Reassessing the safety of dietary emulsifiers through the lens of gut microbiota

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Article

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Abstract

Dietary emulsifiers have been linked to various diseases. The recent discovery of the role of gut microbiota-host interactions on health and disease warrants the safety reassessment of dietary emulsifiers through the lens of gut microbiota. Hydrophilic (lecithin (LEC), sucrose esters (SUC), carboxymethylcellulose (CMC)) and lipophilic (mono- and diglycerides (MDG)) emulsifiers are common dietary emulsifiers with high exposure levels in the population. This study proved that SUC and CMC induced hyperglycemia and hyperinsulinemia. MDG impaired circulating lipid and glucose metabolism. Both hydrophilic and lipophilic emulsifiers changed the intestinal microbiota diversity and induced gut microbiota dysbiosis. Hydrophilic emulsifiers have no impact on mucus–bacterial interactions, whereas MDG tended to cause bacterial encroachment into the inner mucus layer and enhance inflammation potential by raising circulating lipopolysaccharide. Our findings demonstrated the safety concerns associated with using dietary emulsifiers, suggesting that they could lead to metabolic syndromes.

Introduction

Gut microbiota inhabiting the gastrointestinal tract are essential in health and disease. A balanced gut microbiome is functionally beneficial to the body. In contrast, gut microbiota dysbiosis can lead to various illnesses, including cardiovascular disease, obesity, metabolic syndrome, and inflammatory bowel disease, via a metaorganism-pathogenesis pathway involving the gut microbiota, its metabolites, and the host. A thick layer of mucus over the intestinal epithelium prevents translocation of gut microbiota in the body. Colonic bacteria colonizing the outer mucus layer can degrade and utilize mucus glycans as an energy source. Specific mucin-degrading microbiomes have enzyme glycosyl hydrolases that digest specific glycan linkages. A previous study has demonstrated that the intestinal microbiota can influence the properties of the colonic mucus layer. Certain microbes possess various carbohydrate utilization gene clusters that allow them to degrade and metabolize specific glycans in the intestinal mucus layer. Decomposition of the mucus layer leads to gut microbiota encroachment resulting in infection and inflammation. A dysfunctional mucus layer has been found in patients with colitis and rodents. The change in gut microbiome composition directly influences the changes in the mucus layer. Additionally, our dietary habits, such as intake of a high-fat diet, food additives, and prebiotics, directly impact changes in the mucus, which is associated with the development of several diseases. Hence, particular food and food additives can disrupt mucus–bacterial interactions and potentially promote gut inflammation-related diseases.

Food additives, such as instant flavoring agents, preservatives, and emulsifiers, have been used in the food industry to extend the shelf life and improve the appearance, taste, and texture of food products. Emulsifiers might be implicated in the pathogenesis of several diseases, including inflammatory bowel disease and metabolic syndrome. Food additive emulsifiers are classified into hydrophilic and hydrophobic (lipophilic) moieties, which can reduce the interfacial tension between the oil and water phases and incorporate physical force to form a stable emulsion. Carboxymethylcellulose (CMC) and
polysorbate 80 (P80) induce intestinal inflammation and metabolic syndrome by thinning of the intestinal mucus layer and altering gut microbiota composition, increasing the gut epithelial permeability and lipopolysaccharide (LPS) levels\textsuperscript{10}. Moreover, both P80 and CMC can modify the microbiota and elevate pro-inflammatory potential in the mucosal simulator of the human intestinal microbial ecosystem (M-SHIME), and transplantation of emulsifier-treated M-SHIME suspensions to germ-free mice can induce low-grade inflammation-associated phenotypes and metabolic disease\textsuperscript{11}. Another study found that CMC intervention in healthy participants for approximately two weeks can increase postprandial abdominal discomfort and lower gut microbiota diversity, decrease fecal short-chain fatty acids and free amino acids, and enhance microbiota encroachment into the inner mucus layer\textsuperscript{12}. These studies raise safety concerns regarding the use of emulsifiers, which may adversely affect health and lead to chronic diseases.

The classification of emulsifiers as food additives differs among regulatory bodies and across countries. Numerous food additives are recognized as emulsifiers by the Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives (JECFA), Codex Alimentarius (Codex), UK Food Standards Agency (FSA), and US Food and Drug Administration (FDA). However, not all emulsifiers are acknowledged by every organization (Fig. 1a-b). The discrepancies among emulsifier classifications create a challenge for international translation of emulsifier research resulting in differing definitions of a low-emulsifier diet across countries\textsuperscript{9}. Presently, dietary exposure evaluations for emulsifiers that are commonly used in the population are lacking. A recent report has attempted to estimate the dietary exposure to seven emulsifiers, namely mono- and diglycerides (MDGs), lecithin, carboxymethylcellulose (CMC), sucrose esters, polysorbate 80 (P80), stearoyl lactylates (sodium stearoyl lactylate (SSL) and calcium stearoyl lactylate (CSL)), and polyglycerol polyricinoleate (PGPR), during two time periods (1999–2002, 2003–2010) (Fig. 1c). The exposure levels to emulsifiers remained constant during the period. MDGs, lecithin, and CMC had the highest mean and 90th percentile exposures; nevertheless, JECFA has not published any reports on acceptable daily intake (ADI) values for these three emulsifiers. Conversely, sucrose esters, P80, stearoyl lactylates, and PGPR exhibited lower exposure levels than the aforementioned emulsifiers. The exposure estimates to emulsifiers are in line with the market value data (Fig. 1d)\textsuperscript{13}.

Traditionally, the safety evaluation and approval of food additives were based on toxicological evidence following the Generally Recognized As Safe (GRAS) guidelines. However, with recent discoveries of the significant role of gut microbiota in health and diseases, researchers have shifted their focus to studying the adverse health effects of food additives via the metaorganism-pathogenesis pathway and gut microbiota. Thus, it is necessary to explore the safety of food additives concerning the interactions between gut microbiota and the host to fill in the gaps in current literature and support regulations governing food production. Currently, knowledge regarding of the effect of emulsifiers on the development of metabolic disease is lacking. This study aimed to reassess the safety of dietary emulsifiers by examining their impact on gut microbiota and their relation with obesity and metabolic diseases. This study investigated the impact of hydrophilic (LEC, SUC, and CMC) and lipophilic (MDG)
emulsifiers on the development of obesity and metabolic disease through gut microbiota and host interaction, including gut microbiota dysbiosis, changes in the mucus layer, intestinal permeability, and the translocation of gut-derived LPS into the circulation system.

**Results**

**Intake of hydrophilic dietary emulsifiers adversely affected the obesogenic and metabolic biomarkers and induced hyperglycemia and hyperinsulinemia**

We investigated the effect of common hydrophilic dietary emulsifiers on obesogenic, metabolic, gut microbiota, and gastrointestinal changes (Fig. 2a.). Common hydrophilic dietary emulsifiers used for investigation in this study included lecithin (LEC), sucrose fatty acid esters (SUC), and carboxymethylcellulose (CMC). Fifteen-week-old male C57BL/6J mice were fed a regular chow diet with or without hydrophilic emulsifiers supplemented in water for 17 weeks. The dosage design of LEC and SUC followed the report on dietary estimate exposure in humans\(^\text{13}\). The human dosage of emulsifiers was translated to mouse equivalent doses. The dose of hydrophilic emulsifier was mimicked as ten times the daily exposure in humans, and the selected dosage of LEC and SUC were 7523.3 and 1110 mg/kg bw/day, respectively. CMC (1% in drinking water) dosage was set according to a previous study\(^\text{10}\). After ingestion of dietary emulsifier for 17 weeks, the group with hydrophilic emulsifier supplementation showed an increase in weight than the control group (Fig. 2b). At week 17th, SUC and CMC substantially increased the weight gain compared to the CON group (P = 0.1563 and P = 0.0322, respectively) (Fig. 2c). Similarly, the CMC-fed group revealed increased relative fat mass but decreased lean mass. (Fig. 2d-e). Moreover, the CMC group showed a lower serum total cholesterol and triglyceride level (P = 0.0034 and P = 0.0006) than the CON group (Fig. 2f-g), suggesting that CMC may interfere with lipid absorption and metabolism. The hydrophilic emulsifier used in this study did not cause any changes in serum AST and ALT levels, fatty liver, and histopathological characteristics in the liver section (Supplementary Fig. 1). Oral glucose tolerance test (OGTT) revealed no notable variation in the area under the curve (AUC) of OGTT between the hydrophilic emulsifier-fed group and the CON group (Fig. 2h-i). SUC- and CMC-fed groups showed a significant increase in fasting serum glucose (P = 0.0029 and P = 0.0095, respectively) and insulin levels (P = 0.0027 and P < 0.0001, respectively) (Fig. 2j-k). Homeostatic model assessment for insulin resistance (HOMA-IR) revealed that SUC and CMC increased the HOMA-IR index (Fig. 2l), suggesting that the intake of hydrophilic emulsifiers SUC and CMC caused dysregulation of glucose and insulin homeostasis. LEC supplementation in mice tended to increase serum glucose and insulin levels and the HOMA-IR index; however, no significant difference was observed when compared to the CON group.

**Hydrophilic dietary emulsifiers transformed the gut microbiota α- and β-diversity indices**
Because gut microbiota plays an important role in the progression of obesogenic and metabolic diseases, we analyzed the cecal microbiota composition using the V4 16S rRNA gene sequencing to elucidate the effect of hydrophilic dietary emulsifiers on gut microbiota. The QIIME2 platform was used to process the raw sequence for generating the amplicon sequence variance (ASV) table and subsequently aligned against the SILVA database (version 138) to obtain the taxonomic classification of each ASVs. ASVs table comprised 671 ASVs classifying into 122 species and 86 genera. LEC exhibited showed -diversity indices similar to those of the control. The SUC group showed a significant elevation in the observed species (P = 0.0372) and substantially lowered the Shannon diversity index, suggesting that the SUC increased the abundance of the particular gut microbiome, and the microbial community in the cecal microbiome exhibited low evenness (Fig. 3a-c). CMC significantly elevated the Shannon and Simpson diversity indices (P = 0.0229, P = 0.0366, respectively), suggesting CMC could make the diversity of gut microbiota more consistent.

We further computed β-diversity and performed principal coordinate analysis (PCoA) based on the Bray – Curtis distance (Fig. 3d). The data indicated that all hydrophilic emulsifiers affected the gut microbiota shift. PCoA displayed significant separation among all experimental group groups (ANOSIM: R = 0.8205, P < 0·001). The distance of the centroid of the LEC group to the CON group was shortest, indicating LEC did not strongly affect the cecal microbiome. In general, SUC and CMC have a more substantial effect on gut microbiome shift. The SUC group displayed a distinct separation from the CON group counting on the X-axis (PCoA1; 27.32%), whereas the CON, LEC, and CMC were relatively similar to the control. However, the CMC group showed a distinct partition counting on the Y-axis (PCoA2; 20.9%). We also added details of the insulin resistance-related biomarker, including HOMA-IR, blood glucose, and insulin level, in the PCoA plot. The increased circle size for each mouse indicated a higher HOMA-IR value, and the higher intensity of color represented a higher serum glucose or insulin levels. All hydrophilic emulsifier-feeding groups showed increased circle sizes and color intensities, suggesting that insulin-resistance-related parameters may be correlated with the gut microbiota shift. We further estimated the relationship between each genus and the dimensional space of each mouse's gut microbiome. Vectors in the PCoA plot symbolized an associated genus (P < 0.01), and its length presented the strength of the relationship. The CON group associated with the genera Osenella is enriched in lean mass\textsuperscript{14}. The LEC group exhibited a relationship with Faecalibaculum, Enterorhabdus, and Muribaculum. The SUC group correlated with [Eubacterium] xylanophilum group, Clostridium sensu stricto 1, and Akkermansia, whereas the CMC group correlated with Alloprevotella, Acetatifactor, Muribaculaceae, Clostridia vadinBB60 group, Blautia, and UCG – 010. Collectively, hydrophilic emulsifier feeding modified the gut microbiota in terms of both the - and β-diversity indices, and each emulsifier was associated with its particular genus and insulin-resistant biomarkers.

**Hydrophilic dietary emulsifiers adversely affected gut microbiota**

There were 35 significantly different genera among the experimental group. The hierarchical clustering of the genus is classified into three primary clusters characterizing the differences between the hydrophilic
emulsifier-fed and control groups. The results indicate that three kinds of hydrophilic emulsifiers differentiated the cecal microbiota at the genus level (Fig. 3e). The head of the heatmap displays the experimental group and percent of body weight change, insulin, and fasting glucose level, which demonstrated raised values in the three hydrophilic emulsifiers fed group. Compared with the CON group, the LEC, SUC, and CMC groups have 18 (9↑↑9↓), 19 (7↑12↓), and 19 (9↑10↓) significantly different genera (P < 0.05). The LEC group showed increases in the disease-related genera, including *Streptococcus*, [Eubacterium] *coprostanoligenes* group, *Enterobacter*, *Lachnosclostridium*, *Desulfovibrio*, and [Eubacterium] *xylanophilum* group, as well as other genera, such as the *Bifidobacterium*, *Lactobacillus*, and *Candidatus Arthromitus*. LEC also reduced particular possible beneficial bacteria, including *Oscillibacter*, *Parasutterella*, *Dubosiella*, and *Turicibacter*, and other genera, such as the *Prevotellaceae UCG−001*, *Colidextribacter*, *Clostridia vadinBB60* group, *Coriobacteriaceae UCG−002*, and *Tyzzerella*. The SUC-fed group was enriched in the disease-related microbiome, including *Clostridium sensu stricto* 1, *Lachnospiraceae UCG−006*, and [Eubacterium] *xylanophilum* group, and other genera—the [Eubacterium] *ruminantium* group, *Akkermansia*, *Lactobacillus*, UCG−010. SUC also depleted possible beneficial bacteria such as *Muribaculaceae*, *Oscillibacter*, *Faecalibaculum*, *Parasutterella*, *Olsenella*, and other genera—the *Alloprevotella*, *Acetatifactor*, *Prevotellaceae UCG−001*, *Colidextribacter*, *Clostridia vadinBB60* group, *Enterorhabdus*, and *Tyzzerella*. In the CMC group, there was an elevated relative abundance of several disease-associated bacteria, including *Blautia*, *Staphylococcus*, and [Eubacterium] *coprostanoligenes* group, and other genera, such as the *Muribaculaceae*, *Incertae Sedis*, *Clostridia vadinBB60* group, *Enteractinococcus*, *Candidatus Arthromitus*, and UCG−010. Several beneficial microbiontals also lessened in the CMC-treated group, such as *Muribaculum*, *Faecalibaculum*, *Parasutterella*, *Dubosiella*, *Turicibacter*, *Prevotellaceae UCG−001*, *Coriobacteriaceae UCG−002*, *Enterorhabdus*, *Tyzzerella*, and [Eubacterium] *siraeum* group. These data suggested that common dietary hydrophilic emulsifiers adversely affected gut microbiota homeostasis by increasing disease-associated microbiomes but depleting particular beneficial microbiontals.

We additionally analyzed Spearman's correlation to evaluate the association of the meaningful genus with obesogenic and metabolic biomarkers based on the heatmap. Changes in body weight positively correlated with two genera: UCG−010 and the disease-associated genera *Blautia*. Fasting blood glucose positively correlated to *Blautia*, *Incertae Sedis* and the *Clostridia vadinBB60* group. Insulin and HOMA-IR were positively associated with a mucin degrading bacteria—*Akkermansia*. We observed that several beneficial bacteria, including *Muribaculum*, *Faecalibaculum*, *Parasutterella*, *Olsenella*, and *Dubosiella*, were negatively correlated with changes in body weight, fasting glucose levels, insulin levels, or HOMA-IR index. The heatmap and correlation results collectively demonstrated that common hydrophilic emulsifiers adversely affected gut microbiota and may cause microbiota dysbiosis.

**Hydrophilic emulsifiers did not disrupt mucus–bacterial interactions or promote diseases associated with gut inflammation**
The shortening of the colon length is considered to a biomarker of colitis. The colon length in the hydrophilic emulsifiers-fed group was not significantly different from that of the control group (Fig. 3f). We used Periodic acid-Schiff’s (PAS)-stained colon sections to obtain the colonic epithelial damage histological score. The results showed that the intestinal epithelial cells in both the emulsifier-treated and control groups were structurally intact, the number of goblet cells was normal, and the lamina propria and muscular mucosae were not infiltrated by immune cells, suggesting that the administered hydrophilic emulsifiers did not induce colitis (Supplementary Fig. 2). We further investigated whether the hydrophilic dietary emulsifiers affected the intestinal mucus layer changes and localization of bacteria using confocal microscopy. Hydrophilic emulsifier intervention did not result in the thinning of the mucus layer and shortening of the distance of bacteria to intestinal epithelial cells (IEC), indicating there was no invasion of intestinal bacteria to the inner mucus layer, and hydrophilic emulsifiers did not facilitate diseases associated with gut inflammation (Fig. 3g and 3h). Thus, the intake of hydrophilic dietary emulsifier did not disrupt mucus–bacterial interactions. Next, we examined the effect of hydrophilic emulsifiers on intestinal permeability using oral gavage of the FITC-dextran method. SUC group showed increased intestinal permeability (P = 0.1491) compared to the control group, unlike LEC and CMC (Fig. 3i). LPS produced by gut microbiota can translocate to the circulation system through the portal vein and result in low-grade systemic inflammation. Our data showed that the serum LPS level and intestinal permeability were consistent in mice fed with emulsifiers (Fig. 3j); however, the LPS level was not statistically different.

**Lipophilic dietary emulsifier MDG impaired circulating lipid and glucose metabolism**

We investigated the common lipophilic dietary emulsifier MDG (Fig. 4a). Eight-week-old male C57BL/6J mice were fed with a diet containing MDG 5.5% diet. The dosage of MDG used in this experiment followed a prior study that showed no signs of systemic toxicity\(^{15}\). As both soybean oil and MDG are types of fats that provide energy to the body and the control diet contained triacylglycerol mainly derived from soybean oil, we substituted soybean oil with MDG in MDG diet. The mice were fed with CON or MDG diet for 14 weeks. To minimize the effect of different amounts of dietary intake in each mouse, mice were limited to 85 kcal/week\(^{16}\). After intervention with MDG for 17 weeks, no significant differences with respect to changes in body weight at 14 weeks was observed (Fig. 4b-c). However, the relative fat mass of the MDG group during feeding was slightly lower than the that in the CON group, but no significant difference in relative lean mass change was observed (Fig. 4d-e). The MDG intake interfered with blood lipid metabolism by elevating the total serum cholesterol levels (P = 0.0139) and lowering the total triglyceride levels (P < 0.0001), compared to the CON group (Fig. 4f-g). However, MDG did not affect serum AST and ALT levels (Supplementary Fig. 3). OGTT revealed that MDG significantly increased the blood glucose levels. The calculated AUC of OGTT was significantly higher for the MDG group than for the CON (P < 0.0020) (Fig. 4h-i). However, no differences in the fasting serum glucose levels, serum insulin levels, and HOMA-IR index were observed between the MDG and control groups (Fig. 4j-l). These data suggested that MDG intake impaired glucose and lipid metabolism.
MDG reduced the evenness of the gut microbiota community and modified the gut microbiome β-diversity

According to the QIIME2 pipeline, the V4 16S rRNA gene sequences generated 460 ASVs and were assigned to 110 species and 80 genera. MDG did not impact the observed ASVs but reduced the microbiota community evenesss, i.e., the Shannon and Simpson's diversity indices (P = 0.0751 and 0.0285, respectively) (Fig. 5a-c). This data suggests that MDG could allow or inhibit the growth of a particular microbiome resulting in the unevenness of the gut microbiota community. The Bray – Curtis distance-based PCoA demonstrated that MDG simulated the gut microbiota shift (Fig. 5d) with significant separation (ANOSIM: R = 0.9378, P < 0.001). The MDG group displayed a distinguishable separation from the CON group, on the X-axis (PCoA1; 47.88%). Y-axis (PCoA2; 15.96%) showed the effect of the intra-group microbiota shift. The gut microbiota of each mouse in the PCoA plot of the MDG group exhibited a shorter distance toward its group centroid, suggesting MDG firmly adjusted gut microbiota composition with the lower within-group variation. In contrast, the CON group displayed a higher inter-group variation. The PCoA plot also showed the lipid and glucose metabolism biomarkers, including body weight change, AUC of OGTT, and total serum cholesterol. The larger circle indicated a higher body weight increment, and a more intense color showed a more elevated AUC of OGTT and cholesterol level. MDG tended to cause a reduction in the circle's size but increased the color intensity, suggesting that the body weight, lipid, and glucose metabolism biomarkers may be associated with changes in the gut microbiota. According to the vector orientation with statistical significance set at P < 0.05, the MDG group was associated with the genera *Lactococcus, Enterorhabdus*, and *uncultured*. The CON group was associated with *Collinsella, Alistipes, Parabacteroides, Alloprevotella, ASF356, Lachnospiraceae NK4A136 group, Clostridia UCG – 014, Muribaculaceae, Lachnospiraceae UCG – 006, Coriobacteriaceae UCG – 002*, and *Akkermansia*. Therefore, MDG altered the cecal microbiota's -diversity evenness and β-diversity index, which is associated with impaired blood lipid and glucose metabolic biomarkers.

**Lipophilic dietary emulsifier MDG causes gut microbiota dysbiosis**

We further performed the statistical analysis of gut microbiota at the genus level based on the Wilcoxon signed-rank test for comparisons (P< 0.05) of the effect of MDG on gut microbiota (Fig. 5e). Sixteen genera were significantly different from those in the control group. The hierarchical clustering found two main clusters representing the dissimilarities between the MDG-fed and control groups. Eleven genera were significantly reduced in the CON group, whereas five genera were enriched in the MDG-fed group. MDG intake decreased possible beneficial bacteria, including *Muribaculaceae, Parabacteroides, Lachnospiraceae NK4A136 group, Akkermansia*, and *Collinsella*, and few other genera, such as *Alistipes, Alloprevotella, ASF356, Coriobacteriaceae UCG – 002, A2*, and *Lachnospiraceae UCG – 006*. Moreover, the MDG group increased several diseases-related microbiomes such as *Enterorhabdus, Jeotgalicoccus, and Atopostipes*, and few other genera, such as *uncultured* and *Lactococcus*. Hence, consumption of MGD may result in gut microbiota dysbiosis.
Seven genera (Alistipes, Parabacteroides, Lachnospiraceae NK4A136 group, Alloprevotella, ASF356, Lachnospiraceae UCG - 006, and Collinsella) were positively correlated to changes in body weight, whereas Enterorhabdus exhibited a negative correlation. AUC of OGTT was negatively associated with Alloprevotella, ASF356, Akkermansia, and Lachnospiraceae UCG - 006, and Coriobacteriaceae UCG - 002 demonstrated a negative correlation with total blood cholesterol. The heatmap and correlation results collectively showed that the common lipophilic emulsifier MDG adversely affected gut microbiota composition and may be associated with changes in metabolic biomarkers.

**MDG slightly decreased the distance of bacteria to epithelial cells and enhanced inflammation potential by increasing circulating LPS**

Next, we examined how lipophilic emulsifier MDG affected the physiological change in the colon, intestinal permeability, and translocation of the bacterial product. The colon length of mice in the MDG-fed group was not significantly different from that in the CON group (Fig. 5f). The PAS-stained colon sections and colonic epithelial damage histological score exhibited that MDG did not induce colitis (Supplementary Fig. 4). We observed that the distance of bacteria to intestinal epithelial cells tended to be shorter in the mice fed with the MDG diet than in those fed with the CON diet (P = 0.1780) (Fig. 5g and 5h), suggesting the MDG-altered gut microbiome can drive toward the inner layer of the mucus. The reduced distance between the bacterial cell and epithelial cells may promote gut inflammation-associated disease. Additionally, MDG did not affect FITC-dextran-based intestinal permeability (Fig. 5i). However, we found that serum LPS was statistically elevated in mice fed with MDG (Fig. 5j), suggesting the MDG-altered microbiota may be capable of increasing LPS production.

**Discussion**

This study demonstrated that hydrophilic and lipophilic emulsifiers could potentially cause metabolic disorders and gut microbiota dysbiosis, and different emulsifiers showed particular effects on health-related biomarkers. Among the hydrophilic emulsifiers, SUC and CMC showed greater effects on health-related biomarkers than LEC. Intake of SUC and CMC resulted in an adverse effect on obesogenic and metabolic biomarkers and induced hyperglycemia and hyperinsulinemia by increasing weight gain, fasting serum glucose and serum insulin levels, and HOMA-IR index. The CMC group increased the relative fat mass but decreased the lean mass and interfered with lipid absorption and metabolism by lowering serum total cholesterol and triglyceride levels compared to the control group. In contrast, LEC supplementation did not show significant increases in serum glucose and insulin levels and HOMA-IR index. We also examined the lipophilic dietary emulsifier MDG, which affected blood lipid and glucose metabolism by increasing serum cholesterol but reducing triglyceride, thereby raising the blood glucose level of the OGTT.
In the human body, enzymatic digestion of SUC produces sucrose and fatty acids or fructose, glucose, and fatty acids. Intervention with a high-sucrose or high-fructose diet influences blood glucose metabolism by reducing insulin sensitivity and increasing fasting blood glucose and insulin concentration\textsuperscript{17,18}. Therefore, abnormal hyperglycemia and hyperinsulinemia in the SUC group are caused by the increased uptake of sugar and fatty acid. Moreover, dietary sweeteners sucrrose and saccharin supplementation impair glycemic response linked to the microbiome in healthy adults\textsuperscript{19}. CMC and P80 induce metabolic syndrome and intestinal inflammation by thinning the intestinal mucus layer, altering gut microbiota composition, and increasing the gut epithelial permeability and the LPS level\textsuperscript{10}. This shift in gut microbiota induced by CMC and P80 leads to low-grade inflammation-associated phenotypes and metabolic disease in the germ-free mice model\textsuperscript{11}. CMC can increase postprandial abdominal discomfort, affect gut microbiota shift, lower beneficial fecal metabolite, and enhance bacterial encroachment into the inner mucus layer in humans\textsuperscript{12}. Another study found that CMC and P80 negatively affect physiology and behavior, including anxiety-related and social behaviors, along with a shift in gut microbiota via different mechanisms in males and females\textsuperscript{20}. However, not all dietary emulsifiers have an adverse effect on health. Glycerol monodecanoate, a medium-chain monoacylglycerol, positively impacts the gut microbiota and improves lipid metabolism, insulin sensitivity, and inflammation\textsuperscript{21}.

Several recent studies demonstrated that food emulsifiers modify the gut microbiota composition and implicate the progression of several chronic diseases, including inflammatory bowel disease and metabolic syndrome. This study found that hydrophilic and lipophilic emulsifiers reshape the gut microbiota - and β-diversity indices. LEC had lesser influence on gut composition than other hydrophilic emulsifiers, whereas SUC and CMC exhibited a more significant effect on gut microbiota. A previous study reported that LEC did not significantly influence microbiota in ex vivo in the MiniBioReactor Array model\textsuperscript{22}. In contrast, the SUC group increased the observed species in gut microbiota but decreased the evenness index. CMC increased the evenness of the gut microbiota community. β-diversity of hydrophilic emulsifier-treated groups were associated with an insulin-resistant related biomarker. Moreover, hydrophobic emulsifier MDG lessened the evenness of the gut microbiota community and shaped the gut microbiome at β-diversity and was associated with impaired blood lipid and glucose metabolic biomarkers.

In general, hydrophilic and lipophilic dietary emulsifiers adversely impacted gut microbiota at the genus level and caused gut microbiota dysbiosis. A previous study examined the effect of 20 emulsifiers on human microbiota shift in an ex vivo model and revealed that most emulsifiers have detrimental consequences on microbiota composition and function\textsuperscript{22}. A study investigated the effect of five emulsifiers, including CMC, P80, soy lecithin, sophorolipids, and rhamnolipids and revealed that all emulsifiers selectively enriched the abundance of putative pathogens and increased flagellin\textsuperscript{23}. In this study, the LEC group showed enrichment of disease-related genera, including \textit{Streptococcus}, \textit{[Eubacterium] coprostanoligenes group}, \textit{Enterobacter}, \textit{Lachnoclostridium}, \textit{Desulfovibrio}, and \textit{[Eubacterium] xylanophilum group}, but reduced probable healthy bacteria, including \textit{Oscillibacter},
Parasutterella, Dubosiella, and Turicibacter. The disease-related microbiome enriched in the LEC group is described as follows. *Streptococcus pyogenes* is a pathogen that can cause both non-invasive and invasive illnesses, including nonsuppurative sequelae\(^{24}\). The occurrence of colorectal cancer is associated with *Streptococcus gallolyticus* colonization\(^{25}\). A large number of *Eubacterium coprostanoligenes group* is found in homocystinuria patients. Enterobacteriaceae have been reported to be associated with inflammatory bowel disease (IBD) pathogenesis and progression\(^{26}\). *Enterobacter aerogenes* and *Enterobacter cloacae* were found in several outbreaks of hospital-acquired infections\(^{27}\). *Lachnoclostridium* is associated with obesity\(^{28}\), and an abundance of *Lachnoclostridium* linked to a lower level of circulating acetate, which is associated with increased visceral fat in a large population-based-cohort\(^{29}\). *Desulfovibrio* plays a vital role in the pathogenesis of NAFLD by increasing intestinal permeability and hepatic CD36 expression\(^ {30}\). *Eubacterium xylanophilum group* is enriched in high-salt-induced hypertensive mice\(^ {31}\). In contrast, the healthy microbiome depleted in LEC is described as follows. *Oscillibacter* may be a beneficial bacteria because it is found in abundance in diabetic mice fed a high-fat carbohydrate-free diet\(^ {32}\). *Parasutterella* has been reported to play a potential role in bile acid maintenance and cholesterol metabolism\(^ {33}\) and is associated with improving low-density lipoprotein in healthy individuals\(^ {34}\). The relative abundance of *Dubosiella* is found to be decreased in DSS-induced colitis mice and may be used as ulcerative colitis amelioration bacteria\(^ {35}\). *Turicibacter* is more abundant in lean rodents than in obese rodents and may be an anti-inflammatory taxon\(^ {36}\).

The SUC group also showed enrichment of disease-related genera, including *Clostridium sensu stricto 1*, *Lachnospiraceae UCG - 006*, and *Eubacterium xylanophilum group*. The SUC group also showed reduction in possible beneficial genera such as *Muribaculaceae*, *Oscillibacter*, *Faecalibaculum*, *Parasutterella*, and *Olsenella*. Disease-associated genera in the SUC group, such as *Clostridium sensu stricto 1*, are found to have increased abundance in duodenal strictures subjects\(^ {37}\) and mice fed with a high-fat diet increase abundance of *Lachnospiraceae UCG-006*\(^ {38}\). A potential beneficial genus in the SUC group: *Muribaculaceae*, a potential beneficial genus in the SUC group, is enriched in lean mice\(^ {39}\) and may be involved in the degradation of complex carbohydrates\(^ {40}\). *Faecalibaculum* genera such as *Faecalibaculum rodentium* can stimulate epithelial proliferation and turnover through dampening retinoic acid generation that helps the survival of intestinal eosinophils\(^ {41}\).

The CMC group showed enrichment of disease-associated genera *Blautia*, *Staphylococcus*, and *Eubacterium coprostanoligenes group*, whereas several good microbiotas were correspondingly reduced, such as *Muribaculum*, *Faecalibaculum*, *Parasutterella*, *Dubosiella*, and *Turicibacter*. *Blautia* is enriched in NASH patients and is associated with the rise in LPS level\(^ {42}\). Moreover, *Blautia* is associated with an accumulation of visceral fat in adults\(^ {43}\) and obesity and increased blood insulin levels in children\(^ {44}\). *Staphylococcus aureus* is a pathogenic bacterium and that easily colonizes in the infant's intestine because the gut microbiota community has poor competition\(^ {45}\). *Muribaculum* has been speculated to maintain normal conditions of the mouse gut.
Administration of the lipophilic dietary emulsifiers MDG also caused gut microbiota dysbiosis. MDG reduced several potentially beneficial bacteria, including Muribaculaceae, Parabacteroides, Lachnospiraceae NK4A136 group, Akkermansia, and Collinsella. In contrast, MDG intake boosted the growth of several disease-related microbiomes such as Enterorhabdus, Jeotgalicoccus, and Atopostipes. Parabacteroides distasonis have been reported to alleviate obesity and metabolic dysfunctions by producing succinate and secondary bile acid\textsuperscript{46}. Lachnospiraceae NK4A136 group is a potential probiotic, found to be reduced in a high-fat diet mice\textsuperscript{47}. Supplementation with pasteurized Akkermansia muciniphila enhances insulin sensitivity and decreases insulinemia, plasma total cholesterol, and obesity in overweight/obese insulin-resistant volunteers\textsuperscript{48}. Low dietary fiber consumption increases Collinsella, and its abundance correlates with circulating insulin levels in overweight and obese pregnant women\textsuperscript{49}. Coriobacteriaceae UCG – 002 exhibits anti-inflammatory function, and the increased abundance of Coriobacteriaceae UCG – 002 can increase beneficial bacterial metabolite short-chain fatty acid\textsuperscript{50}. In prediabetes patients, the relative abundance of Enterorhabdus is increased\textsuperscript{51}. Jeotgalicoccus is positively correlated with insulin concentration in diabetic rats\textsuperscript{52}. Atopostipes is enriched in carbon tetrachloride-induced hepatic injury mice\textsuperscript{53}. In summary, these data suggested that common dietary hydrophilic and hydrophilic emulsifiers demonstrated an adverse effect on gut microbiome homeostasis by elevating disease-associated bacterial genera and reducing the relative abundance of beneficial microbiomes.

Our study uncovered that hydrophilic emulsifiers did not change the colon length, induce colitis, cause thinning of the mucus layer, or shorten the distance between bacterial and intestinal epithelial cells. SUC tended to increase intestinal permeability and LPS levels. These data suggested that hydrophilic emulsifiers did not disrupt mucus–bacterial interactions or facilitate diseases related to gut inflammation. Similar to hydrophilic emulsifiers, the lipophilic emulsifier MDG did not change the colon length and induce colitis. However, MDG decreased the distance between bacterial and intestinal epithelial cells which may promote gut inflammation-associated diseases. Moreover, MDG did not change intestinal permeability but increased serum LPS levels, suggesting that MDG altered the gut microbiota composition and was capable in increasing LPS generation and may result in systemic inflammation. CMC and P80 promote low-grade inflammation, metabolic syndrome, and colitis in mice by inducing microbiota encroachment, altering bacteria composition, and increasing intestinal permeability and LPS levels. A modified gut microbiome was verified in germ-free mice models with a similar phenotype\textsuperscript{10}. Our study demonstrated that the CMC induced metabolic disorder, altering bacteria composition, but did not reduce the distances of the closest bacterial cells to intestinal epithelial cells. Our data does not agree with the findings of a previous study, possibly due to differences in gut microbiota composition, housing environment, and location. Excessive mucin degradation by gut microbiota may cause intestinal disorders allowing luminal antigens to translocate to the intestinal immune system\textsuperscript{54}. Mucin-degrading microbes possess glycosyl hydrolases that can digest specific glycan linkages. Akkermansia glycaniphila and muciniphila have the mucin-degrading gene\textsuperscript{5}. SUC was increased in Akkermansia; however, we did observe a reduction in the distance between bacterial and intestinal epithelial cells in SUC-fed mice. Beyond the genus Akkermansia, 24 genera of bacteria harboring mucin-degrading glycosyl hydrolase
gene in various phyla have been reported in the literature. Unfortunately, those genera were not detected in MDG-fed mice, showing a reduced distance between bacterial and intestinal epithelial cells.

Although numerous studies have discovered the negative effect of these food additives, including emulsifiers, the usage of food additives has continued to increase in the food industry. Every country has its own regulations to control the use of food additives. Some nations may follow the GRAS guidelines, established using scientific evidence, to approve one substance as a food additive. Since the discovery of the effects of gut microbiota on health in the past decade, researchers have focused on the effects of food additives on gut microbiota, its function, and its metabolites, which may synergistically cooperate with food additives to adversely affect health, eventually leading to the development of chronic diseases via the metaorganism-pathogenesis pathway. Thus, the safety of food additives with respect to the gut microbiota and host interaction needs to be further investigated to fill gaps in the existing literature and support laws governing food production.

Our findings uncovered that the dietary hydrophilic emulsifiers exhibited the potential to induce obesity and metabolic disorders and resulted in hyperglycemia and hyperinsulinemia. On the other hand, lipophilic dietary emulsifiers impaired circulating lipid and glucose metabolism. Both hydrophilic and lipophilic emulsifiers remodeled the complexity of gut microbiota composition, increasing the disease-associated microbiome and enhancing gut microbiota dysbiosis. However, hydrophilic emulsifiers did not affect mucus–bacterial interactions nor facilitate gut inflammation-associated disease. In contrast, the lipophilic emulsifier MDG facilitated bacterial encroachment toward intestinal epithelial cells and enhanced inflammation potential by elevating circulating LPS (Fig. 6). Our study provides information regarding the safety concerns associated with the use of dietary emulsifiers, which may help to reevaluate food safety policies and laws governing food production. However, these outcomes necessitate further verification in humans.

Methods

Hydrophilic dietary emulsifiers-fed C57BL/6J mouse model

The animals were handled following the guidelines of the Institutional Animal Care and Use Committee of National Taiwan University (approval number: NTU107-EL-00121). Male C57BL/6 J mice were purchased from the National Laboratory Animal Center (Taipei, Taiwan). The mice were housed in an animal room with a 12 h light-dark cycle at 23 ± 2°C. After acclimation, fifteen-week-old mice were randomly divided into four experimental groups (n = 3–4/cage): (i) control [CON], (ii) lecithin [LEC, 7523.3 mg/kg bw/day], (iii) sucrose fatty acid esters [SUC, 1110 mg/kg bw/day], and (iv) carboxymethylcellulose [CMC, 1%]. CMC dosage was selected according to a previous study. The dosages of LEC and SUC were selected according to the report on the dietary exposure estimate in humans and were translated into corresponding mouse dosages. The dose of this experiment is mimicked as ten times daily exposure in humans. Emulsifiers were purchased from Gemfont Corporation (Taipei, Taiwan). Mice were fed with a normal chow diet (MFG; Oriental Yeast Co., Ltd., Tokyo, Japan), and all dietary emulsifiers were
supplemented in drinking water. Mice in each experimental group were allowed free access to food and water for 17 weeks. Before euthanization, the mice fasted for 12 h. The mice were subsequently sacrificed using CO₂ asphyxiation. Blood was collected via cardiac puncture using a syringe. Organs were collected for subsequent analysis.

**Lipophilic dietary emulsifiers-fed C57BL/6J mouse model**

The mice were housed in an animal room with the previously described conditions. Six-week-old male C57BL/6J mice were employed for the experiment after an adaptation period of 2 weeks. Eight-week-old male C57BL/6J mice were divided into two groups based on their diet: (i) control diet (CON) group, and (ii) monoglycerides and diglycerides diet (MDG) [5.5%] group. The MDG dosage for this experiment followed a previous study that studied the effect of 5.5% diacylglycerol (DAG), exhibiting no signs of systemic toxicity. The control mice were fed with an AIN-93M mature rodent diet. The MDG diet was customized from the AIN-93M mature rodent diet (#D10012M; Research Diet Inc.) by replacing soybean oil with MDG, and MDG composition was 5.5% in the diet. Mice were limited to 85 kcal/week. Calorie control was implemented to minimize the differences in dietary intake of the mice for solely observing the effect of MDG. The mice were sacrificed after 14 weeks of the experiment. After CO₂ asphyxiation, the blood was collected by cardiac puncture using a syringe and the organs were collected for subsequent analysis.

**Body composition analysis**

Both lean and fat mass body composition was measured using a Minispec LF50 TD-NMR body composition analyzer (Bruker, Billerica, MA, USA). The relative fat or lean mass was calculated as the fat or lean mass to the body weight, respectively.

**Mouse blood biochemistry analysis**

The serum was extracted by centrifuging the blood at 1000 × g for 15 min at 4°C. Serum biochemical biomarkers, including total cholesterol, total triglyceride, high-density lipoprotein (HDL-c), AST, ALT, and glucose, were measured using commercial test strips (Spotchem II reagent strips; Arkray Inc., Kyoto, Japan) in an automatic blood analyzer (Spotchem EZ).

**Oral glucose tolerance test (OGTT)**

The mice were fasting for 5 h before OGTT experiments. A blood sample was collected from the submandibular vein. Then the blood glucose was analyzed using a glucometer (ACCU-CHEK® Performa, Roche, Basel, Switzerland) at 0, 15, 30, 60, 90, and 120 min after oral gavage with 2 g/kg glucose.

**Intestinal permeability analysis**

Intestinal barrier permeability was performed by gavage with intestinal permeability probe–fluorescein isothiocyanate-dextran (FITC-dextran; MW 4,000; Sigma-Aldrich, 46944). After 4 h of fasting, the mice were gavaged with 0.2 mL of FITC-dextran solution (15 mg of FITC-dextran in phosphate-buffered saline). Blood was collected after 3 h. The serum was extracted by centrifuging the blood at 1000 × g for 15 min
at 4°C. Plasma FITC-dextran fluorescent intensity was measured using a fluorometer in black 96-well plates at excitation and emission wavelengths of 485 and 538 nm (Fluoroskan Ascent FL, Thermo Fisher Scientific, USA). FITC–dextran concentrations were measured against a standard curve produced by serial dilution of FITC–dextran in mice serum.

Periodic acid–Schiff staining and histopathological analysis

The mouse colons were removed and fixed in Carnoy's solution for 24 h and subsequently embedded in paraffin. The colon sections were stained with the Periodic acid–Schiff staining method. Hepatic histological scoring was conducted by an experienced pathologist at the College of Veterinary Medicine Animal Disease Diagnostic Center (National Chung Hsing University, Taichung, Taiwan).

Visualized the bacteria co-localized the mucus layer

(i) Paraffin embedding with Carnoy's fixation

The mouse colonic tissues with fecal matter were immersed in Carnoy's solution (60% methanol, 30% chloroform, 10% glacial acetic acid) for 24 h. For embedding, samples were immersed twice in anhydrous methanol for 30 min each, 100% ethanol for 20 min each, and xylene for 15 min each, and finally immersed in paraffin wax (Leica, Germany) at 56–58°C for 3 h each. Blocks were hardened at room temperature. Sections were slid to 4-µm-thickness, floated on a water bath at 40–45°C, and transferred to slides.

(ii) Fluorescent in situ hybridization

Slides were deparaffinized by immersing twice in xylene for 15 min each and 100% ethanol for 5 min each, following which the samples were treated with protease K (5 µg/mL) at 37°C for 15 min and the treated samples were immersed in 0.9 M NaCl, 20 mM Tris pH 7.4 for 10 min in preparation for fluorescence in situ hybridization (FISH). FISH targeting bacterial cells was performed using the oligonucleotide probe EUB338 (5'-GCTGCCTCCGCTAGGAGT-3', with a 5' Alexa 647 label) custom synthesized (Li-Tzung Inc., Taiwan). Sections were incubated in a hybridization buffer (0.9 M NaCl, 0.02 M Tris-HCl pH 7.4, 0.01% SDS, 30% formamide) and 2 µM probe at 46°C for 2.5 h. Samples were washed at 48°C for 15 min excess wash buffer after hybridization. (0.215 M NaCl, 0.02 M Tris-HCl pH 7.5, 5 mM EDTA).

(iii) Fluorescent staining with Hoechst 33258 and wheat germ agglutinin (WGA)

Sections were dyed with Hoechst 33258 (10 µg/mL) and WGA (40 µg/mL) Alexa Fluor 488 conjugate (Thermo-Fisher Inc., USA) in PBS for 15 min and incubated twice for 3 min each in wash buffer (0.112 M NaCl, 20 mM Tris-HCl pH 7.4, 5 mM EDTA, 0.01% SDS). Slides were then washed in water and dried or were submerged for 3 min each into 50%, 80%, and 96% (v/v) ethanol and then dried. Slides were subsequently mounted with Prolong anti-fade mounting media (Life Technologies) and placed in a dark environment at room temperature until the mounting medium solidified. Images were acquired with a
Leica TCS SP5 II confocal microscope (Joint Center for Instruments and Researches, College of Bioresources and Agriculture, National Taiwan University, Taiwan).

(iv) Measurement of mucus thickness

The mucus layer was determined by using Fiji to measure the width of the region brightly stained with WGA and on the interior of the host epithelium. We visualized the border of the host epithelium using differential interference contrast (DIC) and then merged the channel to measure the distance.

**Measurement of serum LPS and insulin concentration**

Pierce LAL Chromogenic Endotoxin Kit (#88282, Thermo Scientific, USA) was used to detect the LPS levels in the serum. Serum insulin levels were measured using commercial kits supplied by Mercodia AB (#10-1247-01, Mouse Insulin ELISA kit, Mercodia, Sweden).

**Gut microbiota analyses**

Cecal samples DNA were extracted according to the manufacturer's instructions using the QIAamp PowerFecal DNA Kit (Qiagen, Netherlands). PCR amplification of 16S rRNA V4 regions was performed using the 515F/806R bacterial primer pair (515F: 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R: 5'-GGACTACHVGGGTWTCTAAT-3'), with a 50 µL reaction volume containing 25 µL 2X Taq Master Mix (Thermo Scientific, USA), 0.2 M of each forward and reverse primer, and 20 ng DNA template. Thermal cycling consisted of initial denaturation at 95°C for 5 min, followed by 25 cycles of denaturation at 98°C for 20 s, annealing at 57°C for 15 s, and elongation at 72°C for 30 s, with a final hold at 72°C for 10 min. Next, amplified products were subjected to 2% agarose gel electrophoresis. The amplified PCR product was attached with Illumina sequencing adapters with a Nextera XT Index kit and then purified using AMPure XP beads. Library quantification was achieved employing the DNA 1000 kit and 2100 Bioanalyzer instrument (Agilent Technologies, USA), and sequencing (single-end reads) was completed using Illumina NextSeq (Illumina, USA). Raw sequences were processed following the QIIME2 pipeline. Raw sequences were quality filtered, trimmed, de-noised, merged, and chimeric sequences removed using the QIIME2 dada2 plugin. The amplicon sequence variances (ASVs) were aligned against the SILVA database (version 138). The alpha diversity, including the observed ASVs and Shannon and Simpson indices, was computed using the vegan package in R. A principal coordinate analysis (PCoA) was conducted using the Bray–Curtis distance. Additionally, an analysis of similarity (ANOSIM) was performed to determine the heterogeneity of gut microbiota among the groups. Finally, a heatmap at the genus level was generated using the heatmap3 package in R.

**Statistical analysis**

Data are presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) with Tukey's range test or Student's t-test was used to determine statistically significant differences between groups. One-way ANOVA followed by Tukey's range test, Student's t-test, Wilcoxon signed-rank test, Kruskal–Wallis test were used to evaluate the gut microbiota dataset. All statistical analyses were analyzed using R Studio (version 1.2.5001), R (version 3.6.1), or GraphPad Prism (version 9.5.1).
Declarations

DATA AVAILABILITY

Gut microbiota-related figures in this study were generated using the raw 16S rRNA sequencing data, which can be accessed at the NCBI Short Read Archive using the corresponding accession numbers: BioProject: PRJNA944655, and BioSample: SAMN33757824.

CODE AVAILABILITY

The "Methods" section of this study provides a description of the bioinformatic tools, software version, parameters, and open-source code utilized. More details regarding the code to reproduce the analyses are available upon request.

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COMPETING INTERESTS

The authors declare that there are no competing interests.

AUTHOR CONTRIBUTIONS

S.P. provided instructions and assisted in the experiments, performed the bioinformatic and statistical analysis, interpreted the results and drafted the manuscript. W.K.W. designed and provided instructions for the experiments, along with reviewing and revising the manuscript. C.T.C. performed the animal experiments. N.W. and H.C.H provided technical support in visualized the bacteria co-localized the mucus layer experiments. R.A.C., H.S.H. and Y.H.C. assisted all the experiments. P.Y.L. supported bioinformatic analysis. H.L.C. provided technical support in mice experiment. T.C.D.S., S.L.T, and C.T.H. critically reviewed the manuscript. M.S.W. and L.Y.S. designed the experiments, provided funding for the study, and revised the manuscript. All authors had full access to all the data in the study and accept responsibility to submit for publication.

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**Figures**
Figure 1

Dietary exposure estimates of common food emulsifiers and market volume of additives classified as food emulsifiers according to regulatory bodies involved in food additive legislation. (a) Food additives are categorized as emulsifiers according to regulatory bodies that govern the use of food additives such as JECFA, Codex, FSA (UK), and FDA (US)\(^9\); (b) Venn diagram representing the overlap between food emulsifier classification by different organizations\(^9\). (c) Dietary exposures of seven common emulsifiers
found in food compared with ADI\textsuperscript{13}; and (d) their market volume\textsuperscript{13}. JECFA: Joint Food and Agriculture Organization/World Health Organization Expert Committee on Food Additives; Codex: Codex Alimentarius; FSA: Food Standards Agency; UK: United Kingdom; FDA: Food and Drug Administration; US: United States; MDGs: mono- and diglycerides; CMC: carboxymethylcellulose; P80: polysorbate 80; SSL: sodium stearoyl lactylate; CSL: calcium stearoyl lactylate; PGPR: polyglycerol polyricinoleate.

Figure 2

Hydrophilic dietary emulsifiers had an adverse effect on obesogenic and metabolic biomarkers and induced hyperglycemia and hyperinsulinemia. (a) Experimental design, (b) changes in weight gain, (c) changes in weight gain at 17th week, (d) changes in relative fat mass, (e) changes in relative lean mass, (f) serum total cholesterol levels, (g) total triglyceride levels, (h) oral glucose tolerance test (OGTT) curve, (i) area under the curve (AUC) of OGTT, (j) serum fasting glucose levels, (k) serum insulin levels, and (l) homeostatic model assessment for insulin resistance (HOMA-IR). Mice were supplemented with or
without different emulsifiers in drinking water for 17 weeks. Dot plots are expressed as the mean ± SD (n=14–15 per group). Statistical analyses were performed using one-way ANOVA with Tukey’s range test for comparisons shown as exact P-values. *, p < 0.05 and **, p < 0.01. CON: control group; LEC: lecithin group; SUC: sucrose fatty acid esters group; CMC: carboxymethylcellulose group.

Figure 3
Hydrophilic dietary emulsifiers transformed the gut microbiota diversity indices, and adversely affected the gut microbiota but did not disrupt mucus–bacterial interactions or promote diseases associated with gut inflammation. (a) Observed amplicon sequence variances (ASVs), (b) Shannon index, (c) Simpson diversity index, and (d) principal coordinate analysis (PCoA) plot based on Bray–Curtis dissimilarity with gut microbiome-associated vector (envfit {vegan}), (e) heatmap of the relative abundances of cecal microbiota with significant difference measured using the Kruskal–Wallis test (P < 0.05) and Spearman’s correlation coefficient between gut microbiome at genera and obesogenic and metabolic biomarkers, (f) colon length, (g) distances of closest bacteria to intestinal epithelial cells (IEC), (h) representative image of immunostaining and localization of bacteria using fluorescent in situ hybridization, (i) FITC-dextran concentration, and (j) serum lipopolysaccharides (LPS) levels. Mice were supplemented with or without different emulsifiers in drinking water for 17 weeks. Dot plots are expressed as the mean ± SD (n=6–8 per group). Statistical analyses were performed using a one-way ANOVA with Tukey’s range test for comparisons shown as exact P-values. Analysis of similarity (ANOSIM) was used to analyze the heterogeneity of the cecal microbiota among the groups in PCoA. Vectors in the PCoA plot show a significant genus (p < 0.01), and its length indicates the strength of the correlation. Pairwise statistical analyses were performed using an unpaired Wilcoxon signed-rank test shown as heatmap (CON vs. LEC; CON vs. SUC; and CON vs. CMC). Confocal microscopy analysis of microbiota localization: mucus layer, green; bacteria, red; and DNA, blue. Scale bar, 25 μm. CON: control group; LEC: lecithin group; SUC: sucrose fatty acid esters group; CMC: carboxymethylcellulose group.
Lipophilic dietary emulsifiers mono- and diglycerides (MDG), impaired circulating lipid and glucose metabolism. (a) Experimental design, (b) changes in weight gain, (c) changes in weight gain at the 14th week, (d) changes in relative fat mass, (e) changes in relative lean mass, (f) serum total cholesterol levels, (g) total triglyceride levels, (h) oral glucose tolerance test (OGTT) curve, (i) area under the curve (AUC) of OGTT, (j) serum fasting glucose levels, (k) serum insulin levels, and (l) homeostatic model assessment for insulin resistance (HOMA-IR). Mice were fed control or MDG diet for 14 weeks. Dot plots are expressed as the mean ± SD (n=14-15 per group). Statistical analyses were performed using an unpaired two-tailed Student’s t-test for comparisons (CON vs. MDG) and shown as exact P-value or symbols (*, p < 0.05). CON: control group; MDG: monoglycerides and diglycerides group.
Lipophilic dietary emulsifiers mono- and diglycerides (MDG) modified the gut microbiome diversity, cause gut microbiota dysbiosis, decreased the distance of bacteria to epithelial cells, and enhanced inflammation potential by increasing circulating LPS. (a) Observed amplicon sequence variances (ASVs), (b) Shannon index, (c) Simpson diversity index, and (d) principal coordinate analysis (PCoA) plot based on Bray–Curtis dissimilarity with gut microbiome-associated vector (envfit {vegan}), (e) heatmap of the
relative abundances of cecal microbiota with a significant difference measured using an unpaired Wilcoxon signed-rank test for comparisons (P < 0.05) and Spearman's correlation analysis between gut microbiota at genera and obesogenic and metabolic biomarkers, (f) colon length, (g) distances of closest bacteria to intestinal epithelial cells (IEC), (h) representative image of immunostaining and localization of bacteria by fluorescent in situ hybridization, (i) FITC-dextran concentration, and (j) serum lipopolysaccharides (LPS) levels. Mice were fed control or MDG diet for 14 weeks. Dot plots are expressed as the mean ± SD (n=6–7 per group). Statistical analyses were performed using an unpaired two-tailed Student's t-test for comparisons (CON vs. MDG) and shown as exact P-value. Analysis of similarity (ANOSIM) was used to analyze the heterogeneity of the cecal microbiome among the groups in PCoA. Vectors in the PCoA plot represented a significant genus (p < 0.05), and its length indicated the strength of the correlation. Confocal microscopy analysis of microbiota localization: mucus layer, green; bacteria, red; and DNA, blue. Scale bar, 25 μm. CON: control group; MDG: monoglycerides and diglycerides group.

**Figure 6**

**Dietary emulsifiers promote metabolic disorders and induce intestinal microbiota dysbiosis.** The study revealed that hydrophilic dietary emulsifiers were found to have a negative impact on glucose and insulin levels, while lipophilic dietary emulsifiers disrupted blood lipid levels and glucose homeostasis. Additionally, both types of emulsifiers caused imbalances in the gut microbiota. Notably, lipophilic emulsifiers further exacerbated the increase of gut-derived LPS in the bloodstream. This study provides insights into the safety risks linked to the consumption of dietary emulsifiers through the lens of gut microbiota, and these findings may help in a reevaluation of existing food safety policies and regulations governing food production.
Supplementary Files

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