Low serum triglyceride response to a high-fat meal is associated with elevated postprandial IL-17 and discriminative gut bacterial features.

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Low serum triglyceride response to a high-fat meal is associated with elevated postprandial IL-17 and discriminative gut bacterial features.

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Abstract

Background

Postprandial lipemia stimulates proinflammatory mediators and is a risk factor for cardiovascular disease. Chronic disease and diet are known to influence the gut microbial community in ways that alter the availability of bioactive compounds capable of influencing the host. The purpose of this study was to identify gut microbiome taxa and inflammatory cytokines differentiating individuals with lower and higher postprandial triglyceridemia.

Methods

A high-fat meal (43.1% fat) was given to 40 healthy, overweight and obese adults to assess the serum triglyceride response in the immediate four-hour postprandial period. Participants were categorized into two groups (high and low) based on serum triglyceride responses. We measured blood lipids, inflammatory cytokines, fat mass, visceral adiposity and used 16S rRNA target amplicon sequencing to identify microbial taxa in human fecal samples distinguishing the two groups. The gut microbiome was assessed using unconstrained ordination, followed by a high-dimensional class comparison to determine discriminative microbial features of the postprandial triglyceride response (ppTG).

Results

High ppTG responders had higher body mass index, visceral adiposity, and fasting serum cholesterol levels than low responders and had a decreased postprandial IL-17 response to the high-fat meal. The overall gut microbiome did not cluster by ppTG response but were found to have four discriminative bacterial features between high and low ppTG. Lower relative abundance of *Clostridium Cluster XIVa* and higher relative abundance of *Pasteurellaceae, Alistipes*, and *Prevotella* was observed in low ppTG relative to high ppTG.

Conclusions

Our findings suggest that specific gut microbial taxa involved in short-chain fatty acid production can discriminate the postprandial triglyceride response in overweight and obese adults. These findings may have implications in how we develop microbial therapies and choose to monitor and treat individuals with hypertriglyceridemia or who may have an increased risk of chronic disease.

Trial Registration:

ClinicalTrials.gov, NCT04128839, Registered October 16, 2019 – Retrospectively registered, https://clinicaltrials.gov/ct2/show/NCT04128839?term=NCT04128839&draw=2&rank=1

Introduction

Adults in the Western world spend most of their waking hours in a postprandial, or post-meal, state relative to a fasting state. Yet, epidemiological studies have routinely used clinical markers from fasted individuals in generation of hazard ratios to predict chronic disease. Growing evidence suggests the magnitude of the postprandial response is more predictive of disease risk than fasting marker concentrations (1-4), with postprandial triglyceride concentrations in particular shown to predict cardiovascular disease (CVD), myocardial infarction, ischemic heart disease, and death (5, 6).

Regular consumption of high fat meals throughout the day can elevate blood lipids for several hours after ingestion. Fasting and postprandial blood lipid concentrations are affected by the macronutrient content of meals and time since last meal. The degree of the postprandial triglyceride response (ppTG) is influenced by dietary and lifestyle factors in addition to genetic polymorphisms and conditions like type 2 diabetes (7). Among dietary factors, the amount of total fat in a meal can have a greater effect on the postprandial response than the type of fat or whether other macronutrients besides fat were ingested (8). Ingested fat is processed through the gastrointestinal (GI) system and packaged into large lipoproteins called chylomicrons, which are delivered to the systemic circulation. Increased free fatty acids from high fat meals can serve as a low intensity stimulus for a range of proinflammatory mediators in the postprandial period (9), which if occurring repeatedly, may contribute to low-grade inflammation seen with many chronic diseases (10).

Diet influences the supply of nutrients to trillions of intestinal microorganisms that comprise the gut microbiome. These gut microbes metabolize numerous dietary compounds including fiber and L-carnitine to produce bioactive compounds capable of influencing the host phenotype (11). Certain bacteria-derived compounds, such as lipopolysaccharide (LPS) and trimethylamine-*N*-oxide (TMAO), have been shown to initiate host inflammation (12, 13).

Products derived from the bacterial fermentation of dietary fiber, such as the short-chain fatty acid (SCFA) butyrate, have anti-inflammatory effects and the ability to modulate expression of genes responsible for barrier function between intestinal epithelial cells (14-16). Changes to the gut microbiome have also been found to affect host inflammation through alterations in tight-junction proteins (17). A compromised GI barrier through tight-junction protein dysfunction allows for increased passive paracellular diffusion of bacterial constituents (i.e., LPS) that promotes inflammation in peripheral tissues, a process that may be exacerbated by increased dietary lipids (12).

The production of SCFAs and gut-derived inflammatory metabolites is dependent on gut microbial composition (12, 18), which is influenced by diet (19, 20). Weight gain from the consumption of a high-fat diet has been shown to be dependent on gut microbiome composition (21) and over the long-term can, alter the composition of the gut microbiome (20, 22-24). Obesity and related health conditions have been linked to phylogenetic and functional alterations in the gut microbial community which include reduced diversity of gut microbiota (25), increased Firmicutes to Bacteroidetes ratio (26, 27), an increased ability for energy harvest (26), reduced butyrate-producers (28), and reduced production of beneficial SCFAs (29, 30). Dietary fat intake, fasting triglycerides (TG), and fasting high-density lipoprotein (HDL) have been correlated with the abundance of several microbial taxa and healthy lipid levels in general are associated with increased microbial diversity (31). The gut microbiome was recently hypothesized to contribute to the sex differences seen in lipid metabolism (32). A growing number of studies have examined glucose metabolism, glycemic response and fasting inflammatory markers and their relation to the gut microbiome (33, 34), but to the best of our knowledge, no study has assessed gut microbial relationships with ppTG. The purpose of this study was to examine fecal microbial composition in relation to ppTG to identify taxa that may contribute to a high ppTG from a high-fat meal challenge in a healthy, overweight and obese human cohort.

Methods

Ethics Statement. The protocol was approved by the Institutional Review Board at Montana State University. Written informed consent was obtained from all participants prior to their participation. This study was retrospectively registered October 2019 at ClinicalTrials.gov (NCT04128839).

Study Population. Potential participants were excluded if they had taken oral antibiotics within 90 days of study enrollment, regular use of anti-inflammatory medications, use of estrogen-only contraceptives, wheat and/or dairy allergies, were pregnant, or had any musculoskeletal, cardiovascular, gastrointestinal, or immunological condition that could interfere with the study (**Supplementary Figure 1**). Forty overweight and obese men and women participated in testing of anthropometric and metabolic markers and ingestion of a 50 g high-fat meal challenge. Participants were 18-55 years old within a BMI 27 - 36 kg/m^2.

Research Design. The study followed a comparative research design. Participants were asked to attend two visits, approximately seven hours in total length. The first visit involved a verbal review of the informed consent, completion of three one-page self-report questionnaires, and analysis of body composition and cardiorespiratory fitness. The second visit occurred within two weeks after the first visit. Participants brought a self-collected stool sample to the visit and then proceeded to blood collection before and after a high fat meal challenge. Postprandial triglyceride responses and body composition measures were used as independent variables in the analysis of microbial composition, obtained through 16S rRNA analysis of stool samples.

Anthropometrics. Measurements were collected from participants using the validated segmental multifrequency bioelectrical impedance analysis (SECA mBCA 515, Hamburg, Germany) (35). Fat mass (%) and estimated visceral adipose (L) were used for analysis.

Cardiorespiratory Fitness. Participants were asked to complete a modified Bruce protocol on a treadmill for determination of calculated absolute VO2 max at their age-predicted heart rate max. The speed and grade of the treadmill (Woodway GmbH D-79576, Weil am Rhein,

Denmark) were manually changed by the researcher with each progressive three-minute stage until the participant reached 85% of their age-predicted maximal heart rate. Expired gases were collected for analysis through a metabolic cart system (ParvoMedics, TrueMax 2400 Metabolic System, Sandy, Utah, USA). Heart rate (bpm) and VO_2 (ml/kg/min) data from each participant were input into a simple linear regression model to predict the absolute VO_2 at the age-predicted maximal heart rate based on the equation presented by Tanaka, Monahan, and Seals (36).

High-Fat Meal Challenge. Total energy content of the high-fat meal challenge was 714 kcal, with 43.1% from fat, with a macronutrient breakdown of 50 g fat, 54 g carbohydrate, and 12 g protein. Water was provided with the meal; caffeinated black tea was provided instead for participants who identified as habitual coffee consumers.

Blood Sampling. Participants were instructed to avoid alcohol consumption and strenuous physical activity in the 24 hours before their second visit and to complete an overnight fast (10 - 12 hours) before blood collection. Participant blood samples were collected by a certified nurse or physician in the morning before ingestion of the meal and hourly for 4 hours after meal ingestion, totaling five time points. Whole blood in serum separating tubes was allowed to clot for 15 minutes before centrifugation at 1200 RPM for 15 minutes with resulting serum aliquoted and stored at -80ºC until analysis.

Determination of blood markers. Blood markers of metabolic syndrome were determined from whole blood run on Picollo Xpress Chemistry Analyzer lipid panels (Abaxis, Union City, USA). Glycated hemoglobin (HbA1c) was determined using the DCA Vantage Analyzer (Siemens Medical Solutions Diagnostics, Cergy-Pontoise, France) performed according to manufacturer instructions. Cytokine measurement was performed using high-sensitivity multiplexing technology (Bio-Rad Bio-Plex® 200 HTS) following procedures by Millipore (EMD Millipore Corporation, Billerica, USA). Classic systemic pro-inflammatory cytokines were measured and include granulocyte macrophage colony stimulating factor (GM-CSF), interleukin (IL)-1β, IL-6, tumor necrosis factor (TNF)-α. Interleukin-17 and IL-23, both of

which serve a pro-inflammatory and regulatory role in the gut mucosa, were also measured. Serum samples at each time point during the high-fat meal challenge were run in duplicate.

Determination of fat responders. Visualization of the fat response for all participants revealed some participants had large increases, and others, a small increase in serum TG (**Supplementary Figure 2**). The absolute change in serum TG from fasting during the high-fat meal challenge was used to group participants into low and high fat responders to the meal. The 18 participants with the lowest absolute change were categorized as having a low ppTG while the 18 participants with the highest change in TG were considered high ppTG.

Stool Sample Collection. Collection kits were provided, and participants were asked to follow included instructions for the self-collection of a stool sample in the 24 hours before their blood collection visit. After initial collection into a sterile disposable commode, a small portion of the sample was transferred into a sterile Eppendorf tube and transported to researchers. Samples were prepared and aliquoted in an anaerobic chamber then frozen at -80ºC until analysis.

Genomic DNA Extraction and Microbial Analysis. Extraction of bulk DNA from fecal samples was performed using Powersoil® DNA Isolation Kit (Mo Bio Laboratories, Inc.) and bead beating. DNA was shipped overnight to the University of Michigan, Michigan Microbiome Project for Illumina MiSeq amplicon sequencing of the 16S rRNA V4 region. After DNA quantification, V4 amplicon libraries were generated with dual-index barcoded primers, then by library purification, pooling, and MiSeq paired-end sequencing. Raw sequencing reads were processed and curated using MOTHUR software (Version 1.35.1) following the MOTHUR standard operating procedure for the MiSeq platform (37). In brief review, paired-end reads were assembled into contiguous sequences and screened for length and quality. The remaining contigs were aligned to the SILVA ribosomal RNA database (Release 132), a comprehensive collection of aligned rRNA sequences. Potentially chimeric sequences were identified and removed using the UCHIME algorithm in MOTHUR. Taxonomic classifications were assigned

using the Bayesian classifier of the Ribosomal Database Project. Non-target reads were removed, and operational taxonomic units (OTU) were assigned using VSEARCH distance-based clustering at the 97% similarity threshold. Alpha-, and β- diversity indices were generated using the *vegan* package in R (38). An OTU-based data matrix was constructed for participants included in the ppTG phenotype.

Statistical Analysis. Analysis was conducted in RStudio (1.2.1335) running 3.6.1 base R (39) and figures were produced using the R package ggplot2 (40). Linear discriminant analysis (LDA) effect size (LEfSe) was performed on the Galaxy platform (41).

To confirm the ppTG phenotyping clearly grouped participants, we utilized the *nlme* package in R to create a linear mixed-effects model examining serum TG as the response by ppTG during the high-fat meal challenge, accounting for subject-to-subject variation in TG at baseline (42). Time was coded as quantitative and subject coded as a factor. A total of 180 observations were included in the analysis. R-squared estimates were generated using the r.squaredGLMM function in the MuMin package (43).

Descriptive statistics of the participants' physical characteristics and metabolic profile by ppTG were analyzed by two sample t-tests. The proportion of men by ppTG was analyzed through a two-sample z-test. Detected group differences were accounted for in subsequent univariate analyses. Inflammatory responses to the high-fat meal challenge by ppTG were additionally analyzed with a non-linear mixed effects model.

Power analysis of alpha-diversity was performed using power.t.test in the *pwr* package (44). Power to detect differences in alpha-diversity with eighteen participants per group was over 99% when $\alpha = 0.05$ and 0.05, delta = 0.24 and 0.029, and estimated sd = 0.064 and 0.0080 respectively for Shannon and Simpson indices. Comparisons of Shannon and Simpson alpha-diversity indices by ppTG were assessed using the Mann-Whitney test. To confirm that alpha-diversity differences were not a result of confounding variables, each index was further assessed through a linear model with the postprandial phenotype as a fixed effect additionally

accounting for confounding variables. Each linear model was screened for shared information among predictor variables using variance inflation factors (VIF) from the *car* package (45), with predictors with VIF > 5 removed from the model.

A non-metric multidimensional scaling (nMDS) was conducted from a Bray-Curtis dissimilarity matrix of gut microbiome data for the ppTG cohort (n=36). Analysis of similarities (ANOSIM) was used to test for microbiome community differences between low and high ppTG and permutational multivariate analysis of variance (PERMANOVA) was used to assess the extent of which ppTG contributed to the variation in the gut microbiome community. Bray-Curtis dissimilarity matrices, nMDS, ANOSIM, and PERMANOVA were determined using the vegan package in R (38), with 10,000 permutations used for analyses.

Relative abundance table derived from total sum scaling normalized count data was uploaded to the Galaxy workflow platform for LEfSe analysis to determine microbial features that discriminate between low and high ppTG (http://huttenhower.sph.harvard.edu/lefse/) (46). Microbial taxa with an LDA score threshold > 2 were determined to be discriminative features, and alpha values were set to 0.05.

Results

Forty participants completed a high-fat meal challenge consisting of three pieces of whole wheat toast (127.5 g; Wheat Montana) and salted butter (58.3 g, Tillamook). Fasting and postprandial serum TG concentrations were measured, with the postprandial blood collection draws occurring 1, 2, 3, and 4 hours after meal ingestion. Four participants whose absolute change (postprandial peak – fasting) in serum TG were not among the 18 highest (+0.81 to +4.13 mmol/L) and lowest (-0.10 to + 0.67 mmol/L) values were designated neither high nor low TG response and not included in subsequent analyses. Participants with the 18 highest and lowest TG responding individuals were found to form two distinct groups in our mixed effects model ($p < 0.0001$), confirming the effectiveness of our ppTG phenotype in separating the ppTG

response (**Figure 1**). Accounting for baseline TG concentrations of each subject increased the variance explained in the mixed effects model from approximately 0.26 to 0.93%. High ppTG participants were found to differ from low ppTG responders in several key physical and metabolic characteristics, including having higher BMIs, visceral adiposity, and fasting serum cholesterol levels (**Table 1**).

Table 1. *Participant characteristics grouped by low and high ppTG.*

	Low (n=18)	**High** (n=18)	***p*-value**
Men/Women	5/13	10/8	0.25
Age (years)	35.4 ± 10.1	38.3 ± 10.8	0.40
BMI (kg/m^2)	30.0 ± 1.8	31.3 ± 1.9	0.04
FM (%)	35.9 ± 6.2	36.7 ± 7.5	0.75
VAT (l)	1.8 ± 0.9	2.9 ± 1.4	0.01
VO_2 (ml/kg/min)	45.3 ± 10.1	40.2 ± 8.5	0.12
HbA1c (%)	5.2 ± 0.3	5.3 ± 0.3	0.50
Fasting GLU (mmol/L)	5.46 ± 0.36	5.40 ± 0.38	0.65
Fasting TG (mmol/L)	1.54 ± 1.12	1.95 ± 1.07	0.27
Fasting CHOL (mmol/L)	4.38 ± 0.83	5.07 ± 0.89	0.02
Fasting HDL (mmol/L)	1.41 ± 0.48	1.29 ± 0.37	0.44

Four participants did not classify into low or high ppTG and were not used for downstream analysis. Data represent mean and standard deviation. P-value for proportion of men vs women was determined through a two-sample z-test. All other characteristic p-values were determined by a two-sample t-test. Abbreviations: low, LOW; high, HIGH; body mass index, BMI; fat mass, FM; visceral adipose tissue, VAT; maximal oxygen consumption, VO_2; hemoglobin A1C, HbA1c; glucose, GLU; triglycerides, TG; cholesterol, CHOL; high-density lipoprotein, HDL.

Inflammation Response to High-fat Meal Challenge: To examine how six pro-inflammatory cytokines in the blood changed in response to the influx of triglycerides from the high-fat meal challenge, fasting and postprandial serum was analyzed through multiplex technology. Mean serum concentrations of IL-17 during the postprandial period varied between high and low ppTG groups ($p = 0.027$), with low ppTG showing a distinct increase in IL-17 one to two hours after the high-fat meal whereas high ppTG show a more gradual increase over the entire 4-hour challenge (**Figure 2**). In our IL-17 mixed effects model, visceral adiposity ($p = 0.065$) and fasting cholesterol ($p = 0.010$) were associated with serum IL-17 concentrations, with higher serum IL-17 concentrations observed with increased visceral adiposity and lower IL-

17 observed with higher cholesterol. A trend was detected in post-prandial serum IL-6 concentrations ($p = 0.068$), with the high ppTG group having a higher mean IL-6 at each time point compared to low ppTG. We did not detect ppTG group differences in IL-1β, IL-23, TNF-α, or GM-CSF.

Gut Microbiome Diversity and Taxa Differences between low and high ppTG: Participant fecal samples were analyzed for gut microbiome composition through Illumina Miseq Amplicon Sequencing. Participants grouped into low or high ppTG were further analyzed for differences in the diversity of their gut microbiome. A total of 1 580 068 raw reads were obtained across all samples that after quality processing, resulted in a total of 387 313 high quality reads, with an average depth of 9 682 reads per sample (range 8 183 – 9 962) that were used for subsequent analyses. This resulted in a γ-diversity of 220 OTUs. A trend was detected in Shannon ($p = 0.065$) and Simpson ($p = 0.073$) α-diversity indices between ppTG groups, with lower α-diversity seen in high ppTG compared to low ppTG individuals (**Figure 3**). However, the trend in α -diversity was not robust after BMI, visceral adiposity, and fasting serum cholesterol were accounted for in the statistical model (Shannon, $p = 0.77$; Simpson, $p = 0.92$). The β-diversity of the gut microbiome of our overweight and obese cohort did not separate between low or high ppTG (ANOSIM $R = -0.02$, $p = 0.64$) (**Figure 4**). The ppTG phenotype was not observed to contribute to the conditional variation of the gut microbial community ($p = 0.62$).

In addition to examining diversity measures, we analyzed whether specific bacterial taxa discriminate between high and low ppTG. Based on our LEfSe analysis, we detected seven differentially abundant taxonomic clades unique to ppTG. Key drivers of ppTG phenotype differences include *Clostridium* cluster XIVa ($p < 0.01$), *Alistipes* ($p = 0.02$), *Prevotella* ($p = 0.04$), and an unidentified genus in *Pasteurellaceae (*$p = 0.04$). Compared to low ppTG, high ppTG individuals had higher relative abundances of *Clostridium* cluster XIVa*,* while low ppTG individuals had higher relative abundances of six clades including *Prevotella, Alistipes,* and an unidentified *Pasteurellaceae* genus (**Figure 5**).

Discussion

Postprandial hyperlipidemia and diet-induced alterations to the gut microbial community have been implicated in disease development and yet, the relationship between the postprandial triglyceride response and the gut microbiome has not, hereunto been explored. Our findings show, for the first time, gut microbial differences between heavier adults who have low and high serum triglyceride responses to a high-fat meal challenge.

In previous human studies, high-fat meals have been used to induce inflammation. We did not observe differences in IL-1β, IL-23, TNF-α, and GM-CSF concentrations between high and low ppTG during the high-fat meal challenge. However, our findings agree with earlier works reporting a postprandial increase in IL-6 (47). The response seen in the participants in this study were unique in that IL-6 concentrations in the high ppTG individuals were elevated at baseline and remained elevated throughout the meal challenge. This was in contrast with the low ppTG individuals who were not elevated prior to the meal, increased post-meal, and then began to return to baseline levels. Dysregulation of IL-6 contributes to the promotion of pro-inflammatory pathways through activation of signal transducers and activators of transcription and mitogen-activated protein kinase (48) and has been implicated in metabolic syndrome and cardiovascular disease (49). Gut bacteria (50) and IL-6 (51) induce T helper 17 (Th17) differentiation, the main source of IL-17. Our low ppTG group had a marked increase in IL-17 in the one- and two-hour postprandial period compared to high ppTG which displayed a slow but steady rise in circulating IL-17. Increases in IL-17 in response to a high-fat meal challenge have been observed in an overweight adult cohort (52). In contrast to its pro-inflammatory role, evidence supports IL-17 as having a role in the upregulation of antimicrobial peptides (53). Further investigation is warranted to determine if the postprandial increase in IL-17 may be a compensatory effect of microbes adjusting to acutely elevated TG in the GI tract.

Exploring the gut microbiome composition of our participants revealed a greater relative abundance of *Alistipes* and *Prevotella* in our low ppTG group. Healthy, obese individuals have

been previously found to have an increased abundance of *Prevotella* but decreased abundance of *Alistipes* relative to healthy, lean individuals (54). A reduction of Prevotella was observed in coronary artery disease patients relative to healthy controls. Both genera are gram-negative anaerobes that can be responsive to changes in diet and inflammation (19, 55) and whose metabolic pathways include acetate and succinate production. Acetate is the most dominant SCFA in systemic circulation and has been shown in murine models to increase with high fat diet. Acetate promotes parasympathetic pathways and ghrelin and glucose-stimulated insulin secretion (29), both factors that promote insulin resistance and the development of Type 2 diabetes and metabolic syndrome. It is worth noting, plasma acetate concentrations in a large human cohort were recently found to have a negative relationship with the metabolic risk factor for CVD (56). The abundance of *Alistipes* increased with an animal-based diet (19, 57) but decreased in response to increased inflammation (55). *Prevotella* has been found to promote pro-inflammatory pathways through activation of toll-like receptor 2 on the surface of dendritic cells (DCs) and subsequent production of IL-1β, IL-6 and IL-23 by DCs and stimulation of Th17 cells (58). A greater abundance of *Prevotella* in our low ppTG group may be linked to the higher inflammation response we observed in the same group and may warrant future exploration.

We also found the family *Pasteurellaceae* to be abundant in low ppTG responders relative to high ppTG. Most members within *Pasteurellaceae* are commensal, but some species are considered pathogenic. Research with the LifeLines-DEEP cohort found *Pasteurellaceae* negatively correlated with fasting TG but not with BMI (31). The same study found fasting TG to be positively correlated with the order *Bacillales*, genera *Eggerthella, Holdemania*, and *Blautia,* and *Stercoris* species (31). The overabundance of *Pasteurellaceae* in low ppTG relative to high ppTG in our study suggests that members of *Pasteurellaceae* may play a role in the regulation postprandial TG in addition to fasting TG.

Surprisingly, overweight and obese individuals who had a high triglyceride response to the high-fat meal were overabundant in *Clostridium* Cluster XIVa, an eclectic microbial group

not restricted to the *Clostridium* genus. *Clostridium* Cluster XIVa is a clade rich with butyrate-producing species such as *Eubacterium rectale* and *Roseburia spp* (59). The increased abundance of *Clostridium* Cluster XIVa in high ppTG would suggest that high ppTG individuals may have enriched butyrate-producing species. Increased butyrate concentrations may help to decrease LPS-secreting bacteria (60), which have been shown to promote endotoxemia and systemic inflammation (12), and to also downregulate LPS-induced proinflammatory mediators in intestinal macrophages (61).

Acetate, propionate, and butyrate make up the majority of SCFA concentrations in humans. Butyrate is perhaps the most important compound of the three, providing energy to colonic epithelial cells, affecting expression of inflammatory cytokines in intestinal macrophages and epithelial tight junction proteins (16, 61), serving as signals to metabolism modulators (62, 63), and regulating cell proliferation (16). Notably, the benefits attributing to exercise's role in altering inflammatory phenotypes has also been observed to involve an increase in butyrate-producing GI microbes (64). Butyrate has promising therapeutic application in metabolic disorders, with murine models finding increased insulin sensitivity during a three-hour insulin tolerance test with butyrate supplementation (63).

Impaired glucose tolerance has been found to better predict CVD than fasting glucose (4), with later research also finding improved CVD predictions with nonfasting triglyceride values (3). *Clostridiaceae* and *Bacteroidaceae* are positively and negatively correlated, respectively, to the area under the curve for insulin (65). Correlations between the postprandial glycemic response to a bread and butter meal and the gut microbiome have been found with bacterial relative abundance obtained from metagenomic sequencing but not from 16S rRNA (33). The meal (110 g white bread; 30 g butter) in the paper by Zeevi and colleagues had positive associations with *Proteobacteria* and *Gammaproteobacteria* and negative associations with the *Bacteroidetes, Bacteroidia, Prevotellaceae,* and *Bacteroidales* (33), findings which were not observed in our study examining the ppTG. The meal in our study had a higher amount of butter

(58.3 vs 30 g) and bread (127.5 g whole wheat vs 110 g white) and sampled time points were specifically used to examine the lipemic response.

Strengths of the present study include comprehensive measurements of pro-inflammatory markers, fecal microbial taxa, and serum metabolites. There are several limitations of the current study. We did not analyze the postprandial TG response after four hours and therefore, missed information about the late postprandial response and return to fasting TG levels. This is relevant in that serum triglyceride concentrations may not have peaked and the 6 - 7 hour postprandial period has been previously shown to have the highest adjusted hazard ratio for CVD (6). Additionally, as we used 16S rRNA gene sequencing, we are not able to identify beyond microbial genera to species and strains and cannot determine microbial mechanisms for differences. Metagenomics would move examination of the microbial community beyond composition to functionality, allowing for a better understanding how microbial metabolic pathways may differ between host phenotypes.

In summary, we identified four bacterial taxa in the fecal microbial community that differ between high and low ppTG to a high-fat meal, three of which contribute to SCFA production in the GI tract. This observation provides insight into how specific members within the gut microbial community may be implicated in postprandial lipemia and affect host inflammation. By identifying microbial taxa implicated in the postprandial period, we can make targeted improvements in how we monitor microbial communities in individuals who have postprandial lipemia. This work has the potential to inform the design of microbial therapies for individuals who have a higher risk for developing chronic disease.

Declarations

Ethics approval and consent to participate: Study was approved by the Montana State University Institutional Review Board (#MM021116-FC). Written informed consent was obtained from all participants before participation.

Consent for publication: Not applicable.

Availability of data and materials: Sequence data that support the findings of this study have been in deposited in the Sequence Read Archive (SRA) with the accession code PRJNA596000. Other participant data is not publicly available due to them containing information that could compromise research participant privacy.

Competing interests: The authors declare that they have no competing interests.

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Author Contributions: The authors' responsibilities were as follows — MPM, CJY, and STW: designed the study and acquired funding; SMW, APM, and MPM: conducted the study; SMW curated the data and generated visualizations. SMW and CJY: advised on or performed statistical analyses; SMW, APM, and MPM: wrote the manuscript; and all authors: read and approved the final paper.

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References

1. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and nonfasting lipid levels: influence of normal food intake on lipids, lipoproteins, apolipoproteins, and cardiovascular risk prediction. Circulation. 2008;118(20):2047-56.

2. Jackson KG, Poppitt SD, Minihane AM. Postprandial lipemia and cardiovascular disease risk: Interrelationships between dietary, physiological and genetic determinants. Atherosclerosis. 2012;220(1):22-33.

3. Bansal S, Buring JE, Rifai N, Mora S, Sacks FM, Ridker PM. Fasting compared with nonfasting triglycerides and risk of cardiovascular events in women. Jama. 2007;298(3):309-16.

4. Tominaga M, Eguchi H, Manaka H, Igarashi K, Kato T, Sekikawa A. Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose. The Funagata Diabetes Study. Diabetes Care. 1999;22(6):920-4.

5. Nordestgaard BG, Benn M, Schnohr P, Tybjaerg-Hansen A. Nonfasting triglycerides and risk of myocardial infarction, ischemic heart disease, and death in men and women. Jama. 2007;298(3):299-308.

6. Mora S, Rifai N, Buring JE, Ridker PM. Fasting compared with nonfasting lipids and apolipoproteins for predicting incident cardiovascular events. Circulation. 2008;118(10):993-1001.

7. Lopez-Miranda J, Williams C, Lairon D. Dietary, physiological, genetic and pathological influences on postprandial lipid metabolism. Br J Nutr. 2007;98(3):458-73.

8. Hansson P, Holven KB, Øyri LKL, Brekke HK, Biong AS, Gjevestad GO, et al. Meals with Similar Fat Content from Different Dairy Products Induce Different Postprandial Triglyceride Responses in Healthy Adults: A Randomized Controlled Cross-Over Trial. The Journal of nutrition. 2019;149(3):422-31.

9. Boden G, She P, Mozzoli M, Cheung P, Gumireddy K, Reddy P, et al. Free fatty acids produce insulin resistance and activate the proinflammatory nuclear factor-kappaB pathway in rat liver. Diabetes. 2005;54(12):3458-65.

10. Duncan BB, Schmidt MI, Pankow JS, Ballantyne CM, Couper D, Vigo A, et al. Low-grade systemic inflammation and the development of type 2 diabetes: the atherosclerosis risk in communities study. Diabetes. 2003;52(7):1799-805.

11. Singh RK, Chang H-W, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med. 2017;15(1):73-.

12. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, et al. Changes in Gut Microbiota Control Metabolic Endotoxemia-Induced Inflammation in High-Fat Diet–Induced Obesity and Diabetes in Mice. Diabetes. 2008;57(6):1470-81.

13. Zhang H, Meng J, Yu H. Trimethylamine N-oxide Supplementation Abolishes the Cardioprotective Effects of Voluntary Exercise in Mice Fed a Western Diet. Frontiers in Physiology. 2017;8:944.

14. Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes. 2007;56(7):1761-72.

15. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature. 2011;472(7341):57-63.

16. Plöger S, Stumpff F, Penner GB, Schulzke JD, Gäbel G, Martens H, et al. Microbial butyrate and its role for barrier function in the gastrointestinal tract. Annals of the New York Academy of Sciences. 2012;1258(1):52-9.

17. Cani PD, Possemiers S, Wiele T, Guiot Y, Everard A, Rottier O. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut. 2009;58.

18. Wu GD, Compher C, Chen EZ, Smith SA, Shah RD, Bittinger K, et al. Comparative metabolomics in vegans and omnivores reveal constraints on diet-dependent gut microbiota metabolite production. Gut. 2016;65(1):63-72.

19. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature. 2014;505(7484):559-+.

20. Bisanz JE, Upadhyay V, Turnbaugh JA, Ly K, Turnbaugh PJ. Meta-Analysis Reveals Reproducible Gut Microbiome Alterations in Response to a High-Fat Diet. Cell Host & Microbe. 2019;26(2):265-72.e4.

21. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proceedings of the National Academy of Sciences. 2007;104(3):979.

22. Xiao L, Sonne SB, Feng Q, Chen N, Xia Z, Li X, et al. High-fat feeding rather than obesity drives taxonomical and functional changes in the gut microbiota in mice. Microbiome. 2017;5(1):43.

23. Lam YY, Ha CWY, Campbell CR, Mitchell AJ, Dinudom A, Oscarsson J, et al. Increased Gut Permeability and Microbiota Change Associate with Mesenteric Fat Inflammation and Metabolic Dysfunction in Diet-Induced Obese Mice. Plos One. 2012;7(3):e34233.

24. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The Effect of Diet on the Human Gut Microbiome: A Metagenomic Analysis in Humanized Gnotobiotic Mice. Sci Transl Med. 2009;1(6):6ra14.

25. Le Chatelier E, Nielsen T, Qin J, Prifti E, Hildebrand F, Falony G, et al. Richness of human gut microbiome correlates with metabolic markers. Nature. 2013;500(7464):541-6.

26. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature. 2006;444.

27. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America. 2005;102(31):11070-5.

28. Tang WHW, Kitai T, Hazen SL. Gut Microbiota in Cardiovascular Health and Disease. Circulation research. 2017;120(7):1183-96.

29. Perry RJ, Peng L, Barry NA, Cline GW, Zhang DY, Cardone RL, et al. Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. Nature. 2016;534(7606):213-+.

30. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F. A metagenome-wide association study of gut microbiota in type 2 diabetes. Nature. 2012;490.

31. Fu J, Bonder MJ, Cenit MC, Tigchelaar EF, Maatman A, Dekens JAM, et al. The Gut Microbiome Contributes to a Substantial Proportion of the Variation in Blood Lipids. Circulation research. 2015;117(9):817-24.

32. Baars A, Oosting A, Lohuis M, Koehorst M, El Aidy S, Hugenholtz F, et al. Sex differences in lipid metabolism are affected by presence of the gut microbiota. Scientific Reports. 2018;8(1):13426.

33. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized Nutrition by Prediction of Glycemic Responses. Cell. 2015;163(5):1079-94.

34. Gérard C, Vidal H. Impact of Gut Microbiota on Host Glycemic Control. Front Endocrinol. 2019;10(29).

35. Bosy-Westphal A, Schautz B, Later W, Kehayias JJ, Gallagher D, Müller MJ. What makes a BIA equation unique? Validity of eight-electrode multifrequency BIA to estimate body composition in a healthy adult population. European Journal Of Clinical Nutrition. 2013;67:S14.

36. Tanaka H, Monahan KD, Seals DR. Age-predicted maximal heart rate revisited. Journal of the American College of Cardiology. 2001;37(1):153-6.

37. Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, et al. Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 2009;75(23):7537-41.

38. Oksanen J, Kindt R, Legendre P, O ' Hara B, Henry M, Stevens H. The Vegan Package2007.

39. Team RC. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2019.

40. Wickham H. Elegant graphics for data analysis. New York: Springer-Verlag; 2009.

41. Dhariwal A, Chong J, Habib S, King IL, Agellon LB, Xia J. MicrobiomeAnalyst: a web-based tool for comprehensive statistical, visual and meta-analysis of microbiome data. Nucleic Acids Research. 2017;45(W1):W180-W8.

42. Pinheiro J, Bates D, DebRoy S, Sarkar D. nlme: Linear and non-linear mixed effects models. R package version 3.1-131 ed2017.

43. Barton K. MuMIn: Multi-Model Inference. R package 1.42.1. ed2018.

44. Champely S. pwr: Basic Functions for Power Analysis. 2018.

45. Fox J, Weisberg S. An {R} companion to applied regression. 2nd Ed. ed. Thousand Oaks, CA: Sage; 2011.

46. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12.

47. Herieka M, Erridge C. High-fat meal induced postprandial inflammation. Mol Nutr Food Res. 2014;58.

48. Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. The Biochemical journal. 2003;374(Pt 1):1-20.

49. Fischer CP, Berntsen A, Perstrup LB, Eskildsen P, Pedersen BK. Plasma levels of interleukin-6 and C-reactive protein are associated with physical inactivity independent of obesity. Scand J Med Sci Sports. 2007;17(5):580-7.

50. Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009;139(3):485-98.

51. Hebel K, Rudolph M, Kosak B, Chang H-D, Butzmann J, Brunner-Weinzierl MC. IL-1β and TGF-β Act Antagonistically in Induction and Differentially in Propagation of Human Proinflammatory Precursor CD4⁺ T Cells. The Journal of Immunology. 2011;187(11):5627.

52. Peluso I, Villano DV, Roberts SA, Cesqui E, Raguzzini A, Borges G, et al. Consumption of Mixed Fruit-juice Drink and Vitamin C Reduces Postprandial Stress Induced by a High Fat Meal in Healthy Overweight Subjects. Curr Pharm Design. 2014;20(6):1020-4.

53. Douzandeh-Mobarrez B, Kariminik A. Gut Microbiota and IL-17A: Physiological and Pathological Responses. Probiotics and Antimicrobial Proteins. 2019;11(1):1-10.

54. Thingholm LB, Rühlemann MC, Koch M, Fuqua B, Laucke G, Boehm R, et al. Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial Functional Capacity and Composition. Cell Host & Microbe. 2019;26(2):252-64.e10.

55. Borton MA, Sabag-Daigle A, Wu J, Solden LM, O'Banion BS, Daly RA, et al. Chemical and pathogen-induced inflammation disrupt the murine intestinal microbiome. Microbiome. 2017;5(1):47-.

56. Kurilshikov A, Munckhof ICLvd, Chen L, Bonder MJ, Schraa K, Rutten JHW, et al. Gut Microbial Associations to Plasma Metabolites Linked to Cardiovascular Phenotypes and Risk. Circulation Research. 2019;124(12):1808-20.

57. Wan Y, Wang F, Yuan J, Li J, Jiang D, Zhang J, et al. Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial. Gut. 2019;68(8):1417.

58. Larsen JM. The immune response to Prevotella bacteria in chronic inflammatory disease. Immunology. 2017;151(4):363-74.

59. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett. 2009;294.

60. Zhai S, Qin S, Li L, Zhu L, Zou Z, Wang L. Dietary butyrate suppresses inflammation through modulating gut microbiota in high-fat diet-fed mice. FEMS Microbiol Lett. 2019;366(13).

61. Chang PV, Hao LM, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. Proceedings of the National Academy of Sciences of the United States of America. 2014;111(6):2247-52.

62. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. Proceedings of the National Academy of Sciences of the United States of America. 2008;105(43):16767-72.

63. Gao Z, Yin J, Zhang J, Ward R. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 2009;58.

64. Miles MP, Wilson S, Yeoman CJ. Physical Activity and Inflammation Phenotype Conversion. Journal of Clinical Exercise Physiology. 2019;8(2):64-73.

65. Kreznar JH, Keller MP, Traeger LL, Rabaglia ME, Schueler KL, Stapleton DS, et al. Host Genotype and Gut Microbiome Modulate Insulin Secretion and Diet-Induced Metabolic Phenotypes. Cell reports. 2017;18(7):1739-50.

FIGURE LEGENDS

Figure 1 | Serum triglyceride response to a high-fat meal challenge grouped by ppTG. Values are mean ± SE. TG, triglycerides; ppTG, postprandial triglyceride response.

Figure 2 | Serum cytokine response to a high-fat meal challenge grouped by ppTG. All cytokine values are in pg/dL and are displayed as mean ± SE. IL-1β; interleukin-1 beta; IL-23, intereukin-23; TNF-α, tumor necrosis factor – alpha; IL-6, interleukin-6; GM-CSF, granulocyte macrophage – colony stimulating factor; IL-17, interleukin-17; ppTG, postprandial triglyceride response.

Figure 3 | Alpha-diversity indices grouped by ppTG. Shannon (A) and Simpson (B) alpha diversity calculations were performed on operational taxonomic units (OTUs).

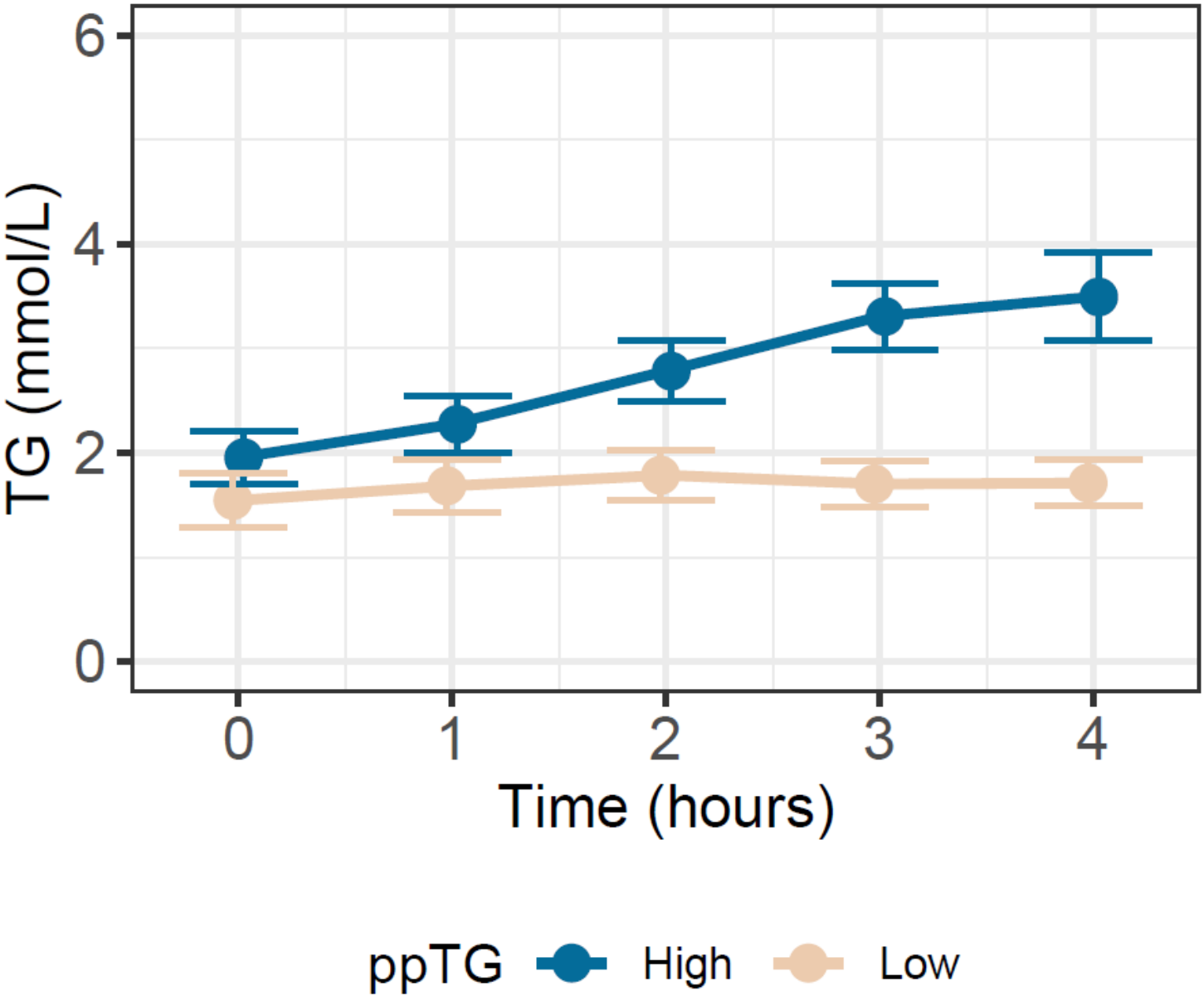
Figure 4 | Non-metric Dimensional Scaling (nMDS) of the gut microbial community. Ellipses indicate low or high ppTG community groupings. As NMDS is not an eigenvector-based method, there is not an available “variability explained” percentage associated with each component. Each symbol represents one participant sample. ppTG, postprandial triglyceride response.

Figure 5 | Discriminatory gut microbial taxa for low and high ppTG phenotypes. Bars represent effect size for each taxon with the length displaying the linear discriminant analysis (LDA) scores after log 10 transformation. Abbreviation in parentheses indicates taxonomic rank. ppTG, postprandial triglyceride response.

List of Abbreviations:

ANOSIM — analysis of similarities
CVD — cardiovascular disease
DCs — dendritic cells
GI — gastrointestinal
GM-CSF — granulocyte macrophage colony stimulating factor
HbA1c — glycated hemoglobin
HDL — high-density lipoprotein
IL — interleukin
LDA — linear discriminant analysis
LEfSe — linear discriminant analysis effect size
LPS — lipopolysaccharide
nMDS — nonmetric dimensional scaling
OTU — operational taxonomic unit
PERMANOVA — permutational multivariate analysis of variance
ppTG — postprandial triglyceride response
SCFA — short chain fatty acid
TG — triglyceride
Th17 — T helper 17
TMAO — trimethylamine-N-oxide
TNF — tumor necrosis factor
VIF — variance inflation factor

Figures



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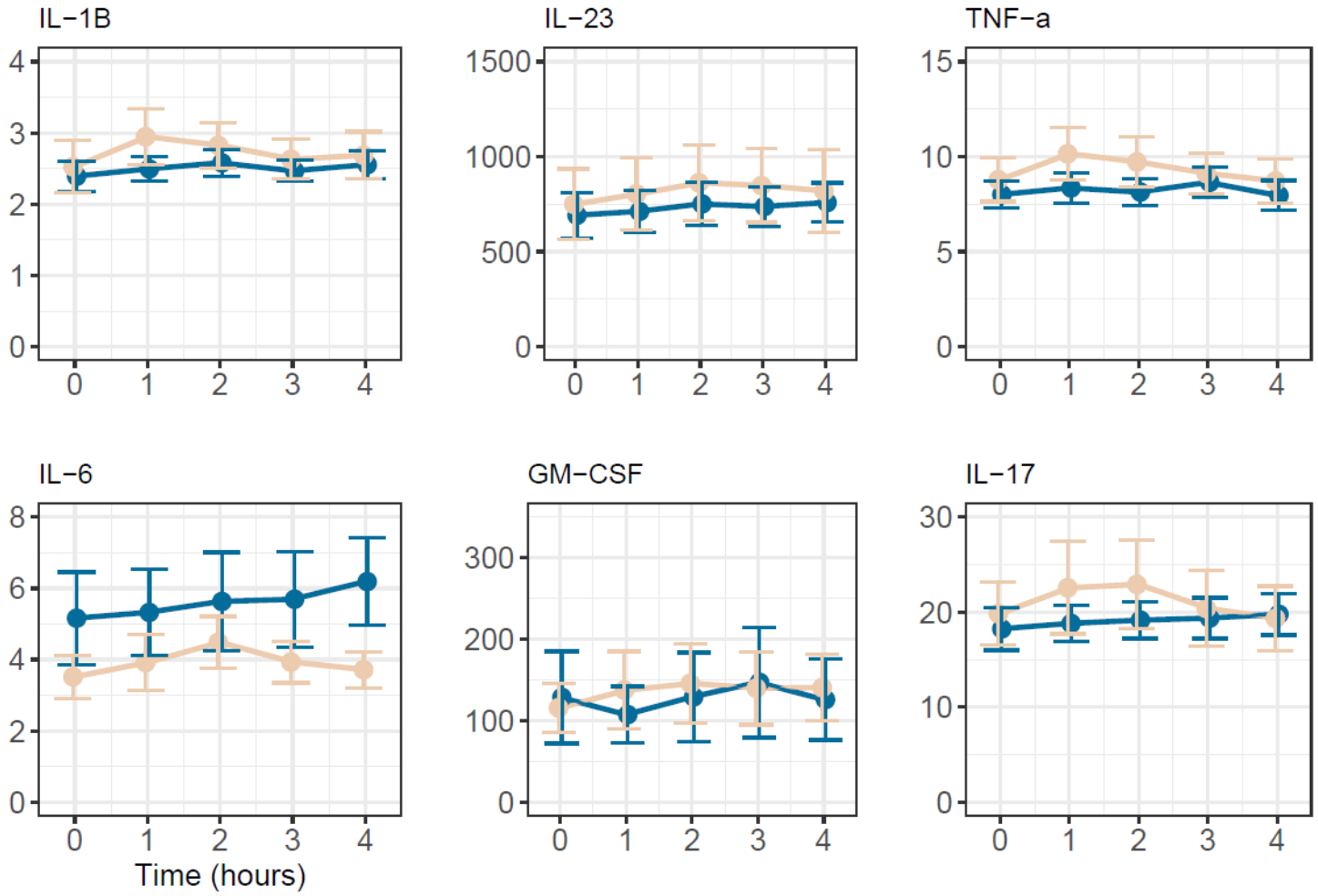
Serum triglyceride response to a high-fat meal challenge grouped by ppTG. Values are mean ± SE. TG, triglycerides; ppTG, postprandial triglyceride response.



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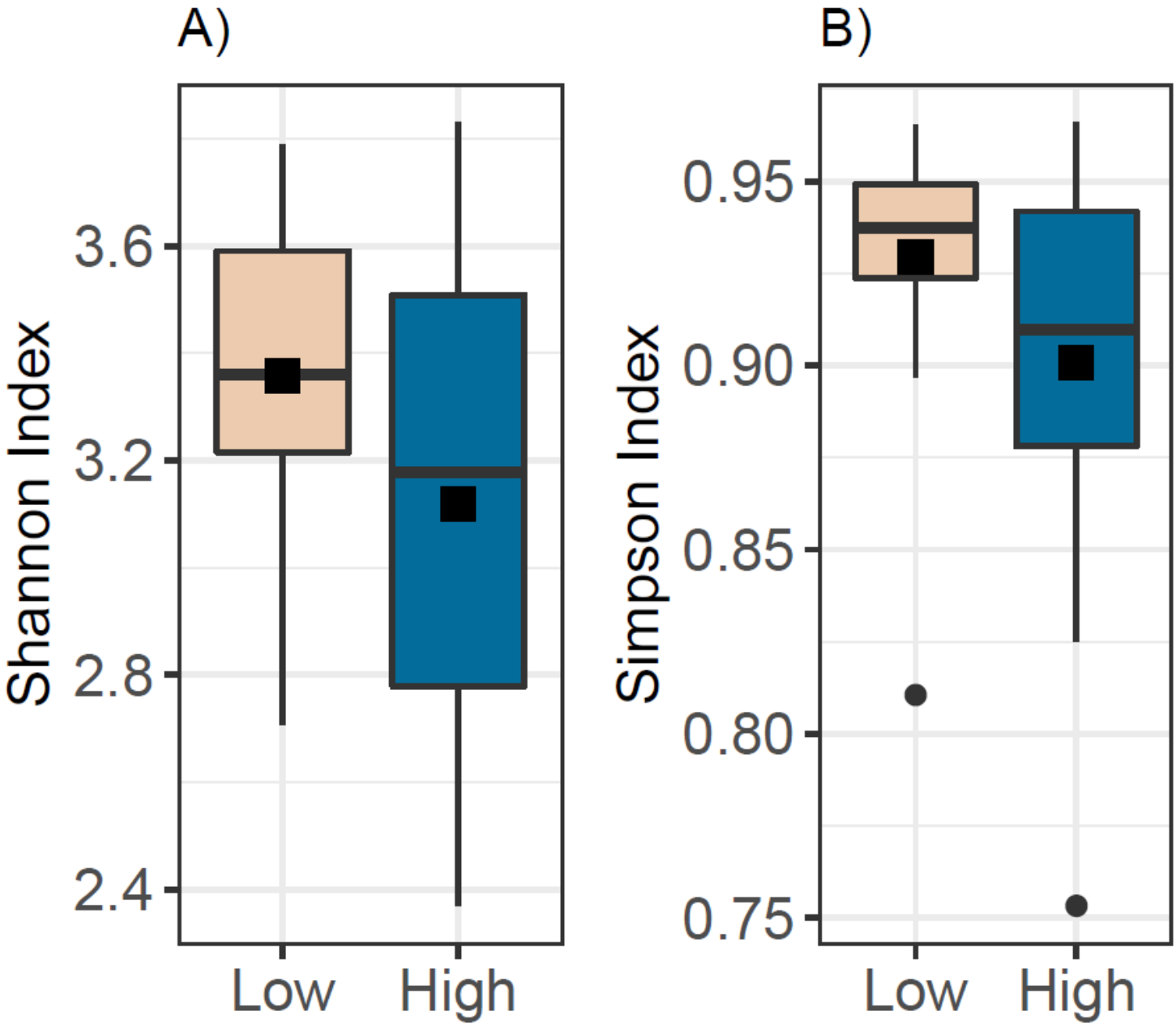

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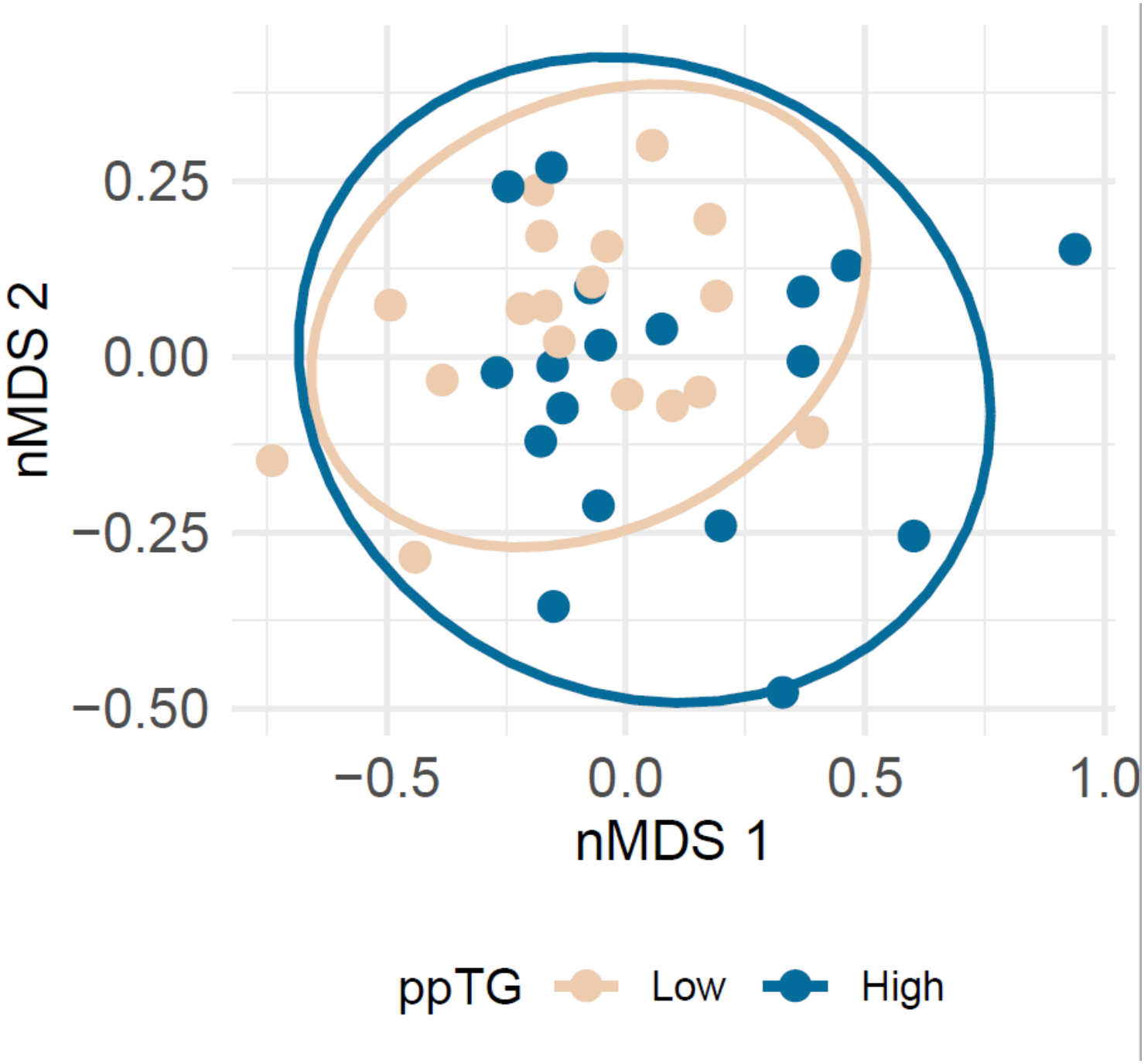

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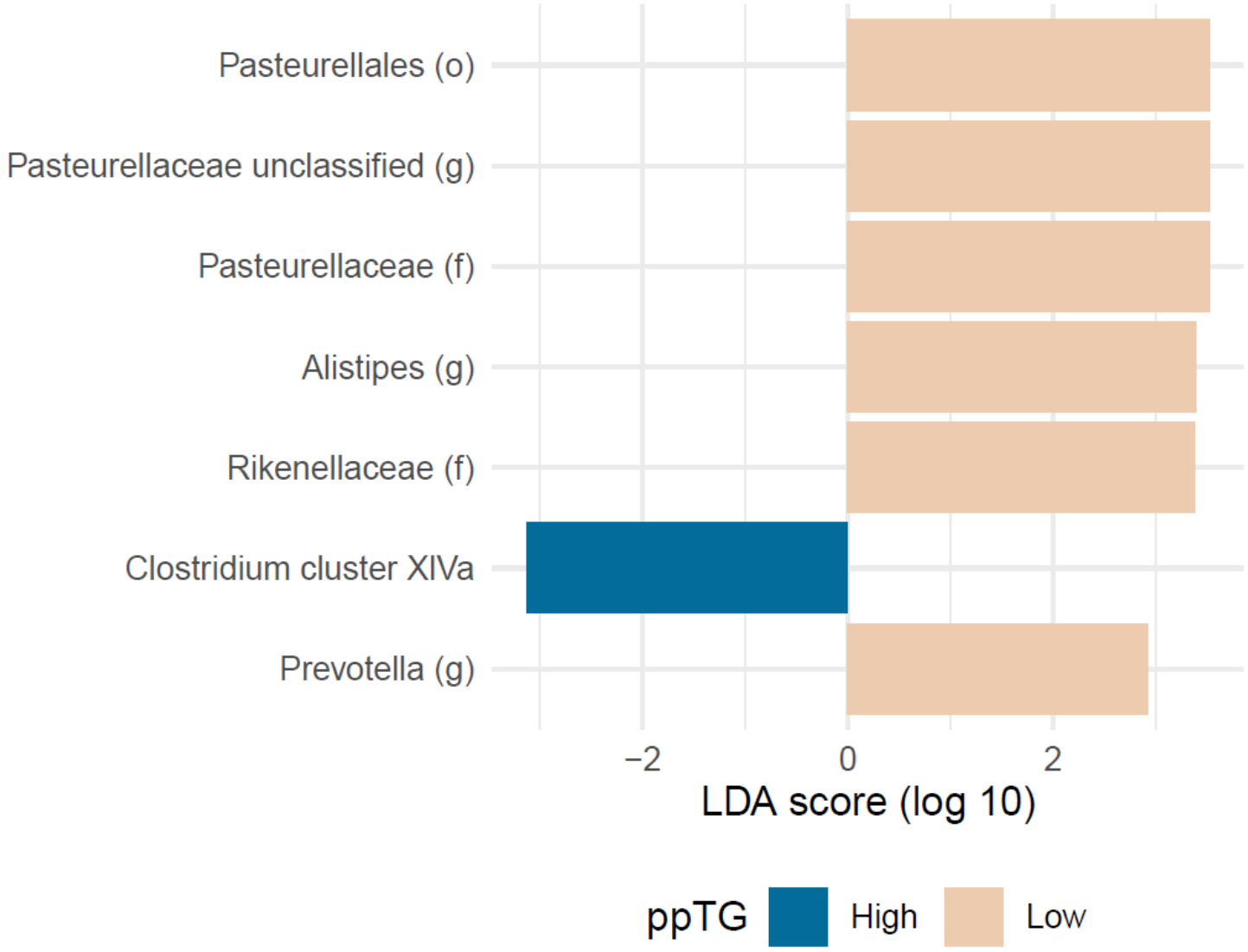

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Supplementary Files

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- ppTGSupplementaryMaterial.pptx