# Summary

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Human gastrointestinal tract is a home for trillions of microbiota which play essential beneficial role including lipid and cholesterol metabolism, SCFA production, modulation of immune response and vitamin synthesis. Disordered microbial "self" leads to a number of diseases like obesity, inflammatory bowel disease and a range of psychiatric diseases which are growing exponentially. Therefore, maintenance of the healthy gut microbiome, possibly by reintroduction of commensal bacteria known as probiotic therapy, may be essential to optimizing the disordered gut flora. These probiotic microorganisms have well documented health benefits when administered in adequate amounts including anti-oxidative activity, stimulation of mucus, suppression of the immune cell proliferation and other gut epithelial barrier protective effects.

The probiotic *E. coli* Nissle 1917 (*Ec*N) used in the present study had incorporated *vgb* and *gfp* which enhanced survival efficiency and also enabled for tracking its colonization in the gut. The aim of the present study was to determine effect of probiotic secreting antioxidant PQQ and producing fructose metabolizing enzyme on fructose induced metabolic syndrome. This strategy not only led to decreased intestinal absorption but also converted fructose into a prebiotic.

PQQ is a potential antioxidant which serves as a co-factor for glucose dehydrogenase protein. *E. coli* genome encodes glucose dehydrogenase apoprotein but lacks the ability to synthesize PQQ co-factor. Enabling *Ec*N to continuously secrete PQQ in gut will reduce dependency of humans for PQQ on plant based diet and as PQQ serves as a co-factor for glucose dehydrogenase it will facilitate *Ec*N to convert glucose to gluconic acid, a prebiotic molecule. This will further be digested by gut microflora resident in lower part of gastrointestinal tract resulting in the production of short chain fatty acids (SCFAs).

In case of fructose mediated disorder in rats, *Ec*N was modified to produce fructose metabolizing enzymes, fructose dehydrogenase and mannitol dehydrogenase. In *E. coli*, fructose is transported in phosphorylated form through PTS system. On the other hand, *Zymomonas mobilis* transports fructose in unphosphorylated form through GLF transporter. GLF transporter was used for the uptake of fructose in unphosphorylated form. Mannitol dehydrogenase (MTLK) was employed for in situ

conversion of fructose to mannitol, a prebiotic molecule. In comparison to *Ec*N producing PQQ, *Ec*N producing PQQ and MTLK was capable of producing two prebiotic molecules, Gluconic acid and Mannitol. This modified *Ec*N transformant decreases the intestinal absorption of fructose by diverting for mannitol formation. Rats fed with *Ec*N producing both PQQ and MTLK was found to exhibit almost normal lipid profile and antioxidant status in both liver and blood. Alternately, fructose dehydrogenase (FDH) converts fructose to 5-ketofructose which is in turn excreted out of the body. Delaying of metabolic syndrome and improving antioxidant status both in blood and liver to almost normal levels in fructose fed rats seems to be combined effect of *Ec*N, PQQ and fructose metabolizing enzymes.

Fructose, apart from leading to metabolic disorder if taken in required amount is beneficial for iron deficiency. To overcome the deleterious effects of fructose, *Ec*N(*pqq-glf-mtl*K) was used as an alternate strategy for enhancing iron absorption. Fructose helps in reducing ferric form of iron which is not easily absorbed to easily absorbable ferrous form. Moreover, dietary fructose is converted to mannitol by *Ec*N*(pqq-glf-mtlK),* which is a prebiotic and is fermented by the colonic microflora to produce SCFAs, which are known to facilitate iron absorption by reducing ferric ions into ferrous forms . Thus, iron absorption gets enhanced by the synergistic beneficial effects of both fructose and SCFA.

Metabolic disorder of fructose is predominantly attributed to increased consumption of HFCS worldwide. HFCS is reported to have traces of mercury which causes many toxic effects including oxidative stress. The current strategy to deal with metal toxicity uses chelators along with potent antioxidant. PQQ served as a potent antioxidant dealing with the metal induced oxidative stress. In addition, *Ec*N (*pqq*-CS-*cit*C) was used to produce PQQ as an antioxidant and citric acid as chelating agent. But, this probiotic did not prove to be very effective as the amount of citric acid produced was not sufficient to chelate mercury. Alternately, PQQ and 2-ketogluconic acid producing probiotic *Ec*N (*pqq-gad*) ameliorated effectively mercury induced metal toxicity.

In conclusion, the present study expanded the potential of genetic modifications of probiotic *Ec*N in overcoming the deleterious effects of sucrose,

ethanol and rotenone to dietary fructose induced metabolic disorders, iron deficiency and mercury induced toxicity.