Title:

Resistant starch does not protect against nephropathy or alter intestinal permeability in an STZ-induced diabetic mouse model

Authors

Matthew Snelson[1]*, Sih Min Tan[1], Runa Lindblom[1], Gavin C. Higgins[1], Charles R. Mackay[2,3], Melinda T. Coughlan[1,4]

Affiliations:

[1]Department of Diabetes, Central Clinical School, Alfred Medical Research and Education Precinct, Monash University, Melbourne, Victoria, Australia.

[2]Infection and Immunity Program, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Australia.

[3]Department of Biochemistry and Molecular Biology, Monash University, Melbourne, Australia.

[4]Baker Heart and Diabetes Institute, Melbourne, Australia.

*matthew.snelson@monash.edu

Keywords

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Abstract

BACKGROUND: Alterations in gut homeostasis may contribute to the progression of diabetic nephropathy (DN). Resistant starch (RS) is a prebiotic fibre that promotes the production of butyrate by the gut microbiota. Butyrate acts as a ligand for G-protein-coupled-receptor (GPR)190a, decreasing intestinal inflammation and promoting gut epithelial barrier integrity in induced-colitis models. This study aimed to assess the effects of: (i) a diet supplemented with the physiological concentration of 12.5% RS and (ii) GPR109a deletion on the progression of DN.

METHODS: Male mice that were homozygous for GPR109a deletion or their wildtype littermates were rendered diabetic with streptozotocin. Mice received either a control diet or an isocaloric diet containing 12.5% RS for 24 weeks. Renal injury was assessed by urine albumin and renal histology. Ileum histology was evaluated and gastrointestinal permeability was determined *in vivo* using the FITC-dextran test and *ex vivo* by expression of ileum tight junction proteins.

RESULTS: Diabetes was associated with increased albuminuria, blood urea nitrogen, glomerulosclerosis index scores and urinary MCP-1, none of which were altered with RS supplementation or GPR109a deletion. Whilst diabetes was associated with alterations in intestinal morphology, intestinal permeability was unaltered and RS supplementation had

no effect on intestinal permeability nor morphology. GPR109a deletion did not worsen DN or alter gastrointestinal homeostasis.

CONCLUSIONS: Twenty-four weeks of supplementation of 12.5% RS does not protect against the development of DN in the STZ-induced diabetic mouse model. Further, GPR109a does not appear to play a critical role in intestinal homeostasis or in the development of DN.

1. Introduction

Diabetic nephropathy (DN) is one of the major microvascular complications of diabetes, occurring in approximately 30% of patients with type 1 diabetes [1]. Concomitant with the rise in diabetes and obesity, the prevalence of DN has been increasing rapidly, with DN now the leading cause of end stage renal disease worldwide (ESRD) [1]. There is need for a greater understanding of therapeutic options to help limit the progression of this disease.

Dietary approaches are often utilised to limit the progression of kidney disease. Recently there has been an increasing amount of research conducted into the diet-gut-kidney axis, whereby elements in the diet alter the composition of the gut microbiota and subsequent production of microbial metabolites with these changes in gut homeostasis having downstream effects on the kidneys [2]. It has been noted that patients with diabetes [3] and ESRD [4, 5] have a contraction in the bacterial taxa that produce beneficial short chain fatty acids (SCFAs). Furthermore, during ESRD, there is an increase in intestinal permeability and subsequent inflammation [6]. SCFAs have been shown to act via local metabolite-sensing receptors to reduce intestinal permeability and inflammation [7, 8]. The use of dietary therapies that directly target the gut microbiota to increase SCFA production, namely probiotics and prebiotics, have recently been investigated as possible interventions to limit DN [9].

Resistant starch (RS) is a type of dietary fibre that acts as a prebiotic. Numerous studies have illustrated that supplementation with RS is associated with an increase in the microbial production of SCFAs, particularly butyrate [9]. A commonly used source of resistant starch is high amylose maize starch (HAMS) and in an obese model of diabetes, the Zucker diabetic fatty rat, six weeks of supplementation with a diet containing 55% HAMS (Amylogel, equivalent to 20% RS) was associated with a reduction in albuminuria [10]. In addition, in the adenine-induced rat model of chronic kidney disease (CKD), supplementation of 59% HAMS diet (Hi-maize 260, equivalent to 27% RS) for three weeks was associated with improvements in creatinine clearance [11]. Conversely however, a study that supplemented a diet containing 55% HAMS (Amylogel, equivalent to 20% RS) for four weeks in male Sprague-Dawley rats with streptozotocin-induced diabetes did not find any renoprotective benefit with RS supplementation [12]. Whilst these results show promise, the concentrations of HAMS used in these studies are likely to be much greater than could be reasonably expected to be consumed by people.

GPR109a is present on a number of cells that form part of the gut including intestinal epithelial cells [13], colonic epithelial cells [14], colonic macrophages and dendritic cells [15]. GPR109a acts as a receptor for the SCFA butyrate, with no binding affinity for the other SCFAs, specifically acetate or propionate [16]. Butyrate is produced by the gut

microbiota and accumulates in high concentrations of up to 10-20 mM in the colonic lumen [14], with the majority of butyrate produced being metabolised by the colonic epithelium [17]. Activation of GPR109a in rodents via butyrate or a high fibre diet reduces the severity of experimental colitis, via the anti-inflammatory effect of GPR109a-dependent IL-18 production [7, 15, 18].

Whilst the majority of the research has been completed in diseases of the gastrointestinal tract, there is evidence that the GPR109a receptor may play a role in metabolic diseases. High glucose conditions are associated with upregulated GPR109a expression *in vitro* in Caco-2 cells [19] and *in vivo* in the retinas of patients with diabetes [20], although there is a reduction in GPR109a protein in pancreatic islets in diabetic mice and humans [21]. Pharmacological activation of the GPR109a receptor reduces glucose stimulated insulin secretion by pancreatic β cells in a GPR109a-dependent manner [22, 23] and transgenic overexpression of GPR109a is associated with reduced fasting insulin levels [24]. GPR109a expression appears to be altered in diabetes, which is postulated could have implications for the progression of the disease and its complications.

Since previous studies exploring the role of resistant starch on the development of renal injury have used supraphysiological doses over a short period of time, we sought to supplement a dose of resistant starch that could be more reasonably expected to be

consumed by people (25% HAMS Hi maize 1043, equivalent to 12.5% RS) over a longer time frame (24 weeks). In the present study, we investigated the effects of RS supplementation, at a dose of ~12.5%, on the development of DN in the STZ-induced diabetic mouse. To provide an insight into the putative mechanisms by which RS may be beneficial, this experiment was conducted in mice with or without deletion of the GPR109a receptor. Renal function and structure were assessed as well as markers of intestinal permeability and morphology.

2. Materials and Methods

2.1. Animals

Male, mice homozygous for a deletion in the GPR109a receptor, were obtained from Professor Charles Mackay (Monash University, Victoria, Australia) [18], and crossbred with wildtype (WT) C57BL6/J mice purchased from The Jackson Laboratory to produce heterozygous mice, which were then mated to produce GPR109a$^{-/-}$ knockout (KO) mice and littermate WT controls. Mice were housed in a climate-controlled animal facility that had a fixed 12-hour light and 12-hour dark cycle and provided with *ad libitum* access to water and chow. All study protocols were conducted in accordance to the principles and guidelines devised by the Alfred Medical Research & Education Precinct Animal Ethics Committee (AMREP AEC) under the guidelines laid down by the National Health and Medical Research Council (NHMRC) of Australia and had been approved by the AMREP AEC (E1487/2014/B).

2.2. Induction of Diabetes

Diabetes was induced at six weeks of age by five daily intraperitoneal injections of streptozotocin (55 mg/kg Sigma Aldrich) in sodium citrate buffer. Diabetes was confirmed by a glycated haemoglobin (GHb) greater than 8%. Two mice failed to meet

this cutoff and were excluded from any further analysis. Of those diabetic mice that were included in the analysis, the mean GHb was 11.7% (median 11.9%).

2.3. Diet Intervention

From six weeks of age, mice received either a custom-made control diet (CON) or a diet supplemented with resistant starch (RS) prepared by Speciality Feeds (Perth, Western Australia, Australia). Both of these semi-pure diets were formulated based on a modified AIN93G growth diet for rodents. These diets were isocaloric, had equivalent protein, provided as 20% g/g casein, and fat, provided as 7% g/g canola oil. Each diet contained 5% g/g sucrose, 13.2% g/g dextrinised starch and 7.4% g/g cellulose. An RS supplemented diet (SF15-015) was formulated with 25% g/g Hi-maize 1043, whilst the CON diet (SF15-021) contained an additional 20% g/g regular starch and 5% g/g cellulose in order to maintain caloric equivalency between diets. Hi-maize 1043, an RS2 starch prepared from high amylose maize starch (HAMS) which contains 50% RS [25], was provided as a raw ingredient by Ingredion (Westchester, IL, USA). Mice received these experimental diets *ad libitum* for 24 weeks.

2.4. Tissue collection

At the end of the study period, mice were anaesthetised by an intraperitoneal injection of 100 mg/kg body weight sodium pentobarbitone (Euthatal; Sigma-Aldrich, Castle Hill, Australia) followed by cardiac exsanguination. Blood was collected in syringes that had been pre-treated with sodium citrate and placed in microcentrifuge tubes containing sodium citrate as an anticoagulant. Following cardiac exsanguination blood was immediately centrifuged at 6000 rpm for 6 minutes and plasma was snap frozen on dry ice and stored at -80°C. Kidney sections were fixed in neutral buffered formalin (10% v/v). The gastrointestinal tract was dissected and the mesentery removed. Sections of the gastrointestinal tract were weighed and length measured. The ileum was flushed with chilled phosphate buffered saline. Ileum sections were fixed in paraformaldehyde (4% v/v) for 24 hours before being transferred to 4% sucrose solution and embedded in paraffin. Ileum sections were snap frozen in liquid nitrogen and stored at -80°C, for ribonucleic acid (RNA) analysis.

2.5. Glycated haemoglobin

Glycated haemoglobin (GHb) was measured in blood collected at cull using a Cobas b 101 POC system (Roche Diagnostics, Forrenstrasse, Switzerland) according to the manufacturer's instructions. The Cobas b 101 POC system has a detection range of between 4-14%, with any sample with a GHb less than 4% designated as low and samples with a GHb of greater than 14% designated high.

2.6. In Vivo Intestinal Permeability Assay

Intestinal permeability was assessed *in vivo* using the previously described dextran FITC technique [26], during the week prior to cull. In brief, mice were fasted for a minimum of four hours and received an oral gavage of a 125 mg/mL solution of dextran FITC equivalent to 500 mg/kg body weight. After one hour, approximately 120 µL was collected from the tail vein using heparinised capillary tubes. Blood was centrifuged at 6000 rpm for 6 minutes, plasma collected and the fluorescence in plasma samples was determined in relation to a standard dilutions set, using a fluorescence spectrophotometer (BMG Labtech, Ortenberg, Germany) set to excitation 490nm, emission 520nm. The intra- and interassay coefficients of variation were 3.2 and 8.9%, respectively.

2.7. Body Composition

Fat mass and lean body mass were determined using a 4-in-1 EchoMRI body composition analyser (Columbus Instruments, Columbus, OH, USA), which measures fat mass, lean mass and total water content using nuclear magnetic resonance relaxometry [27]. After calibration with canola oil, mice were placed in a red-coloured acrylic animal specimen holder, which was placed into the EchoMRI gantry and body composition was measured. The weight of mice prior to being placed in the body composition analyser was used for calculation of percentage fat and lean mass.

2.8. Metabolic Caging, Urine and Plasma Analyses

After 23 weeks of experimental diet, mice were housed individually in metabolic cages (Iffa Credo, L'Arbresle, France) for 24 hours for urine collection and measurement of urine output and food and water intake. The animals received *ad libitum* access to food and water during this period. Urine was stored at -80°C until required for analyses. Urinary albumin was determined using a mouse specific ELISA (Bethyl Laboratories, Montgomery, TX, USA; Cat No. E90-134) according to the kit protocol. The intra- and interassay coefficients of variation were 7.3 and 8.9%, respectively. Urinary and plasma monocyte chemoattractant protein-1 (MCP-1) was measured using a commercially available ELISA kit (R&D Systems, Minneapolis MN, USA; Cat. No. MJE00B) as per the kit protocol. The intra- and interassay coefficients of variation were 2.8 and 4.0%, respectively. Blood urea nitrogen was analysed using a commercially available colorimetric urea assay (Arbor Assays, Ann Arbor, MI, USA; Cat. No. K024-H1) as per the kit protocol. The intra- and interassay coefficients of variation were 4.5 and 3.9%, respectively.

2.9. Kidney and Ileum Histology

Kidneys were fixed in 10% (v/v) neutral buffered formalin prior to embedding in paraffin. Kidney sections (3 μm) were stained with periodic acid-Schiff (PAS) and

assessed in a semiquantitative manner, whereby a blinded researcher assessed the level of glomerulosclerosis for each glomerulus and assigned an integer score of between 1 and 4, indicative of the level of severity of glomerulosclerosis. Twenty-five glomeruli were scored per animal, and these scores were averaged to provide a glomerulosclerosis score index (GSI) for each animal, as previously described [28]. Ileal sections were fixed in 4% paraformaldehylde for 24 hours, followed by a transfer to 4% sucrose, and subsequent embedding in paraffin. Ileal sections (5 μm) were stained with haemotoxylin and eosin (H&E) and images were captured using a brightfield microscope (Nikon Eclipse-Ci; Nikon, Tokyo, Japan) coupled with a digital camera (Nikon DS-Fi3; Nikon, Tokyo, Japan). Morphological measurements of villus height and crypt depth were conducted using ImageJ (Version 1.52a). Villus height was measured from the topmost point of the villus to the crypt transition, whilst the crypt depth was measured as the invagination between two villi to the basement membrane.

2.10. Quantitative RT-PCR

RNA was isolated from snap frozen ileum sections using a phenol-chloroform extraction method and used to synthesise cDNA, as previously described [28]. Gene expression of zonulin and occludin was determined using TaqMan (Life Technologies) and SYBR Green reagents (Applied Biosystems), respectively. Gene expression was normalised to 18S mRNA utilising the ΔΔCt method and reported as fold change compared to WT non-diabetic mice receiving the control diet.

2.11. Statistical Analyses

Data were analysed by two-way ANOVA with the Tukey posthoc test for multiple comparisons. Analyses were performed using GraphPad Prism Version 7.01 (GraphPad Software, La Jolle, CA, USA). Data are shown as mean ± SEM. A value of $p<0.05$ was considered statistically significant.

3. Results

3.1. Metabolic and Phenotypic parameters

As expected, mice with STZ-induced diabetes had greater glycated haemoglobin concentrations and lower body weight (Table 1). Diabetes was also associated with a decrease in relative fat mass and an increased lean mass percentage, and greater 24-hour urine output and water intake (Table 1). Neither deletion of the GPR109a receptor nor supplementation with RS was associated with any alterations in glycated haemoglobin, body weight or body composition. Diabetes was also associated with an increased relative liver weight and a decreased spleen weight (Table 1). Diabetes was associated with an increase in small intestinal length ($p<0.001$, Fig 1A) and caecum length ($p<0.0001$, Fig 1B). Furthermore, diabetes was associated with an increase in the relative weight of the small intestine ($p<0.0001$, Fig 1D), caecum ($p<0.0001$, Fig 1E) and the colon ($p<0.0001$, Fig 1F). Resistant starch was associated with a significant increase in caecal weight and length in diabetic mice but not in non-diabetic mice (Fig 1B, 1E). Resistant starch supplementation was also associated with an increase in colon weight in most groups (Fig 1F).

3.2. Renal Injury and Inflammation

Diabetes was associated with an increase in both albuminuria ($p<0.0001$, Fig 2A) and blood urea nitrogen ($p<0.0001$, Fig 2B), whilst there was no effect of either RS

supplementation nor GPR109a gene deletion on either of these renal functional parameters. Diabetes was associated with an increase in the urinary output of MCP-1 ($p<0.0001$, Fig 2C), whilst there was no observed effect of either RS supplementation nor deletion of the GPR109a receptor on urinary MCP-1 output. Neither diabetes, GPR109a deletion nor resistant starch had an effect of plasma MCP-1 concentrations (data not shown). Diabetes was associated with an increase in proportional kidney weight compared with non-diabetic mice ($p<0.0001$, Fig 2D). Within diabetic mice, deletion of the GPR109a receptor was associated with a further increase in relative kidney weight ($p<0.05$, Fig 2D), an effect that was not observed in non-diabetic mice. Diabetes was associated with an increase in the glomerulosclerotic index histology score ($p<0.0001$, Figure 3A & B). No effect on GSI was observed with either deletion of the GPR109a receptor nor resistant starch supplementation.

3.3. Intestinal Permeability and Morphology

Diabetes was associated with an increase in villi height ($p<0.0001$, Fig 4A & C) and a reduction in crypt depth ($p<0.001$, Fig 4B & C) in the ileum. Resistant starch was associated with a trend towards an increase in villi height in non-diabetic wildtype and the diabetic knockout groups (Fig 4A). In non-diabetic mice, deletion of the GPR109a receptor was associated with a trend towards increased villi height ($p=0.06$, Fig 4A). Interestingly, despite these morphological changes in the ileum with diabetes, diabetes

did not induce any alteration in intestinal permeability, as measured by an *in vivo* intestinal permeability procedure (dextran-FITC, Fig 4D) or by gene expression of the tight junction proteins occludin (Fig 4E) or zonulin (Fig 4F).

4. Discussion

The current study explored the effects of resistant starch supplementation with or without genetic deletion of the butyrate receptor, GPR109a, on the progression of DN. No effect was observed of either RS supplementation nor GPR109a deletion on markers of kidney injury. Furthermore, neither RS nor GPR109a deletion had any effect on intestinal morphology nor permeability.

Whilst RS has been shown to reduce albuminuria in the adenine-induced CKD model [11] and the Zucker diabetic fatty rat, an obesity driven model of diabetes [10], a recent study that utilised the STZ model in Sprague-Dawley rats showed that RS had no protective effect on albuminuria [12]. This is consistent with the findings reported here, that in STZ-induced diabetic mice RS supplementation did not alter albuminuria or blood urea levels. This suggests that while RS may be beneficial at reducing albuminuria in other animal models it does not appear to have a nephroprotective effect in the STZ-induced diabetic model.

The STZ-induced diabetic mouse is a chemically induced model of insulin-deficient diabetes. Other commonly used genetic animal models that have been considered of importance in studying the pathophysiology of type 1 diabetes mellitus (T1DM) include the NOD mouse and the bio breeding diabetes prone (BB-DP) rat [29]. Patients with

T1DM have been shown to have increased intestinal permeability compared with healthy controls [30-32], which has also been observed in both the NOD mouse [33] and BB-DP rat [34-36]. Furthermore, it has been shown that in the BB-DP rat model impairment of the intestinal barrier integrity, as measured in vivo by the lactulose/mannitol ratio, precedes the development of hyperglycaemia [34] and insulitis [35]. Human participants who had a first degree relative with T1DM were screened for autoantibodies to islet cells or insulin, with the presence of two or more autoantibodies indicating preclinical T1DM: in those subjects with "preclinical" T1DM there was increased intestinal permeability when compared with healthy controls [32], suggesting that increased intestinal permeability precedes diabetes development. The use of a hydrolysed casein diet intervention in the BB-DP rat was associated with improvements of both in vivo and ex vivo measurements of intestinal permeability, a decrease in serum zonulin levels and a reduction in the development of autoimmune diabetes [37]. Greater intestinal permeability also correlated with an earlier onset of diabetes in this model [37] and it has recently been suggested that increased intestinal permeability may be an important driver in the progression of T1DM [38]. Given that STZ induces diabetes by directly killing pancreatic β cells, it is possible that there is insufficient opportunity for a gut directed mechanism to alter intestinal homeostasis, thus explaining the lack of an effect of RS in modulating intestinal permeability and renal dysfunction as was observed in studies utilising STZ-induced diabetes. In view of the fact that the intestinal aberrations that occur in the BB-DP rat are recognised to be indicative of the changes that occur in human

T1DM [32], it may be prudent to test RS as a therapeutic intervention in this particular animal model.

Previous animal studies utilising RS have observed differing results in terms of an effect on body weight, with some studies finding no effect of supplementation on body weight [39-41], whereas others observed a decrease in body weight with supplementation [42-44]. It should be noted that those animal studies which observed a decrease in body weight tended to have higher doses of RS, with up to 50% of the diet consisting of HAMS [42]. In the current series of experiments, it was chosen to supplement with 25% HAMS, which is more consistent with what could be tolerated by human participants. Results from human studies have shown that participants receiving RS supplemented diets tend to remain weight stable [45-47]. These results indicate that there are no effects of long-term RS supplementation on body weight or composition.

It was observed that STZ-induced diabetes was associated with an increase in villi height and a reduction in crypt depth in the ileum. Previous studies have reported that STZ-induced model of diabetes is associated with increases in villi height in the jejunum [48-50]. In the ileum, one study found no difference in villi height with STZ-diabetes in Wistar rats [48] whereas another study utilising STZ-induced diabetes in RIP-I/hIFNβ transgenic mice observed that diabetes was associated with an increase in villi height,

however no differences were observed with crypt depth [51]. Intestinal morphological changes observed with STZ-diabetes in the current study are broadly consistent with findings from previously reported studies. It was interesting to note that, despite these changes observed in intestinal morphology, no alterations in intestinal permeability were observed in the current study.

Furthermore, no effect was observed with chronic RS supplementation on either villi height or crypt depth in the ileum. Previous studies have indicated that RS supplementation is associated with increase in colonic crypt depths in pigs [52] and rats [53], although one study in pigs saw a reduction [54]. Notably chronic RS supplementation was not associated with any changes in crypt depth in the ileum or jejunum of pigs [54], and another study which utilised inulin supplementation saw no effect on villi heights in the ileum or jejunum of pigs [55]. RS supplementation was associated with increases in the weight and length of components of the gastrointestinal tract; this effect was most pronounced in the colon and caecum and is consistent with an expansion of saccharolytic bacteria, which has previously been observed to occur with RS supplementation [56, 57], as well as an increase in microbial fermentation [58]. The disparity reported in published studies in morphological changes between the intestine and colon may be due to RS supplementation being associated with bacterial expansion in the colon, as observed in the increased caecal and colon weight observed in the current study.

This is the first study to assess the effects of deletion of the GPR109a receptor on kidney injury and these findings show that deletion of this receptor was not associated with any change in renal injury. Given the hypothesis that renal injury would occur downstream of alterations in intestinal permeability, and there was no effect of GPR109a deletion on in vivo assessment of intestinal permeability, this should perhaps not come as a surprise finding. *In vitro*, GPR109a knockdown inhibits butyrate-induced increases in the tight junction protein Claudin-3, suggesting that GPR109a may have a role in the integrity of the intestinal epithelial barrier [8]. However *in vivo*, whilst deletion of GPR109a was associated with a trend towards an increase in intestinal permeability in an induced food allergy model [59] there was no effect in otherwise healthy mice [7], indicating that deletion of the GPR109a receptor alone is insufficient to alter intestinal permeability. While GPR109a is expressed at a relatively low level in the kidney [60] it is unlikely that butyrate would reach sufficient concentrations in the renal blood flow to effectively induce a biological response as a result of ligation to the receptor. Furthermore, there is redundancy in the metabolite-sensing GPCR family, with butyrate being recognised by GPR109a, GPR41 and GPR43 receptors. Indeed, it has been suggested that given this redundancy, single knockout models are insufficient to fully elucidate the effects of these receptors, and that double or triple knockout models are required [61].

In conclusion, this study shows that long term resistant starch supplementation at a dosage that would reasonably be expected to be consumed by humans, did not alleviate albuminuria in the STZ-induced diabetes model. Whilst diabetes was associated with alterations in intestinal morphology, there was no change in intestinal permeability. It would be pertinent to consider resistant starch supplementation in other rodent models of diabetes that may be more representative of the intestinal changes that occur with diabetes in humans. Finally, this study indicates that GPR109a does not play a critical role in the development of DN nor gastrointestinal homeostasis.

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Declarations of interest

None.

References

1. Alicic, R.Z., M.T. Rooney, and K.R. Tuttle, *Diabetic Kidney Disease: Challenges, Progress, and Possibilities.* Clin J Am Soc Nephrol, 2017. **12**(12): p. 2032-2045.
2. Evenepoel, P., R. Poesen, and B. Meijers, *The gut-kidney axis.* Pediatr Nephrol, 2017. **32**(11): p. 2005-2014.
3. Forslund, K., et al., *Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota.* Nature, 2015. **528**: p. 262.
4. Wong, J., et al., *Expansion of Urease- and Uricase-Containing, Indole- and p-Cresol-Forming and Contraction of Short-Chain Fatty Acid-Producing Intestinal Microbiota in ESRD.* American Journal of Nephrology, 2014. **39**(3): p. 230-237.
5. Jiang, S., et al., *Alteration of the gut microbiota in Chinese population with chronic kidney disease.* Scientific Reports, 2017. **7**: p. 2870.
6. Meijers, B., et al., *Intestinal Barrier Function in Chronic Kidney Disease.* Toxins, 2018. **10**(7): p. 298.
7. Chen, G., et al., *Sodium Butyrate Inhibits Inflammation and Maintains Epithelium Barrier Integrity in a TNBS-induced Inflammatory Bowel Disease Mice Model.* EBioMedicine, 2018. **30**: p. 317-325.
8. Feng, W., et al., *Sodium Butyrate Attenuates Diarrhea in Weaned Piglets and Promotes Tight Junction Protein Expression in Colon in a GPR109A-Dependent Manner.* Cellular Physiology and Biochemistry, 2018. **47**(4): p. 1617-1629.

9. Snelson, M., M.T. Coughlan, and N.J. Kellow, *Modulation of the Gut Microbiota by Resistant Starch as a Treatment of Chronic Kidney Diseases: Evidence of Efficacy and Mechanistic Insights.* Advances in Nutrition 2019. **10**(2): p. 303-320.

10. Koh, G.Y., et al., *Dietary Resistant Starch Prevents Urinary Excretion of Vitamin D Metabolites and Maintains Circulating 25-Hydroxycholecalciferol Concentrations in Zucker Diabetic Fatty Rats.* The Journal of Nutrition, 2014. **144**(11): p. 1667-1673.

11. Vaziri, N.D., et al., *High Amylose Resistant Starch Diet Ameliorates Oxidative Stress, Inflammation, and Progression of Chronic Kidney Disease.* PLoS ONE, 2014. **9**(12): p. e114881.

12. Koh, G.Y., et al., *Consumption of Dietary Resistant Starch Partially Corrected the Growth Pattern Despite Hyperglycemia and Compromised Kidney Function in Streptozotocin-Induced Diabetic Rats.* Journal of Agricultural and Food Chemistry, 2016. **64**(40): p. 7540-7545.

13. Smith, P.M., et al., *The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic Treg Cell Homeostasis.* Science, 2013. **341**(6145): p. 569-573.

14. Thangaraju, M., et al., *GPR109A is a G-protein-coupled receptor for the bacterial fermentation product butyrate and functions as a tumor suppressor in colon.* Cancer research, 2009. **69**(7): p. 2826-2832.

15. Singh, N., et al., *Activation of Gpr109a, receptor for niacin and the commensal metabolite butyrate, suppresses colonic inflammation and carcinogenesis.* Immunity, 2014. **40**(1): p. 128-139.

16. Kasubuchi, M., et al., *Dietary Gut Microbial Metabolites, Short-chain Fatty Acids, and Host Metabolic Regulation.* Nutrients, 2015. **7**(4): p. 2839-2849.

17. Hamer, H.M., et al., *Review article: the role of butyrate on colonic function.* Alimentary Pharmacology & Therapeutics, 2008. **27**(2): p. 104-119.

18. Macia, L., et al., *Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome.* Nat Commun, 2015. **6**.

19. Wong, T., L. Chan, and P. Leung, *Involvement of the Niacin Receptor GPR109a in the LocalControl of Glucose Uptake in Small Intestine of Type 2Diabetic Mice.* Nutrients, 2015. **7**(9): p. 7543-7561.

20. Gambhir, D., et al., *GPR109A as an Anti-Inflammatory Receptor in Retinal Pigment Epithelial Cells and Its Relevance to Diabetic Retinopathy.* Investigative Ophthalmology & Visual Science, 2012. **53**(4): p. 2208-2217.

21. Wang, N., et al., *Niacin receptor GPR109A inhibits insulin secretion and is down-regulated in type 2 diabetic islet beta-cells.* General and Comparative Endocrinology, 2016. **237**: p. 98-108.

22. Li, M.H.-M., et al., *Nicotinic Acid Inhibits Glucose-Stimulated Insulin Secretion Via the G Protein-Coupled Receptor PUMA-G in Murine Islet β Cells.* Pancreas, 2011. **40**(4): p. 615-621.

23. Chen, L., et al., *Niacin-induced hyperglycemia is partially mediated via niacin receptor GPR109a in pancreatic islets.* Molecular and Cellular Endocrinology, 2015. **404**: p. 56-66.

24. Masuda, Y., N. Kurikawa, and T. Nishizawa, *Overexpressing human GPR109A leads to pronounced reduction in plasma triglyceride levels in BAC transgenic rats.* Atherosclerosis, 2018. **272**: p. 182-192.

25. Le Leu, R., et al., *Effect of high amylose maize starches on colonic fermentation and apoptotic response to DNA-damage in the colon of rats.* Nutrition & Metabolism, 2009. **6**(1): p. 1-9.

26. Cani, P.D., et al., *Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability.* Gut, 2009. **58**(8): p. 1091-1103.

27. Lancaster, G.I. and D.C. Henstridge, *Body Composition and Metabolic Caging Analysis in High Fat Fed Mice.* JoVE, 2018(135): p. e57280.

28. Coughlan, M.T., et al., *Deficiency in Apoptosis-Inducing Factor Recapitulates Chronic Kidney Disease via Aberrant Mitochondrial Homeostasis.* Diabetes, 2016. **65**(4): p. 1085-98.

29. Graham, M.L. and H.-J. Schuurman, *Validity of animal models of type 1 diabetes, and strategies to enhance their utility in translational research.* European Journal of Pharmacology, 2015. **759**: p. 221-230.

30. Damci, T., et al., *Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in Type 1 diabetic patients.* European Journal of Clinical Investigation, 2003. **33**(5): p. 397-401.

31. Secondulfo, M., et al., *Ultrastructural mucosal alterations and increased intestinal permeability in non-celiac, type I diabetic patients.* Digestive and Liver Disease, 2004. **36**(1): p. 35-45.

32. Bosi, E., et al., *Increased intestinal permeability precedes clinical onset of type 1 diabetes.* Diabetologia, 2006. **49**(12): p. 2824-2827.

33. Lee, A.S., et al., *Gut barrier disruption by an enteric bacterial pathogen accelerates insulitis in NOD mice.* Diabetologia, 2010. **53**(4): p. 741-748.

34. Watts, T., et al., *Role of the intestinal tight junction modulator zonulin in the pathogenesis of type I diabetes in BB diabetic-prone rats.* Proceedings of the National Academy of Sciences of the United States of America, 2005. **102**(8): p. 2916-2921.

35. Meddings, J.B., et al., *Increased gastrointestinal permeability is an early lesion in the spontaneously diabetic BB rat.* American Journal of Physiology-Gastrointestinal and Liver Physiology, 1999. **276**(4): p. G951-G957.

36. Neu, J., et al., *Changes in intestinal morphology and permeability in the biobreeding rat before the onset of type 1 diabetes.* J Pediatr Gastroenterol Nutr, 2005. **40**(5): p. 589-95.

37. Visser, J.T., et al., *Restoration of impaired intestinal barrier function by the hydrolysed casein diet contributes to the prevention of type 1 diabetes in the diabetes-prone BioBreeding rat.* Diabetologia, 2010. **53**(12): p. 2621-8.

38. Mu, Q., et al., *Leaky Gut As a Danger Signal for Autoimmune Diseases.* Frontiers in immunology, 2017. **8**: p. 598-598.

39. Haenen, D., et al., *A Diet High in Resistant Starch Modulates Microbiota Composition, SCFA Concentrations, and Gene Expression in Pig Intestine.* The Journal of Nutrition, 2013. **143**(3): p. 274-283.

40. Abell, G.C.J., et al., *Dietary resistant and butyrylated starches have different effects on the faecal bacterial flora of azoxymethane-treated rats.* British Journal of Nutrition, 2011. **105**(10): p. 1480-1485.

41. Henningsson, Å.M., et al., *Influences of dietary adaptation and source of resistant starch on short-chain fatty acids in the hindgut of rats.* British Journal of Nutrition, 2003. **89**(03): p. 319-327.

42. Aziz, A.A., et al., *Dietary starch type affects body weight and glycemic control in freely fed but not energy-restricted obese rats.* The Journal of Nutrition, 2009. **139**(10): p. 1881-9.

43. Belobrajdic, D.P., et al., *Dietary resistant starch dose-dependently reduces adiposity in obesity-prone and obesity-resistant male rats.* Nutr Metab (Lond), 2012. **9**(1): p. 93.

44. Lerer-Metzger, M., et al., *Effects of long-term low-glycaemic index starchy food on plasma glucose and lipid concentrations and adipose tissue cellularity in normal and diabetic rats.* Br J Nutr, 1996. **75**(5): p. 723-32.

45. de Roos, N., et al., *Resistant starch has little effect on appetite, food intake and insulin secretion of healthy young men.* Eur J Clin Nutr, 1995. **49**(7): p. 532-41.

46. Robertson, M.D., et al., *Insulin-sensitizing effects of dietary resistant starch and effects on skeletal muscle and adipose tissue metabolism.* Am J Clin Nutr, 2005. **82**(3): p. 559-67.

47. Johnston, K.L., et al., *Resistant starch improves insulin sensitivity in metabolic syndrome.* Diabet Med, 2010. **27**(4): p. 391-7.

48. Thulesen, J., et al., *Diabetic intestinal growth adaptation and glucagon-like peptide 2 in the rat: effects of dietary fibre.* Gut, 1999. **45**(5): p. 672-8.

49. Min, X.H., et al., *Abnormal differentiation of intestinal epithelium and intestinal barrier dysfunction in diabetic mice associated with depressed Notch/NICD transduction in Notch/Hes1 signal pathway.* Cell Biol Int, 2014. **38**(10): p. 1194-204.

50. McAnuff, M.A., et al., *Alterations in intestinal morphology of streptozotocin-induced diabetic rats fed Jamaican bitter yam (Dioscorea polygonoides) steroidal sapogenin extract.* Nutrition Research, 2003. **23**(11): p. 1569-1577.

51. Domènech, A., et al., *Morphofunctional changes underlying intestinal dysmotility in diabetic RIP-I/hIFNβ transgenic mice.* International journal of experimental pathology, 2011. **92**(6): p. 400-412.

52. Mentschel, J. and R. Claus, *Increased butyrate formation in the pig colon by feeding raw potato starch leads to a reduction of colonocyte apoptosis and a shift to the stem cell compartment.* Metabolism, 2003. **52**(11): p. 1400-1405.

53. Paturi, G., et al., *Effects of Potato Fiber and Potato-Resistant Starch on Biomarkers of Colonic Health in Rats Fed Diets Containing Red Meat.* Journal of Food Science, 2012. **77**(10): p. H216-H223.

54. Nofrarías, M., et al., *Long-term intake of resistant starch improves colonic mucosal integrity and reduces gut apoptosis and blood immune cells.* Nutrition, 2007. **23**(11): p. 861-870.

55. Lacorn, M., M. Goerke, and R. Claus, *Inulin-coated butyrate increases ileal MCT1 expression and affects mucosal morphology in the porcine ileum by reduced apoptosis.* Journal of Animal Physiology and Animal Nutrition, 2010. **94**(5): p. 670-676.

56. Bird, A.R., et al., *Two high-amylose maize starches with different amounts of resistant starch vary in their effects on fermentation, tissue and digesta mass accretion, and bacterial populations in the large bowel of pigs.* British Journal of Nutrition, 2007. **97**(1): p. 134-144.

57. Brown, I., et al., *Fecal Numbers of Bifidobacteria Are Higher in Pigs Fed Bifidobacterium longum with a High Amylose Cornstarch Than with a Low Amylose Cornstarch.* The Journal of Nutrition, 1997. **127**(9): p. 1822-1827.

58. Charrier, J.A., et al., *High fat diet partially attenuates fermentation responses in rats fed resistant starch from high-amylose maize.* Obesity (Silver Spring), 2013. **21**(11): p. 2350-5.

59. Tan, J., et al., *Dietary Fiber and Bacterial SCFA Enhance Oral Tolerance and Protect against Food Allergy through Diverse Cellular Pathways.* Cell Reports, 2016. **15**(12): p. 2809-2824.

60. Rajkumar, P., et al., *Identification and Characterization of Novel Renal Sensory Receptors.* PLoS ONE, 2014. **9**(10): p. e111053.

61. Tan, J.K., et al., *Metabolite-Sensing G Protein–Coupled Receptors—Facilitators of Diet-Related Immune Regulation.* Annual Review of Immunology, 2017. **35**(1): p. 371-402.

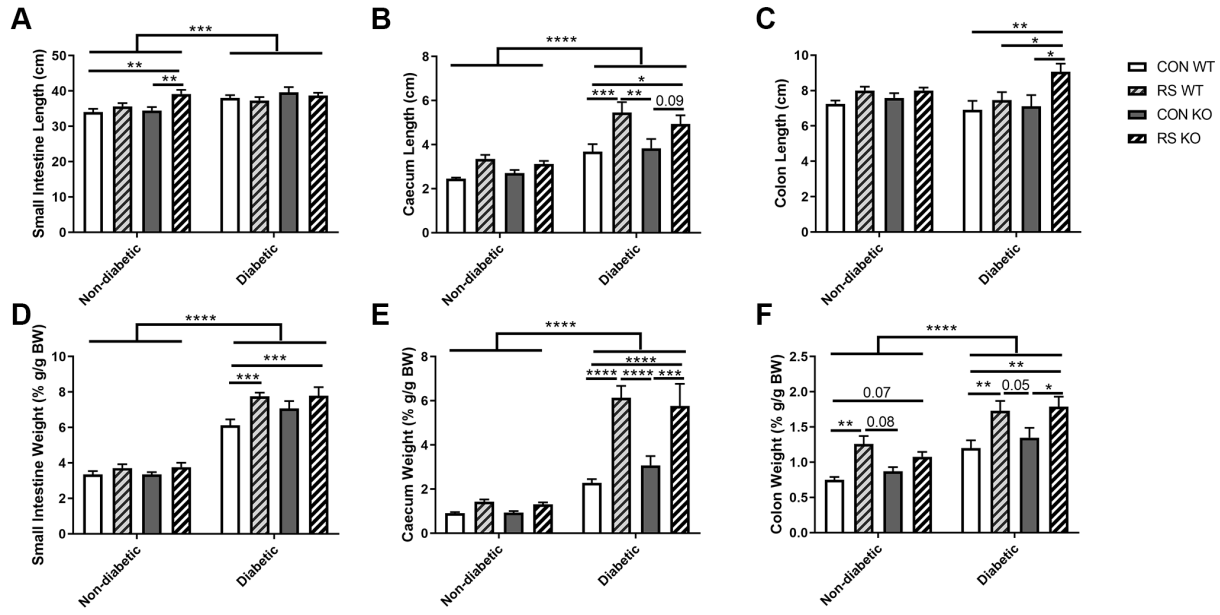

Figure 1: Intestinal Anatomy

A Small intestine length, **B** caecum length, **C** colon length, **D** small intestine weight, **E** caecum weight, **F** colon weight. Data are expressed as mean ± S.E.M. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. n = 7-12. CON = Control Diet, RS = Resistant Starch Supplemented Diet, WT = wildtype, KO = knockout.

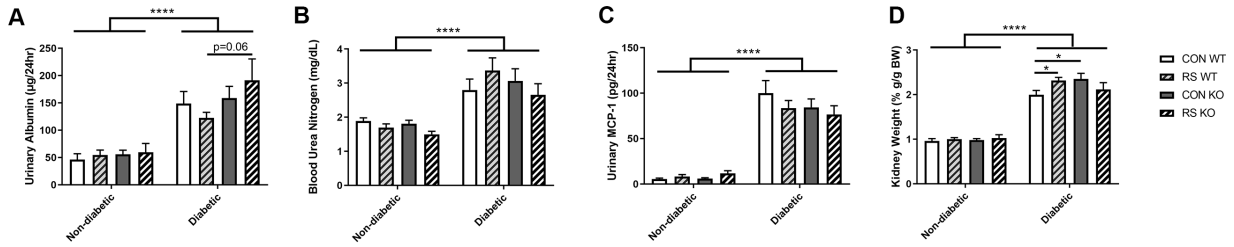

Figure 2: Renal Injury and Inflammation

A 24 hour urinary albumin, **B** blood urea nitrogen, **C** 24 hour urinary MCP-1, **D** Relative kidney weight. Data are expressed as mean ± S.E.M. * $p<0.05$, **** $p<0.0001$. n = 7-14. CON = Control Diet, RS = Resistant Starch Supplemented Diet, MCP-1 = Monocyte Chemoattractant Protein-1, WT = wildtype, KO = knockout.

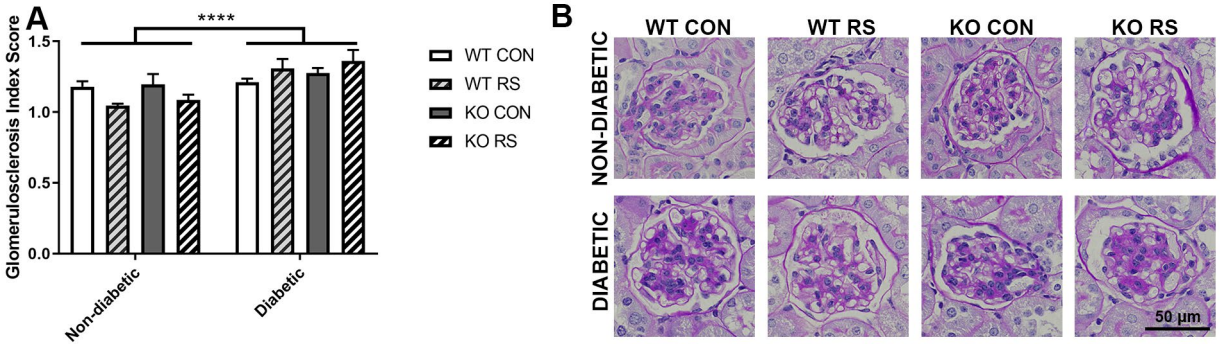

Figure 3: Renal Structural Changes

A Glomerulosclerotic Index Score, **B** representative PAS stained images. Data are expressed as mean ± S.E.M. **** $p<0.0001$. n = 7-12. CON = Control Diet, RS = Resistant Starch Supplemented Diet, WT = wildtype, KO = knockout.

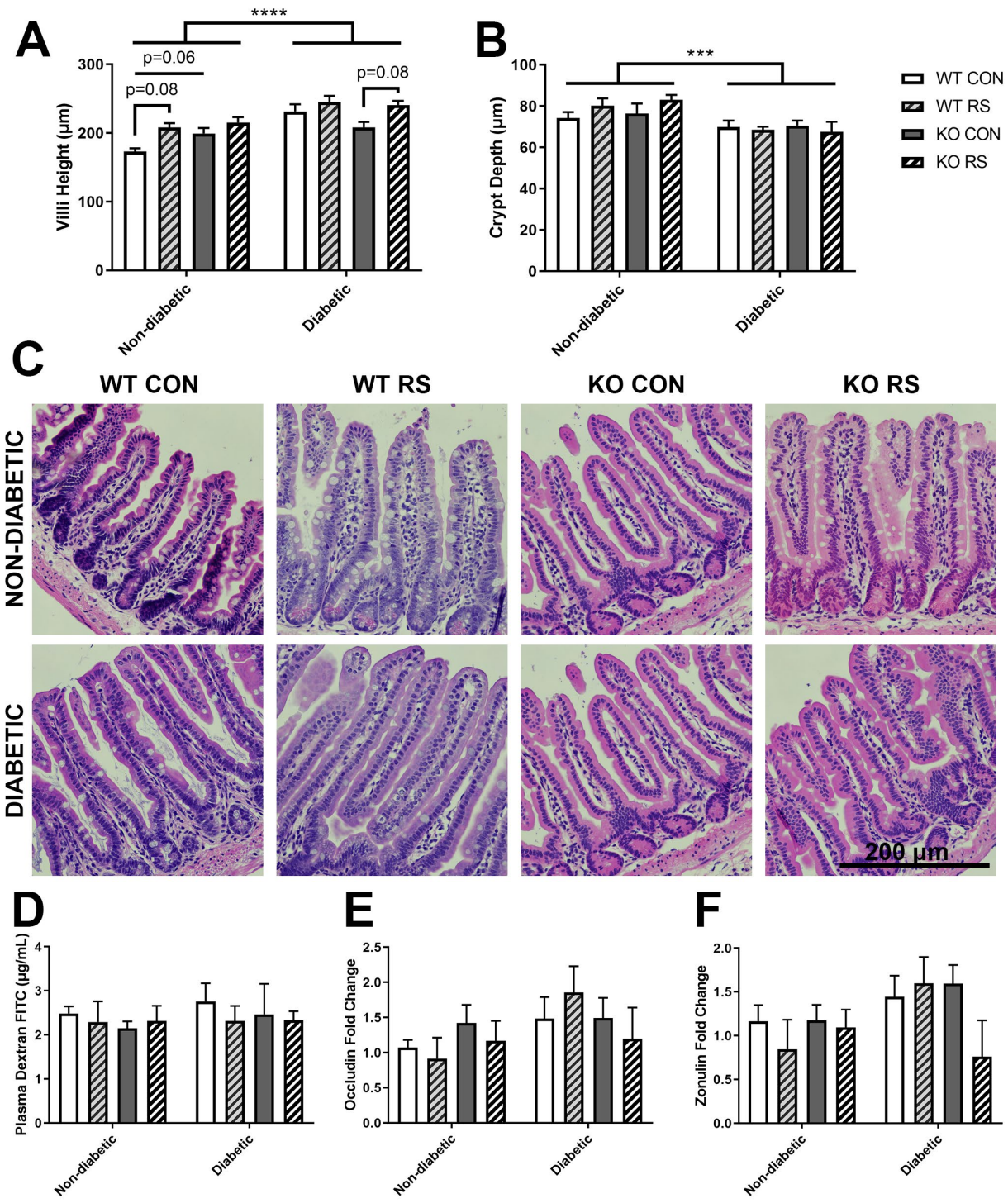

Figure 4: Intestinal Permeability and Morphology

A Ileum villi height, **B** Ileum crypt depth, **C** representative H&E stained images, **D** Plasma dextran-FITC intestinal permeability assay, **E** Ileum expression of Occludin, **F** Ileum expression of Zonulin. Data are expressed as mean ± S.E.M. *** $p<0.001$, **** $p<0.0001$. n= 7-12. CON = Control Diet, RS = Resistant Starch Supplemented Diet, WT = wildtype, KO = knockout.

	NON-DIABETIC				DIABETIC						
	WT CON	**WT RS**	**KO CON**	**KO RS**	**DIAB WT CON**	**DIAB WT RS**	**DIAB KO CON**	**DIAB KO RS**	**Interaction**	**Genotype / Diet**	**Diabetes**
Body weight (g)	35.6 ± 1.2	34.9 ± 1.6	37.7 ± 2.1	35.6 ± 1.4	24.3 ± 0.8	21.9 ± 0.6	22.3 ± 1.2	24.9 ± 1.0	NS	NS	****
GHb (%)	4.0 ± 0.0	4.3 ± 0.2	4.1 ± 0.1	4.0 ± 0.1	11.9 ± 0.5	12.3 ± 0.3	11.6 ± 0.5	11.8 ± 0.5	NS	NS	****
Fat Mass (% BW g/g)	19.4 ± 1.7	19.5 ± 2.5	22.0 ± 1.7	20.7 ± 2.1	2.1 ± 0.5	1.4 ± 0.5	1.1 ± 0.5	1.0 ± 1.0	NS	NS	****
Lean Mass (% BW g/g)	77.1 ± 1.6	77.0 ± 2.3	74.5 ± 1.7	75.8 ± 2.1	90.1 ± 0.5	90 ± 0.6	87.5 ± 0.7	89.7 ± 0.6	NS	NS	****
Urine Output	1.8 ± 0.2	2.0 ± 0.2	1.7 ± 0.1	1.8 ± 0.2	5.3 ± 0.3	5.6 ± 0.2	5.1 ± 0.3	4.8 ± 0.4	NS	NS	****
Water Intake	0.7 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	19.6 ± 2.2	18.7 ± 1.0	17.7 ± 2.0	16.4 ± 2.6	NS	NS	****
Liver (%BW g/g)	3.9 ± 0.2	4.3 ± 0.2	4.4 ± 0.2	4.2 ± 0.2	5.4 ± 0.2	5.5 ± 0.2	6.0 ± 0.4	6.0 ± 0.4	NS	NS	****
Spleen (%BW g/g)	0.3 ± 0.0	0.3 ± 0.0[a]	0.3 ± 0.0	0.4 ± 0.1[a]	0.3 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	NS	NS	*

Table 1: Phenotypic and Biochemical Characteristics of Mice

Two-way ANOVA followed by Tukey's multiple comparisons test. Data are expressed as mean ± S.E.M. * $p<0.05$, **** $p<0.0001$. For post hoc test, cells within rows sharing the same superscript are significantly different from each other ([a] $p<0.05$). n = 7-14. GHb = Glycated Haemoglobin, CON = Control Diet, RS = Resistant Starch Supplemented Diet, WT = wildtype, KO = knockout.