LACTOBACILLUS PENTOSUS STRAIN S-PT84 ATTENUATES LIPOTOXICITY-INDUCED HEPATIC INSULIN RESISTANCE AND STEATOHEPATITIS BY MAINTAINING GUT PERMEABILITY AND INDUCING MACROPHAGE ALTERNATIVE ACTIVATION IN MICE

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Introduction:
Nonalcoholic fatty liver disease (NAFLD), a form of lipotoxic liver injury that can impair systemic insulin resistance, and can progress to nonalcoholic steatohepatitis (NASH). We previously demonstrated that excessive hepatic lipid accumulation promotes the activation of macrophages/Kupffer cells, resulting in exacerbated insulin resistance and hepatic inflammation (Ota T et al, Gastroenterology 132:282, 2007, Hepatology 46:1392, 2007, Endocrinology 156:987, 2015). Meanwhile, obesity or a high-fat diet enhances gut permeability and metabolic endotoxemia, which can trigger insulin resistance and NASH. However, there are few promising treatments targeting lipotoxicity-mediated endotoxemia and hepatic activation/polarization of macrophages in NASH. The Lactobacillus pentosus strain S-PT84 has immunomodulatory functions including Th1/Th2 balance modulatory effects and natural killer (NK)/NKT cell activation. Here, we examined the effect of S-PT84 on diet-induced NASH to test the hypothesis that S-PT84 can attenuate immune cell-mediated insulin resistance and NAFLD progression to NASH.

Methods:
C57BL/6 mice were fed a high-cholesterol/high-fat diet (CL) alone or with S-PT84 (CL + S-PT) for 22 weeks. Liver histology, insulin sensitivity and endotoxemia were examined. Next, we quantified intrahepatic immune cells using a fluorescence-activated cell sorter (FACS). To investigate the effects of S-PT84 on intestinal permeability, we performed a fluorescence carboxyfluorescein assay by orally gavaging mice with 1 mg/kg of body weight of 5(6)-carboxyfluorescein and collecting plasma after 2 hr.

Results:
After 22 weeks of feeding, histological examination revealed hepatic steatosis and inflammation in mice fed the CL diet. The mice exhibited hyperinsulinemia and glucose intolerance, indicating that the development of NASH associated with insulin resistance. S-PT84 administration improved hepatic steatosis by decreasing TG and FFA levels by 34% and 37%, respectively. S-PT84 also inhibited the development of hepatic inflammation and fibrosis, lowering F4/80+ macrophage/Kupffer cell infiltration and the hydroxyproline content of the liver. S-PT84 administration in mice fed a CL diet led to improved glucose intolerance and hyperinsulinemia as well as enhanced hepatic insulin signal assessed by phospho-Akt. These changes were associated with attenuated excess lipid peroxidation (TBARS) and MAPK (JNK/p38MAPK) and NF-kB activation in the liver. FACS analysis revealed that CD11c+CD206+ M1 or ‘classically activated’ pro-inflammatory macrophages were not significantly altered in the CL + S-PT group compared with mice fed the CL diet. However, mice fed the CL + S-PT had 71% more CD11c+CD206+ M2 or ‘alternatively activated’ non-inflammatory macrophages than mice fed the CL diet, resulting in a predominance of the M2 over the M1 macrophage population. S-PT84, however, showed little effect on NK/NKT cell and T regulatory cell populations in the liver. Furthermore, S-PT84 induced significant improvements in intestinal epithelial barrier permeability. Importantly, plasma lipopolysaccharide binding protein (LBP) levels were markedly increased in mice fed the CL diet compared with normal chow, while S-PT84 markedly diminished plasma LBP levels.

Discussion:
A CL diet enhanced intestinal permeability and metabolic endotoxemia, contributing to hepatic insulin resistance and inflammation in NASH. S-PT84 treatment attenuated lipotoxicity-induced hepatic insulin resistance and steatopheetitis by maintaining gut permeability and inducing alternative
activation of liver macrophages. Further studies are required to determine whether S-PT84 can directly influence the gut immune system.

**Keywords:** Lactobacillus pentosus strain S-PT84, Nonalcoholic steatohepatitis (NASH), Insulin resistance, Probiotics, Lipopolysaccharide binding protein (LBP), Macrophage

**Citation:**