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Therapeutic Potential of Inulin-Coated MCT Microcapsules in Modulating the Gut Microbiome for Effective Treatment of Diet-Induced Obesity

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Obesity, a global epidemic, leads to metabolic dysregulation and systemic inflammation. Recently, therapies targeting the gut microbiome have garnered attention for metabolic health regulation. This study evaluates the potential of inulin-coated medium-chain triglyceride (InuMCT) microcapsules in rats with diet-induced obesity (DIO). Inulin prebiotic fibers have been shown to promote the gut microbiome, while the digestion products of medium chain triglycerides (MCTs), free fatty acids, and mono-/diglycerides, can attenuate pro-inflammatory outcomes. It is hypothesized that encapsulating MCTs within inulin via spray drying creates a solid dosage form that can exert multifunctional effects in ameliorating inflammation in DIO. Inulin and InuMCT treatments not only reduce DIO weight gain but also improve metabolic markers in high-fat diet (HFD) fed rats. Specifically, inulin attenuates the reduction of high-density lipoprotein (HDL) by 55% and lowers glucose levels by 21%. Meanwhile, InuMCT increases HDL by 23% and reduces glucose levels by 15%. Furthermore, inulin decreases serum proinflammatory tumor necrosis factor-alpha (TNF-α) by 35%, while InuMCT further reduces TNF-α to normal diet levels within 21 days. These results highlight InuMCT's superior efficacy, offering a promising strategy for combating obesity and related metabolic diseases.

1. Introduction

Obesity, commonly regarded merely as a condition of excess body weight, is now recognized as a multifaceted health problem that extends far beyond weight gain. Recent scientific explorations have identified obesity as a state of systemic inflammation derived from complex biochemical pathways rather than simple caloric excess.[1] This paradigm shift implicates alternative biological mechanisms, notably disturbances in the gut microbiome, which lead to metabolic endotoxemia a condition where bacterial toxins enter the bloodstream, promoting inflammation and metabolic disruptions associated with obesity.[2]

Despite this improved understanding, current pharmacological treatments for obesity often ignore the important role that the gut microbiome plays in this metabolic disease, instead focusing on the downstream effects of the disease, such as body weight and hyperglycemia.[3] Furthermore, existing anti-obesity pharmacotherapies often perturb gut microbiome diversity either via the active drug molecule or the excipients utilized within the formulation, potentially exacerbating long-term systemic inflammation and weight gain.[4] Ultimately, this highlights an urgent need for therapeutic strategies that not only address the metabolic and inflammatory manifestations of obesity but also target the underlying microbial disruptions within the gastrointestinal tract (GIT).

In recent years, significant attention has been afforded to the therapeutic modulation of the gut microbiome, serving as either a standalone or adjunct therapy in treating obesity.[5]

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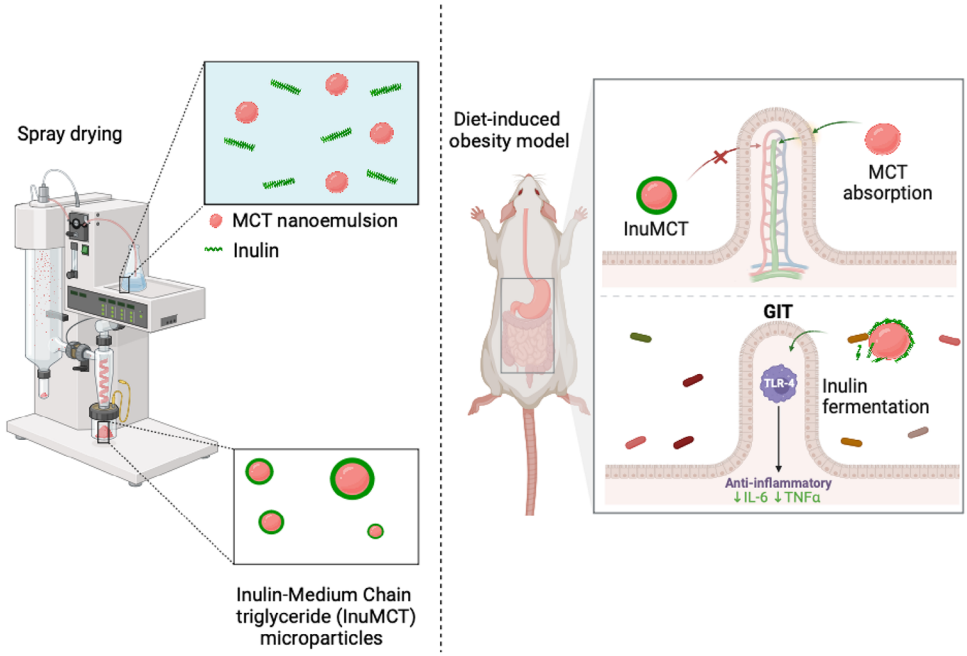

Scheme 1. A graphical schematic depicting the spray drying process of an aqueous dispersion of inulin and an MCT emulsion. Spray-dried InuMCT reaches the GIT, where inulin is utilized by the microbiome and MCT exerts its anti-inflammatory effects.

Various food-grade biomaterials such as prebiotics, probiotics, and synbiotics have been explored for their potential to address the multifaceted aspects of obesity through their interaction with the gut microbiome.[6] Prebiotics such as inulin are polysaccharides resistant to digestion by the host. The prebiotics are instead fermented exclusively by the GIT microbiome, promoting the diversity and abundance of commensal niches.[7] This fermentation process produces short-chain fatty acids (SCFAs) such as acetic acid, propionic acid, and butyric acid, which play crucial roles in regulating inflammatory pathways implicated in various metabolic diseases.[8] Furthermore, emerging evidence highlights the role that SCFAs play in regulating the release of gut hormones such as glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), which help reduce food intake and promote satiety.[9] Such modulation of the gut microbiome via prebiotic intake has been associated with reduced adiposity and improved metabolic health in overweight and obese individuals, thereby offering a promising therapeutic strategy.[10]

Bioactive lipids, such as medium-chain triglycerides (MCTs), have been extensively studied as integral dietary macronutrients that regulate metabolic and inflammatory health. Kono et al. (2016) highlighted the benefits of an MCT-enriched diet in preventing metabolic endotoxemia in rats, demonstrating a significant reduction in proinflammatory tumor necrosis factor-alpha (TNF-α) production in liver macrophages, which indicates the anti-inflammatory properties of MCTs and their role in improving liver health.[11] Xu et al. (2018) supported the previous findings in an *ex vivo* intestinal model and found that the predominate mechanism of anti-inflammatory action is via the inhibition of toll-like receptor (TLR)−4, agonism of free fatty acid receptors (FFARs) and necroptosis signaling pathways.[12] Other studies have found that MCT dosed in an in vitro intestinal cell model suppresses IL-8 secretion at the transcriptional levels, highlighting MCT's localized action on intestinal cell lines.[13]

However, the rapid absorption of MCT's digestion products in the upper GIT limits their residence time, consequently limiting their gut activity and potential benefits.[14] Studies have shown that MCTs are absorbed quickly in the upper GIT, particularly in the small intestine, which reduces their availability to interact further across the GIT, where an abundant microbiome exists that readily utilizes inulin.[15] Microencapsulating MCTs with prebiotics that are not digested in the small intestine is believed to extend GIT residency time, which may enhance their anti-inflammatory effects. Given inulin's effectiveness as a wall material for microencapsulation via spray drying, this study aimed to develop inulin-medium chain triglyceride (InuMCT) microcapsules as a multifunctional formulation that modulates the gut microbiome and delivers MCT to the GIT for enhanced anti-inflammatory effects (**Scheme 1**). Importantly, Inulin and MCT are both categorized as Generally Recognized as Safe (GRAS) by the US Food and Drug Administration, exhibiting a high safety profile that is well tolerated even when administered at high doses.[16]

Previous research demonstrated that InuMCT microcapsules enhance the bioavailability of oral antipsychotics and positively modulate the gut microbiome.[17] This study focused on preparing a viable solid dosage form via spray drying and explored InuMCT's therapeutic potential for diet-induced obesity (DIO) in Sprague Dawley rats.[18] By examining inflammatory, metabolic, and microbial markers, the study aimed to demonstrate that InuMCT offers a novel and effective approach to managing DIO and its inflammatory outcomes.

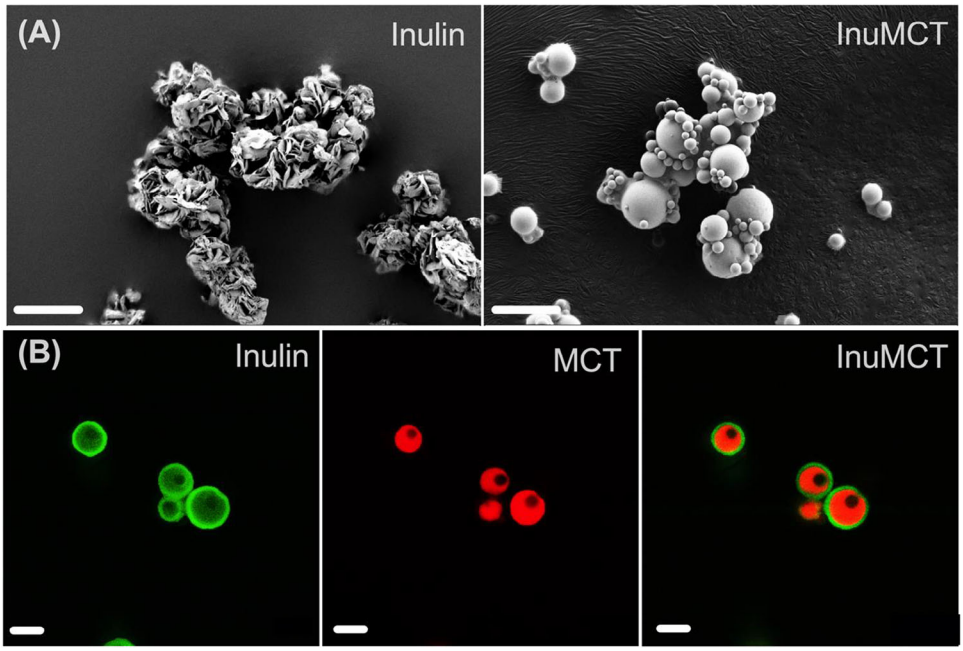

Figure 1. A) A scanning electron microscope (SEM) to reveal the formation of uniformly spherical microcapsules of 2–6 µm in size (right panel), whereas the inulin precursor appeared to be a size irregular 15–20 µm aggregate (left panel). B) Confocal fluorescence images of Inulin (left panel), MCT (middle panel), and InuMCT (right panel) demonstrated that the spray-dried matrix featured an Inulin shell and a MCT core. Scale bars represent 10 µm.

2. Results and Discussion

2.1. Spray Drying Induces Inulin Microcapsules with an MCT Core

The production of inulin microcapsules with an MCT core was successfully achieved by spray drying an MCT nanoemulsion with inulin dispersed in the aqueous phase (1:1 wt. ratio). The dehydration process led to the formation of smooth, uniformly spherical microcapsules, ranging in diameter from 2–6 µm (**Figure 1**A). In contrast, the precursor inulin was observed to form randomly arranged aggregates of≈15–20 µm in size. Confocal fluorescence imaging revealed a distinct inulin-coated shell surrounding an inner MCT core (Figure 1B). The unique matrix arrangement of the InuMCT microcapsules was hypothesized to be particularly important for enhancing the GIT residency time of MCT by providing a barrier against complete upper GIT absorption, enhancing its anti-inflammatory properties in the process.[19] The chemical characteristics of inulin, particularly its average degree of fructan polymerization, play a crucial role in this system as the molecular weight of inulin has been demonstrated to be inversely proportional to the rate of its enzymatic hydrolysis.[20] Long-chain inulin (average degree of polymerization >10), with its more complex chemical structure, transits further to the distal GIT region before being completely utilized by the microbiome.[21] Consequently, the MCT core from the InuMCT is potentially delivered to more parts of the GIT, in order to exert its anti-inflammatory effects.[11] Nevertheless, the multifunctional targeting of the GIT by InuMCT is especially important for diseases where the abundance and species of commensal taxa in the microbiome are perturbed, as in metabolic and gastrointestinal diseases.[22]

2.2. Microbial Abundance in HFD Fed Rats is Promoted by InuMCT Administration

Following the 21-day treatment phase, the administration of inulin and InuMCT significantly increased the fecal microbial abundance of commensal microbial taxa, particularly *Bifidobacteriaceae* (**Figure 2**A). Inulin alone promoted the relative abundance of species from the *Bifidobacteriaceae* family by 11- and 7-$\log_2$ folds compared to the HFD and normal diet groups, respectively. InuMCT also demonstrated substantial increases, with 8.3- and 4.3- $\log_2$ fold enhancements over the HFD and normal diet groups. Additionally, MCT improved the abundance of commensal taxa such as *Lactobacillaceae* and *Akkermansiaceae* by 2.3 and 3.9 $\log_2$ folds respectively, compared to the HFD group.

The increase in *Bifidobacteriaceae* due to inulin administration may be attributed to its prebiotic properties, which nourish commensal species of the gut microbiome. *Bifidobacterium* strains have been shown to enhance the intestinal barrier by increasing the expression and/or localization of tight junction proteins, which are crucial for maintaining the integrity of the gut barrier and preventing pathogens from entering the bloodstream.[23] Moreover, some strains of *Bifidobacterium* can mitigate weight gain and alter fat distribution. For instance, *Bifidobacterium M13-4* was found to improve weight gains in obese rats induced by a high-fat diet, while *Bifidobacterium L66-5* induced a decrease in body weight.[24] All tested strains helped reduce serum and liver triglycerides and significantly alleviated lipid deposition in the liver.

The elevation of *Lactobacillaceae* and *Akkermansiaceae* due to MCT treatment suggests a protective role of these lipids on the intestinal epithelium. *Akkermansia muciniphila* within the *Akkermansiaceae* family is known for its ability to degrade mucin,

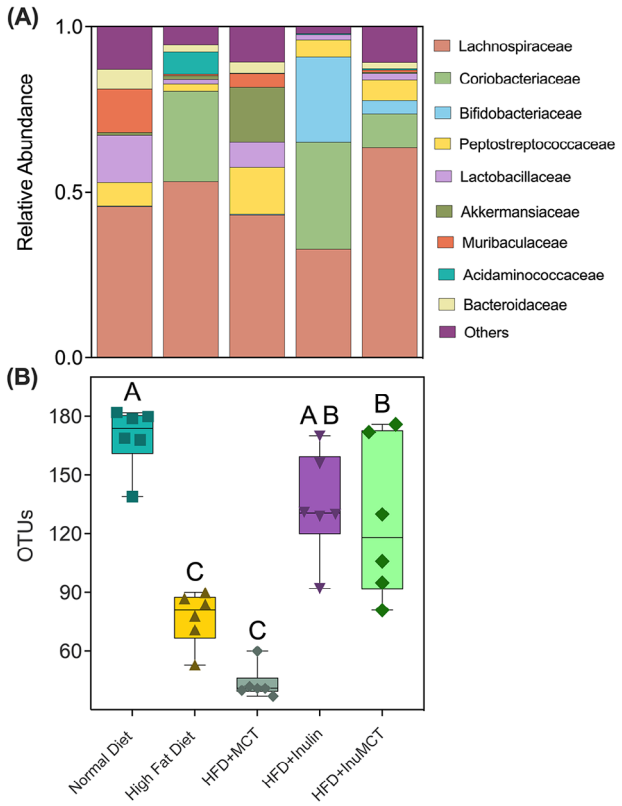

Figure 2. A) Data represents the relative abundance of microbiome families collected from rat's fecal samples at the end of the 3-week treatment phase. Both Inulin and InuMCT administration enhanced the B) OTUs (operational taxonomic units) represented unique species, whilst only inulin recovered levels to that of normal diet-fed rats. Statistical significance was derived using a one-way ANOVA test, followed by a Tukey's post hoc test for multiple comparisons between all means. The means represented by a different letter indicate significant differences between treatments ($p < 0.05$). An *n* of 6 was used for each group.

which helps in maintaining a healthy mucosal layer and has been associated with improved metabolic profiles in obesity models.[25] Similarly, *Lactobacillus acidophilus AD031* significantly decreased triglyceride levels in the liver, whilst *Lactobacillus fermentum* has attenuated obesity by modulating lipid metabolism, improving glucose tolerance, and reducing inflammation.[26]

The effects of inulin and InuMCT on the fecal microbial richness, as measured by operational taxonomic units (OTUs), revealed significant increases in the number of detectable taxa after 21 days of treatment in HFD-fed rats. Specifically, inulin administration resulted in a 43% increase in OTUs, successfully enhancing microbial richness to levels comparable with those observed in rats fed a normal diet (Figure 2B). InuMCT treatment also led to a significant enhancement, with a 39% increase compared to the HFD group, although it did not fully recover OTU numbers to normal diet levels. Inulin promotes the growth of microbial taxa with known health benefits, such as those in the *Bifidobacteriaceae* family, which may contribute to the observed increase in microbial richness.[27] The significant but slightly lesser increase in OTUs with InuMCT treatment compared to inulin alone might be attributed to the additional MCT component in InuMCT, which has been shown to adversely affect gut microbial diversity and increase proinflammatory IL-1β concentration in rats.[28] Hence, inulin in the InuMCT treatment may mitigate MCT's negative impact on the microbiome, whilst affording its multifunctionality in promoting beneficial commensal taxa and producing an anti-inflammatory effect in the GIT.

2.3. InuMCT Protects Against HFD-Induced Gut Microbiome Perturbations in Rats

The impact of a HFD on fecal microbial alpha diversity, quantified using Shannon's index, caused a significant 53% reduction, demonstrating the detrimental effects of such diets on gut microbial richness (**Figure** 3A). This reduction in alpha diversity is often associated with metabolic health and is typically observed in metabolic diseases, such as obesity.[29] The results of the current study align with previous findings which highlighted that a Western diet rich in saturated fats can substantially alter the GIT microbiome, leading to decreased microbial richness and diversity in mice.[30] Conversely, treatments with inulin and InuMCT significantly enhanced the alpha diversity by 35% and 42%, respectively.

InuMCT treatment induced a significantly unique community structure in the microbiome, as quantified using the Bray-Curtis beta-diversity plot (Figure 3B). The unique microbial community structure induced by InuMCT suggests that the MCT component in InuMCT, is altering the gut environment in a way that supports the growth of distinct bacterial populations not typically promoted by inulin alone, such as those from the commensal *Lactobacillaceae, Akkermansiaceae, and Lachnospiraceae* families (Figure 2A). Hence, MCTs may further enhance the resilience of the gut microbiome against dysbiotic shifts induced by HFDs, as well as potentially promote a unique microbial niche that could have distinct metabolomic outcomes beneficial to the host.

2.4. InuMCT has an Additive Impact on Promoting Commensal Microbial Taxa

The differential abundance analysis following a 21-day HFD intervention highlighted significant shifts in the gut microbiome linked to metabolic function (**Figure** 4A). Notably, the reduction of *A.muciniphila* by 14 $\log_2$ folds when compared to the normal diet group, may have detrimental effects on metabolic health, as these species are known for improving gut barrier function against inflammatory agents in animal models.[31] Similarly, the decrease in several species from the genus *Blautia* of 11 $\log_2$ folds is noteworthy, as certain strains have demonstrated a strong inverse association with obesity and metabolic syndrome.[32] *Blautia* has also been inversely associated with visceral fat accumulation.[33] The finding in the current study further substantiates the protective role of *Blautia* against metabolic markers of health. The addition of MCT to a HFD notably altered the gut microbiome in rats, impacting species implicated in obesity and metabolic diseases (Figure 4B). Specifically, MCT reversed some of the perturbations of the HFD on the microbiome, by promoting *A.muciniphila* by 15 $\log_2$ folds and *Blautia coccoides* by 9.6 $\log_2$ folds. The latter species has previously been associated with improved lipid metabolism and reduced systemic inflammation in HFD-mice.[34]

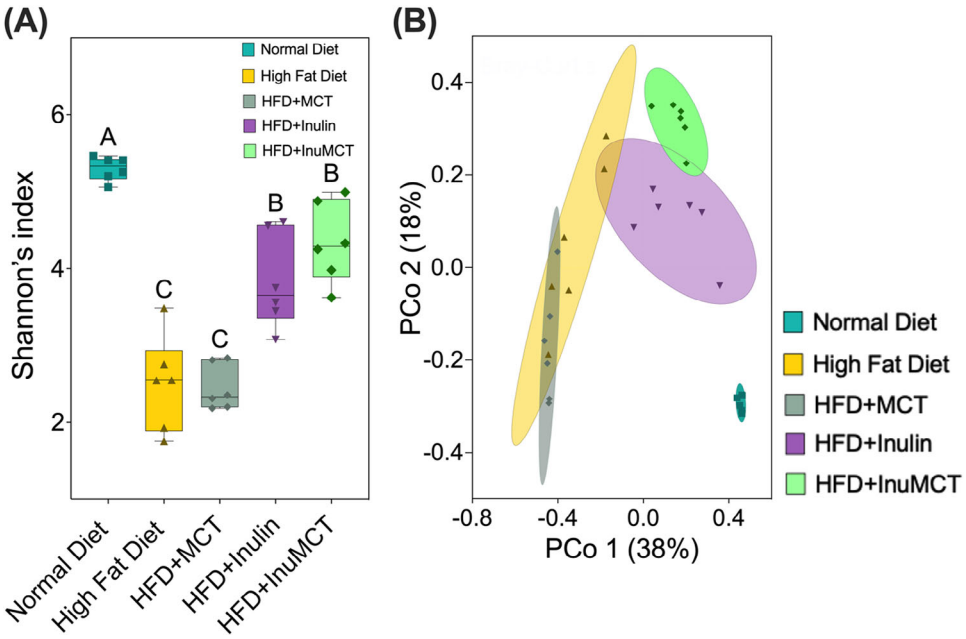

Figure 3. Representative graph for A) Alpha diversity (Shannon's index) depicting the variety of species within each sample from treatment groups, indicating richness and evenness. B) Beta diversity (Bray-Curtis) compares species differences across samples from different treatment groups. Inulin and InuMCT administration to HFD-rats significantly enhanced the diversity of microbial species, whilst promoting a microbial community that is unique from that of HFD group. Bray-Curtis diversity for each treatment is grouped using a 95% confidence ellipse. PCoA, principal coordinates. Statistical significance was derived using a one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons between all means. For the PCoA plot, a PERMANOVA test was used with Bonferroni-corrected *p*-values for multiple comparisons between all means. The means represented by a different letter indicate significant differences between treatments ($p < 0.05$). An *n* of 6 was used for each group.

The administration of inulin significantly enhanced the expansion of various *Bifidobacterium* species in rats, with increases ranging from 5.2 to 18 $\log_2$ folds (Figure 4C). These species include *Bifidobacterium longum, B. adolescentis, B. scaligerum, B. globosum, B. breve,* and *B. animalis*. This potent bifidogenic effect of inulin is well-documented in the literature, where it is recognized for promoting several commensals that contribute to improved gut health and protection against metabolic endotoxemia.[7a,35] Studies have demonstrated that increased *Bifidobacterium* populations can enhance gut barrier function, produce metabolism-regulating SCFAs, and reduce pathogen colonization, mitigating inflammation.[36] However, InuMCT not only further enhanced these bifidogenic effects but also promoted *Blautia* species between 13,14 $\log_2$ folds (Figure 4D). It is important to note that the 16S rRNA gene sequencing method used in this study may not have sufficient resolution for precise species-level identification.[37] This limitation may affect the accuracy of species-specific findings, and further validation with higher-resolution methods, such as whole-genome sequencing, would be beneficial to confirm these results.[38] Nevertheless, these findings indicate a potential additive effect of InuMCT, enhancing the overall prebiotic impact of the treatment.

2.5. Anti-Inflammatory SCFAs are Promoted by the Addition of Inulin to the High Fat Diet

After 21 days of dietary intervention, significant changes were observed in the fecal SCFA profiles of rats. Notably, the HFD did not significantly alter the SCFA concentrations, suggesting that a short-term HFD environment does not impact the GIT metabolome in rats (**Figure 5**). However, the addition of inulin significantly enhanced the production of acetic acid and propionic acid by 12 and 4.2 folds, respectively (Figure 5A & B). Similarly, the administration of InuMCT significantly increased acetic acid concentrations by 3.5-fold compared to the HFD group (Figure 5A).

These findings are consistent with previous research indicating that inulin, as a readily fermentable polysaccharide, significantly boosts the production of SCFAs.[7b] Inulin is primarily fermented by the gut microbiome into acetic acid and, to lesser extents, into butyric acid and propionic acid (or their conjugate bases).[39] The production of these SCFAs enhances the intestinal barrier by protecting tight junctions and promoting mucus production, which is crucial for protecting against pathogen invasion and maintaining gut integrity.[40] The increase in SCFAs, specifically acetic and propionic acids, reflects the fermentation capacity of the gut microbiome enhanced by inulin, which can lead to positive outcomes in metabolic health by improving glucose homeostasis and reducing inflammation.[41] InuMCT enhances acetic acid production, but not propionic or butyric acids, indicating that its MCT component may specifically promote acetic acid-producing species in the GIT, though the mechanism remains unclear.

The role of SCFAs in metabolic disease and inflammation is further supported by studies demonstrating their effects on weight loss, insulin sensitivity, and inflammation reduction.[8,42] For instance, propionate and butyrate protect against DIO and

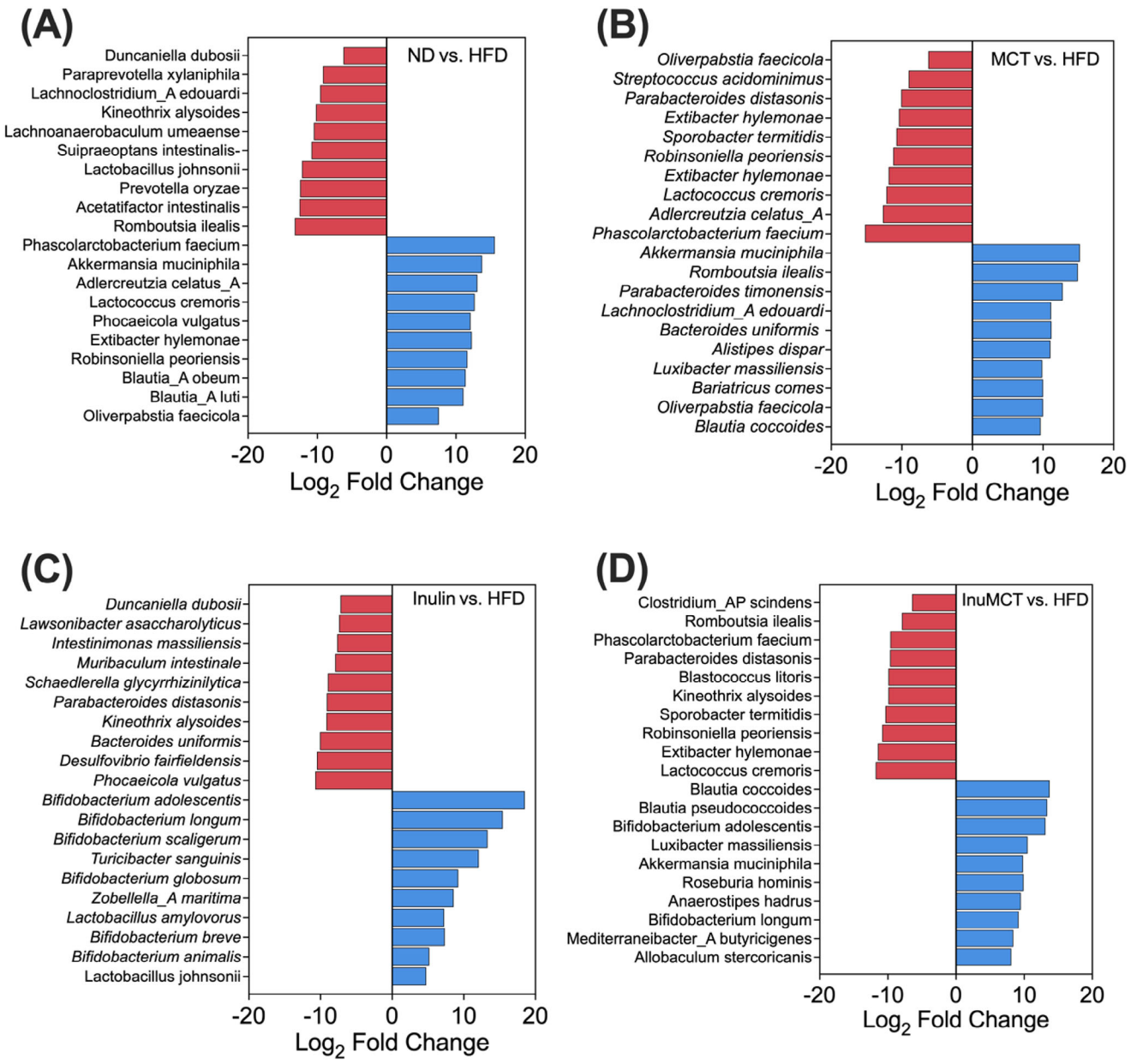

Figure 4. Microbial species significantly changed between A) Normal Diet, B) MCT, C) Inulin, D) InuMCT and HFD group. Whilst a HFD depletes species involved in the regulation of intestinal epithelial barrier protection against inflammatory agents and SCFA production, the combination of MCT and Inulin in InuMCT has an additive impact in promoting their abundance. A Likelihood Ratio test was used in the across-groups (ANOVA-like) comparison to determine statistical significance in differential abundance between the two treatments. An *n* of 6 was used for each group.

insulin resistance by regulating gut hormones and reducing food intake independently of their interactions with FFARs.[43] Sodium butyrate administration in DIO mice significantly reduced body weight, hepatic triglycerides, serum IL-6, and TNF-α levels while improving intestinal integrity and modulating gut microbiota composition.[44] SCFAs act as agonists of FFAR1-4 that are highly expressed in intestinal enteroendocrine cells, exerting improvements in insulin sensitivity, weight loss, and inflammation by modulating inflammatory pathways in patients with type-2 diabetes.[45] These results highlight the importance of such novel hybrid material therapies in targeting microbial disturbances that occur in HFD diets by modulating gut microbial SCFA production, suggesting a potential therapeutic strategies as oral drug carriers in obesity and other metabolic diseases.

2.6. InuMCT Protects Against diet-Induce Metabolic Dysfunction

The change in body weight was measured in rats across 21 days (**Figure** 6A), which demonstrated that DIO significantly increases the area under the curve (AUC) for rodent weight gain by 10% (Figure 6B). Inulin and InuMCT significantly reduced the AUC

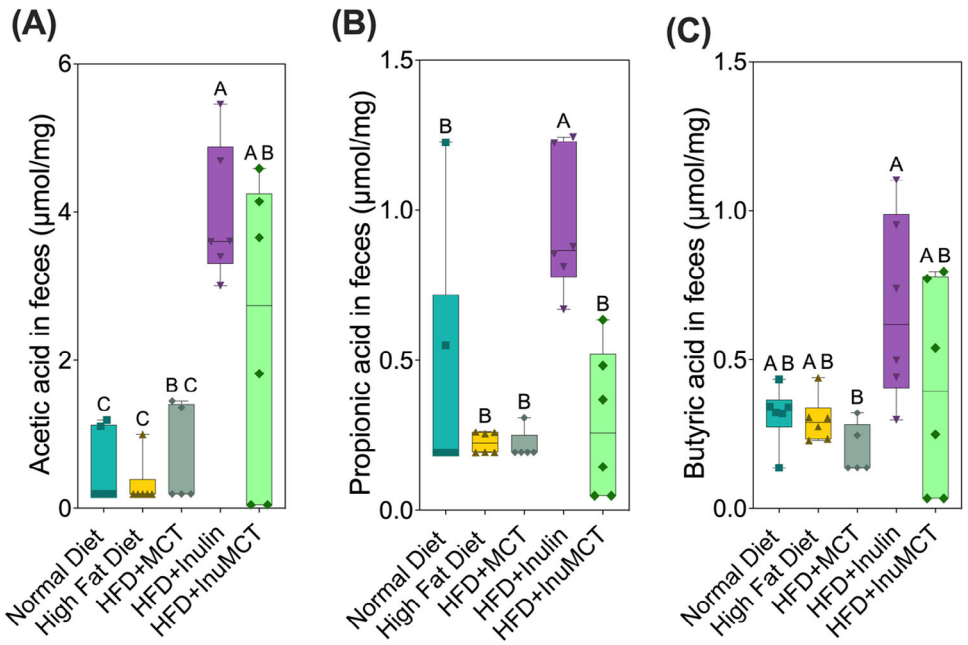

Figure 5. The metabolites of the microbiome, SCFAs are promoted by the addition of fermentable inulin in HFD-fed rats. Fecal A) acetic acid, B) propionic acid, and C) butyric acid concentrations at the end of the three-week treatment phase. Statistical significance was derived using a one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons between all means. The means represented by a different letter indicate significant differences between treatments ($p < 0.05$). An *n* of 6 was used for each group.

for HFD-induced weight gain, however, MCT administration did not result in bodyweight changes. This is in agreement with our previous study demonstrating that long-chain (average degree of polymerization of 27) inulin treatment (1 g kg^{-1} body weight/day) induced a significant reduction in HFD-rat weight gain across 21 days.[7b]

InuMCT treatments were found to protect against HFD-induced elevations in blood glucose levels to those comparable with rats on a normal diet (Figure 6C). This normalization of glucose levels is attributed to inulin's capacity to modulate the gut microbiome and enhance its metabolomic profile of SCFAs production that improving insulin sensitivity.[46] One such mechanism facilitated by SCFAs, particularly butyrate and propionate, is the enhanced production and secretion of GLP-1 from the intestinal L-cells. GLP-1 is a potent incretin hormone that enhances glucose-stimulated insulin secretion from the pancreas, thereby improving glucose homeostasis and insulin sensitivity.[47] Such beneficial effects of inulin on glucose metabolism are corroborated by findings in human studies that inulin-type fructans improve blood glucose levels.[48]

InuMCT treatment attenuated high-density lipoprotein (HDL) levels when compared to the HFD group. In contrast, inulin alone significantly reduced triglycerides by 69% and reduced HDL by 45% compared to the normal diet baseline (Figure 6D & E). These enhancements by inulin are likely due to their impact on lipid metabolism and fat absorption, which is particularly significant in the context of MCTs known for their rapid metabolism and energy utilization properties that reduce fat storage. SCFAs production is enhanced by the presence of inulin utilization in the GIT, stimulating the release of appetite-regulating hormones such as PYY and GLP-1.[49] Moreover, inulin intake has been linked to changes in lipid metabolism, which includes reductions in blood triglycerides and improvements in overall lipid profiles.[50] These effects are potentially mediated through the action of SCFAs on liver function, where they can inhibit fat synthesis and promote lipid oxidation, further contributing to reduced adiposity and prevention of obesity. Whilst the weight gain reduction induced by InuMCT treatment is modest, its application may better be suited as an adjuvant to conventional obesity treatment, to further enhance their therapeutic effect. Furthermore, our previous work has also demonstrated the effective use of InuMCT as a delivery system for drugs with poor aqueous solubility.[17,51] As such, InuMCT presents novel avenues toward its adjuvant use or as a drug carrier for drugs with pharmaceutical challenges.

2.7. HFD-Induced Bodyweight Gain is Correlated with Microbiome Diversity

A significant linear correlation was observed between rat bodyweight change at day 21 and alpha diversity, as measured by Shannon's index (**Figure** 7A). This strong correlation suggests that higher microbiome diversity is associated with less bodyweight gain in HFD-fed rats. This finding aligns with previous research indicating that gut microbiome diversity can be an effective biomarker of obesity in mice.[52] Higher alpha diversity is often linked with a more resilient and stable gut microbiome, which can maintain metabolic homeostasis.[53] The diverse microbiome can outcompete pathogenic bacteria (i.e., colonization resistance), reduce inflammation, and enhance energy extraction from food, thus preventing metabolic stresses.[54]

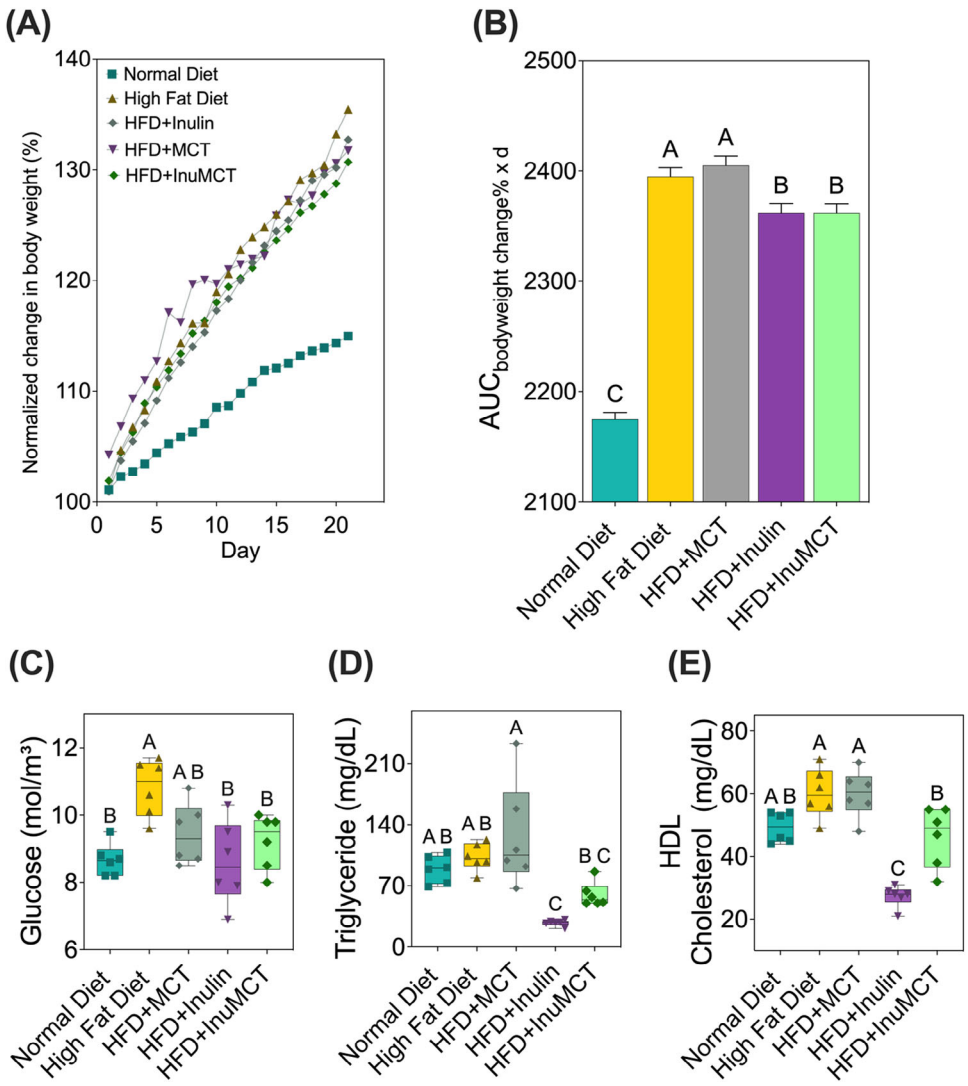

Figure 6. A) The normalized change in rat body weight was measured daily over the 21-day treatment period. B) The area under the curve (AUC) for the change in body weight revealed a high-fat diet (HFD) significantly induces weight gain, but treatment with MCT, Inulin, or InuMCT has no significant effect on rodent weight gain. Inulin administration to HFD-fed rats significantly attenuates metabolic markers of health after 3 weeks. C) Blood glucose, D) triglyceride, and E) HDL cholesterol levels were measured at the end of the treatment phase (at 1 g kg^{-1} bodyweight/d). InuMCT treatment significantly reduces glucose and HDL cholesterol but not triglyceride levels at the end of 3 weeks. Statistical significance was derived using a one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons between all means. The means represented by a different letter indicate significant differences between treatments ($p < 0.05$). An *n* of 6 was used for each group.

Within the microbiome community altered by HFD-induced bodyweight change, the *Lactobacillus* genera exhibited the strongest inverse correlation with bodyweight change ($R^2 = 0.76$) (Figure 7B). Specifically, three species from the *Lactobacillus* genera *Lactobacillus johnsonii*, *L. amylovorus*, and *L. ultunensis* – exhibited significant correlations with bodyweight change at day 21 (Figs. 7C,D, and E). The strength of this association, measured by the slope of the linear correlation, was in the order of *L. ultunensis* > *L. amylovorus* > *L. johnsonii* (−32, −76, −99). The inverse relationship between these *Lactobacillus* species and body weight gain is significant because *Lactobacillus* is known for its probiotic properties, contributing to improved gut health and metabolic outcomes. *L. johnsonii* has been shown to reduce fat mass accumulation and metabolism in pigs.[55] Likewise, *L. amylovorus* exhibits beneficial effects on weight loss in overweight adults when combined with a hypocaloric diet.[56] A study by Park et. al. found that *L. amylovorus KU4* promoted adipose browning, a strategy used to treat diet-associated weight gain, through Peroxisome proliferator-activated receptor delta (PPARβ) signaling, inhibiting DIO in mice.[57] The impacts of *L. ultunensis* have been less studied, but its strong correlation with reduced weight gain suggests it may have potent anti-obesity effects. These findings highlight the crucial role of specific gut microbial profiles in regulating body weight and metabolic health. By modulating the gut environment and promoting commensal species, interventions targeting the microbiome offer promising strategies for managing obesity and other metabolic diseases.

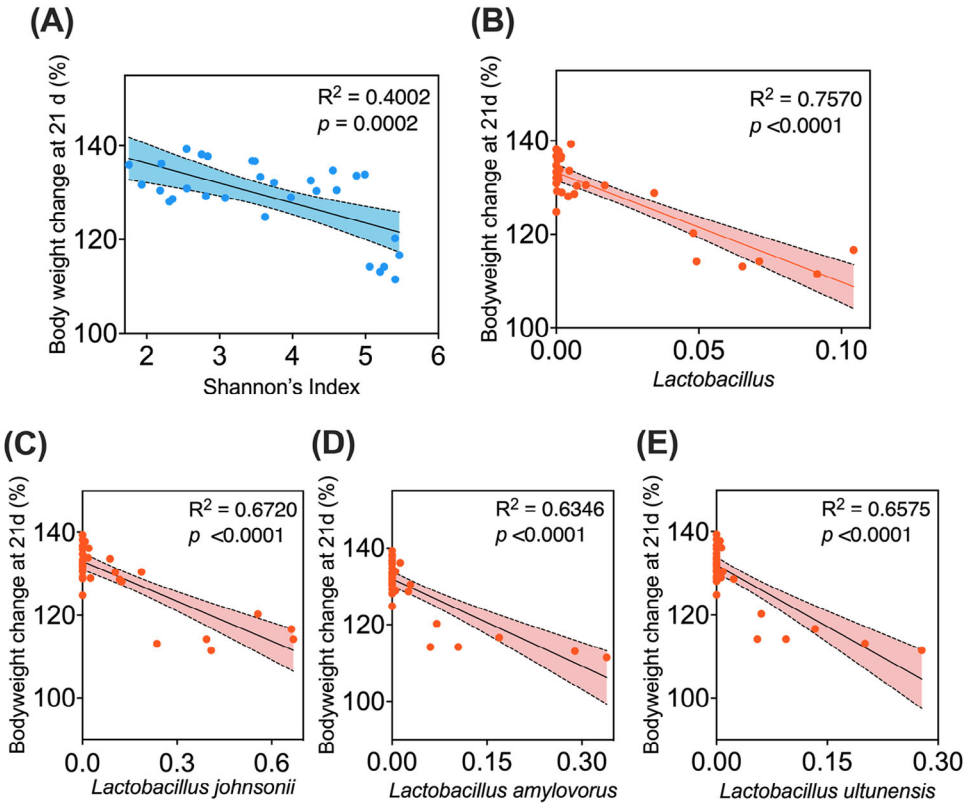

Figure 7. Significantly non-zero linear regressions were observed between bodyweight gain at 21 days for HFD-rats, and A) Shannon's index, B) *Lactobacillus*, C) *L. johnsonii*, D) *L. amylovorus*, and E) *L. ultunensis*.

2.8. HFD-Associated Proinflammatory and Liver Biomarkers Attenuated by InuMCT

In the investigation of inulin and MCTs on pro-inflammatory cytokine release, distinct impacts on IL-6 and TNF-α levels were observed in HFD-rats. After 21 days of treatment, inulin significantly reduced serum IL-6 concentrations by 61% compared to untreated HFD rats (**Figure** 8A). Furthermore, inulin lowered TNF-α concentrations by 35%. However, the additive effect of MCTs with inulin in the InuMCT normalized TNF-α levels back to those of the normal diet rat group (Figure 8B). Inulin's ability to reduce IL-6 may be attributed to its impact on the gut microbiome and the resultant increase in the production of SCFAs, which possess anti-inflammatory properties. SCFAs, particularly butyrate, have been shown to inhibit the production of pro-inflammatory cytokines like IL-6 by down-regulating nuclear factor kappa B (NF-κB) pathways, which are critical in the inflammatory response.[58] Additionally, inulin enhances the production of regulatory anti-inflammatory cytokines such as IL-10, which further helps in mitigating inflammatory responses in the body.[59] Similarly, MCTs have demonstrated a capacity to influence immune responses, potentially through mechanisms involving the modulation of lipid metabolism and direct effects on inflammatory cell function.[60] MCTs can reduce the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and the expression of pro-inflammatory cytokines in various cells, including macrophages.[61] This mechanism is thought to contribute to their effect in normalizing TNF-α levels when used in conjunction with inulin. The synergistic effect observed with InuMCT in normalizing TNF-α levels to those of the normal diet rat group baseline suggests an interplay between inulin's microbiome-modulated anti-inflammatory properties and MCT's metabolic and direct immunomodulatory actions. More importantly, it supports the initial hypothesis that the inulin coating in spray-dried InuMCT may enhance the anti-inflammatory properties of MCT by enhancing its GIT residency time.

InuMCT administration to HFD-fed rats for 21 days significantly lowered levels of liver lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) by 47%, 24%, and 22%, respectively (Figure 8C,D, and E). These findings suggest a protective effect of InuMCT on liver health against the disruptions caused by an HFD. LDH, AST, and ALT are key markers of liver function, often elevated in states of hepatic injury, inflammation, and metabolic disorders such as obesity and non-alcoholic fatty liver disease (NAFLD).[62]

Elevated LDH, released during cell lysis, along with AST and ALT, enzymes involved in amino acid metabolism, signal hepatocellular damage.[63] These elevations are common in obese individuals due to liver lipid accumulation and inflammation.[64] InuMCT's ability to lower these liver enzymes suggests a reduction in liver damage and inflammation. MCTs in InuMCT are rapidly metabolized for energy and have proven effective at significantly reducing fat accumulation in the liver of obese rats.[65] Other animal models with induced liver injury have demonstrated the hepatoprotective effects of inulin, in significantly reducing ALT, AST, ALP levels between 49% and 67%.[66] The

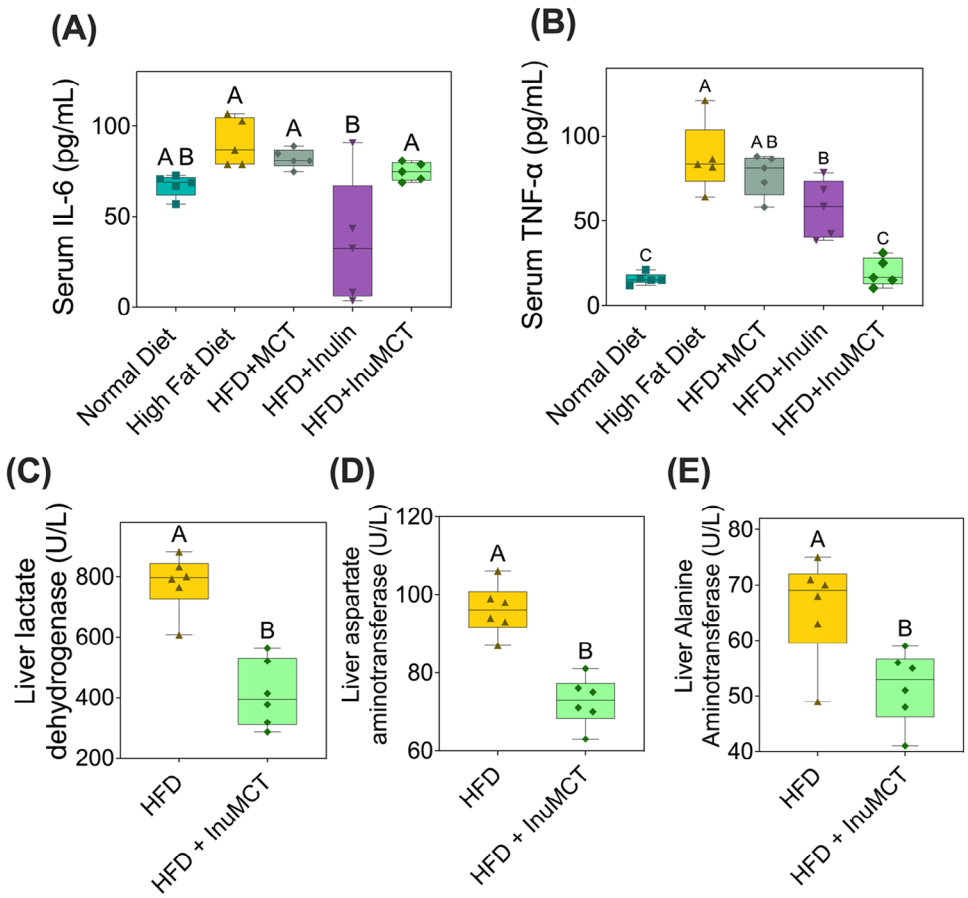

Figure 8. Proinflammatory cytokines and liver enzyme levels associated with metabolic diseases were measured in rats. A) IL-6 levels were fully recovered by Inulin to those of normal diet rats. In contrast, B) TNF-α levels were significantly reduced in HFD-rats by Inulin, while InuMCT recovered TNF-α levels to those of normal diet-fed rats. C) Lactate dehydrogenase (LDH), D) aspartate aminotransferase (AST), and E) alanine aminotransferase (ALT) levels were all significantly reduced by the administration of InuMCT to HFD-fed rats over a 21-day period. Statistical significance for all data was derived using a one-way ANOVA test followed by Tukey's post hoc test for multiple comparisons between all means. Statistical significance between the two treatments was derived using paired t-tests. The means represented by a different letter indicate significant differences between treatments ($p < 0.05$). An *n* of 6 was used for each group.

combined effects of inulin and MCTs in the spray-dried InuMCT treatment likely contribute to the observed decrease in liver LDH, AST, and ALT levels. Notably, while our previous research has demonstrated the potential of InuMCT as a multifunctional drug delivery vehicle, this study is the first to reveal its efficacy as a standalone therapy for treating metabolic disorders related to microbiome dysbiosis, highlighting its therapeutic potential beyond drug delivery applications.

3. Conclusion

Inulin and InuMCT microcapsules were explored in HFD-induced rat obesity model, for their potential to address metabolic and inflammatory markers of disease. InuMCT provided additive benefits by promoting the growth of commensal communities associated with both MCT and inulin treatments individually, such as *Bifidobacterium* and *Blautia*. Both Inulin and InuMCT effectively attenuated diet-induced weight gain and improved blood HDL and glucose profiles. Whilst Inulin significantly reduced serum TNF-α levels in rats on a high-fat diet, InuMCT administration further lowered TNF-α concentration to levels comparable to those in rats on a normal diet. As such, the combined inflammatory and microbial benefits of InuMCT highlighted its therapeutic potential over administering MCT or inulin alone for obesity and other diet-induced metabolic diseases. Future work should explore the potential of InuMCT as a standalone or adjuvant therapy in a more clinically relevant model of disease. Investigating the clinical multifunctional benefits of InuMCT could further accelerate its role in managing obesity and other related metabolic diseases, offering a novel and effective therapeutic approach.

4. Experimental Section

Materials: Inulin extracted from chicory root (average polymerization degree of 14), and phosphate-buffered saline (PBS) were sourced from Merck (Bayswater, Australia). Miglyol 812, a blend of mono-diglyceride of medium chain fatty acids (mainly caprylic and capric), was sourced from Hamilton Laboratories (Adelaide, Australia). All utilized chemicals and solvents were of analytical grade and were used without further

purification. Throughout the study, high-purity Milli-Q water was consistently used.

Fabrication of Inumct Microcapsules via Spray Drying: InuMCT microcapsules were prepared using a spray drying technique, following previously established methods.[17,67] Initially, Capmul MCM (5 g) and lecithin (6% w/w) were mixed using sonication for 30 min. After thorough mixing, water (100 mL) was added to create a 5% *w/v* lipid-in-water emulsion. This emulsion was then subjected to sonication for an additional hour to achieve size reduction and form a stable nanoemulsion.

Next, an aqueous solution of inulin (2% *w/v*) was prepared and combined with the MCT lipid-in-water nanoemulsion in a 1:1 weight ratio. The resulting nanoemulsion was then spray-dried using a Mini Spray Dryer B-290 (Büchi, Flawil, Switzerland). The spray drying parameters were set as follows: emulsion flow rate at 0.5 mL mi^{-1}n, air flow rate at 0.6 mL mi^{-1}n, inlet temperature at 200 °C, outlet temperature at 110 °C, and an aspirator setting at 100%.

Scanning Electron Microscopy (SEM) Imaging: Scanning electron microscopy (SEM) images of the fabricated InuMCT and precursor inulin were obtained using a Zeiss Crossbeam 540 system (Oberkochen, Germany) to elucidate the size and morphological impacts of spray drying. To prepare the samples for SEM analysis, small amounts of InuMCT and inulin were placed on carbon tape fixed to SEM stubs. The samples were then sputter-coated with a thin layer of platinum, ≈10 nm in thickness before imaging at an accelerating voltage of 8 kV. SEM micrographs were analyzed using the AztecOne software (Oxford Instruments, Abingdon, United Kingdom).

Confocal Fluorescence Imaging Analysis: To understand the matrix formed from the spray drying of Inulin with MCT, confocal fluorescence imaging was performed using a Zeiss LSM800 microscope (Oberkochen, Germany). Before the spray drying process, 0.1 wt.% of rhodamine-labeled triglyceride, specifically 1,2-dioleoyl-3-[16-N-(lissamine rhodamine B sulfonyl) amino]palmitoyl-sn-glycerol, was added to the lipid phase, and 0.1 wt.% FITC-inulin was incorporated into the inulin phase to label the components in the spray-dried InuMCT matrix. During confocal microscopy, the rhodamine label was detected using an excitation wavelength of 556 nm and an emission wavelength of 580 nm, while the FITC label was detected with an excitation wavelength of 495 nm and an emission wavelength of 519 nm. The resulting confocal images displayed the rhodamine-labeled components in red and the FITC-labeled components in green, allowing for clear differentiation and detailed visualization of the labeled structures.

In Vivo Study Design: The in vivo study received approval from the Animal Ethics Committee at the University of South Australia (approval #24-21), adhering to the NIH's Principles of Laboratory Animal Care (NIH publication #85-23), revised in 1985) and the Australian code for the care and use of animals for scientific purposes (8th edition 2013, revised 2021), and was conducted and reported in accordance with ARRIVE guidelines. 6–8-week-old Sprague Dawley rats were procured from the Animal Resource Centre (Perth, Australia). This study utilized a rodent model on a high-fat diet (HFD), based on established obesity models.[7b,68] A total of 24 male Sprague–Dawley rats were used in this study. Rats were randomly assigned to one of four groups ($n = 6$ per group) using a computer-generated random number sequence to minimize selection bias: 1. a normal diet group receiving 5% of kcal from fat; 2. an HFD group receiving 44% of kcal from fat; 3. an HFD group supplemented with inulin (1 g kg^{-1} bodyweight/ d), and 4. a HFD group supplemented with InuMCT microcapsules (1 g kg^{-1} body weight/ d). Groups of three rats were housed in separate cages for an acclimation period of one week under standard laboratory conditions (12 h light/dark cycle, controlled temperature 22 ± 2 °C, and humidity 55 ± 10%). At the conclusion of the treatment period (21 days), the rats underwent a 24 h fasting period before being anesthetized with isoflurane. A cardiac puncture was performed to collect ≈3 mL of blood, which was then analyzed for metabolic and inflammatory biomarkers using enzyme-linked immunosorbent assay (ELISA, (ThermoFisher Scientific, Australia)), followed by cervical dislocation. Only healthy rats were included in the study, and those showing signs of illness or abnormal behavior during the acclimation period were excluded. Blinding was implemented during the allocation of animals to treatment groups and throughout the data collection process. The researchers administering diets and treatments, as well as those assessing outcomes, were blinded to the group allocations to prevent bias.

Post-in Vivo Study 16s rRNA Analysis of Fecal Samples: Following humane euthanasia, fecal samples were collected, and underwent DNA extraction and 16S rRNA gene sequencing (Australian Genomics Research Facility, Brisbane, Australia). The sequencing focused on the hypervariable V3–V4 regions. The sequences were clustered at a 97% similarity threshold into operational taxonomic units (OTUs) using QIIME 2 and the Silva reference database.[29,30] Taxonomic assignments for the OTUs were made using QIAGEN CLC Genomics Workbench Version 23.0.4 and the QMI-PTDB.[31] Diversity within the microbial communities was analyzed using Shannon's Index for alpha diversity Bray–Curtis dissimilarity and Jaccard indices for beta diversity through Principal Coordinate Analyses (PCoAs). Statistical differences in beta diversity were assessed using Permutational Multivariate ANOVA (PERMANOVA) and PCoA plots were grouped with a 95% confidence ellipse to illustrate the variation and clustering among treatment groups.[34]

Fecal Short-Chain Fatty Acid Quantification: SCFA concentrations, including acetic, butyric, and propionic acids, were determined using a Shimadzu gas chromatography–mass spectrometry system (GCMS-QP2010 SE, Kyoto, Japan). SCFA extraction and GCMS analysis were conducted using a Zebron (Phenomenex, Washington, United States) ZB-FFAP column (30 m x 0.25 mm x 0.25 µm) and according to the parameters specified in a previously established protocol.[69]

Serum Proinflammatory Cytokine Quantification: Following the centrifugation of whole blood at 2000 g for 20 min at 4 °C, the serum supernatant was collected, and then stored at −80 °C until analysis. The levels of interleukin-6 (IL-6) and TNF-α in the rat serum were quantified using ELISA kits, following the manufacturer's detailed protocols. Data were analyzed using standard curves generated from known concentrations of IL-6 and TNF-α standards. Due to the presence of outliers, the sample sizes for these analyses ranged from four to six for each ELISA kit, ensuring accurate and reliable results. All assays were performed following the standard operating procedures to maintain consistency and repeatability.

Statistical Analysis: Experimental data, excluding 16S rRNA gene sequencing, were analyzed using GraphPad Prism Version 8.0 (Boston, MA, USA). Statistical significance was assessed using one-way ANOVA with Tukey's post hoc test for multiple comparisons, paired t-tests, and linear regressions. Results were reported as mean ± standard deviation (SD) and means represented by different letters used in alphabetical order (i.e., A, B, C, and D) to indicate significant differences between treatments ($p < 0.05$).

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Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

gut health, inflammation, inulin, mct, metabolic disease, obesity

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