (0.82 to 1.46) P=0.13	Men 1.72 (1.24 to 2.37) P<0.001 Women 1.07 (0.80 to 1.43) P=0.19	
							Rectal cancer	Men 267 Women 398	Men 1.01 (0.68 to 1.48) P=0.72 Women 1.10 (0.81 to 1.51) P=0.70	Men 1.05 (0.71 to 1.56) P=0.89 Women 1.08 (0.79 to 1.49) P=0.84	
Romaguera et a., 2021[38]	Case-control study (Multi-Case-Control) Spain 49.4% female 62.9 y (SD 12.0)	3543 controls	N/A	FFQ (1 year recall) NOVA	Colonoscopy and histology (cases only)	OR (95% CI) Q3 (>14.6% of food weight as UPF) Reference Q1 (<6.9% of food weight as UPF)	Colorectal cancer	1852	1.44 (1.24 to 1.67) P<0.001	1.30 (1.11 to 1.51) P=0.001	Sex, age, study area, education, BMI, physical activity, smoking, NSAIDs, family history, energy intake, ethanol intake
Kinany et al, 2022[39]		1453 controls	N/A			OR (95% CI)	Colorectal cancer	1453	1.28 (1.13 to 1.46)	1.40 (1.22 to 1.61)	Age, education, family history of CRC,

	Matched case-control study Morocco 50.7% female 56 .0 y (SD 13.8)			FFQ (1 year recall) NOVA	Colonoscopy and histology (cases only)	Q3 (≥37.3 g/d UPF) Reference Q1 (<3.9 g/d UPF)	Colonic cancer	729	1.26 (1.06 to 1.51)	1.36 (1.12 to 1.66)	smoking status, physical activity, BMI, energy intake
							Rectal cancer	724	1.31 (1.09 to 1.57)	1.44 (1.18 to 1.76)	
Fliss-Isakov et al, 2020[40]	Case-control study Egypt 49.2% female 58.5 y (SD 6.6)	358 controls	N/A	FFQ (1 year recall) NOVA	Colonoscopy in all and histology in cases	OR (95% CI) Q3 (≥44.9% of total E from UPF) Reference Q1 (<30.4% of total E from UPF)	Adenoma	294	N/R	1.75 (1.14 to 2.68) P=0.009	Age, gender, BMI, energy intake, aspirin use, indication for colonoscopy
							Non-advanced adenoma	147	N/R	1.31 (0.76 to 2.25) P=0.325	
							Advanced adenoma	147	N/R	2.17 (1.29 to 3.65) P=0.003	
							Proximal adenoma	143	N/R	2.38 (1.37–4.11) P=0.002	
							Distal adenoma	151	N/R	1.39 (0.82–2.34) P=0.212	
Zhong et al, 2023 [41]	Cohort study (PLCO) USA 52.5% female 65.6 y mean (SD 5.7)	98,265	871040 person years (mean 8.9 y, SD 1.9)	FFQ (1 year recall) NOVA	Self-report, followed by medical record confirmation in all positive cases where possible	HR (95% CI) Q4 (>3.7 servings/d of UPF, energy adjusted) Reference Q1 (<0.9 servings/d of UPF)	Pancreatic cancer	387	1.47 (1.10 to 1.97) P=0.012	1.49 (1.07 to 2.07) P=0.021	Age, sex, race, smoking, alcohol, BMI, aspirin, diabetes, family history of pancreatic cancer, energy intake

BMI, body mass index

FFQ, food frequency questionnaire

N/A, not applicable

NR, not reported

OR, odds ratio; RR, relative risk; HR, hazard ratio

Where quantiles are used, data represents the highest quantile reported (e.g. Q3 is tertile 3, Q4 is quartile 4, Q5 is quintile 5) compared with the reference quantile Q1

Table 2 - In vitro, animal- and human research studies investigating the impact of dietary emulsifiers on gastrointestinal microbiology and health

Reference	Model	Emulsifier studied	Key findings related to gut health
Swidsinski et al, 2009[67]	*in vivo* - IL10-/- mice	Carboxymethylcellulose	- Bacterial overgrowth in the small intestine - Evidence of small intestinal inflammation in a subset of animals
Roberts et al, 2010[68]	*in vitro* - M-cell monolayer	Polysorbate-80	- 2-fold increase in translocation of *E. coli* across M cell monolayer in the presence of polysorbate 80
Maronpot et al, 2013[69]	*in vivo* - WT rats	Gum ghatti	- No major differences compared to control diet
Chassaing et al, 2015[70]	*in vivo* - WT, TLR5-/- and IL10-/- mice	Carboxymethylcellulose Polysorbate 80	- Alterations in microbiota composition and localisation in proximal colon - Metabolic dysregulation and chronic low-grade intestinal inflammation in WT mice and TLR5-/- mice - Increase colitis incidence and severity in IL10-/- mice
Lecomte et al, 2016[71]	*in vivo* - WT mice	Milk-derived polar lipid emulsifier Soybean lecithin	- Metabolic dysregulation and chronic low-grade inflammation in WT mice consuming soybean lecithin
Viennois et al, 2017[72]	*in vivo* - WT mice (model of colorectal cancer)	Carboxymethylcellulose Polysorbate 80	- Alterations in microbiota composition and pro-inflammatory potential - Increase susceptibility to chemically induced colorectal cancer

Chassaing et al, 2017[73]	*in vitro* - mSHIME system *in vivo* - WT mice	Carboxymethylcellulose Polysorbate 80	- Direct impact of carboxymethylcellulose and polysorbate on the human intestinal microbiota, with alterations in composition and pro-inflammatory potential - Human microbiota that had been emulsifier-treated *in vitro* and transferred to germ-free mice, resulted in promotion of metabolic dysregulations and chronic low-grade intestinal inflammation
Jiang et al, 2018[74]	*in vivo* - WT mice	Glycerol Monolaurate	- Metabolic dysregulation, alterations in microbiota composition and chronic low-grade inflammation
Lock et al, 2018[75]	*in vitro* - porcine mucus *in vitro* - Caco-2 and HT29-MTX cells	Carboxymethylcellulose Polysorbate 80	- Carboxymethylcellulose impacted mucus pore size and significantly decreased *E. coli* speed and particle diffusion rates through mucus - Polysorbate 80 increased *E. coli* speed in mucus. - Both emulsifiers altered mucus quantity and thickness *in vitro* in mucus-producing cell cultures and *in vivo* in rats.
Laudidi et al, 2019[76]	*in vivo* - WT mice (DSS model of colitis)	Maltodextrin	- exacerbated intestinal inflammation - reduction of mucin-2 expression
Holder et al, 2019[77]	*in vivo* - WT mice	Carboxymethylcellulose Polysorbate 80	- Alterations in microbiota composition - Metabolic deregulations and chronic low-grade intestinal inflammation - Alterations in anxiety-like behaviour and social behaviour - Altered expression of neuropeptides implicated in modulation of feeding
Temkin et al, 2019[78]	*in vivo* - WT mice	Dioctyl sodium sulfosuccinate	- in male offspring of treated dams, observation of metabolic dysregulation and increased markers of chronic inflammation
Furuhashi et al, 2020[79]	*in vivo* - WT mice (indomethacin-induced lesions model)	Polysorbate 80	- Alterations in small intestinal microbiota composition - Exacerbation of indomethacin-induced small-intestinal lesions - Elevation in interleukin-1β expression

Zhao et al, 2020[80]	*in vivo* - WT mice (diet-induced obesity model)	Glycerol monolaurate	- Impact on microbiota composition - In high-fat diet-treated mice, glycerol monolaurate reduced body weight and visceral fat deposition, improved hyperlipidaemia and hepatic lipid metabolism, and ameliorated glucose homeostasis and inflammation
Sandall et al, 2020[81]	*in vivo* - WT mice Humans with Crohn's disease	Carboxymethylcellulose Polysorbate 80	- Metabolic dysregulation and reduced colonic weight (evidence of chronic low-grade intestinal inflammation) - In Crohn's disease, dietary emulsifier restriction is feasible
Miclotte et al, 2020[82]	*in vitro* - mSHIME in vitro microbiota system	Carboxymethylcellulose Polysorbate 80 Soy lecithin Sophorolipids Rhamnolipids	- Alterations in microbiota composition and gene expression, in a compound-dependant manner - Alterations in microbiota pro-inflammatory potential, in a compound-dependant manner
Nishimura et al, 2020[83]	*in vivo* - WT mice	Polysorbate 80	- Polysorbate 80 consumption increase intestinal permeability and circulating level of lipopolysaccharide - Polysorbate 80 consumption induce skeletal muscle inflammation
Viennois et al, 2020[84]	*in vitro* - adherent-invasive *E. coli* strains *in vivo* - WT and IL10-/- mice	Carboxymethylcellulose Polysorbate 80	- Combination of intestinal colonization with adherent-invasive E. coli strain and dietary emulsifier consumption is sufficient to induce chronic intestinal inflammation - Exposure of adherent-invasive E. coli to emulsifiers in vitro increases its motility and ability to adhere to intestinal epithelial cells. - Emulsifiers directly induce expression of clusters of genes that mediate adherent-invasive E. coli virulence and promotion of inflammation
Viennois et al, 2021[85]	*in vivo* - APCmin mice (model of spontaneous intestinal adenoma)	Carboxymethylcellulose Polysorbate 80	- Alterations in microbiota composition - Increased small intestinal tumour development

Naimi et al, 2021[86]	*in vitro* - MiniBioReactor Array (MBRA) in vitro microbiota system	Carboxymethylcellulose, Polysorbate 80, Soy lecithin Sunflower lecithin, Maltodextrin, Propylene glycol alginate, Iota carrageenan, Kappa carrageenan, Lambda carrageenan, Xantham gum, Gum Arabic, Guar gum, Locust bean gum, Agar, DATEM, Hydroxypropyl methylcellulose, Sorbitan monostearate, Mono- and diglycerides, Glyceryl Stearate, Glyceryl Oleate	- Alterations in microbiota composition and gene expression, in a compound-dependant manner - Alterations in microbiota pro-inflammatory potential, in a compound-dependant manner
Um et al, 2021[87]	Human - healthy prospective cohort	Dietary emulsifiers estimated from six 24-h dietary recalls	- Greater emulsifier intake was not associated with antibodies to flagellin and/or to lipopolysaccharide - Greater emulsifier intake positively associated with the inflammatory biomarker glycoprotein acetyls (GlycA)
Rousta et al, 2021[88]	*in vivo* - WT mice humanized with microbiota from patients with inflammatory bowel disease	Carboxymethylcellulose Polysorbate 80	- in ex-germ-free (GF) IL10$^{-/-}$ mice colonized by faecal transplant with microbiota from donors with active IBD, carboxymethylcellulose increased intestinal inflammation - Carboxymethylcellulose and polysorbate 80 altered microbiota composition
Jin et al, 2021[90]	*in vivo* - WT mice	Polysorbate 80	- Maternal consumption of polysorbate 80 induced low-grade intestinal inflammation in offspring. - Maternal consumption of polysorbate 80 exacerbated dextran sulphate sodium (DSS)-induced colitis in adult offspring.

Chassaing et al, 2022[89]	Human - healthy prospective cohort	Carboxymethylcellulose	- In healthy humans, short-term consumption of carboxymethylcellulose promoted postprandial abdominal discomfort and impacted intestinal microbiota and metabolome
Daniel et al, 2023 [91]	*in vivo* - WT mice	Carboxymethylcellulose Polysorbate 80	- Alterations in microbiota composition and localisation in proximal colon, which can be prevented through daily consumption of probiotic *Akkermansia muciniphila* - Metabolic dysregulation and chronic low-grade intestinal inflammation in WT mice consuming CMC or P80, which can be prevented through daily consumption of probiotic *Akkermansia muciniphila*
Kordahi et al, 2023[92]	*in vivo* - WT mice	Carboxymethylcellulose Polysorbate 80	- Alterations in microbiota localisation within the proximal colon and increased microbiota pro-inflammatory potential that can all be prevented through immunisation against purified bacterial flagellin - Metabolic dysregulation and chronic low-grade intestinal inflammation in WT mice consuming CMC or P80 that can be prevented through immunisation against purified bacterial flagellin

1491 DATEM, Diacetyl tartaric acid ester of mono- and diglycerides

1492

Table 3 Animal and mechanistic studies investigating the impact of artificial sweeteners on gut health, including microbiota composition, intestinal permeability, gene expression, inflammation and colitis

Reference	Model	Artificial sweetener	Key findings relating to gut health
Hanawa et al[105]	C57BL/6 mice	Acesulfame-K	**Microbiota diversity & composition** – Acesulfame-K reduced diversity. Significant changes in many genera noted compared to controls. **Intestinal permeability, inflammation, colitis & carcinogenesis** - Acesulfame increased gut permeability and caused histological damage. Levels of IFN-γ, IL-1β and TNF-α were significantly higher in acesulfame treated mice, and had a higher expression of MAdCAM-1.
Bian et al[106]	CD1 mice	Acesulfame-K	**Microbiota diversity & composition** - Changes in the relative abundance of Bacteroides, Anaerostipes and Sutterella in male mice. Female mice had a decrease in Lactobacillus, Clostridium, an unassigned Ruminococcaceae and Oxalobacteraceae, and Mucispirillum increased. **Bacterial translocation, gene regulation and bacterial cell-to-cell communication** - Genes involved in LPS synthesis, flagella components and bacterial toxin synthesis increased in a gender specific manner.
Wang et al[107]	C57BL/6 mice	Acesulfame-K, sucralose, saccharin, rebaudioside A	**Microbiota diversity & composition** - Acesulfame-K, sucralose, saccharin and rebaudioside-A (active component of stevia) had bacteriostatic effects on different Escherichia coli strains. Sucralose did this in solid media and in liquid culture. Mice fed sucralose showed a significant increase in change in abundance of Firmicutes.
Van den Abbeele et al [108]	*ex vivo*	Acesulfame-K, stevia, sucralose	**Microbiota diversity & composition –** Acesulfame-K and sucralose resulted in similar microbial diversity, composition, and metabolite production to controls. Stevia increased *Bifidobacterium longum* and *B. adolescentis*, *Parabacteroides distasonis, Blautia obeum* and *Faecalibacterium prausnitzii,* which increased acetate, propionate and butyrate.
Palmnas et al[109]	Sprague Dawley rats	Aspartame	**Microbiota diversity & composition** - After 8 weeks, aspartame induced gut microbiota changes including an increase in Enterobacteriaceae and Clostridium leptum and increased the Firmicutes:Bacteroidetes ratio, an elevation in Roseburia ssp, as well as large elevations in serum levels of the SCFA propionate.
Chi et al[110]	CD1 mice	Neotame	**Microbiota diversity & composition** - After 4 weeks CD1 mice exhibited decreased α and β diversities of the mouse gut microbiome, a higher microbial dysbiosis index than controls and an enriched Bacteroidetes. **Bacterial translocation, gene regulation and bacterial cell-to-cell communication** - Reduction in butyrate synthesis genes.
Bian et al[111]	C57BL/6 mice	Saccharin	**Microbiota diversity & composition** - Eleven genera were significantly altered, some considered pro-inflammatory such as Corynebacterium, Turicibacter, Anaerostipes, Dorea, Roseburia and Ruminococcus. **Bacterial translocation, gene regulation and bacterial cell-to-cell communication** - Upregulation of several bacterial genes (LPS, flagella, fimbriae and bacterial toxins). **Intestinal permeability, inflammation, colitis & carcinogenesis** - TNF-α and iNOS were significantly elevated in saccharin-treated mice.
Anderson et al[112]	Male rats	Saccharin	**Microbiota diversity & composition** - The caecal population of aerobes and equivalent numbers of anaerobes was higher in the saccharin group compared controls, leading to a downward shift of the anaerobe/aerobe ratio.
Becker et al[113]	C57BL/6 mice	Saccharin and stevia	**Microbiota diversity & composition** - Relative abundance of Firmicutes increased from start to finish in the saccharin and stevia groups. Relative abundance of Bacteroidetes, Actinobacteria increased in the high fat (HF) and saccharin and HF and

			stevia groups. Verrucomicrobia increased in relative abundance in HF and saccharin groups and increased in the low-fat group. Tenericutes decreased in HF, saccharin and stevia groups. Proteobacteria increased in all groups.
Suez et al[114]	C57BL/6 mice	Saccharin, sucralose, aspartame	**Microbiota diversity & composition** - Mice given saccharin clustered separately from controls and their starting microbiome configuration. Compared to controls, there was significant dysbiosis, with more than 40 OTUs significantly altered in abundance. Many taxa that increased in relative abundance belonged to the Bacteroides genus and Clostridiales order. SCFAs propionate and acetate were significantly higher.
Shil et al[115]	Caco-2 cell model	Saccharin, sucralose, aspartame	**Microbiota diversity & composition** - Exposure of E.coli to saccharin led to reduced *E.coli* growth. All three sweeteners significantly increased *E.coli* biofilm formation. Only aspartame led to a significant increase in *E.faecalis* biofilm formation. **Intestinal permeability, inflammation, colitis & carcinogenesis** - All three sweeteners increased the adhesion properties of *E.coli* and more dramatically with *E.faecalis*. Sucralose and Aspartame increased the ability of E.coli and *E.faecalis*, but saccharin only had this effect on *E.faecalis*.
Rodrigues-Palacios et al[116]	SAMP mice	Sucralose	**Microbiota diversity & composition** - Six weeks exposure to sucralose did not worsen ileitis severity, but caused a dysbiosis in SAMP mice and the control mice strain AKR/J. In SAMP mice only, there was a significant increase of *Proteobacteria*. **Intestinal permeability, inflammation, colitis & carcinogenesis** - Increased myeloperoxidase activity and larger clusters of bacteria within the villi, suggesting sucralose may affect individuals predisposed to developing CD.
Abou-Donia et al[117]	Sprague-Dawley rats	Sucralose	**Microbiota diversity & composition** - Faecal pH increased significantly. Faecal bacteria continued to increase in number in the control groups. In groups fed sucralose, total anaerobes and aerobic bacteria decreased after initial administration of sucralose. At the lowest dose of sucralose (100 mg/kg) the number of anaerobes reduced by 49.8% relative to control samples. Total anaerobes remained suppressed after the 12-week recovery period. Counts of lactobacilli, bifidobacteria and *Bacteroides* decreased in all sucralose groups. **Intestinal permeability, inflammation, colitis & carcinogenesis** - In rats given sucralose there were histological changes such as lymphocytic infiltration into the epithelium, mild depletion of goblet cells, epithelial scarring.
Li et al[118]	C57BL/6 DSS induced colitis	Sucralose	**Microbiota diversity & composition** - All groups developed a dysbiosis compared to controls. Compared to the just AOM/DSS group, the addition of sucralose caused significant increases in *Ficmicutes, Actinomycetes, Peptostreptococcus stomatis, Clostridium symbiosum*, and *Peptostreptococcus anaerobius* and a decrease in Proteobacteria. **Intestinal permeability, inflammation, colitis & carcinogenesis** - The AOM/DSS group demonstrated higher levels of faecal trypsin and chymotrypsin than controls, a decrease in B-glucuronidase, reduced occludin and increased claudin-1 and claudin-4, suggesting gut barrier dysfunction. Sucralose aggravated DSS-induced colitis and led to higher numbers and greater size of AOM/DSS induced colorectal cancers, more severe weight loss, more blood in stools, more shortening of the colon, and a higher mortality. The sucralose group demonstrated significantly higher levels of TNF-α and IL-6, and lower levels of IL-10 and TRAF-6.
Guo et al[119]	C57BL/6 mice	Sucralose	**Microbiota diversity & composition** - Bacteroidetes and *Faecalibacterium prausnitzii* decreased with sucralose, and pro-inflammatory bacteria such as Pseudomonas aeruginosa increased. **Intestinal permeability, inflammation, colitis & carcinogenesis** - Decreased β-glucuronidase activity, which negatively correlates with trypsin and chymotrypsin activity, decreased expressions of claudin. Sucralose decreased expressions of MUC-2, ZO-1, and TFF3, indicating more severe intestinal barrier breakdown. Sucralose exacerbated colitis, with a decrease in body weight, worsening disease activity indices, activation of the TLR5-MyD88-NF-κB signalling pathway. Sucralose increased the levels of cytokines such as TNF-α and IL-1β while the levels of IL-10, NLRP12, and immune cell Th1 decreased.

Bian et al [120]	C57BL/6 mice	Sucralose	**Microbiota diversity & composition** - Changes in gut bacteria composition (14 genera, including those associated with inflammation such as *Ruminococcaceae Ruminococcus*) The fecal metabolome was perturbed as well as amino acid derivatives involved in tryptophan metabolism such as L-tryptophan, quinolinic acid, kynurenic acid, and 2-aminomuconic acid. **Bacterial translocation, gene regulation and bacterial cell-to-cell communication** - Genes related to LPS and flagella protein and fimbriae synthesis increased significantly after 6 months as well as bacterial toxin genes, such as toxic shock syndrome toxin-1.
Zheng et al [121]	C57BL/6 mice	Sucralose	**Microbiota diversity & composition –** Mice given sucralose had a reduced caecal abundance of *Lachnoclostridium* and *Lachnospiraceae* and increased abundance of *Tenacibaculum*, *Ruegeria*, and *Staphylococcus* in the jejenum, ileum and colon (compared to controls). **Intestinal permeability, inflammation, colitis, and carcinogenesis –** Mice given sucralose developed lymphocyte aggregation in the ileum and colon, with histological signs of severe colitis.
Zani et al [122]	C57BL/6 mice	Sucralose	**Microbiota diversity & composition –** There was no consistent shift in gut microbiota after sucralose exposure. **Intestinal permeability, inflammation, colitis & carcinogenesis –** After sucralose exposure, there was no change in weight or length of the caecum. There were also no signs of diarrhoea (watery stool) in the mice. In a model of T-cell induced colitis, sucralose reduced inflammatory T cells.
Uebanso et al[123]	C57BL/6 mice	Sucralose, acesulfame	**Microbiota diversity & composition** - Sucralose decreased the relative concentration of butyrate and the relative amount of Clostridium cluster XIVa (which produce butyrate) in the faecal microbiome. Acesulfame did not cause any significant changes.
Shil et al[124]	Caco-2 cell model	Sucralose, aspartame	**Intestinal permeability, inflammation, colitis & carcinogenesis** - Sucralose and aspartame influence claudin-3 and claudin-15 (tight junction proteins and regulate permeability). Sucralose and aspartame decreased Caco-2 cell viability at a dose of ≥1000 μM but saccharin only had this effect at a dose of 10, 000 μM[15]. Aspartame increased reactive oxygen species production.
Escoto et al[125]	CD1 mice	Sucralose, sucrose, stevia	**Microbiota diversity & composition** - After 12 weeks of exposure, mice fed sucrose and sucralose led to decreased bacterial diversity, whereas stevia increased diversity.
Rosales-Gomez et al[126]	CD1 mice	Sucralose, sucrose and stevia	**Intestinal permeability, inflammation, colitis & carcinogenesis** - Stevia increased B cells, and IgA, with an increase in the presence of IL-4 and IL-10 (anti-inflammatory cytokines), but in the lamina propria triggered an inflammatory response with increased TNF-α. Sucralose decreased humoral immunity, decreased IgA plasma cells in Peyer's patches, but increased the B cells, IgA and IL-4 in the lamina propria and thus also decreased TNF-α secretion.

Abbreviations - IFN-g - Interferon-γ, IL-1β - Interleukin-1β, TNF-α - Tumour Necrosis Factor-α, MAdCAM-1 -Mucosal vascular dressin cell adhesion molecule-1, LPS - lipopolysaccharide, iNOS – inducible Nitric Oxide Synthase, OTU – Operational Taxonomic Units, SCFA- short chain fatty acids , AOM/DSS – azoxymethane/dextran sodium sulphate, ZO-1 – Zonula Occludens-1, TFF3 – Trefoil Factor-3, TLR5-MyD88-NF-κB – Toll-Like Receptor-5-Myeloid Differentiation factor-88-Nuclear Factor- κB , NLRP-NACHT Leucine-rich Repeat and pyrin domain containing protein-3, IgA – Immunoglobulin-A

Table 4 Human studies investigating the impact of artificial sweeteners on gut health

Reference	Population	Artificial sweetener	Key findings relating to gut health
Gerasimidis et al[133]	13 Healthy volunteers	Aspartame, stevia, sucralose	**Microbiota diversity & composition** - Sucralose induced a significant shift in β-diversity. Aspartame promoted the growth of *B. coccoides.* Shannon α-diversity increased with Stevia, sucralose shifted microbiome structure, increased the abundance of Escherichia/Shigella and Bilophila.
Suez et al[114]	7 Healthy volunteers	Saccharin	**Microbiota diversity & composition** - Healthy volunteers who did not normally consume artificial sweeteners were given 6 mg/kg/bw saccharin (FDA's maximal ADI). Those who developed poorer glycaemic responses (whose microbiomes clustered differently to non-responders) had stool transferred to a germ-free mouse, which recapitulated the glucose intolerance and dysbiosis seen in humans (20-fold increase in *Bacteroides fragilis, Weissella cibari;* 10-fold increase in *Candidatus arthromatus).*
Thomson et al[134]	34 Healthy volunteers	Sucralose	**Microbiota diversity & composition** - Individuals consumed sucralose or placebo for 7 days at equivalent of 75% ADI per day (15mg/kg/day). There were no major changes in the gut microbiome.
Ahmad et al[135]	17 Healthy volunteers	Aspartame, sucralose	**Microbiota diversity & composition** - Randomized double-blind crossover trial of sucralose and aspartame. There were no changes in microbiota structure induced by either sweetener, no difference in SCFAs, and no differences found in median relative proportions of the most abundant bacterial taxa, suggesting no effect of sweeteners on gut microbiota composition or their metabolites.
Frankenfeld et al[136]	31 Healthy volunteers	Acesulfame-K, aspartame	**Microbiota diversity & composition** – No difference in bacterial abundance between consumers and non-consumers, but bacterial diversity was lower in consumers of acesulfame-K and aspartame than non-consumers.
Serrano et al[137]	54 Healthy volunteers	Saccharin	**Microbiota diversity & composition** - Volunteers received maximum ADI for 2 weeks. There was no change in bacterial diversity or composition.
Mendoza-Martinez et al[138]	137 Healthy volunteers	Acesulfame-K, aspartame, saccharin, sucralose	**Clinical symptoms –** Volunteers were randomised to a sweetener-containing diet or sweetener-free diet. Those consuming sweeteners developed symptoms including diarrhoea, post-prandial discomfort, constipation; those in sweetener-free diet experienced improvements in abdominal pain, post-prandial discomfort and early satiety.
Suez et al[139]	120 Healthy volunteers	Aspartame, saccharin, sucralose, stevia	**Microbiota diversity & composition** - Two week randomized-controlled trial of four sweeteners in doses lower than ADI. Each sweetener distinctly altered the stool and oral microbiome and plasma metabolome.

Abbreviations – ADI – acceptable daily intake, SCFA – short-chain fatty acids.

1504 **Table 5 Summary of clinical trials of dietary restriction of UPF or food additives in the management of gut disease**

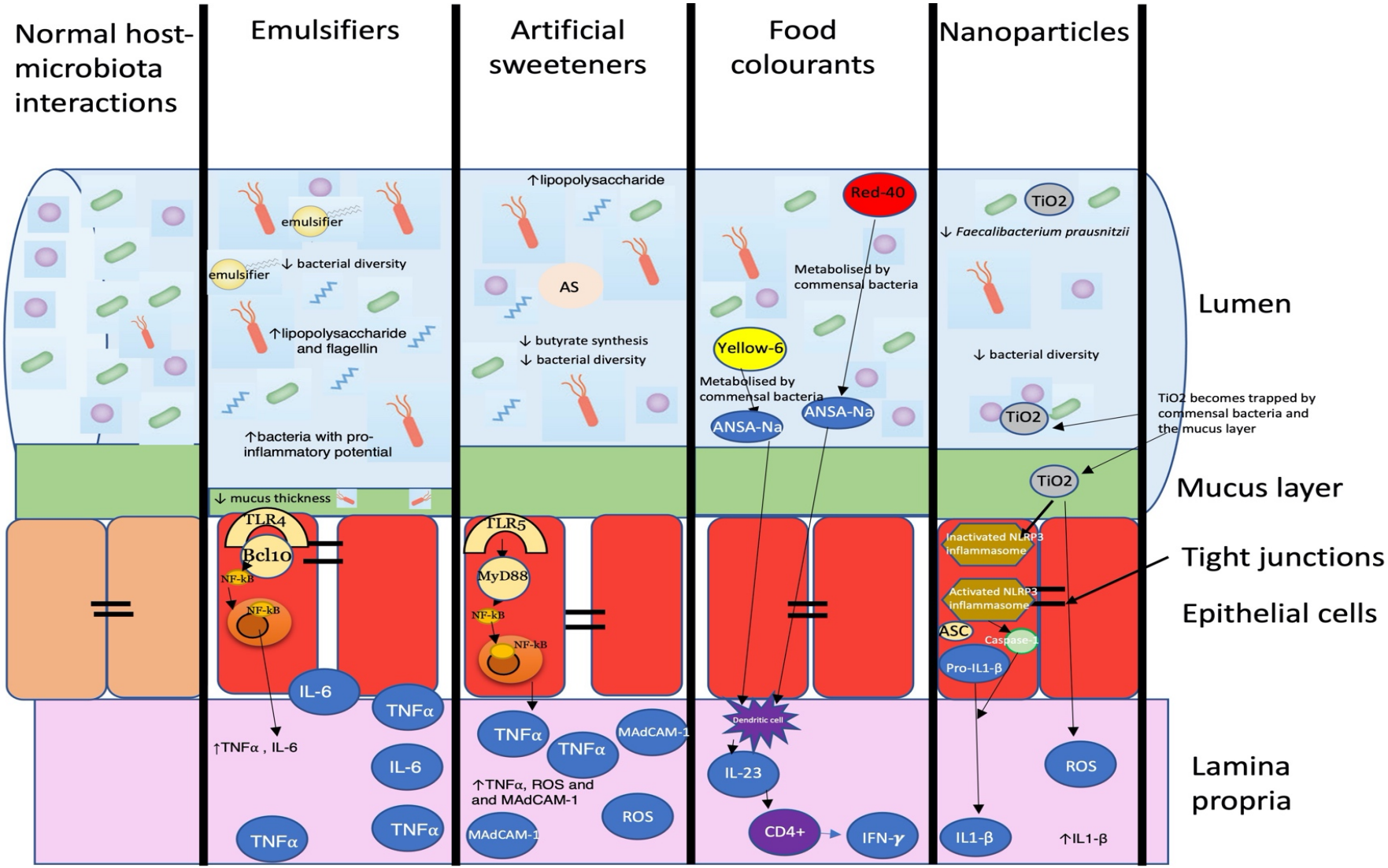
Reference	Diet	Population	Intervention	Delivery	Duration and study design	Control	Key findings relating to gut disease
Clinical trials of diets that intentionally restrict UPF or food additives							
Bhattacharyya et al, 2017[96]	Low carrageenan	12 patients with quiescent UC	No-carrageenan diet plus placebo capsule (n=7)	Dietetic counselling plus placebo capsule	52-week randomised placebo-controlled trial	No carrageenan diet plus 200 mg/d carrageenan capsule (n=5)	Relapse in 0/7 (low carrageenan) vs 3/5 (control) (p=0.046). SCCAI 0.86 (low carrageenan) vs 4.20 (control) (p=0.05)
Sandall et al, 2020[81]	Low emulsifier	20 patients with Crohn's disease	Low emulsifier diet designed to exclude 65 emulsifiers	Dietetic counselling, educational booklet, smartphone application	14 days open label feasibility trial	No control group	95% adherence to diet; emulsifier intake reduced from 2.3 per day to 0.0 per day (p < 0.001). Food-related-QoL improved from median 81.5 to 90.0 (p=0.028) Clinical symptoms (PRO2) reduced from 3.0 to 1.4, p=0.006 IBD control increased from 13.5 to 15.5p=0.026
Mendoza-Martinez et al 2022[138]	Artificial sweetener-free diet	137 healthy volunteers (95 included in analysis; 53 had GI symptoms at baseline)	<10 mg/d sweeteners (n=45, 34 analysed)	Dietary advice	5-week randomised controlled trial	supplemented group (50-100mg non calorie sweetener (80%sucralose and 20% aspartame, acesulfame K and saccharin) /day).	The percentage of participants with diarrhoea (p = 0.02), post-prandial discomfort (p = 0.02), constipation (p < 0.01), and burning p < 0.01) increased in the sweetener group. Whereas, abdominal pain (p = 0.04), post-prandial discomfort (p = 0.02), burning (p = 0.02), early satiety (p < 0.01), and epigastric pain (p < 0.01) decreased in the sweetener free group
Clinical trials of diets that intentionally restrict UPF or food additives in addition to other dietary components							
Levine et al, 2019[163]	CDED (children)	78 children with active Crohn's disease	CDED plus 50% energy from EEN (n=40)	Dietetic counselling plus support	6-weeks randomised open-label comparative trial	100% EEN	Tolerability CDED 97.5% (CDED) vs 73.6% (EEN) (p=0.002)

Levine et al, 2019[163]	CDED (children)	78 children post induction	CDED plus 25 % calories from EEN	Dietetic counselling plus support	6-weeks open label maintenance extension	25% partial enteral plus free diet	Steroid-free remission in 75.6% CDED vs 45.1% (free-diet) (P=0.01)
Yanai et al, 2022[164]	CDED (adult)	44 adults with active CD	CDED plus partial enteral nutrition (PEN) (n=20; ITT 19)	Dietetic counselling plus support	24-weeks randomised open label comparative trial	CDED (n=24, ITT 21)	Remission at week 6 68% (CDED plus PEN) vs 57% (CDED) (p=0·4618). Endoscopic remission at week 24, 6 (CDED plus PEN) vs 8 (CDED)
Clinical trials of diets that will likely reduce intakes but as part of wider dietary intervention not specifically targeting UPF or food additives							
Cox et al, 2020 [165]	Low FODMAP	52 patients with quiescent IBD (26 UC, 26 CD) and functional GI symptoms)	Low FODMAP diet (n=27)	Dietetic counselling plus support	4-weeks randomised controlled trial	Sham control diet (n=25)	IBS-SSS change of -67 in low FODMAP group and -34 in control (p=0.07). Adequate symptom relief in 14/27 (52%) low FODMAP and 4/25 (16%) control (p=0.007). IBD-control score was higher following low FODMAP (88.3) compared to sham diet (74.3, P=.028). No impact on disease activity
Svolos et al, 2020 [166]	CD-TREAT	5 children with active Crohn's disease	CD-Treat	Prepared food delivered to patients	8-weeks open label trial	No control group	Clinical response in 4 patients; remission in 3 patients. Fall in wPCDAI from 32.5 to 7.5 (p = 0.005) at 8 weeks. FCP 918+/- 555 mg/kg
Lewis et al, 2021 [167]	CD DINE	194 CD patients with sCDAI 175-400 47% had inflammation at baseline	Specific carbohydrate diet (n=101)	6 weeks prepared food delivered to participants and 6 weeks dietary advice / meal plans with dietetic support	RCT	Mediterranean diet (n=93)	Remission at week 6: MD, 43.5%; SCD, 46.5%; P = .77). No change in overall CRP. Fall in FCP in SCD group. FC response was achieved in 8 of 23 participants (34.8%) with the SCD and in 4 of 13 participants (30.8%) with the MD (P = .83). CRP response was achieved in only 2 of 37 participants (5.4%) with the SCD and in 1 of 28 participants (3.6%) with the MD (P = .68)

Konijeti et al, 2017 [168]	CD AID	15 patients (9 CD and 6 UC) with active IBD Harvey–Bradshaw index ≥ 5 or partial Mayo score ≥3 and erosions on endoscopy and/or elevated fecal calprotectin	Anti inflammatory diet	6 weeks induction and 5 weeks maintenance	Single centre open label cohort.	N/A	Remission at week 6 and 11 in 11/15 (73%; 6 CD and 5 UC) Among those with a baseline FC >50 µg/g, mean FC decreased from 701 to 139 (P = 0.09)
Albenberg et al, 2019[169]	FACES	214 patients with CD in remission (sCDAI<150) who consume meat at least once per week	High meat (at least 2 servings red or processed meat / week) n=118	49 weeks		Low meat (no more than one serving red or processed meat per week) n=95	Any and moderate to severe relapse occurred in 62% of participants in the high-meat group and 42% of participants in the low-meat group. There were no significant differences in time to any (P = .61) or moderate/severe (P = .50) relapse

Abbreviations - QoL – quality of life, UC – ulcerative colitis, SCCAI – simple clinical colitis activity index, CDED – Crohn's disease exclusion diet, EEN – exclusive enteral nutrition, PEN – partial enteral nutrition, FODMAP – Fermentable Oligosaccahrides Disaccharides Monosaccharides and Polyols , CD – Crohn's disease, FC – faecal calprotectin, sCDAI – short Crohn's Disease Activity Index, AID - Anti-Inflammatory Diet

Figure 1 Different effects of emulsifiers, sweeteners, colours and nanoparticles on the microbiome, mucous, barrier and inflammation in the gut



Legend for Figure 1 Many food additives have been shown to alter gut luminal and mucosal homeostasis. (A) Emulsifiers alter bacterial diversity and gene regulation, decrease mucus thickness, increase gut permeability by having a negative effect on tight junction proteins, and upregulate bacteria with pro-inflammatory potential, which can trigger inflammatory pathways and lead to colitis. (B) Artificial sweeteners may decrease bacterial diversity and have deleterious effects on short-chain fatty acids such as butyrate, as well as increasing gut permeability, which can lead to triggering of inflammation via pathways such as the colitis-associated NF-κB pathway, Tumour Necrosis Factor-α (TNF-α) and Mucosal vascular dressin cell adhesion molecule-1 (MAdCAM-1) secretion. (C) Food colours are metabolised by the gut microbiota, leading to metabolites such as ANSA-Na that may trigger Interleukin-23R (IL-23R) dependent inflammation. (D) Nanoparticles influence bacterial diversity, including reduction of *Faecalibacterium prausnitzii*, and have been shown to trigger the NACHT Leucine-rich Repeat and Pyrin domain containing protein-3 (NLRP3) inflammasome, thus activating cytokines such as IL-1β and creating reactive oxygen species.

Box 1. Examples of common classification systems used in epidemiological research and public communication regarding the food processing concept, including the definition of the most processed categories

NOVA[3]

(1) Unprocessed and minimally processed foods
(2) Processed culinary ingredients
(3) Processed food products
(4) Ultra-processed products (defined as "Formulations of ingredients, mostly of exclusive industrial use, typically created by series of industrial techniques and processes"

IARC-EPIC[4]

(1) Foods with unknown process
(2) Non processed foods consumed raw
(3) Moderately processed foods
    i. Modest processing, no further cooking
    ii. Cooked foods from raw to moderately processed foods
(4) Highly processed foods (defined as "Foods that have been industrially prepared, including those from bakeries and catering outlets, and which require no or minimal domestic preparation apart from heating and cooking")

IFIC[5]

(1) Minimally processed
(2) Processed for preservation
(3) Mixtures of combined ingredients
    i. Packaged mixes, jarred sauce
    ii. Mixtures, home prepared
(4) "Ready-to-eat" foods
    i. Packaged ready-to-eat foods
    ii. Mixtures, store prepared
(5) Prepared foods and meals (defined as "Foods packaged to stay fresh and save time")