**Development of a long acting FGF21 analogue-albumin fusion protein for the treatment of MASLD**

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**Background and aims.** Metabolic dysfunction-associated steatotic liver disease (MASLD) affects 30% of the global population and involves factors such as insulin resistance and dyslipidemia. Fibroblast growth factor 21 (FGF21) improves insulin sensitivity and lipid metabolism by acting on major target organs such as adipose tissue and the liver. However, its clinical use is limited by poor stability and a short half-life. To overcome these limitations, we developed HSA-mFGF21, a fusion protein combining human serum albumin (HSA) with a stabilized FGF21 mutant (mFGF21), and evaluated its efficacy against hepatic steatosis by enhancing tissue retention and systemic stability.

**Methods.** HSA-mFGF21 was expressed using the *Pichia pastoris* expression system. Pharmacokinetic profiles of 125I-labeled HSA, FGF21 and HSA-mFGF21 were evaluated following intravenous administration in healthy mice. The therapeutic effect of HSA-mFGF21 on obesity was evaluated using HFD-60-induced MASLD model mice.

**Results.** SDS-PAGE analysis of purified HSA-mFGF21 revealed a single band at the expected molecular weight of 86 kDa. Pharmacokinetic studies demonstrated that HSA-mFGF21 exhibited a 20-fold longer half-life than native FGF21, owing to albumin fusion. In MASLD model mice, HSA-mFGF21 administration led to significant reductions in serum lipid and glucose levels. In adipose tissue, the treatment enhanced glucose uptake and thermogenesis. In the liver, it decreased hepatic triglyceride content, alleviated insulin resistance, and improved plasma ALT. Histological analysis using H&E and Oil Red O staining confirmed a marked reduction in hepatic lipid droplet accumulation.

**Conclusion/Discussion.** The findings reported in this study indicate that HSA-mFGF21 suppresses hepatic lipid accumulation, improved obesity and insulin resistance in high-fat diet-induced MASLD model mice. In addition, HSA-mFGF21 ameliorated liver damage. HSA-mFGF21 represents a potentially useful therapeutic agent for the treatment of MASLD in the future.