High dose saccharin supplementation does not induce gut microbiota dysbiosis or glucose intolerance in healthy humans and mice

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Research

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Abstract

Background: Non-caloric artificial sweeteners (NCAS) are widely used as a substitute for dietary sugars to control body weight or glycemia. Paradoxically, saccharin and other NCAS have been reported to induce glucose intolerance in mice fed a high-fat diet and in a subset of humans by directly inducing unfavorable changes in gut microbiota. These findings have raised concerns about NCAS and called into question their broad use. Whether these results can be generalized to healthy populations consuming conventional diets is unknown. It is also unclear how different NCAS, that do not share a common chemical structure, can produce identical direct effects on

gut microbiota. A common feature of all NCAS is their strong affinity for sweet taste receptors (STRs) which are expressed in the intestine. However, their role in mediating NCAS-induced effects has not been addressed.

Results: We conducted a double-blind, placebo-controlled, parallel arm study exploring the effects of saccharin on gut microbiota and glucose tolerance in healthy men and women. Participants were randomized to placebo, saccharin, lactisole (STR inhibitor), or saccharin with lactisole administered in capsules twice daily to achieve the maximum acceptable daily intake for two weeks. In parallel, we performed a ten-week study administering high-dose saccharin in the drinking water of chow-fed mice with genetic ablation of STRs (T1R2-KO) and wild-type (WT) littermate controls. In humans and mice alike, none of the interventions affected glucose or hormonal responses to a glucose tolerance test, nor *ex vivo* glucose absorption in mice. Similarly, saccharin supplementation did not alter microbial diversity or abundance at any taxonomic level in humans or mice. No treatment effects were also noted in readouts of microbial activity such as fecal metabolites or short chain fatty acids (SCFA). However, compared to WT, T1R2-KO mice were protected from age-dependent increases in fecal SCFA and the development of glucose intolerance.

Conclusions: In the absence of other permissive conditions, short-term saccharin consumption at the maximum recommended levels does not alter gut microbiota or induce glucose intolerance and, thus, it may be safely included in the diet of healthy individuals who wish to substitute sugars for weight management or caloric control.

Trial registration number NCT03032640, Registered 26 January 2017.

https://clinicaltrials.gov/ct2/show/NCT03032640

Keywords: artificial sweeteners; saccharin; sweet taste receptors; gut microbiota; glucose intolerance; short-chain fatty acids; fecal metabolomics; T1R2; dysbiosis

Background

Non-caloric artificial sweeteners (NCAS) are often consumed as a substitute for dietary sugars, limiting the caloric content of food without compromising its palatability. Six NCAS are approved as food additives in the United States (saccharin, aspartame, acesulfame potassium, sucralose, neotame, and advantame) by the Food and Drug Administration (FDA). The use of NCAS has increased dramatically over the past decade [1, 2], due to growing awareness of the negative health outcomes associated with sugar overconsumption [3]. Strikingly, NCAS use in children has tripled in a decade [4] with recent estimates suggesting that 25% of children and 41% of adults in the United States are daily consumers of NCAS [4]. Paradoxically, some epidemiological and experimental studies suggest that consumption of NCAS is associated with metabolic syndrome, weight gain, obesity, and non-alcoholic fatty liver disease (reviewed in [5, 6]). These findings have raised concerns among consumers and health professionals alike that NCAS may not be physiologically inert, as originally thought, and that their general use may lead to adverse public health outcomes. While there is some evidence supporting this viewpoint, many critical questions must be answered before concluding that these concerns apply to the general population and, ultimately, setting health policy to guide optimal NCAS use [7].

Among the pathophysiological mechanisms hypothesized to underlie the adverse effects attributed to NCAS consumption, one of the most plausible is the suggestion that consumption of various NCAS such as saccharin, aspartame or sucralose can directly alter gut microbiota composition to cause glucose intolerance in both mice and humans [8]. Indeed, seven days of saccharin supplementation in humans produced dysbiosis and glucose intolerance, but only in a subset of participants that also had a distinct microbiota composition prior to the intervention [8]. Similarly in mice, ten weeks of saccharin supplementation caused microbiota-induced glucose intolerance when accompanied by glucose consumption (in the water) or by high fat diet feeding [8]. As both sucrose consumption and a high-fat diet can independently alter the gut microbiome [9], these confounding factors may have played a permissive role allowing the manifestation of NCAS-induced effects on gut microbiota and glucose homeostasis. Due to the absence of

interventional studies that specifically test the effects of NCAS consumption as an independent modulator of gut microbiome and glucose tolerance, it is unclear whether conclusions from prior studies can be extrapolated to healthy populations that consume NCAS as part of a standard western diet. Finally, it is perplexing how compounds that are unrelated in chemical structure, such as saccharin, aspartame, and sucralose, can cause homogeneous changes in gut microbiota without a common mechanism that involves the host. NCAS are *bona fide* ligands for sweet taste receptors (STRs) which, beyond the tongue, are expressed in a variety of tissues including the gastrointestinal tract. Intestinal STRs play a role in regulating metabolic responses to the ingestion of sugars [10], so it is reasonable to speculate that STR-mediated chemosensation in the gut may provide a mechanistic link between NCAS-induced metabolic dysfunction and gut microbiota.

To circumvent the limitations of prior studies and explore the potential role of chemosensory STRs in the gut, we conducted a comprehensive translational investigation using humans and rodents. First, we performed a randomized, double-blind, placebo-controlled interventional study during which the diet of healthy participants was supplemented for two weeks with capsules that contained saccharin at the maximum acceptable daily intake (ADI), lactisole (a human specific inhibitor of human STRs), saccharin with lactisole, or placebo. To address potential adverse effects that may require higher NCAS dose and time of exposure and to shed light on possible mechanistic effects of saccharin on gut microbiota and glucose homeostasis, we performed a corresponding study in chow-fed mice with a genetic ablation of STRs (T1R2-KO) or wild-type controls (WT) aiming to exceed the maximum saccharin ADI for 10 weeks.

Results

Human participants

A total of fifty-four participants were randomized to four treatment groups. Forty-six subjects completed the study and were included in all analyses. Eight participants were excluded from the analysis due to non-compliance (**Supp. Figure.1**). The clinical characteristics of all participants

are summarized in **Supp. Table.1**. At baseline, no differences in basic anthropometric and metabolic parameters were noted between treatment groups (**Table.1**). The remaining participants of all groups met the expected dose requirement for the treatment period (**Supp. Table.2**). No adverse effects of the treatments were reported.

Glucose tolerance and *ex vivo* intestinal function

Two weeks of continuous saccharin supplementation at a dose equal to ADI [11] did not alter glucose responses to a 75g oral glucose tolerance test (OGTT) among participants (**Figure.1A**). To test for possible delayed effects of the treatment, we assessed glucose tolerance after a two-week recovery period during which all groups received placebo. No differences in glucose excursions were observed between the post-treatment and recovery (washout) periods (ANCOVA repeated measures p=0.99; **Supp. Figure.2**). Similar to glucose responses, plasma excursions of insulin, C-peptide, glucagon or glucagon-like peptide 1 (GLP-1) were not different between groups with treatment or after the wash out period (**Figure.1B-E and Table.2)** (**Supp. Figure.2**). Next, we addressed the long-term effects of high-dose saccharin supplementation on glucose tolerance in mice and specifically explored the role of NCAS sensing by intestinal STRs. *Ad libitum* chow-fed WT and T1R2-KO mice were supplemented with saccharin in the drinking water for 10 weeks to achieve daily consumption equal to 4 times the human ADI adjusted for mouse body surface area [12]. The actual saccharin consumption slightly exceeded the target consumption for both genotypes (**Supp. Figure.3A**), but without affecting food intake (**Supp. Figure.3B**). Saccharin consumption did not cause differences in body weight gain compared to water alone in either genotype (**Supp. Figure.3C**). As we observed in humans, saccharin treatment had no effect on glucose tolerance in WT or T1R2 mice assessed after two or ten weeks of treatment (**Figure.2A-B**). However, we did observe age-dependent increases in intra-gastric GTT (IGGTT) responses in WT mice. Notably, these effects were absent in T1R2-KO mice, which also had reduced IGGTT responses compared to WT littermates [13] (**Figure.2A**).

Although saccharin treatment was unsuccessful in modifying IGGTT responses, it may have induced localized intestinal changes that may contribute to long-term metabolic susceptibility. To address this possibility we assessed post-treatment *ex vivo* glucose transport using intact intestines (Ussing chamber) and found no effect of saccharin supplementation in the transport of the non-metabolizable glucose analog 3-O-methyl-glucose (3-OMG) (**Figure.2C**), but we observed decreased glucose transport in T1R2-KO intestines, consistent with the IGGTT data and our previous studies [13]. In addition, saccharin supplementation did not change the expression of glucose transporters or of STRs (**Supp. Figure.3D**). Because saccharin treatment was shown to disrupt epithelial cell barrier in Caco-2 cell monolayers [14], we assessed *ex vivo* FITC-dextran (4kDa) flux in treated intact intestines and found no differences in gut permeability (**Figure.2D**).

Gut microbiota

Saccharin-induced glucose intolerance was previously shown to be contingent upon direct changes in gut microbiota composition [8], so we performed 16S rRNA sequencing of fecal samples from the human and mouse studies to investigate whether alterations in microbial communities are induced in response to treatments despite the absence of metabolic responses. All human subjects had similar microbiota composition prior to the interventions (**Supplemental Figure.4A**). Also, no sex-dependent differences in Shannon diversity index at baseline were noted (**Supplemental Figure.4B).** None of the treatments affected relative microbial abundances at any taxonomic levels (Family taxa are shown; **Figure.3A**) or the degree of microbial diversity (**Figure.3B**). Multivariate analysis by non-metric multidimensional scaling (NMDS) also showed no clear clustering by treatment (**Figure.3C**). Taxonomic distributions were equivalent across treatments when we performed pre-post analysis of variance to account for between subjects differences in microbial communities within a treatment group (Two-way ANOVA; $p>0.05$ for each treatment). In mice, we did not observe a genotype or gender effect on gut microbiota composition or taxonomic diversity at baseline (**Supplemental Figure.4C-F**). Despite the larger dose and

longer duration of treatment in mice, saccharin did not produce any change in microbial abundances at any taxonomic level (Family taxa are shown; **Figure.3D**) or diversity (**Figure.3E**). Similar to humans, no clustering effect was observed for post-treatment groups by multivariate analysis (**Figure.3F**). Also, no changes were noted in within-subject microbial abundances, as assessed by pre-post analysis of variance (Two-way ANOVA; $p>0.05$ for each treatment at family level).

Fecal metabolomics

Although the interventions did not induce substantial shifts in the gut microbial communities in either humans or mice, we tested whether saccharin might have instead altered the intestine's metabolic profile by performing untargeted metabolomics of fecal samples.

Multivariate analysis showed that human participants had similar metabolomics profiles at baseline (**Supplemental Figure.4G**) and none of the interventions affected the fecal metabolome (**Figure.4A**). Importantly, we did observe saccharin in feces from participants assigned to the corresponding intervention groups, indicating that the saccharin dose was sufficient to reach the intestinal microbiota (**Figure.4B**). All mice had similar baseline fecal metabolome, excluding potential genotype effect (**Supplemental Figure.4H**). Initial analysis showed an effect of saccharin treatment compared to water, with a moderate predictive value in the orthogonal partial least squares discriminant analyses (OPLS-DA; **Figure.4C**). Subsequent metabolite distribution and identification (S-plot analysis) revealed that the presence of saccharin itself in the feces was the only metabolite responsible for the clustering effect (**Figure 4D-E**). Hence, removal of saccharin from the model abolished the clustering effects, eliminating any independent treatment effects on the fecal metabolome (**Figure 4F**). In addition, we specifically assessed fecal glucose content in all human and mouse samples, but found no treatment or genotype differences excluding major defects in glucose absorption. (**Supplemental Figure.4I-J**). Finally, we independently measured short-chain fatty acids (SCFA) in feces and found no treatment effect in

human participants (**Figure.4G**). However, we noticed an age-dependent increase in SCFA in WT mice, but these effects were absent in T1R2-KO mice (**Figure.4H**).

Discussion

Concerns and confusion about the general safety of NCAS can be attributed, in part, to the amount and quality of the available evidence. A critical knowledge gap has been the lack of interventional studies designed to rigorously investigate whether consumption of NCAS *per se* is sufficient to cause deterioration of glucose homeostasis in healthy individuals. Using a randomized, placebo-controlled design, we clearly show that daily consumption of saccharin at maximum ADI for 2 weeks is inadequate to alter fecal microbiota composition and metabolites or affect glucose tolerance in healthy participants. Notably, identical results were recapitulated in chow fed mice that consumed saccharin equal to 4-times the human ADI for 10 weeks.

Over the past 30 years, a number of cross-sectional and observational studies have reported positive correlations between NCAS consumption and outcomes such as metabolic syndrome and weight gain (reviewed in [6, 15]). These findings have alarmed both consumers and health care professionals, despite the fact that health and other lifestyle-related characteristics of the populations might have influenced these outcomes through reverse causality or residual confounding. For instance, positive associations between NCAS consumption (estimated from soda consumption) and metabolic syndrome were noted in a recent cross-sectional study [16], but after careful adjustment for age, dietary quality and physical activity these associations disappeared. A paucity of well-controlled interventional studies has also contributed to confusion in the field.

In this regard, an elegant report by Suez et al (2014) [8] appeared to establish a causative relationship between the consumption of NCAS (i.e. saccharin) and the development of glucose intolerance through direct modification of gut microbiota composition. This report, mainly conducted in mice, revived concerns about the use of NCAS and long-term health implications. However, in this study only 3 out of the 7 human participants developed glucose intolerance in

response to 3-7 days of NCAS use. In contrast, we exposed 23 healthy lean participants in 2 separate cohorts (Saccharin, or Saccharin plus Lactisole groups) to 15 days of daily saccharin consumption at the maximum ADI levels. None of the treated subjects, who were also not regular NCAS users, developed glucose intolerance or showed altered endocrine responses during an OGTT, but it is reasonable to speculate that the treatment effects of NCAS supplementation may be delayed. However, OGTT responses remained unaltered after 2 additional weeks of placebo treatment following the main intervention. In agreement with our findings in healthy lean participants, 12 weeks of NCAS supplementation using sweetened beverages did not change glucose tolerance in healthy overweight or obese individuals [17]. This suggests that the development of glucose intolerance in response to NCAS use is independent of obesity status *per se* and may instead require the presence of other, yet unknown, underlying risk factors. For instance, the saccharin responders in Suez et al (2004) [8] had different baseline microbiome compared to non-responders; a factor shown to confound outcomes of dietary interventions [18]. We circumvented these issues since all participants contained similar basal gut microbiota composition. This similarity is partially due to the enforcement of comprehensive inclusion and exclusion criteria including dietary habits that were consistent with the typical macronutrient intake of healthy US adults. Thus, saccharin treatment did not alter gut microbiota composition compared to other interventions, but also did not induce any relative changes in treated participants (i.e. within-subject pre-post analyses). Although gut microbiota abundances were mainly unaltered by the treatments, marginal shifts in some species or changes in microbial metabolism [19] might predispose the host to dysbiosis [20]. This effect is unlikely, as neither saccharin nor any other treatment significantly altered fecal metabolite profiles or induced any relative changes in treated participants. The microbiota-induced pathophysiology is often linked to SCFA changes in microbial production and availability [21], but saccharin did not alter fecal SCFA in humans and mice alike, mirroring the null effect observed in untargeted metabolite profiles. However, the age-dependent increase in SCFA in the WT mice is consistent with the

age-dependent development of glucose intolerance in the same mice and it is in agreement with findings showing that increased fecal SCFA correlate with age, obesity and metabolic dysregulation [22]. Notably, in T1R2 mice the absence of SCFA increases with aging correlates with the absence of glucose intolerance. These associations require further investigation since fecal concentrations of SCFA can be affected by several factors including transit time [23] and colonic clearance [24].

Interestingly, saccharin was detected in the feces of several saccharin- or saccharin plus lactisole-treated participants. From a clinical perspective, this observation is very significant because about 90% of ingested saccharin is absorbed in the small intestine and eliminated in the urine without biotransformation, while the remainder excreted in the feces [25]. Thus, only a small portion of ingested saccharin can reach and potentially be metabolized by the microbes at the large intestine. Similar to Suez et al [8], we administered saccharin equivalent to the ADI [11], suggesting that saccharin bioavailability was not a limiting factor for gut microbes in our population. Nevertheless, even in high saccharin consumers (>90th percentile) the average intake is only about 2mg/kg/d, a minor fraction of the ADI (5mg/kg/d) [26]. Taken together with our findings, it is reasonable to suggest that typical saccharin use is unlikely to induce adverse alterations in the gut microbiota of the general healthy consumer.

On the other hand, the absence of effects following short-term NCAS supplementation in our study cannot exclude the possibility that the deleterious consequences of NCAS consumption might require higher doses and/or longer durations. Because of safety limitations regarding the dose and duration of treatment involving human participants, we supplemented C57Bl\6J mice with saccharin for 10 weeks using a target dose that exceeded the human ADI by 4 times adjusted for mouse body surface area to discern possible mechanistic effects that might have not been apparent in the human study. Surprisingly, but in agreement with the human findings, glucose tolerance, gut microbiota composition and fecal metabolite profiles were unaffected by the higher saccharin dose and extended treatment in chow fed mice. As in humans, saccharin appeared in

the feces of almost all treated mice, confirming saccharin's bioavailability for microbial metabolism. In contrast to our findings, mice fed chow diet and supplemented with 10% solution of commercial saccharin, which contained 95% glucose by mass, or mice fed high-fat and supplemented with pure saccharin, developed glucose intolerance mediated by unfavorable changes in gut microbiota [8]. Similarly, 12 weeks of saccharin supplementation in chow-fed ICR/HaJ mice caused marginal glucose intolerance, but responsive mice also showed increased food intake and weight gain [27]. Our saccharin-fed mice consumed similar amount of chow and experienced the same age-related increases in body weight compared to water control littermates. Taken together, these findings suggest that high saccharin consumption may exert negative health outcomes only in the presence of other permissive conditions such as underlying risk factors, caloric overload, or dietary regiments known to independently alter gut microbiota and induce dysbiosis of the host [28]. Finally, because plasma glucose excretion can be modulated by the rate of intestinal glucose absorption, we tested whether saccharin treatment tampered this process and compensated for systemic effects in glucose homeostasis. No differences in the expression of the main glucose transporters, *ex vivo* intestinal glucose transport, gut permeability or glucose malabsorption were noted in mice supplemented with saccharin, excluding secondary effects on glucose assimilation.

In the presence of other permissive dietary factors, saccharin may be able to cause glucose intolerance by directly altering gut microbiota. However, it is still perplexing how other NCAS, such as aspartame or sucralose, can demonstrate identical effects [8] considering that they share no structural similarities to suggest their intersection of common pathways of microbial metabolism. NCAS are *bona fide* ligands for STRs, so it is reasonable to speculate that if consumption of all NCAS leads to specific metabolic effects, such as glucose intolerance, a common underlying mechanism should exist. Thus, a secondary aim of our studies was to test whether STR partially mediate the effects of NCAS feeding. Participants that consumed lactisole, a human specific inhibitor of STRs, or mice with genetic ablation of STRs had no differences in glucose tolerance

or gut microbiota in response to saccharin feeding, which suggests that in the absence of a primary effect of NCAS consumption the role of STR signaling is not apparent. Nevertheless, we observed a genotype effect in mice independent of treatment. T1R2-KO mice had reduced IGGTT responses and *ex vivo* glucose transport compared to WT littermates, confirming our previous findings [13]. Interestingly, although WT mice developed mild age-related glucose intolerance, T1R2-KO mice were resistant to these effects. We previously showed that T1R2-KO mice were also protected against metabolic derangements induced by high-fat diet [29], suggesting that STR signaling may be involved in age- and diet-dependent deterioration of glucose homeostasis.

Although we report no adverse effects of short-term NCAS consumption on the glycemic responses in healthy lean participants and mice, our study has some notable limitations. First, we tested saccharin as a representative NCAS but it is unknown whether our results can be extrapolated to all NCAS. Since the six FDA-approved NCAS have different metabolic fates and bioavailability [30], potential health implications relevant to their consumption must be addressed separately. Second, the duration of treatment in humans was limited to two weeks, which may have been inadequate to induce physiological effects in a healthy young population. This does not preclude the possibility that years of chronic high use of saccharin or of other NCAS may eventually lead to slow maladaptive responses or predispose consumers to the development of disease. Third, we focused on a number of outcomes based on previous reports and specific objectives. Thus, saccharin might have altered other physiological parameters that, if measured, may have helped identify other adverse health conditions linked to NCAS consumption.

Conclusions

We clearly show that short-term saccharin supplementation *per se* is insufficient to alter gut microbiota or induce glucose intolerance in apparently healthy humans and mice on conventional diets. The clinical significance of our findings should not be underestimated since it emphasizes that the recommended saccharin use is safe for healthy consumers that wish to substitute dietary sugars for weight management or caloric control. Our findings also do not contradict previous

reports showing harmful effects of saccharin. On the contrary, together they highlight that the potential harmful effects of chronic NCAS use are likely contingent upon permissive physiological or lifestyle features in vulnerable populations. Therefore, for individuals who lack these characteristics - such as those studied here - consumption of NCAS is likely innocuous, but for susceptible populations NCAS use may be contraindicated. Consequently, it is imperative that future studies concentrate in isolating and identifying the critical underlying pathophysiology or conditions that may render specific NCAS as harmful.

Methods

Experimental design

Human Studies

We conducted a randomized, placebo-controlled, double-blind, interventional study (NCT02835859) at the Advent-Health Translational Research Institute (TRI) in healthy lean male and female participants who were randomly assigned to four intervention groups. Recruitment, enrollment and all study-related visits, including specimen collection and point-of-care laboratory testing, took place at Advent-Health. Subjects were recruited between January 2017 and February 2018. The study was approved by the Institutional Review Board at Advent-Health and all participants signed an informed consent.

Healthy men and women 18-45 years of age were recruited from volunteer lists and by social media to participate in the study. Only subjects who consumed less than a can of diet beverage or a spoonful of NCASs weekly (or the equivalent from foods) during the past month, whose body mass index (BMI) ≤ 25.0 kg/m^2, and who were weight stable (± 3 kg) during the 3 months prior to enrollment were included. Subjects with acute or chronic medical conditions that would contraindicate participation in the research testing or that were taking medications that could potentially affect metabolic function were excluded. Specifically, individuals with diabetes, bariatric surgery, inflammatory bowel disease or a history of malabsorption and pregnant or

nursing women were excluded. A complete list of inclusion and exclusion criteria are available (**Supp. methods**).

Participants were randomized into four treatment groups and were instructed to consume capsules containing: 1) Pulp filler/placebo (1000mg/day 1) Sodium saccharin (400mg/day), 3) Lactisole (670mg/day) or 4) Sodium saccharin (400mg/day) + lactisole (670mg/day) twice daily for two weeks. A sealed envelope with the randomization allocation sequence (SAS procedure PROC PLAN) was given to the pharmacist who prepared and provided the appropriate treatment. The pharmacist was the only un-blinded member of the study. Diet-related instructions were provided to avoid additional consumption of NCASs for the duration of the study. Participants were asked to give blood samples and stool samples during their visits. The investigation agents, saccharin and lactisole, were formulated in capsules for oral delivery (Compounding Pharmacy, Advent-Health) at the maximum acceptable daily intake (ADI) [11].

A schematic of the experimental design is shown in **Supp. Figure 5A**. At visit 1 (pre-intervention), participants arrived at the TRI after a 10-hour overnight fast omitting breakfast and the following procedures were performed: 1) Stool sample collection. 2) Assessment of dietary compliance; 3) Vital signs; 4) Measurements of weight; 5) Insertion of an intravenous (IV) catheter for blood draws; 6) Baseline blood sampling (t = -10, 0 min); 7) Oral consumption of a 75g glucose solution (300mL) to assess glucose tolerance (i.e. OGTT); 8) OGTT blood sampling (t = 10, 20, 30, 45, 60, 90, 120, 180 min); 9) Participants were provided with 2-week supply of treatment capsules and were instructed to consume 2 capsules a day (morning and evening) with water until the night before their next visit. At visit 2 (post-treatment), the same procedures as listed above were repeated. All groups were subjected to additional 2 weeks of pulp filler/placebo capsule treatment (blinded for participants) and at visit 3 (recovery) the same procedures were performed.

Blood was collected in K_2EDTA tubes with a cocktail of protease, esterase and DPP-IV inhibitors (BD^{TM} P800 blood collection system; BD Bioscience, CA). Glucose concentrations were

measured by a point of care device (NOVA StatStrip Meter); insulin, C-peptide, total GLP1, and glucagon concentrations by immunoassay (Milliplex Map Kit, Millipore, MA).

Mouse studies

All animal experimental procedures were approved by Institutional Animal Care and Use Committee (IACUC) committee of The Ohio State University. Whole body T1R2 deficient mice (T1R2-KO; a gift of Dr. Zuker) were used with WT littermates back-crossed on the C57Bl\6J strain for at least 10 generations. After weaning, all mice were housed individually in ventilated caging with limited shared environmental exposure and placed on standard polysaccharide chow diet (Teklad #2016) for 4-5 weeks. Eight week-old mice were randomly assigned to one of the following treatment groups for additional 10-weeks (**Supp. Figure.5B**): 1) drinking water only (control), 2) drinking water plus saccharin. All groups were on standard chow diet and saccharin concentrations were adjusted based on pilot studies aiming to a) avoid taste aversive effects (<0.3% saccharin in water) [31], b) ensure equal consumption between genotypes since WT mice can taste saccharin but T1R2-KO cannot, and c) to achieve an average daily dose equal to 4 times (250mg/kg) the human ADI (62mg/kg) adjusted for mouse body surface area [12]. An intra-gastric GTT (IGGTT) was performed at baseline, week 2 and week 10 of the intervention. Fecal pellets we collected at baseline and at week 10 of the intervention for each mouse. The IGGTT was performed in 5-hour fasted mice (h) which received 1g/kg body weight (BW) of glucose. For the saccharin treated groups, saccharin was maintained in the drinking water during the fasting period prior to testing. A baseline IGGTT was performed the day following the initiation of the interventions to account for possible acute effects of saccharin feeding on the test. Blood glucose was sampled from the tail and analyzed with an AlphaTRAK blood glucose monitoring meter (North Chicago, IL). Glucose tolerance curves over time are shown in absolute values. Area under curve (AUC) was calculated using the trapezoid method adjusted for fasted baselines.

Ussing Chamber

Ex vivo glucose transport was measured in intact intestinal sections by monitoring short-circuit current and measuring ^{14}C isotopic flux of 3-O-methyl-glucose ([^{14}C]-3-OMG), exactly as described previously [13]. To assess gut permeability, 0.2mg/ml of 4kDa FITC-dextran (Sigma) was added to the donor chamber of pre-equilibrated jejunums and FITC flux to the acceptor side was assessed every 15 min for 1.5h in a fluorimeter at 485nm excitation and 528nm emission.

Fecal Microbiota

Genomic DNA was isolated from mouse and human feces using QiaAmp DNA stool kit (QIAGEN), with an additional step of bead beating for 5 min with 0.1 mm beads to ensure maximum lysis of bacterial cells. Multiplexed libraries were prepared according to the protocol from Illumina using V3-V4 region of 16S rRNA and HiFi HotStart DNA Polymerase (Kapa Biosystems) for amplification. Final amplified products were quantified by ABI Prism library quantitation kit (Kapa Biosystems). Each sample was diluted to 10 nM, and equal volume from each sample was pooled. The quality of the library was checked by Bio-Rad Experion bioanalyzer (Bio-Rad). Illumina MiSeq platform was used for sequencing (Novogene Bioinformatics Technology Co., Ltd).

Raw FASTQ sequences were quality checked with FastQC v0.11.5. Raw sequences were trimmed with 'cutadapt' v2.6 to remove low quality bases and adaptor sequences. The trimmed FASTQ files were converted into a Qiime2 v2019.1 file format PairedEndFastqManifestPhredd33. The imported forward and reverse reads were merged using 'vsearch' with a minimum sequence length of 200 base pairs. Joined pairs were quality trimmed using Qiime2 'quality filter' with an average quality score of 20 (Q20) over a 3 base pair sliding window and removing trimmed reads having less than 75% of their original length. 'Deblur 16S rRNA positive filter' was used as a final quality control step by dereplicating and removing chimera sequences from each sample; reads were trimmed to a final length of 195 base pairs. Taxonomic analysis and Operational Taxonomic Unit (OTU) tables were created with Qiime2 and converted using biom format is Qiime1. All statistics were ran in Graphpad Prism v8 unless specified otherwise. Alpha and beta diversity measurements were calculated using Microbiomeanalyst.ca with no filtering. Alpha diversity

calculations were based on Shannon diversity index with Mann-Whitney test and figures were plotted in Graphpad Prism. All boxplot data were evaluated with median and minimum/maximum values. Statistical analysis of the multiple group comparisons was performed using one-way analysis of variance (ANOVA) followed by Tukey post-hoc test; when two groups were compared, the nonparametric t-test was performed. For mouse genotypes at 0 weeks, a one-way ANOVA with Tukey post-hoc test was performed to determine initial genotypic effects on microbiome. Results were considered significant with P-value < 0.05. Beta diversity was calculated on 16S rRNA OTU data using Bray-Curtis dissimilarity and NMDS figure created using R package 'vegan'. Permutational multivariate ANOVA based on NMDS ordination distances was used to calculate community composition. Based on OTU data produced by Qiime2, a relative abundance bar chart was created using Microbiomeanalyst.ca. For abundances statistical analysis, each individual in human and mouse population was tested with a t-test and two-way ANOVA for each family level classification for pre and post treatment.

Fecal Metabolomics

The nuclear magnetic resonance (NMR) spectra of aqueous fecal extracts were acquired at 298K on a Bruker Avance III 800 MHz spectrometer equipped with a TCI probe (Bruker Biospin, Germany). The ^{1}D ^{1}H NMR experiments were conducted using the first increment of the nuclear Overhauser enhancement spectroscopy (NOESY) pulse sequence with presaturation for water suppression (Relaxation delay-90-t1-90-mixing time-90-Free induction decay). The acquisition parameters were as follows: 64 scans and 4 dummy scans, 64K data points, 90° pulse angle (11.3 us), relaxation delay of 3 s and a spectral width of 14 ppm. The spectra were acquired without spinning the NMR tube in order to avoid spinning side bands artifacts. The free induction decays were multiplied by a decaying exponential function with a 1 Hz line broadening factor prior to Fourier transformation. The ^{1}H NMR spectra were corrected for phase and a polynomial fourth-order function was applied for base-line correction. Chemical shifts are reported in ppm as referenced to Trimethylsilylpropanoic acid ($\delta = 0$). NMR signal were assigned using a range of

2D NMR spectra, namely $^{1}H-^{1}H$ correlation spectroscopy, $^{1}H-^{1}H$ total correlation spectroscopy , $^{1}H-^{13}C$ edited heteronuclear single quantum correlation, and $^{1}H-^{13}C$ heteronuclear multiple bond correlation spectra. 1D and 2D NMR spectra were processed using TopSpin 3.2 (Bruker Biospin, Germany).

The spectral region δ 0.50−10.0 was integrated into regions with equal width of 0.005 ppm using the AMIX software package (V3.8, Bruker-Biospin). The region δ 4.70−4.90 was discarded due to imperfect water saturation. Prior to statistical data analysis, each bucketed region was normalized to the total sum of the spectral intensities to compensate for the overall concentration differences.

Multivariate statistical analysis was carried out with SIMCA-P+ software (version 14.1, Umetrics, Sweden). Data were mean-centered and scaled using the Pareto method, while log-transformation was applied to achieve an improved normal distribution of the data. Principal component analysis (PCA) and orthogonal projection to latent structures with discriminant analysis (OPLS-DA) were conducted on the scaled data. The OPLS-DA model's confidence level for membership probability was set to 95% and was validated using a 7-fold cross validation method. The quality of the model was assessed by the values of R^2Y and Q^2. The R^2Y metric describes the percentage of variation explained by the model; Q^2 shows the predictive ability of the model. The difference between these metrics describes the model's fitness.

Fecal Short Chain Fatty Acids

Liquid chromatography tandem mass spectrometry (LC-MS/MS) methods for SCFA were performed as described [32]. Briefly, samples of mouse and human feces were thawed on ice. Samples were then homogenized in 50% acetonitrile, containing ^{13}C-propionate as an internal standard at a ratio of 10 µL solvent per 1 mg fecal sample. Fecal samples were then derivatized as described previously [32]. Samples were sealed and stored at 4 ºC until analyses, and throughout LC-MS/MS quantification. All LC-MS/MS analyses were performed within 24 hours of sample creation. Samples were analyzed on an Agilent 6460 QQQ LC-MS/MS system, using a

Poroshell EC-C18 column (3.0 x 50 mm). Collision energies were 10 for butyric acid, 5 for propionic acid, and 15 for acetic acid. Retention times and mass transitions for each SCFA monitored were: Butyrate: 7.138 min., 222→137; Propionate: 5.097 min., 208→165, 208→137; ^{13}C Propionate: 5.097 min., 209→165, 209→137; Acetate: 2.754 min., 194→137. SCFA levels were quantified using standard curves generated using authentic standards and normalized using ^{13}C propionate as an internal standard. Data was analyzed using the Agilent MassHunter Quantitative Analysis software suite.

Gene expression

Gene expression of scraped mucosa from mouse intestines was performed as described [13] using the following genes: t1r2 (forward: GAACTGCCCACCAACTACAA, reverse: CCATCGTGGACAGACATGAA), t1r3 (forward: CCAGTGAGTCTTGGCTGACA, reverse: TTCAGTGAGGCACAGAATGC), sglt1 (forward: TGGAGTCTACGCAACAGCAAGGAA, reverse: AGCCCACAGAACAGGTCATATGCT), glut2 (forward: CCCTGGGTACTCTTCACCAA, reverse: GCCAAGTAGGATGTGCCAAT).

Statistical analyses

For human studies, sample size calculation (PROC GLMPOWER, SAS) was based on the minimal detectable difference of glycemic responses (area under curve) during an OGTT performed before and after 7-days of saccharin treatment (Figure.4B of reference [8]), using an ANCOVA model with baseline as covariate to provide 80% statistical power for one-sided 0.05 significance level test. Differences between groups in glycemic and hormonal responses (i.e. AUC) during the OGTT were tested via ANCOVA with the baseline AUC as the covariate, followed by post-hoc multiple comparisons. To investigate the treatment effect at the different visits, we built repeated measures ANCOVA with treatment, time and treatment x time interaction as main effects, along with baseline AUC as a covariate, followed by post-hoc multiple comparisons. For mouse studies, differences between groups in glycemic responses during the OGTT and ex vivo intestinal transport and gene expression were tested by two-way ANOVA. A p-value <0.05 was

considered statistically significant. All analyses will be performed with SAS version 9.4 (SAS Institute Inc).

Abbreviations

3-OMG 3-O-methyl-glucose

ADI Acceptable daily intake

ANCOVA Analysis of covariance

ANOVA Analysis of variance

AUC Area under the curve

BW Body weight

FDA Food and Drug Administration

GLP-1 Glucagon-like peptide 1

IGGT Intragastric glucose tolerance test

KO knockout

NCAS Non-caloric artificial sweeteners

NMDS non-metric multidimensional scaling

OGTT Oral glucose tolerance test

OPLS-DA Ortigonal partial least squares discriminant analysis

OTU Operational taxonomic unit

SCFA Short term fatty acids

STRs Sweet taste receptors

TRI Translational research institute (Advent-Health)

WT Wild-type

Declarations:

Ethics approval and consent to participate

The clinical study was performed in accordance with the requirements of Good Clinical Practice and the Revised Declaration of Helsinki. All participants provided written informed consent to

participate after receiving verbal and written information about the study. The protocol was approved by the Institutional Review Board of Advent-Health and registered at IRBNet (#982524). The study was registered on ClinicalTrials.gov on the 26th of January of 2017 (NCT03032640). All the studies in mice were performed in accordance to NIH and institutional guidelines of the Ohio State University Institutional Animal Care and Use Committee.

Consent for publication

Not applicable

Availability of data and materials

The raw sequence data from 16S rRNA gene amplicon sequencing were submitted to NCBI BioProject under accession number PRJNA605207
https://www.ncbi.nlm.nih.gov/bioproject/PRJNA605207

Competing interests

The authors declare no competing interests.

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Author contributions

JS designed research studies, performed experiments, analyzed data, and wrote the manuscript. KRS, VS, JB, EH, performed experiments and analyzed data. VV, TEL, LMD, VS, FT, LG performed experiments. ALC, FY, SNP, MA analyzed data and edited the manuscript. REP designed research studies and edited the manuscript. GAK conceived the project, designed research studies, analyzed data, and wrote the manuscript.

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Figure Legends

Figure 1. Effects of saccharin and/or lactisole treatment on glucose tolerance in humans

Plasma excursions of **(A)** glucose, **(B)** insulin, **(C)** C-peptide, **(D)** glucagon, and **(E)** GLP-1 during an oral glucose challenge after 2 weeks of treatment. Two-way ANOVA repeated measures ($p>0.05$). N=10-13.

Figure 2. Effects of saccharin treatment on glucose homeostasis in mice

(A) Glucose responses during an i.g.GTT expressed as area under curve (AUC) before (0 weeks), 2 and 10 weeks after saccharin treatment. Two-way ANOVA main effect; *$p<0.05$, WT 10-week vs. T1R2 10-week, **$p<0.01$ WT 0-week vs. WT 10-week). **(B)** Glucose excursions during an i.g.GTT in response to 10 weeks of saccharin treatment (2-way ANOVA repeated measures, $p=0.0007$). **(C)** *Ex vivo* glucose flux using 3-O-methy-glucose (3-OMG) in intact mouse intestines following 10 weeks of saccharin treatment. Two-way ANOVA, **$p<0.01$. **(D)** *Ex vivo* intestinal permeability assessed by FITC-dextran (4kDa) flux in intact mouse intestines

following 10 weeks of saccharin treatment. Two-way ANOVA, p>0.05. N=23-28 for *in vivo* studies, n=6-11 for *ex vivo* studies.

Figure 3. Taxonomic abundances and diversity of gut microbiota in response to treatments in humans and mice

(A and D) Bar chart summary showing relative abundance at the family level post-treatment in human participants or in WT and T1R2 mice. Each bar represents abundances of one subject. **(B and E)** Alpha diversity box plot (Shannon diversity metric) showing community richness between groups post-treatment in human participants (Mann-Whitney U Test; p=0.156, U= 5.22) or in WT and T1R2 mice (Mann-Whitney U Test; p=0.987, U=152). **(C and F)** Nonmetric multidimensional scaling (NMDS) plot showing community similarities between groups post-treatment in human participants (p< 0.999, NMDS stress = 0.2274) or WT and T1R2 mice (p< 0.111, NMDS stress = 0.209). NMDS ordination was derived from pairwise Bray-Curtis distances and statistical inferences made using PERMANOVA. N=11-13 for human studies, n=8-11 for mouse studies.

Figure 4. Fecal metabolomics in response to treatments in humans and mice

(A) Differences in human fecal metabolites between treatment groups using orthogonal partial least squares discriminant analyses (OPLS-DA). **(B)** Post-treatment saccharin presence in human fecal samples. Dashed lines represent average noise ± SD. **(C)** Differences in WT and T1R2 fecal metabolites following saccharin treatment using OPLS-DA. **(D)** Post-treatment saccharin presence in mouse fecal samples. Dashed lines represent average noise ± SD. **(E)** Metabolite distribution (S-plot) in fecal mouse samples. Metabolites attributed to saccharin shown in red. **(F)** Differences in WT and T1R2 fecal metabolites following saccharin treatment using OPLS-DA after removal of saccharin signals. **(G)** Assessment of short chain fatty acids (SCFA) following treatments in human samples. One-way ANCOVA baseline as covariate. **(H)** SCFA in mouse feces before (pre) and after (post) treatment. Two-way ANOVA repeated measures with post-hoc. N=11-13 for human studies, n=8 for mouse studies.

Table.1

TABLE 1

Baseline characteristics of intervention groups

	Placebo	Saccharin	Lactisole	Sac + Lac	
Total, n	11	13	12	10	p
Age, y	24.91 ± 1.59	28.91 ± 2.60	32.92 ± 2.78	28.80 ± 2.91	0.199
Height, cm	166.61 ± 2.37	169.03 ± 3.31	164.54 ± 1.92	172.53 ± 2.23	0.494
Weight, kg	59.00 ± 1.81	64.52 ± 3.49	62.13 ± 1.90	66.57 ± 2.64	0.305
BMI, kg/m2	21.29 ± 0.62	22.40 ± 0.53	22.93 ± 0.47	22.38 ± 0.78	0.261
Glucose, mg/dL	87.55 ± 2.40	92.00 ± 2.41	91.63 ± 2.64	90.00 ± 1.23	0.519
Triglycerides, mg/dL	72.36 ± 7.93	71.82 ± 13.88	87.42 ± 12.85	65.70 ± 7.57	0.605
Total cholesterol, mg/dL	166.91 ± 9.38	163.82 ± 10.09	182.33 ± 8.25	154.60 ± 7.47	0.333
HDL, mg/dL	66.55 ± 4.12	57.55 ± 3.74	62.58 ± 4.60	64.80 ± 3.96	0.236
LDL, mg/dL	85.91 ± 7.92	91.82 ± 7.47	102.25 ± 7.29	76.50 ± 9.24	0.162
Cholesterol/HDL	2.58 ± 0.18	2.95 ± 0.27	3.08 ± 0.28	2.49 ± 0.20	0.103
LDL/HDL	1.36 ± 0.17	1.67 ± 0.22	1.77 ± 0.25	1.26 ± 0.19	0.115

All values are mean ± SEM. Baseline differences between groups were assessed by ANCOVA using sex as covariate. BMI, body mass index; HDL, high density cholesterol; LDL, low density cholesterol. AUC, area under the curve; Sac, saccharin; Lac, lactisole.

Figure 1

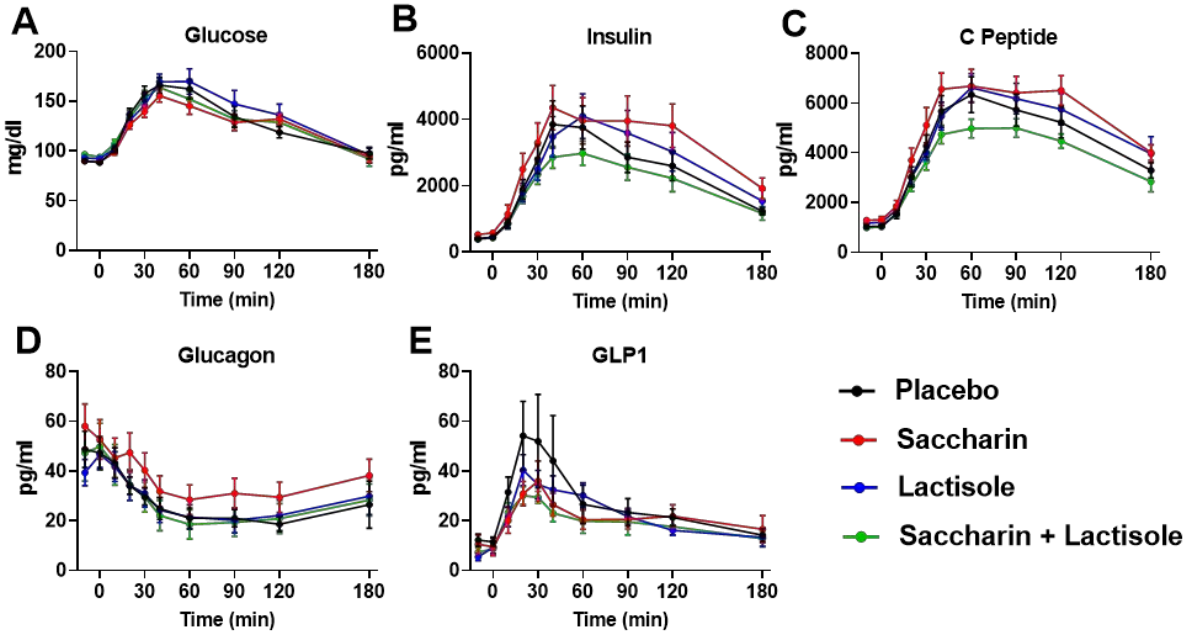

A
Glucose
mg/dl
200
150
100
50
0
0 30 60 90 120 180
Time (min)
B
Insulin
pg/ml
6000
4000
2000
0
0 30 60 90 120 180
Time (min)
C
C Peptide
pg/ml
8000
6000
4000
2000
0
0 30 60 90 120 180
Time (min)
D
Glucagon
pg/ml
80
60
40
20
0
0 30 60 90 120 180
Time (min)
E
GLP1
pg/ml
80
60
40
20
0
0 30 60 90 120 180
Time (min)
Placebo
Saccharin
Lactisole
Saccharin + Lactisole

Table 2.

TABLE 2

Glucose and hormonal excursions during an OGTT after the intervention

	Placebo	Saccharin	Lactisole	Sac + Lac	p
Glucose (AUC)	7136.4 ± 943.6	6606.9 ± 951.7	7571.0 ± 1289.2	6203.8 ± 1084.2	0.6018
Insulin (AUC)	362518.7 ± 55694.9	476124.6 ± 77735.0	412000.5 ± 69663.6	299078.8 ± 41026.4	0.7627
C peptide (AUC)	645605.0 ± 73479.8	747311.4 ± 68855.8	677199.5 ± 61852.4	525798.3 ± 28535.9	0.6034
Glucagon (AUC)	-3993.5 ± 771.9	-3226.6 ± 1306.1	-3768.0 ± 984.4	-4504.1 ± 1528.9	0.8632
GLP1 (AUC)	2734.8 ± 983.2	2195.4 ± 410.7	2294.1 ± 384.5	1862.9 ± 520.9	0.0662

All values are mean ± SEM. Treatment effects between groups were assessed by ANCOVA using the baseline glucose tolerance test AUC as a covariate. OGGT, oral glucose tolerance test; AUC, area under curve; Sac, saccharin; Lac, lactisole

Figure 2

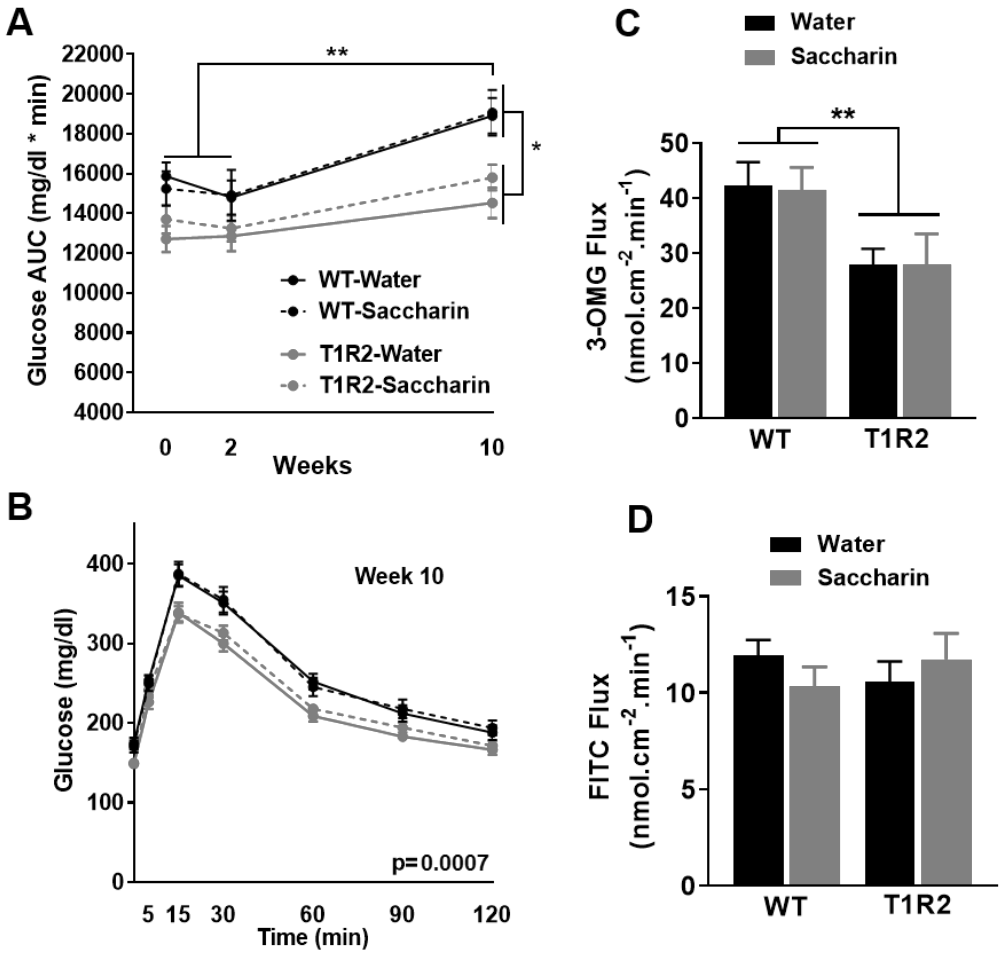

A
Glucose AUC (mg/dl * min)
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
**
*
WT-Water
WT-Saccharin
T1R2-Water
T1R2-Saccharin
0
2
10
Weeks
B
Glucose (mg/dl)
400
300
200
100
0
Week 10
p=0.0007
5
15
30
60
90
120
Time (min)
C
Water
Saccharin
3-OMG Flux
(nmol.cm-2.min-1)
50
40
30
20
10
0
**
WT
T1R2
D
Water
Saccharin
FITC Flux
(nmol.cm-2.min-1)
15
10
5
0
WT
T1R2

Figure 3

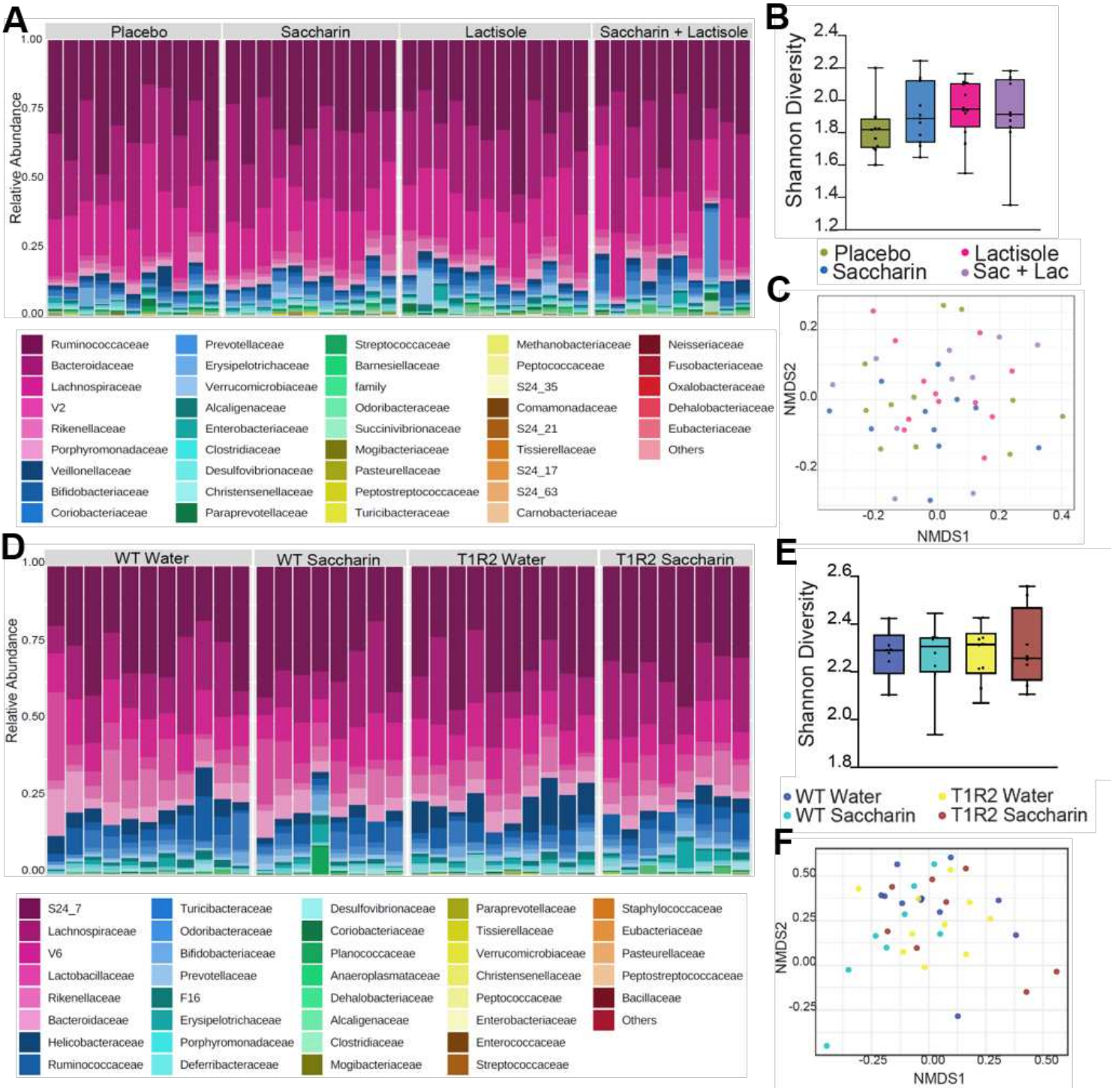
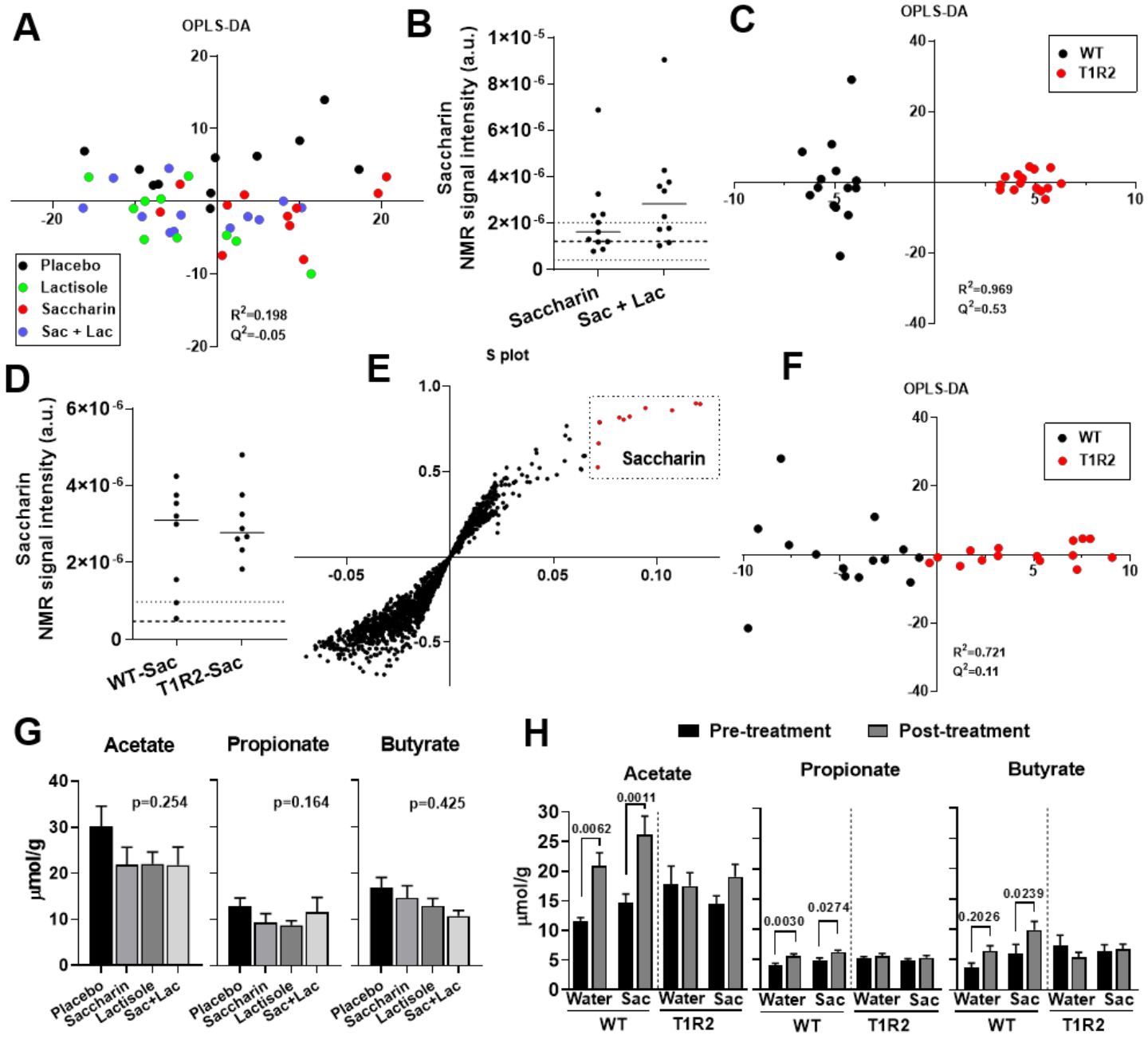

A
Placebo
Saccharin
Lactisole
Saccharin + Lactisole
Relative Abundance
1.00
0.75
0.50
0.25
0.00
Ruminococcaceae
Bacteroidaceae
Lachnospiraceae
V2
Rikenellaceae
Porphyromonadaceae
Veillonellaceae
Bifidobacteriaceae
Coriobacteriaceae
Prevotellaceae
Erysipelotrichaceae
Verrucomicrobiaceae
Alcaligenaceae
Enterobacteriaceae
Clostridiaceae
Desulfovibrionaceae
Christensenellaceae
Paraprevotellaceae
Streptococcaceae
Barnesiellaceae
family
Odoribacteraceae
Succinivibrionaceae
Mogibacteriaceae
Pasteurellaceae
Peptostreptococcaceae
Turicibacteraceae
Methanobacteriaceae
Peptococcaceae
S24_35
Comamonadaceae
S24_21
Tissierellaceae
S24_17
S24_63
Carnobacteriaceae
Neisseriaceae
Fusobacteriaceae
Oxalobacteraceae
Dehalobacteriaceae
Eubacteriaceae
Others
B
Shannon Diversity
2.4
2.2
2.0
1.8
1.6
1.4
1.2
Placebo
Saccharin
Lactisole
Sac + Lac
C
NMDS2
0.2
0.0
-0.2
-0.2
0.0
0.2
0.4
NMDS1
D
WT Water
WT Saccharin
T1R2 Water
T1R2 Saccharin
Relative Abundance
1.00
0.75
0.50
0.25
0.00
S24_7
Lachnospiraceae
V6
Lactobacillaceae
Rikenellaceae
Bacteroidaceae
Helicobacteraceae
Ruminococcaceae
Turicibacteraceae
Odoribacteraceae
Bifidobacteriaceae
Prevotellaceae
F16
Erysipelotrichaceae
Porphyromonadaceae
Deferribacteraceae
Desulfovibrionaceae
Coriobacteriaceae
Planococcaceae
Anaeroplasmataceae
Dehalobacteriaceae
Alcaligenaceae
Clostridiaceae
Mogibacteriaceae
Paraprevotellaceae
Tissierellaceae
Verrucomicrobiaceae
Christensenellaceae
Peptococcaceae
Enterobacteriaceae
Enterococcaceae
Streptococcaceae
Staphylococcaceae
Eubacteriaceae
Pasteurellaceae
Peptostreptococcaceae
Bacillaceae
Others
E
Shannon Diversity
2.6
2.4
2.2
2.0
1.8
WT Water
WT Saccharin
T1R2 Water
T1R2 Saccharin
F
NMDS2
0.50
0.25
0.00
-0.25
-0.25
0.00
0.25
0.50
NMDS1

Figure 4


A
OPLS-DA
Placebo
Lactisole
Saccharin
Sac + Lac
R²=0.198
Q²=-0.05
B
Saccharin
NMR signal intensity (a.u.)
Saccharin
Sac + Lac
C
OPLS-DA
WT
T1R2
R²=0.969
Q²=0.53
D
Saccharin
NMR signal intensity (a.u.)
WT-Sac
T1R2-Sac
E
S plot
Saccharin
F
OPLS-DA
WT
T1R2
R²=0.721
Q²=0.11
G
Acetate
Propionate
Butyrate
µmol/g
p=0.254
p=0.164
p=0.425
Placebo
Saccharin
Lactisole
Sac+Lac
H
Pre-treatment
Post-treatment
Acetate
Propionate
Butyrate
µmol/g
0.0062
0.0011
0.0030
0.0274
0.2026
0.0239
Water
Sac
WT
T1R2

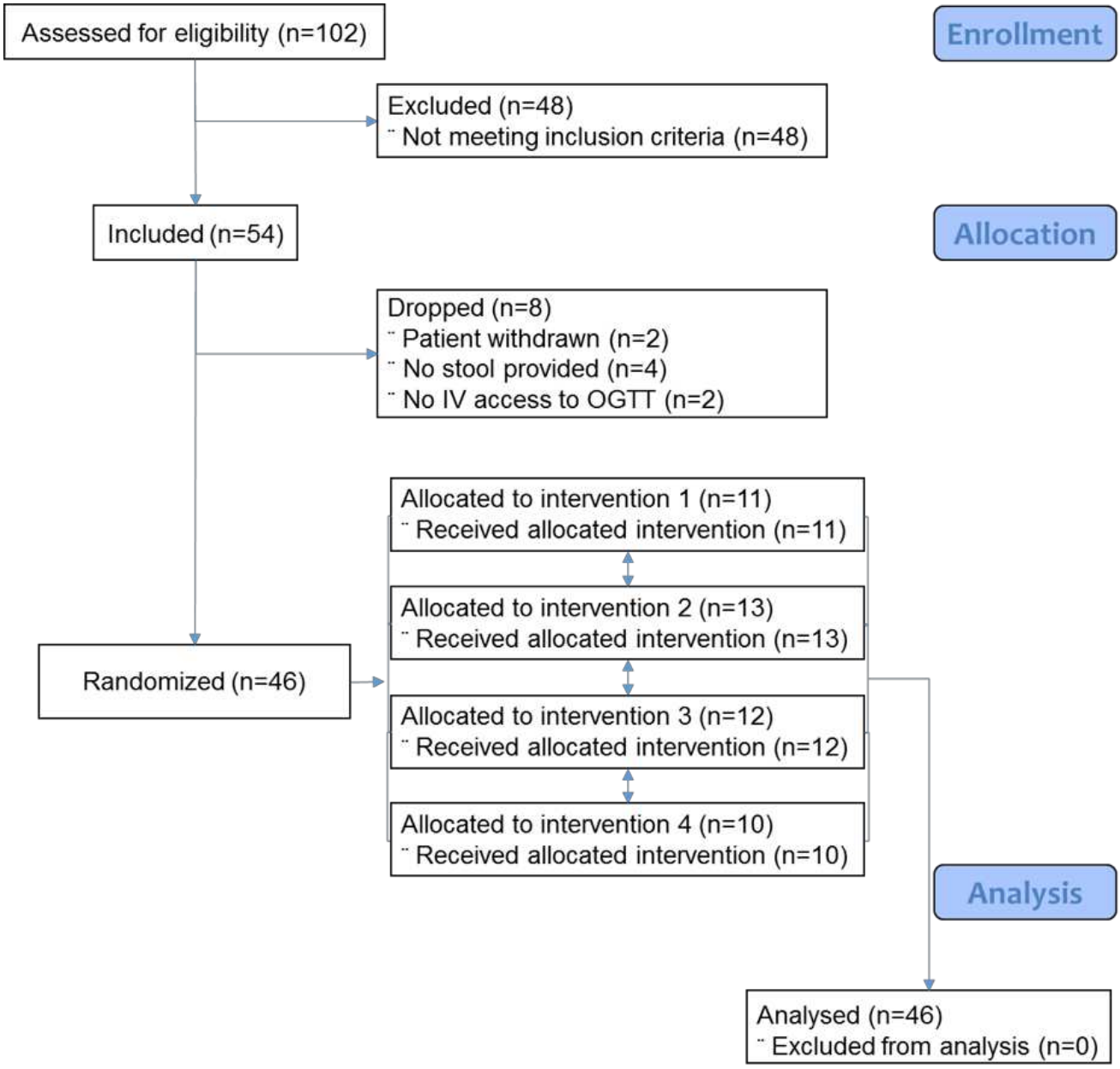
Supplemental methods

Inclusion and exclusion criteria

Inclusion criteria: Age 18-45 years apparently healthy; Consumption of less than a can of diet beverage or a spoonful of NCASs weekly (or each equivalent from foods) during the past month; Weight stable (± 3 kg) during the 3 months prior to enrollment; Body Mass Index (BMI) ≤ 25.0 kg/m^2.

Exclusion Criteria: Acute or chronic medical conditions or medications that would contraindicate participation in the research testing or could potentially affect metabolic function including, but not limited to: Known coronary artery disease, angina or congestive heart failure; Type 1 or Type 2 Diabetes (A1c ≥6.5%); Bleeding disorders; Hemoglobin level < 11.5 g/dL for women, < 12.0 g/dL for men; Acute or chronic infections; Hepatitis and/or cirrhosis (AST or ALT 2.5 times the upper limit of normal); Severe asthma or chronic obstructive pulmonary disease; Renal insufficiency or nephritis (creatinine > 1.6 mg/dl); Prior bariatric surgery; Inflammatory bowel disease or malabsorption; Cancer within the last 3 years (except non-melanoma skin cancer or treated cervical carcinoma in situ); Psychiatric disorders or eating disorders; Cushing's disease or syndrome; Untreated or inadequately controlled hypo- or hyperthyroidism (abnormal TSH); Active rheumatoid arthritis or other inflammatory rheumatic disorder; Pregnant or nursing women; Smoking (smoking within the past 3 months); Less than 4 bowel movements per week; Dietary habits exceeding the 10^{th}-90^{th} percentile of age- and gender-dependent usual macronutrient intake (31); Known hypersensitivity to saccharin, lactisole or any of its excipients. Excluded medications include but are not limited to: Anti-diabetic agents; Oral, injected or chronic topical steroids (inhaled steroids for mild asthma are acceptable); Antibiotic use (within the past 3 months) (32); Other drugs known to affect immune or metabolic function; Orlistat, phenteramine, topirimate or other weight loss or anorectic agents (tricyclic antidepressants, atypical antipsychotics or other psychiatric drugs with effects on body weight).

Supplemental Fig .1



Supplemental figure 1. Participant inclusion flowchart
A total of 54 subjects out of the 102 screened were allocation among the four experimental groups. Eight non-compliant individuals were removed from the study at various stages. Forty-six randomized subjects completed the interventions. (Intervention 1: Placebo; Intervention 2: Saccharin; Intervention 3: Lactisole; Intervention 4: Saccharin and Lactisole; IV, Intravenous; OGTT, oral glucose tolerance test).

Supplemental Table 1

TABLE S1

Baseline characteristics of participants

	Value
Total (male/female), n	46 (14/32)
Age, y	28.98 ± 1.29
Height, cm	168 ± 1.29
Weight, kg	62.95 ± 1.29
BMI, kg/m2	22.26 ± 0.30
Glucose, mg/dL	90.33 ± 1.14
Triglycerides, mg/dL	74.82 ± 5.53
Total cholesterol, mg/dL	167.55 ± 4.56
HDL, mg/dL	62.82 ± 2.07
LDL, mg/dL	89.70 ± 4.09
Cholesterol:HDL ratio	2.79 ± 0.12
LDL:HDL ratio	1.53 ± 0.11

All values are mean ± SEM. BMI, body mass index; HDL, high density cholesterol; LDL, low density cholesterol.

Supplemental Table.2

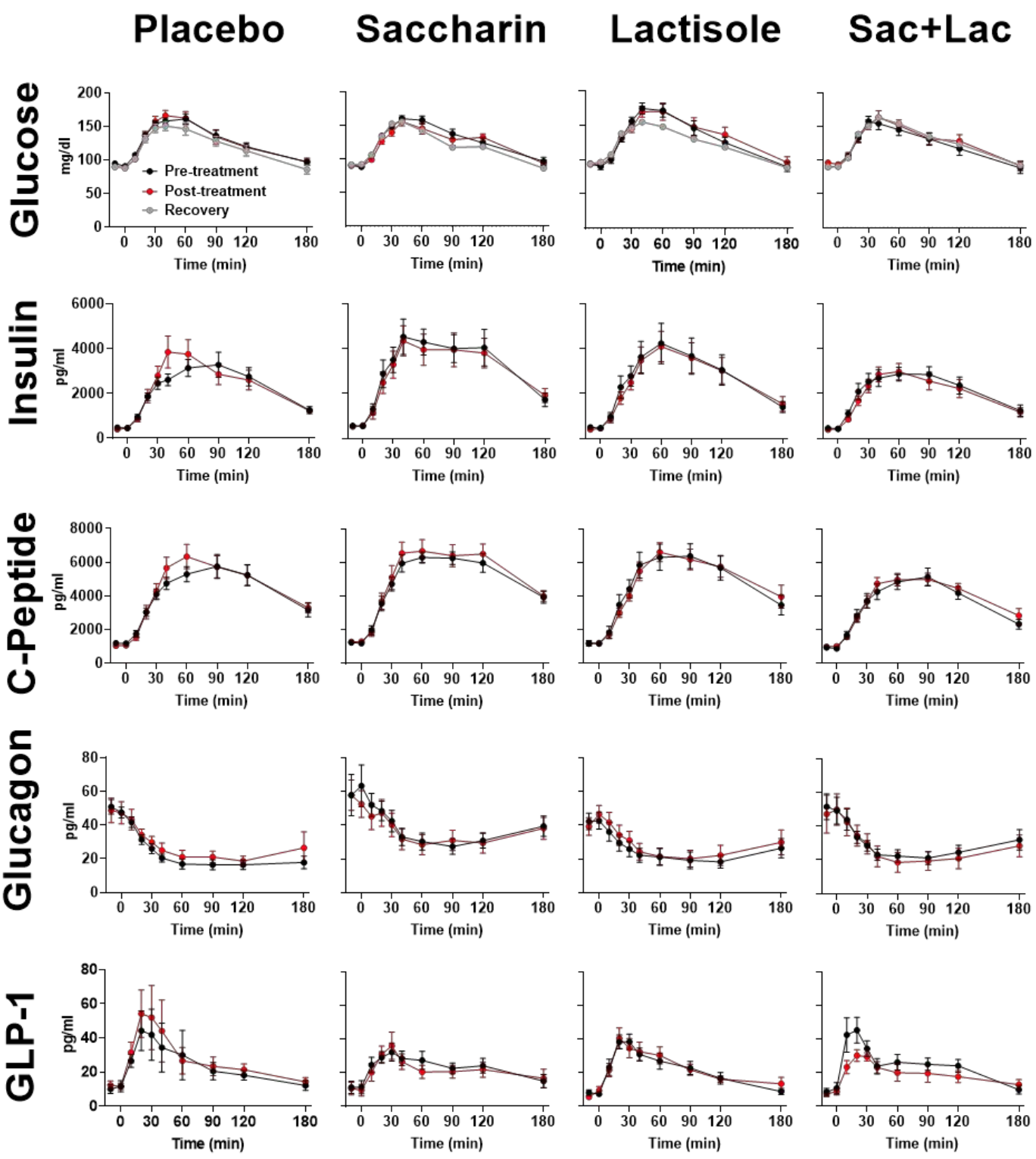
TABLE S2

Participant compliance

Treatment group	Length (d)	Compliance (%)
Placebo	14.2 ± 0.5	100.4 ± 3.7
Saccharin	14.2 ± 0.3	100.4 ± 1.8
Lactisole	14.1 ± 0.3	100.7 ± 2.2
Saccharin + Lactisole	13.7 ± 0.3	97.4 ± 2.4

Values are mean ± SEM

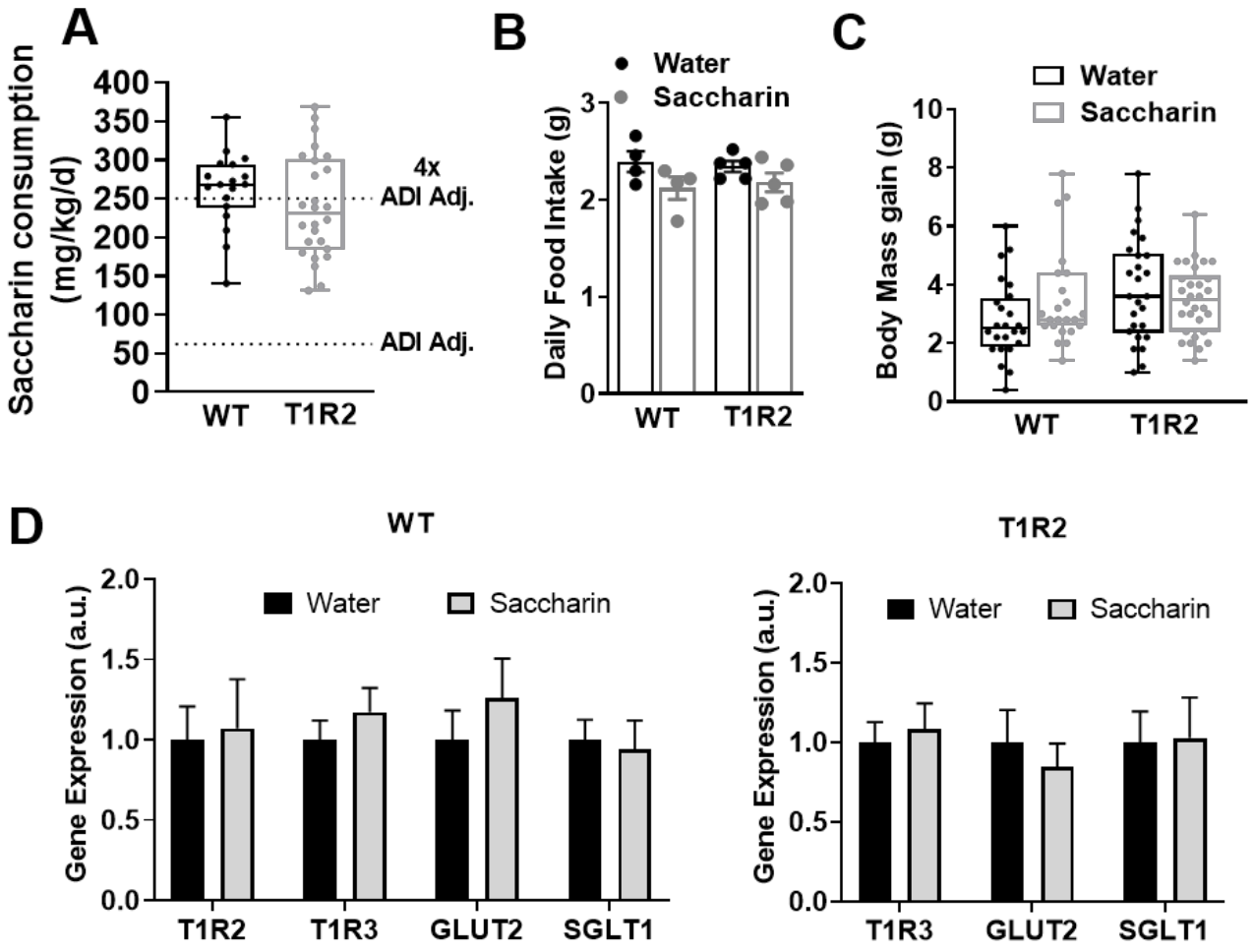
Supplemental Fig. 2



Supplemental figure 2. Longitudinal treatment effects in plasma glucose and hormonal excursions during an OGTT

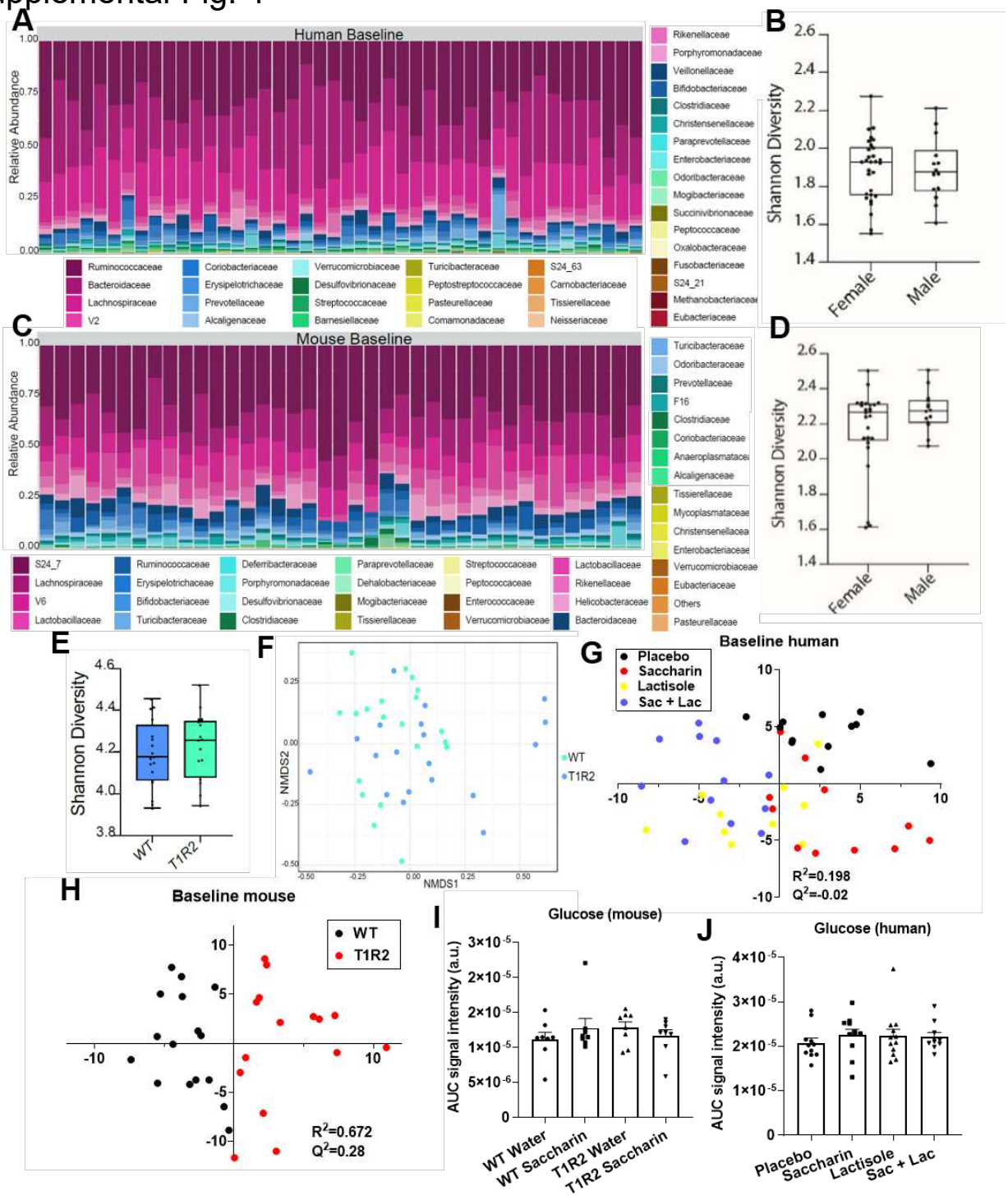
Excursions of glucose, insulin, C-peptide, glucagon and GLP-1 during OGTTs before the intervention (pre-treatment), after 2 weeks of intervention (post-treatment), and after the wash-out period (recovery, only for glucose). Two-way ANOVA repeated measures, $p>0.05$.

Supplemental Fig. 3



Supplemental figure 3. Treatment compliance and intestinal gene expression in mice
Average daily consumption of **(A)** saccharin or **(B)** food intake in WT and T1R2 mice. Dotted horizontal lines show saccharin consumption equivalent to human ADI or 4x ADI adjusted for body surface. **(C)** Body mass gain in response to treatment in mice. **(D)** Gene expression of T1R2, T1R3, SGLT1 and GLUT2 in jejunal mucosa of mice following treatment. Student's t-test, $p>0.05$ (A and D). Two-way ANOVA, $p>0.5$ (B and C).

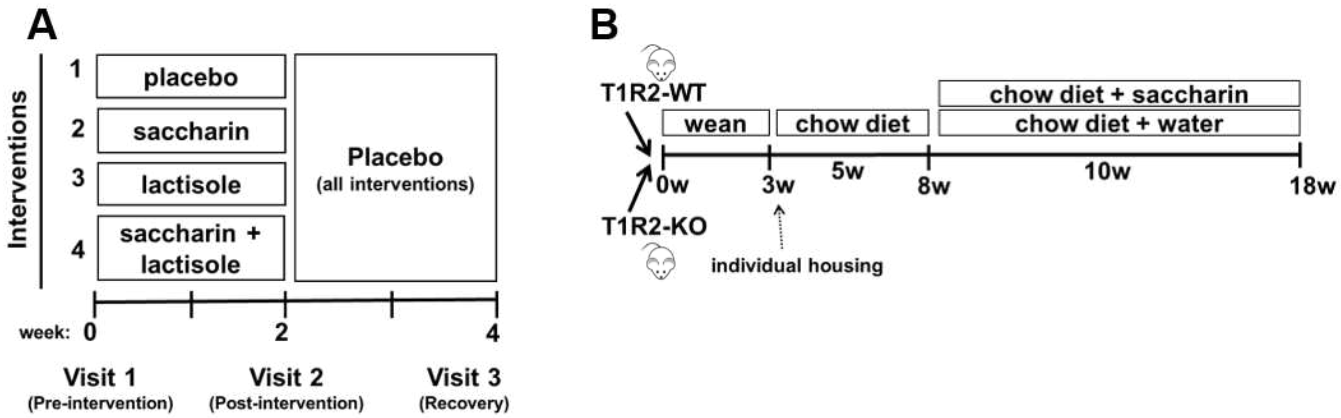
Supplemental Fig. 4



Supplemental figure 4. Assessment of baseline taxonomic abundances and diversity of gut microbiota and metabolomics in humans and mice.

(A and C) Bar chart summary showing relative abundance at the family level before treatment (baeline) in human participants or in WT and T1R2 mice. Each bar represents abundances of one subject. **(B and D)** Alpha diversity box plot (Shannon diversity metric) showing community richness between gender in human participants (Mann-Whitney U Test; p=0.77, U= 222) or in WT and T1R2 mice (Mann-Whitney U Test; p=0.987, U=152). **(E)** Alpha diversity box plot (Shannon diversity metric) showing community richness before treatment between WT and T1R2 mice (Mann-Whitney U Test; p=0.34, U=142). **(F)** Nonmetric multidimensional scaling (NMDS) plot showing community similarities between groups before treatment in WT and T1R2 mice (p< 0.19, NMDS stress = 0.209). NMDS ordination was derived from pairwise Bray-Curtis distances and statistical inferences made using PERMANOVA. **(G and H)** Differences in human and mouse fecal metabolites before treatment using orthogonal partial least squares discriminant analyses (OPLS-DA). **(I and J)** Post-treatment glucose presence in human and mouse fecal samples.

Supplemental Fig. 5



Supplemental figure 5. Experimental design of the human and mouse studies
Diagram showing the experimental design of the **(A)** human and **(B)** mouse studies.

Figures

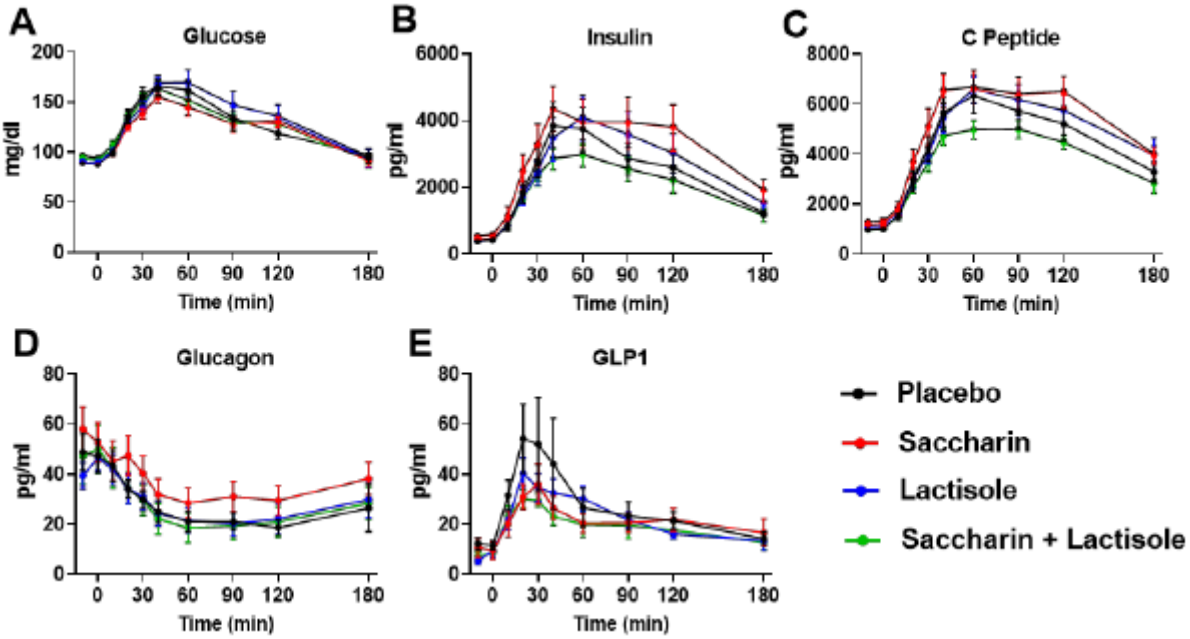


Figure 1

Effects of saccharin and/or lactisole treatment on glucose tolerance in humans Plasma excursions of (A) glucose, (B) insulin, (C) C-peptide, (D) glucagon, and (E) GLP-1 during an oral glucose challenge after 2 weeks of treatment. Two-way ANOVA repeated measures ($p>0.05$). N=10-13.

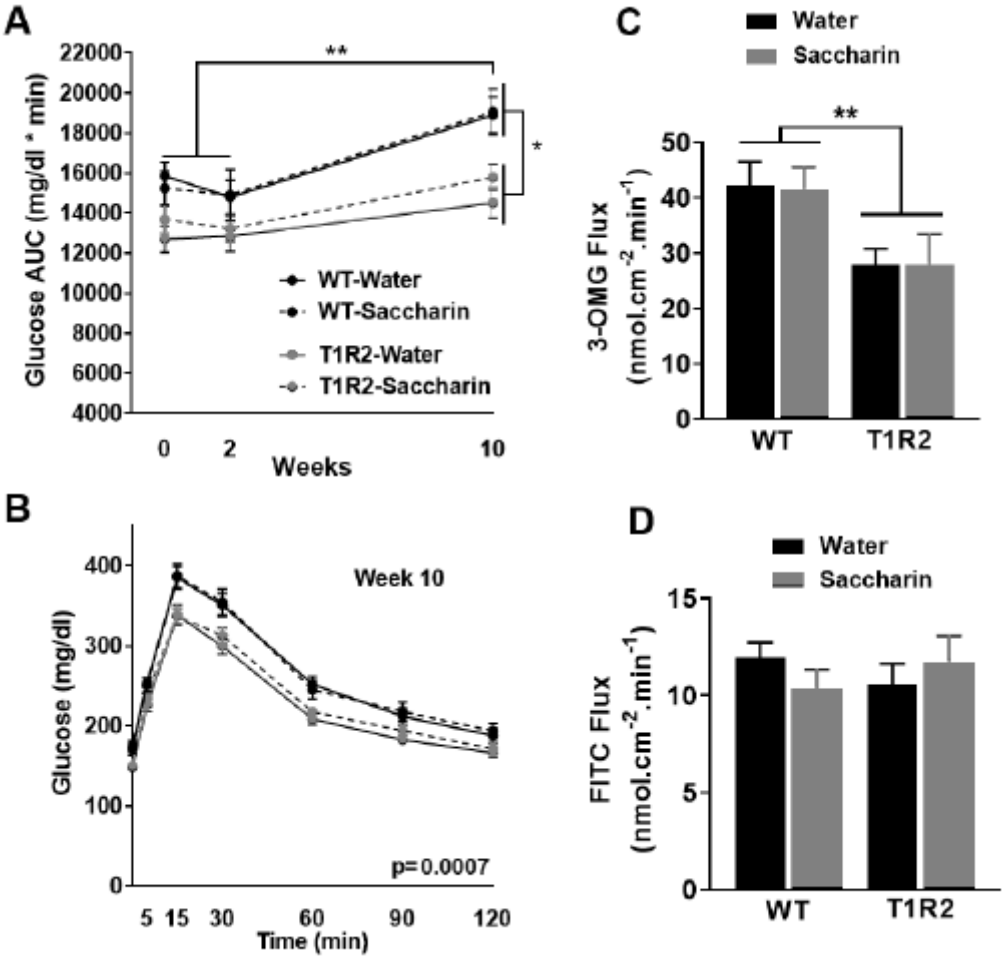


Figure 2

Effects of saccharin treatment on glucose homeostasis in mice (A) Glucose responses during an i.g.GTT expressed as area under curve (AUC) before (0 weeks), 2 and 10 weeks after saccharin treatment. Two-way ANOVA main effect; *p<0.05, WT 10-week vs. T1R2 10-week, **p<0.01 WT 0-week vs. WT 10-week). (B) Glucose excursions during an i.g.GTT in response to 10 weeks of saccharin treatment (2-way ANOVA repeated measures, p=0.0007). (C) Ex vivo glucose flux using 3-O-methy-glucose (3-OMG) in intact mouse intestines following 10 weeks of saccharin treatment. Two-way ANOVA, **p<0.01. (D) Ex vivo intestinal permeability assessed by FITC-dextran (4kDa) flux in intact mouse intestines following 10 weeks of saccharin treatment. Two-way ANOVA, p>0.05. N=23-28 for in vivo studies, n=6-11 for ex vivo studies.

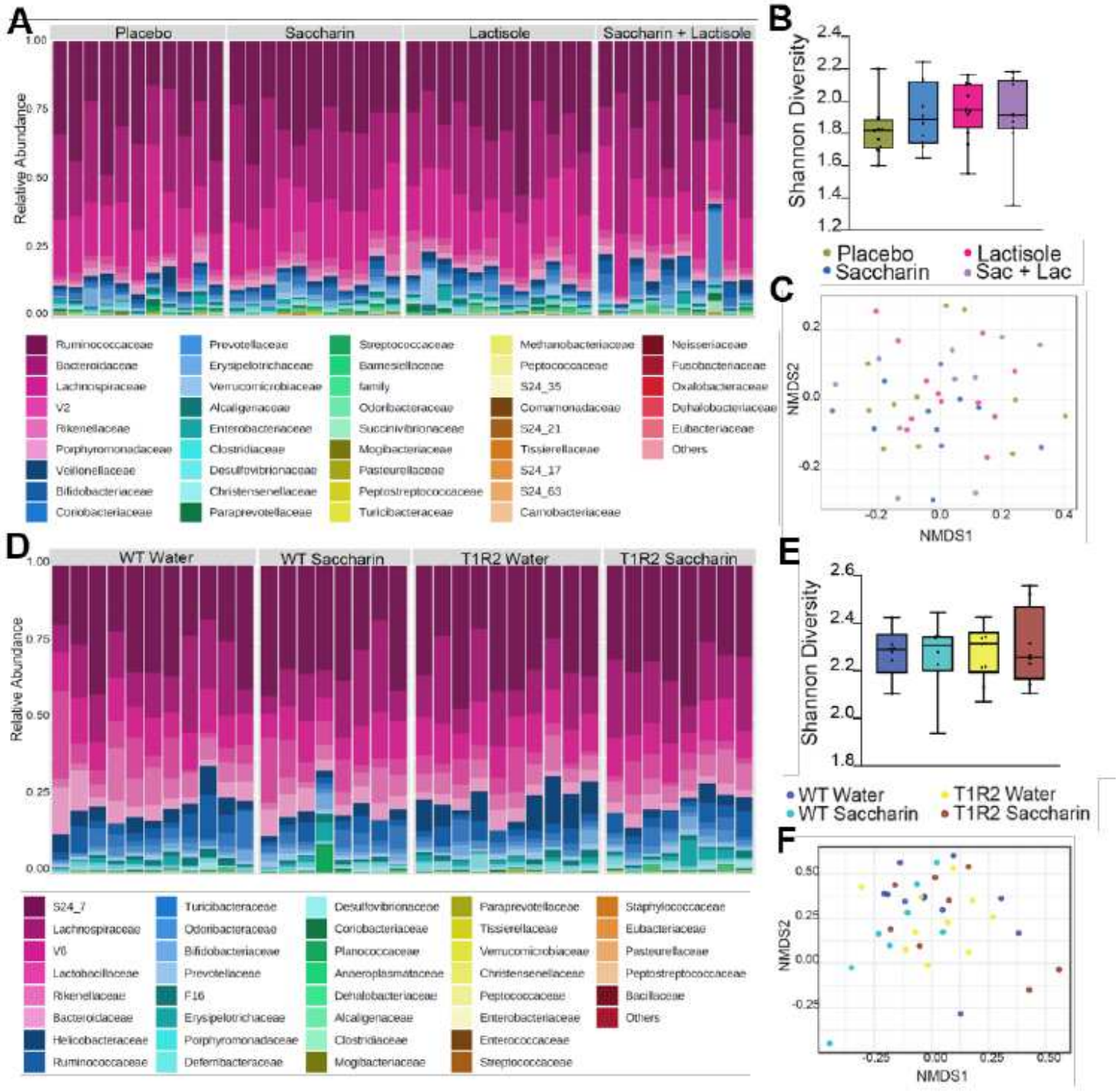


Figure 3

Taxonomic abundances and diversity of gut microbiota in response to treatments in humans and mice (A and D) Bar chart summary showing relative abundance at the family level post-treatment inhuman participants or in WT and T1R2 mice. Each bar represents abundances of one subject. (B and E) Alpha diversity box plot (Shannon diversity metric) showing community richness between groups post-treatment in human participants (Mann-Whitney U Test; p=0.156, U= 5.22) or in WT and T1R2 mice (Mann-Whitney U Test; p=0.987, U=152). (C and F) Nonmetric multidimensional scaling (NMDS) plot showing community similarities between groups post-treatment in human participants (p< 0.999, NMDS

stress = 0.2274) or WT and T1R2 mice (p< 0.111, NMDS stress = 0.209). NMDS ordination was derived from pairwise Bray-Curtis distances and statistical inferences made using PERMANOVA. N=11-13 for human studies, n=8-11 for mouse studies.

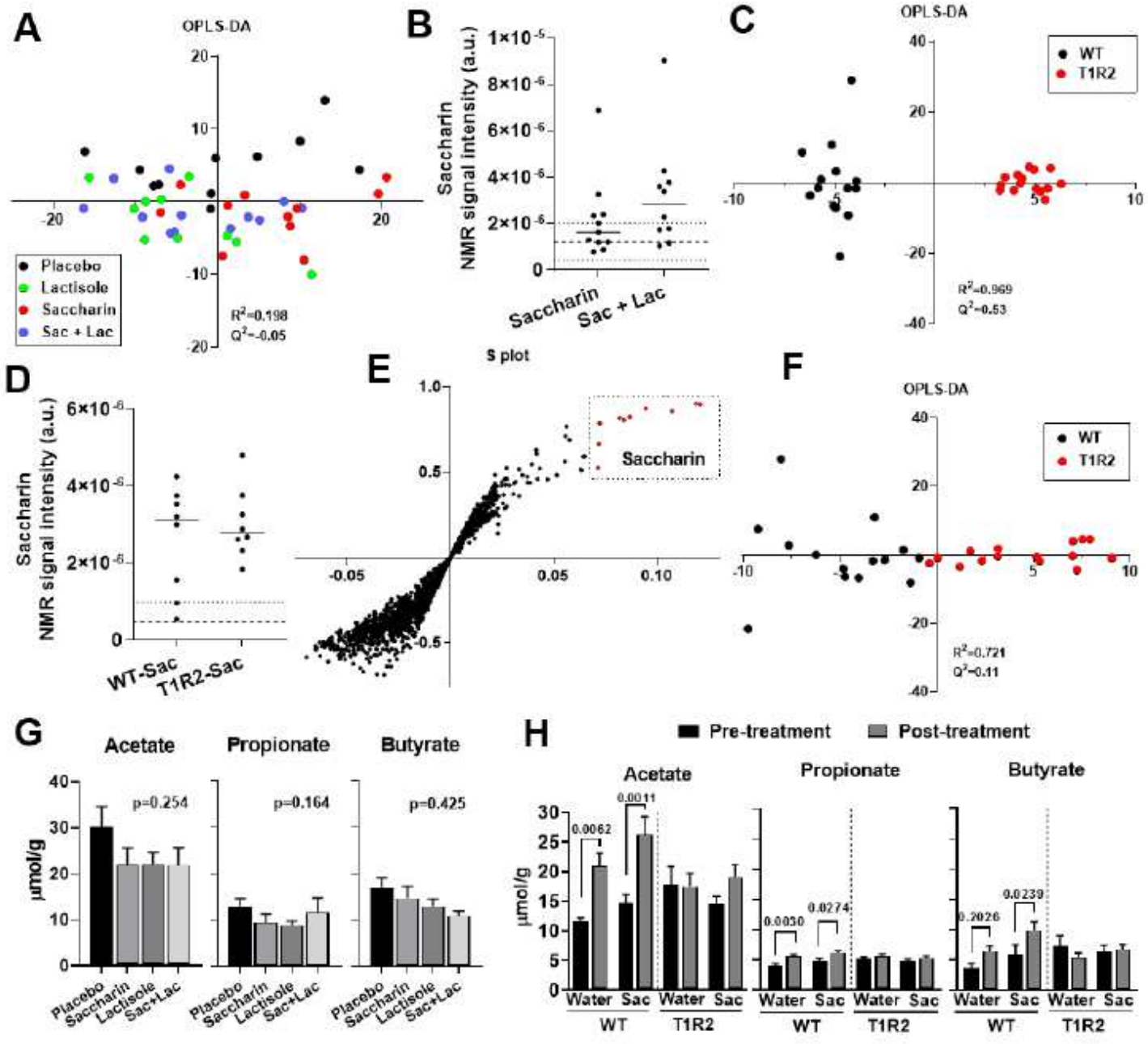


Figure 4

Fecal metabolomics in response to treatments in humans and mice (A) Differences in human fecal metabolites between treatment groups using orthogonal partial least squares discriminant analyses (OPLS-DA). (B) Post-treatment saccharin presence in human fecal samples. Dashed lines represent average noise ± SD. (C) Differences in WT andT1R2 fecal metabolites following saccharin treatment using OPLS-DA. (D) Post-treatment saccharin presence in mouse fecal samples. Dashed lines represent

average noise ± SD. (E) Metabolite distribution (S-plot) in fecal mouse samples. Metabolites attributed to saccharin shown in red. (F) Differences in WT and T1R2 fecal metabolites following saccharin treatment using OPLS-DA after removal of saccharin signals. (G) Assessment of short chain fatty acids (SCFA) following treatments in human samples. One-way ANCOVA baseline as covariate. (H) SCFA in mouse feces before (pre) and after (post) treatment. Two-way ANOVA repeated measures with post-hoc. N=11-13 for human studies, n=8 for mouse studies.