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Full Length Article

Probiotics Supplementation Reduces High Fat High Sugar Diet-Associated Oxidative Stress at Intestinal Epithelial Cells, Nephrons and Hepatocytes in Rat Model

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Abstract

Non-alcoholic fatty liver disease, characterized by abnormal fat accumulation in the liver that manifests many metabolic diseases, targets mainly the gut-liver axis and outcomes into non-alcoholic steatohepatitis, necrosis and fibrosis that ultimately lead towards cirrhosis. Probiotics, being "live microorganisms", strengthen the immune system (both innate and adaptive) and are used in the prevention and treatment of many metabolic diseases. The present study aimed to explore the protective effects of three important strains, commonly used as probiotics, *i.e.*, *Lactobacillus* spp., *Bifidobacteria sp.* and *Streptococcus sp.* against high fat high sugar diet-associated oxidative stress in conjunction with histopathological changes in intestinal epithelial cells, nephrons and hepatocytes in rat model. In this study, probiotics (2×10^6 colony forming units) therapeutic potential was evaluated on gut-liver and kidney axis using *in-vivo* rat models. At the end of the study, serum was separated from blood for biochemical analysis while tissue samples of liver, kidney and intestine were collected for histopathological analyses. The results of cholesterol, triglyceride, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, creatinine, urea and uric acid levels suggested ameliorative effects of probiotics in metabolic disease caused by high fat high sugar diet. Moreover, the entire antioxidant capacity was improved by probiotics administration as measured by serum total antioxidant capacity, total oxidant status, malondialdehyde, paraoxonase and arylesterase levels and by histopathological analysis of liver, gut and kidneys. These results suggest that the protective effects of probiotics supplementation might be mediated through gut microbiota modification. It was concluded that probiotics comprising these three strains are potential candidates for prevention or adjuvant treatment of metabolic diseases involving oxidative stress. © 2020 Friends Science Publishers

Keywords: Probiotics; Oxidative Stress; Non-alcoholic fatty liver disease; Metabolic disorders; Gut-liver and kidney axis

Introduction

The gut-liver axis is considered an important player in mediating the obesity, non-alcoholic fatty liver disease (NAFLD) and the metabolic syndrome (Wiest *et al.* 2017). High fat high sugar diet causes gut dysbiosis and leads to production of lipopolysaccharides by Gram negative gut bacteria. The intestinal microbiota have potential role in controlling obesity, metabolic diseases and NAFLD (Mouzaki *et al.* 2013) through increased intrahepatic fat accumulation (Tilg and Moschen 2010). The hepatocellular inflammation and kidney injuries (Nallu *et al.* 2017) are secondary to altered intestinal permeability through toxins generated by gram negative bacteria (Schnabl and Brenner 2014). The endotoxins produced by intestinal microbiota activate hepatic stellate cells to induce liver fibrosis (Miura *et al.* 2010). The microbiota under normal physiological condition produces endotoxins, that are absorbed through hepatic portal circulation and cleared by Kupffer cells (Zhang *et al.* 2007). Oxidative stress (Rolo *et al.* 2012) and low-grade inflammation in gastrointestinal tract are common features of metabolic diseases mediated *via* gut dysbiosis (Turnbaugh *et al.* 2006). The chronic kidney diseases (CKD) in association with dyslipidemia, oxidative stress, insulin resistance, obesity and NAFLD through gut host microbial interaction are well studied (Xu *et al.* 2017). Dietary interventions, systemic infection and liver diseases cause an increased endotoxin levels (Yatsunenko *et al.* 2012) that lead to increase intestinal permeability (Frazier *et al.* 2011), enhance Gram negative bacterial population and compromise Kupffer cells phagocytic abilities (Han 2002). Gut dysbiosis, Firmicutes to Bacteriodetes ratio (Elabd *et al.* 2018), cause metabolic diseases associated with high fat and high sugar diets (Turnbaugh *et al.* 2009). High fat diet causes ectopic fat accumulation (Wiedemann *et al.* 2013), inflammation (Williams *et al.* 2014), oxidative stress (Sies,

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2015), obesity (Murphy *et al.* 2013) and ultimately NAFLD (Valenzuela *et al.* 2012). The NAFLD often converts into more severe forms like non-alcoholic steatohepatitis, necrosis, fibrosis, cirrhosis and ultimately the hepatocellular carcinoma (Zhu *et al.* 2013).

The gut microbiota maintain intestinal permeability, gut immunity, fat regulation and metabolism (Alonso and Guarner 2013). The intestinal lumen presents large surface area for microbial proliferation and their number (4×10^3) is even greater than that of total body eukaryotic cells (3×10^3) (Sender *et al.* 2016). The gut microbiota is considered as separate endocrine organ (Clarke *et al.* 2014), which maintains the host homeostasis and immunity through molecular crosstalk with management and detoxifying organs like kidneys and liver (Kieffer *et al.* 2016). Probiotics, being "live microorganisms", strengthen the immune system, reduce inflammatory cytokines (Bernini *et al.* 2016) and cholesterol deposition (Sharma *et al.* 2016) in the blood and hepatocytes (Ma *et al.* 2013). Probiotics of specific strains control oxidants/antioxidant levels, support intestine through mucin production and modify the proinflammatory chemokines and cytokines (Sánchez *et al.* 2017). Previous studies have shown significant effects of *Lactobacilli* spp. *(L. brevis, L. ruminis, L. casei, L. Plantarum, L. rhamnosus GG, L. farciminis, L. lactis L. acidophilus* and *L. Pentosus)* and *Bifidobacteria* spp. *(B. longum, B. bifidum, B. adolescentis* and *B. polyfermenticus*) in the attenuation of colitis, diabetes and pancreatitis (Fang *et al.* 2017).

The pathogenesis of NAFLD through gut microbiota (Boursier and Diehl 2015) is multifaceted and complicated, regarding our hypothesis "two hints" evolved. The first one is due to accumulation of fat in hepatic cells and hypercholestremia, while the other one due to the mediation of oxidative stress (Deng *et al.* 2019). Therefore, in this study, the mechanisms of anti-oxidants production and to minimize free radical through probiotics are investigated. The intestinal integrity plays role in lowering the endotoxin production and impaired lipid metabolism, which are considered as major players for pathogenesis of NAFLD and non-alcoholic steatohepatitis (Shen *et al.* 2018). As the probiotics are associated with gut health, so the impact of probiotics on gut-liver and kidney axis is also being investigated in the present study.

Materials and Methods

Probiotics

The lyophilized mixed therapeutic grade bacterial strains of *Lactobacilli* spp., *Bifidobacteria* spp. and *Streptococcus thermophilius* containing 2×10^6 colony forming units (CFU)/g (Amybact Powder, ICI Pakistan Nutraceutical) were purchased from medicine market, Faisalabad, Pakistan.

Experimental design and animals used

Total twenty four, 4 week old male albino rats were kept at animal housing facility of Institute of Pharmacy, Physiology and Pharmacology, University of Agriculture Faisalabad, Pakistan at 25 ± 2°C with relative humidity 55-60%, equal distribution of light and dark cycle, rat chow (Table 1) and water *ad-libitum*. Minimum pain and stress to the animals was assured during experimentation. The study was approved by Institutional Biosafety and Bioethics Committee (IBBC) Approval Number ORIC 499/19, University of Agriculture, Faisalabad.

Four groups were made by random distribution as follows: (1) Vehicle group, *ad libitum* standard rat chow and water; (2) Probiotics group, rat chow with probiotics dose 2×10^6 CFU/rat/day for 18 weeks of commercially available therapeutic grade probiotics mixture containing *Lactobacillus* sp., *Bifidobacteria* spp. and *Streptococcus thermophilus* group; (3) High fat high sugar (HFHS) group, fed high fat (36%) and high sugar (40%) for 14 weeks with standard feed to develop the NAFLD model; (4) HFHS-Probiotics group, fed as in group (3) along with therapeutic grade probiotics *(Lactobacillus* spp., *Bifidobacteria* spp. and *Streptococcus thermophilus* mixture) with the dose 2×10^6 CFU/rat/day for 4 weeks. The body weight of each rat was measured fortnightly.

Blood and organ collection

Blood collection was done at the 18th experimental week. Rats were anesthetized with chloroform before sacrificing and blood of each rat was taken in separate vacutainers for serum collection in platelet activator gel. Serum was separated using centrifuge machine (Centrifugal Machine, China) at 1010 x g for 15 minutes and stored in the biomedical freezer (Sanyo Japan) at -20°C for biochemical analysis by using automated serum analyser (Bio-Ray 310 diagnostic). Liver, kidneys and intestine of each rat was separated and preserved in 10% neutral buffered formalin solution for tissue analysis.

Serum biochemical analysis

The stored serum was thawed and analyzed for bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), cholesterol, uric acid, urea, creatinine, albumin and globulin through commercially available bio-kits (Merck, Pvt., Ltd.) according to the given protocols. The oxidant and antioxidant status were assessed through measuring total oxidant status (TOS), total antioxidant capacity (TAC), malondialdehyde (MDA), superoxide dismutase (SOD) and arylesterase levels through calorimetric method using spectrophotometer (Thermo Scientific Multiskan GO™ with SkanIt software 4.1) according to manufacturer guidelines (Juretić *et al.* 2006).

Tissue analysis

The histopathological analysis of liver, intestine and kidney

tissues was performed by preparing the slides according to protocols mentioned in the literature (Bedossa *et al.* 2012). The histological images of liver, intestine and kidney sections were taken with the camera (TOUPCAM, ToupTek Photonics Co., Ltd.; China) attached to a light microscope (Model IM-910 IRMECO GmbH & Co.; Germany). The degree of histopathological alterations (Brown and Kleiner 2016) was evaluated for each group of liver, intestine and kidney and classified according to the severity such as 0 for normal limits, 1 for minimal, 2 for slight, 3 for moderate and 4 for severe as mentioned in previous study (Mann *et al.* 2012).

Statistical analysis

The SPSS software (version 16.0) was used for data analysis to calculate significance ($P \leq 0.05$) difference through one way ANOVA following post hoc assessment by DMR test. For histopathologic statistical analysis, Kruskal-Wallis test was applied in order to determine the effects of treatments on each experimental parameter. All results were expressed as Mean ± SE.

Results

Probiotics supplementation restores the HFHS diet-associated alterations in serum biochemical parameters.

As expected, high fat and high sugar diet administration for 4 weeks resulted in increased serum lipid profile (Moreno-Fernández *et al.* 2018). Thereafter, probiotics supplementation for 4 weeks significantly ameliorated the HFHS diet-induced hypercholesterolemia and elevated triglyceride levels, total protein and globulin as shown in Fig. 1.

Probiotics improve HFHS diet-induced hepatic dysfunction

The higher values of ALT, AST and ALP in HFHS group suggested hepatic malfunction. Probiotics supplementation in HFHS-Probiotics group significantly restored AST and ALP levels whereas non-significant reduction was observed in ALT and bilirubin levels (Fig. 2). The histopathological analysis of vehicle group (Left panel) showed normal hepatocytes, hepatic triad and no fat accumulation. On the other hand, histopathological analysis of HFHS diet-treated group (Middle Panel) showed abnormal hepatic triad, cytoplasmic vacuolation, perivascular and portal cell infiltration, fat accumulation in hepatocytes, eccentric and pyknotic nuclei. The HFHS-Probiotics group (Right panel) showed restoration of liver parenchyma suggesting ameliorative effects of probiotics on HFHS diet-induced alterations in liver tissue (Fig. 3, Table 2).

Probiotics improve HFHS-induced alteration in intestinal architecture

The histopathological analysis of images of vehicle group (Left panel) showed the normal epithelial lining, villi structure, glands and intestinal mucosa. The HFHS group (Middle panel) showed fat accumulation in ilial region and damaged gut mucosa and villi. The thick epithelium showed pyknotic and eccentric nuclei. The HFHS-Probiotics group (Right panel) showed rare cytoplasm vacuolation, normal villi and glandular epithelium suggesting ameliorative effects of probiotics on gut health (Fig. 4, Table 3).

Table 1: Rat diet composition

Feed Constituents	Normal Diet	High Fat and High Sugar (HFHS) Diet
Fat	6 %	36%
Sucrose	Nil %	40%
Crude protein	20 %	8.75%
Crude fiber	4.5 %	1.23%
Ash	6 %	0.9%
Nitrogen free extract	63.5%	13.12%

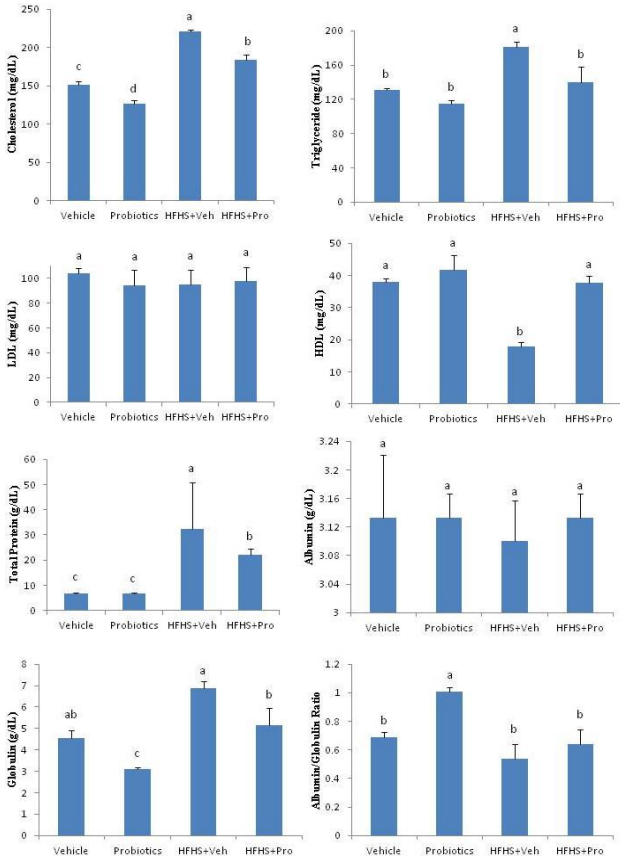

Fig. 1: Effect of HFHS-diet and probiotics on serum lipid and protein profile. Results are expressed as Mean ± SE. Different alphabets show statistically significant at $P < 0.05$

Probiotics alleviate renal damage associated with HFHS diet

The serum biomarkers for renal injury including creatinine, urea and uric acid level showed significant difference in HFHS group (Fig. 5). Histopathological analysis of vehicle

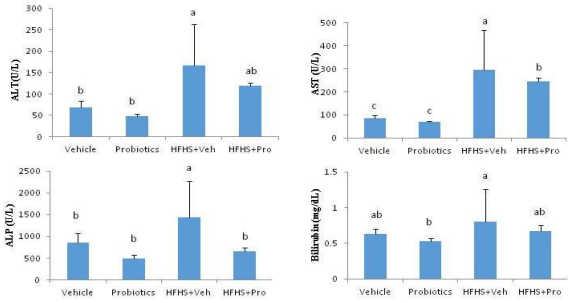

Fig. 2: Effect of HFHS-diet and probiotics on liver function markers. Results shown are the Mean ± SE, while different alphabets suggest statistical significance at P < 0.05

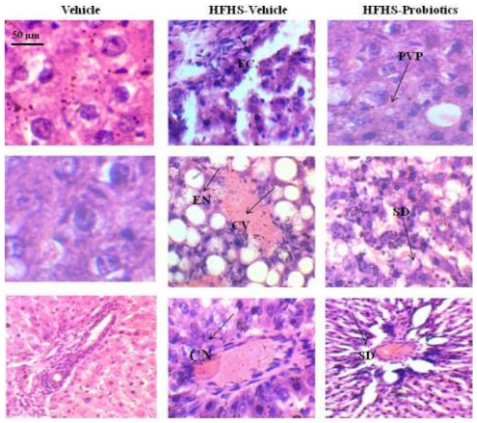

Fig. 3: Effect of HFHS-diet and probiotics on liver histology. The H&E stained images of liver histopathology (Magnification 400x). Three representative images from respective group showing different areas of liver tissue. CN, centrilobular necrosis; CV, cytoplasmic vacuolation; EN, eccentric nuclei; FC, focal hepatic necrosis; PVP, peri-vascular and portal cell infiltration; SD, sinusoidal dilatation

group (Left panel) showed normal Bowman's capsule and proximal convoluted tubular structure. The HFHS group (Middle panel) showed distorted glomeruli with increased Bowman capsular space. The histological analysis of HFHS-Probiotics group (Right panel) showed renal architecture comparable to that in vehicle-treated group. Probiotics restored serum biomarker levels in HFHS-Probiotics group and ameliorated the overall renal architecture as observed by histological analysis (Fig. 6; Table 4).

Probiotics reduce oxidative Stress associated with HFHS diet

The TOS, TAC and MDA assay showed significant difference in HFHS group as compared to that of vehicle group. Probiotics supplementation reduced the HFHS-induced elevated oxidative stress markers (TOS, MDA) and increased the antioxidant parameter (TAC). Results of paraoxinase and arylesterase activity showed non-significant difference among the groups (Fig. 7).

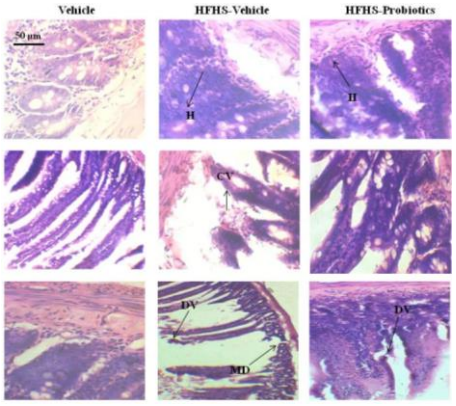

Fig. 4: Effect of HFHS-diet and probiotics on ilium histology. Three representative images from respective group showing different areas of ilium. CV, cytoplasmic vacuolation; DV, damaged villi; H, hemorrhages; MD, mucosal damaged; TI, thickened intestinal muscle layer

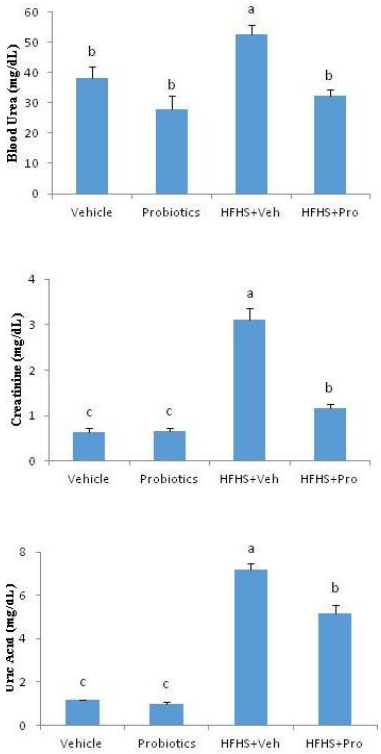

Fig. 5: Effect of HFHS-diet and probiotics on renal function markers. Results shown are the Mean ± SE, while different alphabets suggest statistical significance at *P* < 0.05

Discussion

Liver and gut are anatomically and physiologically connected organs (Zhao *et al.* 2019). The gut microbiome play significant role in maintaining host immunity (Kau *et al.* 2011) and metabolism (Mazidi *et al.* 2016). Experimental data on gut-liver axis has provided important mechanistic

Table 2: Probiotics supplementation improves histological structure of liver in HFHS-diet associated NAFLD

Histology parameters	Vehicle	HFHS-Vehicle	HFHS-Probiotics	Kruskal-Wallis Test for global comparison of organ lesion among groups
				Asymptotic Significant ($P < 0.05$)
Cytoplasmic vacuolation	0.33 ± 0. 08	1.5 ± 0.01	0.75 ± 0.11	0.01
Focal hepatic necrosis	0.16 ± 0.08	1.5 ± 0.06	0.91 ± 0.16	0.001
Centro-lobular necrosis	0.16 ± 0.08	1.80 ± 0.08	0.91 ± 0.16	0.001
Eccentric nuclei	0.25 ± 0.08	2.00 ± 0.17	1.00 ± 0.22	0.001
Pyknotic nuclei	0.53 ± 0.01	1.52 ± 0.01	0.91 ± 0.11	0.001
Sinusoidal dilatation	0.25 ± 0.10	1.43 ± 0.12	0.83 ± 0.01	0.006

Table 3: Probiotics supplementation improves histology structure of ilium in HFHS-diet associated impaired ilium

Histology parameters	Vehicle	HFHS-Vehicle	HFHS-Probiotics	Kruskal-Wallis Test for global comparison of organ lesion among groups
				Asymptotic Significant ($P < 0.05$)
Cytoplasmic vacuolation	0.25 ± 0.08	1.75 ± 0.12	0.91 ± 0.11	0.001
Damaged villi	0.25 ± 0.08	2.0 ± 0.11	0.91 ± 0.16	0.001
Thickened intestinal muscle layers	0.33 ± 0.14	1.66 ± 0.08	0.91 ± 0.11	0.001

Table 4: Probiotics supplementation improves histological structure of kidney HFHS-diet associated renal damaged

Histology parameters	Vehicle	HFHS-Vehicle	HFHS-Probiotics	Kruskal-Wallis Test for global comparison of organ lesion among groups
				Asymptotic Significant ($P < 0.05$)
Cytoplasmic vacuolation of tubular epithelium	0.25 ± 0.08	1.91 ± 0.08	0.83 ± 0.14	0.011
Tubular necrosis	0.25 ± 0.08	1.58 ± 0.21	1.00 ± 0.11	0.008
Tubular thickening	0.166 ± 0.11	1.50 ± 0.06	1.08 ± 0.11	0.002
Interstitial cell infiltration	0.166 ± 0.11	1.75 ±0.06	0.91 ± 0.16	0.004

insights into pathophysiology of liver diseases (Tilg *et al.* 2016). The hepatic gene expression of obese individuals suggest endoplasmic reticulum stress and periportal inflammation in mediating the NAFLD (Soderborg *et al.* 2018). Therapeutic strategies are being explored to modulate gut microbiota, gut permeability and bile acid signaling (Jiao *et al.* 2018) in preventing NAFLD. The HFHS diet produces NAFLD (Sellmann *et al.* 2015) by increasing fat accumulation, inflammation, neutrophils infiltration, necrosis, fibrosis at hepatocellular level (Xu *et al.* 2003) and can be indicated by higher levels of ALT and AST in serum (Hussain *et al.* 2019). Several studies have demonstrated ameliorative effects of probiotics in liver diseases with different dosage in experimental models (Cho *et al.* 2018; Hong *et al.* 2018). The current study aimed to investigate the protective effects of probiotics in HFHS-induced NAFLD in rat model.

Lipid homeostasis is regulated through balance between lipid generation and lipid utilization. In liver diseases, dysfunction in lipolytic and lipogenic pathways occurs (Liu *et al.* 2017). Consistent with previous literature, the increased burden of triglycerides (Marchesini *et al.* 1999), cholesterol, low density lipids and drop down in high density lipids was accompanied with the dysfunction of liver as suggested from increased activities of liver function enzymes (Weber *et al.* 2003). The results from our research model indicated successful induction of disease. Probiotics in HFHS-Probiotics group lowered the triglycerides burden, decreased the cholesterol level, increased the HDL level and lowered the LDL level suggesting the role of probiotics in regulating lipid metabolism. The possible mechanisms of probitics include: 1) reduced the toxins generated by food components and host microbiota through alterations in the levels of ROS, TNF-α, LPS and SCFA 2) modulates host innate and adaptive immunity 3) maintains host pathogenic and commensal microbial balance (Al-Muzafar and Amin 2017).

HFHS-diet causes gut dysbiosis, which increase LPS generation and intestinal permeability that are associated with metabolic disturbances. Furthermore, these metabolic disturbances may hamper fat and glucose metabolism through which hypercholestremia, hyperglycemia and ultimately hepatosteatosis occurs (Al-Muzafar and Amin 2017). The elevated levels of AST, ALT and ALP have also been noticed in previous studies of liver disease models (Rahmat *et al.* 2014). These biochemical parameters have considered as baseline parameters to declare hepatic impairment (Gazwi and Mahmoud 2019). In our study, elevated levels of ALT, AST, ALP and bilirubin strongly validated the studied models of liver diseases. The ameliorative effects of probiotics in fatty liver diseases might be ascribed to their antilipogenic properties (Moratalla *et al.* 2014). Probiotics have played role in normalizing the liver enzyme levels to lessen the disease burden (Nabavi *et al.* 2014). In liver diseases, irregularities are frequently observed in hexagonal hepatic lobule, portal triad, central vein and centric nuclei (Hussain *et al.* 2019). These irregularities along with fat accumulation and necrosis have also been observed in our study. Probiotics lowered the lipid peroxidation at hepatic cellular levels through which reduced inflammation and restored hepatic portal triad, central lobular architecture alongwith absence of fat accumulation occurred.

The systemic vasodilation, increased nitric oxide (NO), reduced renal blood flow and glomerular filtration in liver

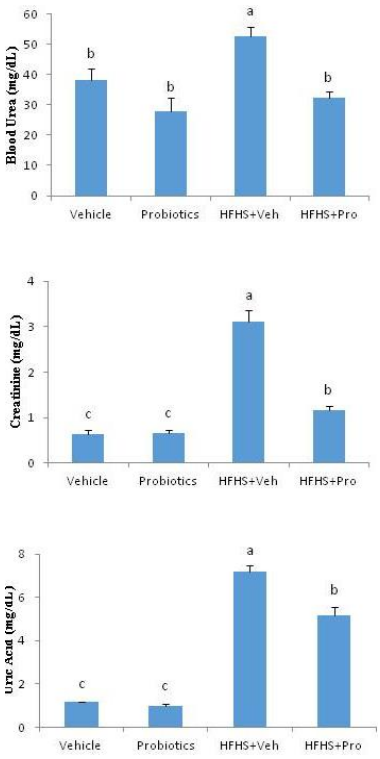

Fig. 5: Effect of HFHS-diet and probiotics on renal function markers. Results shown are the Mean ± SE, while different alphabets suggest statistical significance at $P < 0.05$

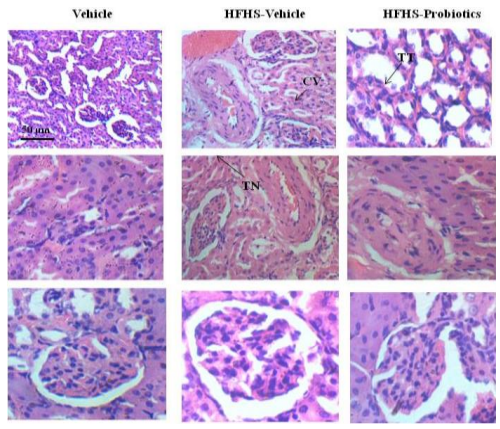

Fig. 6: Effect of HFHS-diet and probiotics on renal histology. Three representative images from respective group showing different areas of renal tissue. CV, cytoplasmic vacuolation of tubular epithelium; ICI, interstitial cell infiltration; TN, tubular necrosis; TT, tubular thickening

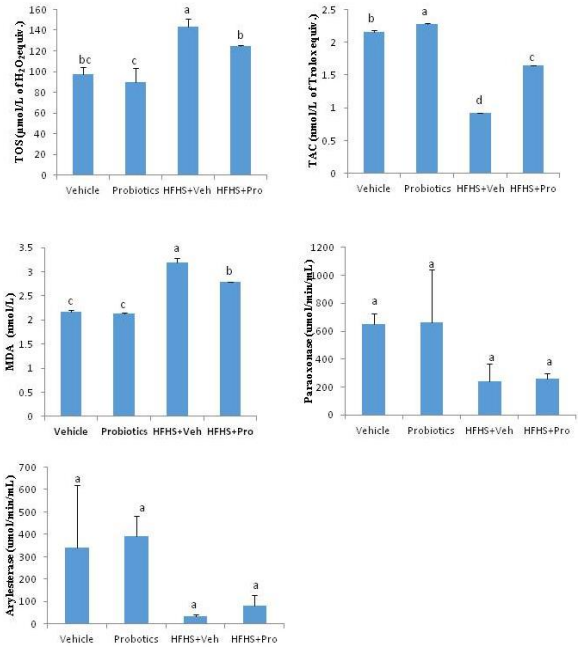

Fig. 7: Probiotics ameliorate HFHS-diet associated oxidative stress. The oxidant and anti-oxidant status.
Results shown are the Mean ± SE. Different alphabets indicate statistical significance at $P < 0.05$

diseases could be due to hypoalbuminemia. The role of albumin to enhance the sodium retension, vasoconstriction, arterial pressure and in reducing rennin aldosterone level and ascites, secondary to the cirrhosis are most frequently observed (Walayat *et al.* 2017). The total protein contents (Castro *et al.* 2013) including albumin and globulin varied significantly consistent with HFHS group induced impaired liver functioning. The supplementation of probiotics in HFHS-Probiotics group has restored the hepatic and renal function markers. The probiotics of different starins in previous study suggested decreased morbidity and mortality in patients suffering from hepatic malfunctioning (Velayudham *et al.* 2009).

Detoxification of gut derived pathogens presented through hepatic portal system becomes weaker in liver disease (Schnabl and Brenner 2014). Innate immunity response and intestinal integrity to maintain gut barrier for bacterial translocation are well studied (Jiang *et al.* 2015). The healthy mucosa limits intestinal colonization of opportunistic bacteria. In liver diseases, the congestion of enetric veins leads to necrosis and apoptosis of enterocytes (Wang *et al.* 2012). The gut microbiota affects cellular metabolism in hepatic and adipose tissue (Griffiths *et al.* 2004). Probiotics beneficial effects help the intestinal mucosal lining to provide maximum gut barrier function for immunity, moreover direct regulation, production and secretion of gut peptide hormones from enteroendocrine cells in controlling the satiety as (Li *et al.* 2019).

The undigested food in colon upon fermentation generated phenols, indoles and amines (Guldris *et al.* 2017). These endogenous toxins increase the gut permeability, inflammation and oxidative stress related chronic kidney diseases. Whereas, the probiotics tranformed the undigested food into short chain fatty acids (Flint *et al.* 2008), thereby exert anti-inflammatory action through lowering IL17A and

TNFα (Moya-Pérez *et al.* 2015). Uremic toxins accompanied with liver diseases accumulated renal waste (Bammens *et al.* 2006). The malfunctioning in emulsification of fats and excretion results in urates deposition in nephron and ultimately renal damage concomitant with liver disease (Palanisamy *et al.* 2008; Sasson and Cherney 2012). In our study the higher levels of uremic toxins (uric acid, urea and creatinine) along with the observed degeneration in Bowman's capsule and excessive neutrophils infiltration indicate renal damage. Probiotics improved the serum uremic levels and histological architecture of nephron possibly through lowering the inflammatory and pro-inflammatory cytokines as supported with the previous studies (Pei *et al.* 2018; Jia *et al.* 2018).

Supplementation of probiotics attenuated oxidative stress induced by high fat and high sugar diet consistent with previous literature (Asemi *et al.* 2012; Ejtahed *et al.* 2012; Mehmood *et al.* 2018; Hafez and Gad 2018). The plausible mechanisms could be the decrease methylation of MutL homolog 1 (MLH1) promoter (Yang *et al.* 2013) and 8-hydroxy-20-deoxyguanosine level in plasma (Sáez-Lara *et al.* 2016), significantly increase in superoxide dismutase (SOD) activity (Hariri *et al.* 2015) in probiotics group. The different strains of probiotics supplementation like *Lactobacillus*, *Bifidobacteria*, *Bacillus* or *Enterococus* result in decreasing the ammonia level in feces and blood through binding the endotoxins (Zhao *et al.* 2019). Although, oxidative stress produces hepatic impairment in NAFLD, the generation of antioxidants enzyme maintain cumulative redox balance (Armutcu *et al.* 2005). As also, probiotics in our study help in maintaining the anti-oxidant capacity and lowering the oxidative stress.

Conclusion

We evidenced that probiotics have therapeutic role in ameliorating HFHS-associated NAFLD. As it inflict, probiotics improve serum biomarkers and the histopathological architecture of liver, intestine and kidneys. It is plausible to use probiotics in the prevention and treatment of metabolic syndrome associated with HFHS-diet involving gut-liver and kidney axis.

Acknowledgments

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References

Alonso VR, F Guarner (2013). Linking the gut microbiota to human health. *Braz J Nutr* 109:21–26

Al-Muzafar HM, KA Amin (2017). Probiotic mixture improves fatty liver disease by virtue of its action on lipid profiles, leptin, and inflammatory biomarkers. *BMC Compl Altern Med* 17:43–54

Armutcu F, O Coskun, A Gürel, M Kanter, M Can, F Ucar (2005). Thymosin alpha 1 attenuates lipid peroxidation and improves fructose-induced steatohepatitis in rats. *Clin Biochem* 38:540–547

Asemi Z, S Jazayeri, M Najafi, M Samimi, V Mofid, F Shidfar (2012). Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: A randomized controlled clinical trial. *Ann Nutr Metab* 60:62–68

Bammens B, P Evenepoel, H Keuleers, K Verbeke, Y Vanrenterghem (2006). Free serum concentrations of the protein-bound retention solute p-cresol predict mortality in hemodialysis patients. *Kidney Intl* 69:1081–1087

Bedossa P, C Poitou, N Veyrie, JL Bouillot, A Basdevant, V Paradis (2012). Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology* 56:1751–1759

Bernini LJ, ANC Simão, DF Alfieri, MAB Lozovoy, NL Mari, CHBD Souza (2016). Beneficial effects of *Bifidobacterium lactis* on lipid profile and cytokines in patients with metabolic syndrome: A randomized trial. Effects of probiotics on metabolic syndrome. *Nutrition* 32:716–719

Boursier J, AM Diehl (2015). Implication of Gut Microbiota in Nonalcoholic Fatty Liver Disease. *PLoS Pathol* 11:1–6

Brown GT, DE Kleiner (2016). Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Metabolism* 65:1080–1086

Castro UGMD, RASAD Santos, ME Silva, WGD Lima, MJ Campagnole-Santos, AC Alzamora (2013). Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids. *Health Dis* 12:136

Cho MS, SY Kim, KT Suk, BY Kim (2018). Modulation of gut microbiome in nonalcoholic fatty liver disease: pro-, pre-, syn-, and antibiotics. *J Microbiol* 56:855–867

Clarke G, RM Stilling, PJ Kennedy, C Stanton, JF Cryan, TG Dinan (2014). Minireview: Gut microbiota: the neglected endocrine organ. *Mol Endocrinol* 28:1221–1238

Deng P, J Barney, MC Petriello, AJ Morris, B Wahlang, B Hennig (2019). Hepatic metabolomics reveals that liver injury increases PCB 126-induced oxidative stress and metabolic dysfunction. *Chemosphere* 217:140–149

Ejtahed HS, J Mohtadi-Nia, A Homayouni-Rad, M Niafar, M Asghari-Jafarabadi, V Mofid (2012). Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* 28:539–543

Elabd EMY, SM Morsy, HA Elmalt (2018). Investigating of *Moringa oleifera* Role on Gut Microbiota Composition and inflammation associated with obesity following high fat diet feeding. *Open Access Maced J Med Sci* 6:1359–1364

Fang D, D Shi, L Lv, S Gu, W Wu, Y Chen, J Guo, A Li, X Hu, F Guo, J Ye, Y Li, L Li (2017). *Bifidobacterium pseudocatenulatum* LI09 and *Bifidobacterium catenulatum* LI10 attenuate D-galactosamine-induced liver injury by modifying the gut microbiota. *Sci Rep* 7:1–13

Flint HJ, EA Bayer, MT Rincon, R Lamed, BA White (2008). Polysaccharide utilization by gut bacteria: potential for new insights from genomic analysis. *Nat Rev Microbiol* 6:121–131

Frazier TH, JK DiBaise, CJ McClain (2011). Gut microbiota, intestinal permeability, obesity-induced inflammation, and liver injury. *J Parent Enter Nutr* 35:14–20

Gazwi HSS, ME Mahmoud (2019). Restorative activity of aqueous extract *Mangifera indica* leaves against CCl_4 induced hepatic damage in rats. *J Pharm Biomed Anal* 164:112–118

Griffiths EA, LC Duffy, FL Schanbacher, H Qiao, D Dryja, A Leavens (2004). *In Vivo* effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. *Dig. Dis Sci* 49:579–589

Guldris SC, EG Parra, AC Amenós (2017). Gut microbiota in chronic kidney disease. *Nefrología* 37:9–19

Hafez MH, SB Gad (2018). Zinc oxide nanoparticles effect on oxidative status, brain activity, anxiety-like behavior and memory in adult and aged male rats. *Pak Vet J* 38:1–5

Han DW (2002). Intestinal endotoxemia as a pathogenetic mechanism in liver failure. *World J Gastroenterol* 8:961–965

Hariri M, R Salehi, A Feizi, M Mirlohi, R Ghiasvand, N Habibi (2015). A randomized, double-blind, placebo-controlled, clinical trial on probiotic soy milk and soy milk: effects on epigenetics and oxidative stress in patients with type II diabetes. *Genes Nutr* 10:52

Hong M, DH Han, J Hong, DJ Kim, KT Suk (2018). Are Probiotics Effective in Targeting Alcoholic Liver Diseases? *Prob Antimicrob Proteins* 11:335-347

Hussain Z, JA Khan, A Arshad, P Asif, H Rashid, MI Arshad (2019). Protective effects of *Cinnamomum zeylanicum* L. (Darchini) in acetaminophen-induced oxidative stress, hepatotoxicity and nephrotoxicity in mouse model. *Biomed Pharmacother* 109:2285–2292

Jia L, Q Jia, J Yang, R Jia, H Zhang (2018). Efficacy of probiotics supplementation on chronic kidney disease: a systematic review and meta-analysis. *Kidney Blood Press Res* 43:1623–1635

Jiang W, N Wu, X Wang, Y Chi, Y Zhang, X Qiu, Y Hu, J Li, Y Liu (2015). Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep* 5:1–7

Jiao, N, SS Baker, A Chapa-Rodriguez, W Liu, CA Nugent, M Tsompana (2018). Suppressed hepatic bile acid signalling despite elevated production of primary and secondary bile acids in NAFLD. *Gut* 67:1881–1891

Juretić D, A Motejlkova, B Kunović, B Rekić, Z Flegar-Mestrić, L Vujić (2006). Paraoxonase/arylesterase in serum of patients with type II diabetes mellitus. *Acta Pharm* 56:59–68

Kau AL, PP Ahern, NW Griffin, AL Goodman, JI Gordon (2011). Human nutrition, the gut microbiome and the immune system. *Nature* 474:327–336

Kieffer DA, RJ Martin, SH Adams (2016). Impact of dietary fibers on nutrient management and detoxification organs: *Gut Liver Kidneys Adv Nutr* 7:1111–1121

Li Y, L Lv, J Ye, D Fang, D Shi, W Wu (2019). Bifidobacterium adolescentis CGMCC 15058 alleviates liver injury, enhances the intestinal barrier and modifies the gut microbiota in d-galactosamine-treated rats. *Appl Microbiol Biotechnol* 103:375–393

Liu Q, R Pan, L Ding, F Zhang, L Hu, B Ding (2017). Rutin exhibits hepatoprotective effects in a mouse model of non-alcoholic fatty liver disease by reducing hepatic lipid levels and mitigating lipid-induced oxidative injuries. *Intl Immunopharmacol* 49:132–141

Ma YY, L Li, CH Yu, Z Shen, LH Chen, YM Li (2013). Effects of probiotics on nonalcoholic fatty liver disease: A meta-analysis. *World J Gastroenterol* 19:6911–6918

Mann PC, J Vahle, CM Keenan, JF Baker, AE Bradley, DG Goodman, T Harada, R Herbert, W Kaufmann, R Kellner, T Nolte, S Rittinghausen, T Tanaka (2012). International harmonization of toxicologic pathology nomenclature: an overview and review of basic principles. *Toxicol Pathol* 40 (4 Suppl):7S–13S

Marchesini G, M Brizi, AM Morselli-Labate, G Bianchi, E Bugianesi, AJ McCullough (1999). Association of nonalcoholic fatty liver disease with insulin resistance. *Amer J Med* 107:450–455

Mazidi M, P Rezaie, AP Kengne, MG Mobarhan, GA Ferns (2016). Gut microbiome and metabolic syndrome. *Diabetes Metab Syndr Clin Res Rev* 10:150–157

Mehmood K, H Zhang, MK Iqbal, MU Rehman, K Li, S Huang, M Shahzad, F Nabi, M Iqbal, J Li (2018). Tetramethylpyrazine mitigates toxicity and liver oxidative stress in tibial dyschondroplasia chickens. *Pak Vet J* 38:76–80

Miura K, Y Kodama, S Inokuchi, B Schnabl, T Aoyama, H Ohnishi (2010). Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 139:323–334

Moratalla A, I Gómez-Hurtado, A Santacruz, A Moya, G Peiró, P Zapater (2014). Protective effect of *Bifidobacterium pseudocatenulatum* CECT7765 against induced bacterial antigen translocation in experimental cirrhosis. *Liver Intl* 34:850–858

Moreno-Fernández S, M Garcés-Rimón, G Vera, J Astier, JF Landrier, M Miguel (2018). High fat/high glucose diet induces metabolic syndrome in an experimental rat model. *Nutrients* 10:1–15

Mouzaki M, EM Comelli, BM Arendt, J Bonengel, SK Fung, SE Fischer, ID McGilvray, JP Allard (2013). Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 58:120–127

Moya-Pérez A, A Neef, Y Sanz (2015). *Bifidobacterium pseudocatenulatum* CECT 7765 Reduces obesity-associated inflammation by restoring the lymphocyte-macrophage balance and gut microbiota structure in high-fat diet-fed mice. *PLoS One* 10:1–28

Murphy EF, PD Cotter, A Hogan, O O'Sullivan, A Joyce, F Fouhy (2013). Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. *Gut* 62:220–226

Nabavi S, M Rafraf, MH Somi, A Homayouni-Rad, M Asghari-Jafarabadi (2014). Effects of probiotic yogurt consumption on metabolic factors in individuals with nonalcoholic fatty liver disease. *J Dairy Sci* 97:7386–7393

Nallu A, S Sharma, A Ramezani, J Muralidharan, D Raj (2017). Gut microbiome in chronic kidney disease: Challenges and opportunities. *Transl Res* 179:24–37

Palanisamy N, P Viswanathan, CV Anuradha (2008). Effect of genistein, a soy isof lavone, on whole body insulin sensitivity and renal damage induced by a high-fructose diet. *Ren Fail* 30:645–654

Pei M, L Wei, S Hu, B Yang, J Si, H Yang, J Zhai (2018). Probiotics, prebiotics and synbiotics for chronic kidney disease: protocol for a systematic review and meta-analysis. *BMJ Open* 8:1–5

Rahmat AA, FA Dar, IM Choudhary (2014). Protection of CCl_4-induced liver and kidney damage by phenolic compounds in leaf extracts of *Cnestis ferruginea* (de Candolle). *Pharmacogn Res* 6:19–28

Rolo AP, JS Teodoro, CM Palmeira (2012). Role of oxidative stress in the pathogenesis of nonalcoholic steatohepatitis. *Free Rad Biol Med* 52:59–69

Sáez-Lara MJ, C Robles-Sanchez, FJ Ruiz-Ojeda, J Plaza-Diaz, A Gil (2016). Effects of probiotics and synbiotics on obesity, insulin resistance syndrome, Type 2 diabetes and non-alcoholic fatty liver disease: A review of human clinical trials. *Intl J Mol Sci* 17:1–15

Sánchez B, S Delgado, A Blanco-Míguez, A Lourenço, M Gueimonde, A Margolles (2017). Probiotics, gut microbiota, and their influence on host health and disease. *Mol Nutr Food Res* 61:1–15

Sasson AN, DZ Cherney (2012). Renal hyperfiltration related to diabetes mellitus and obesity in human disease. *World J Diab* 3:1–6

Schnabl B, DA Brenner (2014). Interactions between the intestinal microbiome and liver diseases. *Gastroenterology* 146:1513–1524

Sellmann C, J Priebs, M Landmann, C Degen, AJ Engstler, CJ Jin (2015). Diets rich in fructose, fat or fructose and fat alter intestinal barrier function and lead to the development of nonalcoholic fatty liver disease over time. *J Nutr Biochem* 26:1183–1192

Sender R, S Fuchs, R Milo (2016). Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164:337–340

Sharma S, AV Kurpad, S Puri (2016). Potential of probiotics in hypercholesterolemia: A meta-analysis. *Ind J Publ Health* 60:280–286

Shen TCD, N Pyrsopoulos, VK Rustgi, 2018. Microbiota and the liver: Microbiota and the Liver. *Liver Transpl* 24:539–550

Sies H, 2015. Oxidative stress: a concept in redox biology and medicine. *Redox Biol* 4:180–183

Soderborg TK, SE Clark, CE Mulligan, RC Janssen, L Babcock, D Ir, B Young, N Krebs, DJ Lemas, LK Johnson, T Weir, LL Lenz, DN Frank, TL Hernandez, KA Kuhn, A D'Alessandro, LA Barbour, KCE Kasmi, JE Friedman (2018). The gut microbiota in infants of obese mothers increases inflammation and susceptibility to NAFLD. *Nat Commun* 9:1–12

Tilg H, AR Moschen (2010). Evolution of inflammation in nonalcoholic fatty liver disease: the multiple parallel hits hypothesis. *Hepatology* 52:1836–184

Tilg H, PD Cani, EA Mayer (2016). Gut microbiome and liver diseases. *Gut* 65:2035–2044

Turnbaugh PJ, M Hamady, T Yatsunenko, BL Cantarel, A Duncan, RE Ley (2009). A core gut microbiome in obese and lean twins. *Nature* 457:480–484

Turnbaugh PJ, RE Ley, MA Mahowald, V Magrini, ER Mardis, JI Gordon (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 444:1027–1031

Valenzuela R, A Espinosa, D González-Mañán, A D'Espessailles, V Fernández, LA Videla, G Tapia (2012). N-3 Long-chain polyunsaturated fatty acid supplementation significantly reduces liver oxidative stress in high fat induced steatosis. *PLoS One*, 7:1–8

Velayudham A, A Dolganiuc, M Ellis, J Petrasek, K Kodys, P Mandrekar (2009). VSL#3 probiotic treatment attenuates fibrosis without changes in steatohepatitis in a diet-induced nonalcoholic steatohepatitis model in mice. *Hepatology* 49:989–997

Walayat S, D Martin, J Patel, U Ahmed, MN Asghar, AU Pai (2017). Role of albumin in cirrhosis: from a hospitalist's perspective. *J Commun Hosp Intern Med Perspect*, 7:8–14

Wang Y, Y Liu, A Sidhu, Z Ma, C McClain, W Feng (2012). Lactobacillus rhamnosus GG culture supernatant ameliorates acute alcohol-induced intestinal permeability and liver injury. *Amer J Physiol Gastrointest Liver Physiol* 303:32–41

Weber LWD, M Boll, A Stampfl (2003). Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model. *Crit Rev Toxicol* 33:105–136

Wiedemann MSF, S Wueest, F Item, EJ Schoenle, D Konrad (2013). Adipose tissue inflammation contributes to short-term high-fat diet-induced hepatic insulin resistance. *Amer J Physiol Endocrinol Metab* 305:388–395

Wiest R, A Albillos, M Trauner, JS Bajaj, R Jalan (2017). Targeting the gut-liver axis in liver disease. *J Hepatol* 67:1084–1103

Williams LM, FM Campbell, JE Drew, C Koch, N Hoggard, WD Rees, T Kamolrat, HT Ngo, IL Steffensen, SR Gray, A Tups (2014). The development of diet-induced obesity and glucose intolerance in C57Bl/6 mice on a high-fat diet consists of distinct phases ed. Michael Müller. *PLoS One* 9:1–19

Xu A, Y Wang, H Keshaw, LY Xu, KS Lam, GJ Cooper (2003). The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J Clin Invest* 112:91–100

Xu KY, GH Xia, JQ Lu, MX Chen, X Zhen, S Wang, C You, J Nie, HW Zhou, J Yin (2017). Impaired renal function and dysbiosis of gut microbiota contribute to increased trimethylamine-N-oxide in chronic kidney disease patients. *Sci Rep* 7:1–12

Yang T, JL Owen, YL Lightfoot, MP Kladde, M Mohamadzadeh (2013). Microbiota impact on the epigenetic regulation of colorectal cancer. *Trends Mol Med* 19:714–725

Yatsunenko T, FE Rey, MJ Manary, I Trehan, MG Dominguez-Bello, M Contreras (2012). Human gut microbiome viewed across age and geography. *Nature* 486:222–227

Zhang HY, DW Han, ZF Zhao, MS Liu, YJ Wu, XM Chen (2007). Multiple pathogenic factor-induced complications of cirrhosis in rats: A new model of hepatopulmonary syndrome with intestinal endotoxemia. *World J Gastroenterol* 13:3500–3507

Zhao ZH, JKL Lai, L Qiao, JG Fan (2019). Role of gut microbial metabolites in nonalcoholic fatty liver disease. *J Digest Dis* 181–188

Zhu L, SS Baker, C Gill, W Liu, R Alkhouri, RD Baker (2013). Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. *Hepatology*, 57:601–609