TOTUM-63 reduces hepatic steatosis in obese mice

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Background.

Prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD) is very high worldwide, increasing in parallel with that of obesity and diabetes. NAFL is mainly characterized by abnormal lipid accumulation in hepatocytes (steatosis), mostly under the form of triglycerides, without any evidence of hepatocellular injury. Hepatic steatosis represents a high risk factor for developing Non-Alcoholic Steato-Hepatitis (NASH), which can further lead to fibrosis, cirrhosis, hepatocellular carcinoma (HCC), and liver failure. We have developed TOTUM-63, candidate for managing metabolic disorders related to insulin resistance, such as NAFLD, and prevent the onset of severe diseases including NASH.

Methods.

To determine whether TOTUM-63 may have beneficial effects on hepatic steatosis progression, C57BL/6JRj male mice were fed a High-Fat-Diet (60%, 260HFD, Safe, France) with or without TOTUM-63 (2,7%) supplementation for 16 weeks. A third group was used as control and fed a control diet (A03 enriched with 3% corn oil, Safe, France). Body weight and composition (EchoMRI) were measured at the end of the study, along with fasting glycemia and plasma insulin levels. In the 15th week of the experiment, an oral glucose tolerance test (OGTT) was carried out, allowing to calculate the insulin sensitivity index. H&E and Oil Red O staining have been done in liver sections. Triglycerides amount was quantified by colorimetric assay. RT-qPCR analysis have also been conducted in liver tissue to quantify expression of several transcription factors and other genes involved in the regulation of hepatic lipid metabolism.

TOTUM-63 showed a strong effect to prevent hepatic steatosis development on this diet-induced NAFLD mice model. Mechanisms may involve modulation of key transcription factors linked to NAFLD pathophysiology, like PPARδ, PPARγ or FXR, leading to reduced accumulation of hepatic lipid droplets. TOTUM-63 is a promising candidate to prevent hepatic steatosis which is a strong risk factor to develop NASH.

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Results.

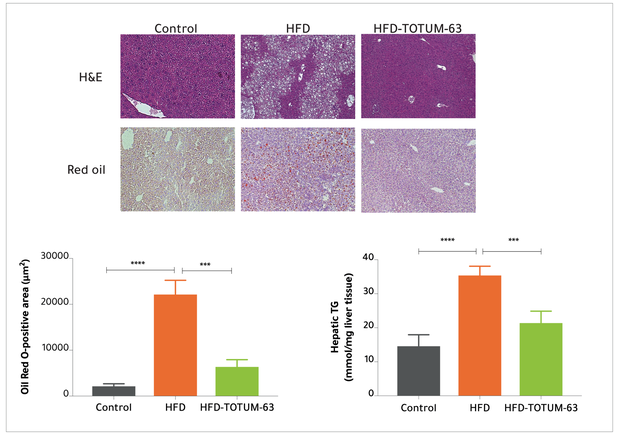

Figure 1: TOTUM-63 strongly reduces hepatic steatosis in HFD-fed mice. H&E and Oil Red O staining in liver sections revealed a strong reduction of lipid droplets accumulation in hepatocytes. The mean area of lipid droplets was reduced by 70% (p<0.001) with TOTUM-63 supplementation. Colorimetric measurements of hepatic triglycerides confirmed this result (-40%, p<0.001).

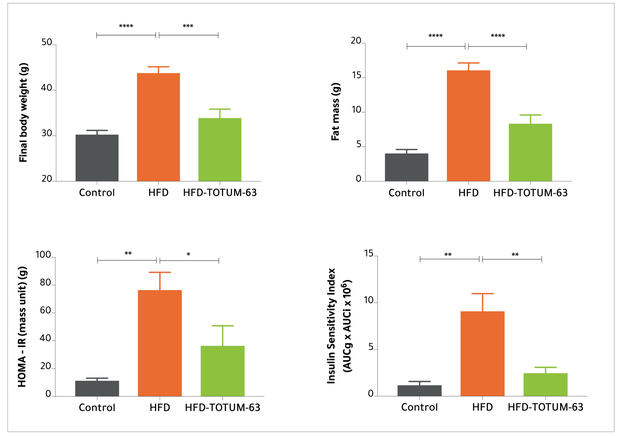

Figure 2: TOTUM-63 reduces weight gain, fat mass and insulin resistance, key factors in hepatic steatosis pathogenesis. Body weight gain was strongly reduced following TOTUM-63 supplementation (-23%, p<0.001). That was explained by lower fat mass (-48%, p<0.0001). HOMA-IR (-52%, p<0.05) and insulin sensitivity index during OGTT (-72%, p<0.01) also revealed that TOTUM-63 strongly reduced insulin resistance.

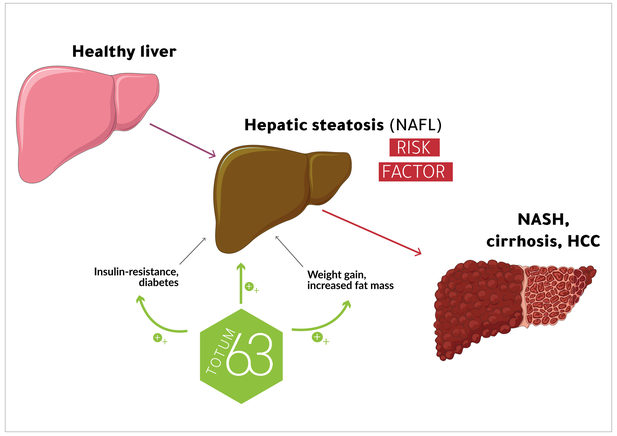

Figure 3: Effects of TOTUM-63 on NAFLD pathophysiology. TOTUM-63 strongly reduces hepatic steatosis, insulin resistance and fat mass, all of them key factors in NAFLD pathophysiology. Hepatic steatosis is a major risk factor for developing NASH and subsequently cirrhosis and HCC. TOTUM-63 is a promising candidate to manage NAFLD, with a high potential to reduce the risk of NASH.

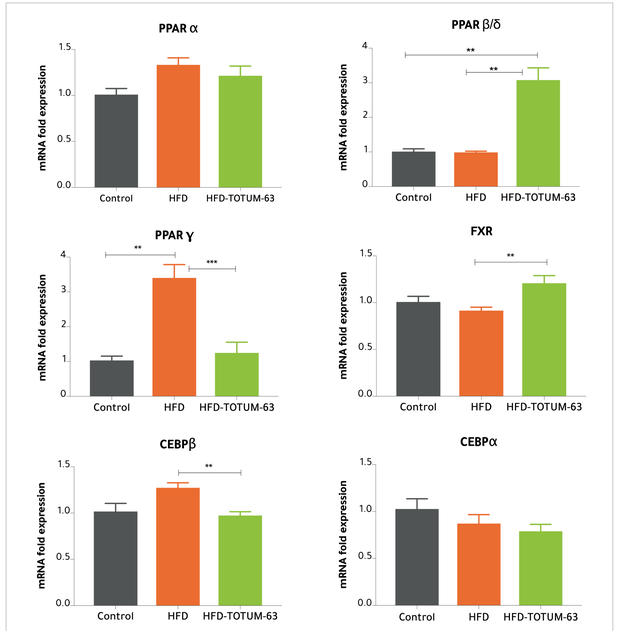

Figure 4: TOTUM-63 alters expression of key transcription factors involved in hepatic steatosis regulation. TOTUM-63 increased PPARδ (+314%, p<0.01) and FXR (+132%, p<0.05) gene expression, and reduced PPARγ (-64%, p<0.001) and C/EBPβ (-23%, p<0.01) gene expression in HFD-fed mice. These transcription factors are key regulators of hepatic lipid metabolism.

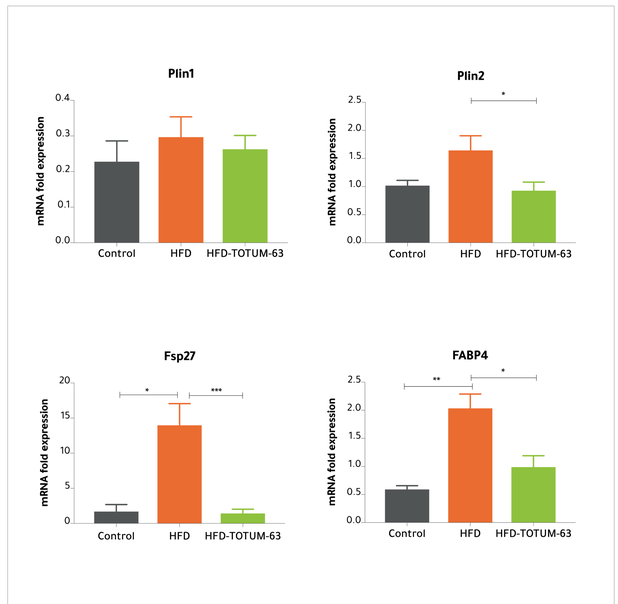

Figure 5: TOTUM-63 regulates the expression of several genes involved in hepatic lipid storage. TOTUM-63 supplementation lowered Plin-2 (-40%, p<0.05), Fsp27 (-90%, p<0.001) and FABP4 (-52%, p<0.05) gene expression in HFD-fed mice. These proteins are known to be up-regulated in hepatic steatosis condition and are involved in lipid droplets accumulation in the liver.

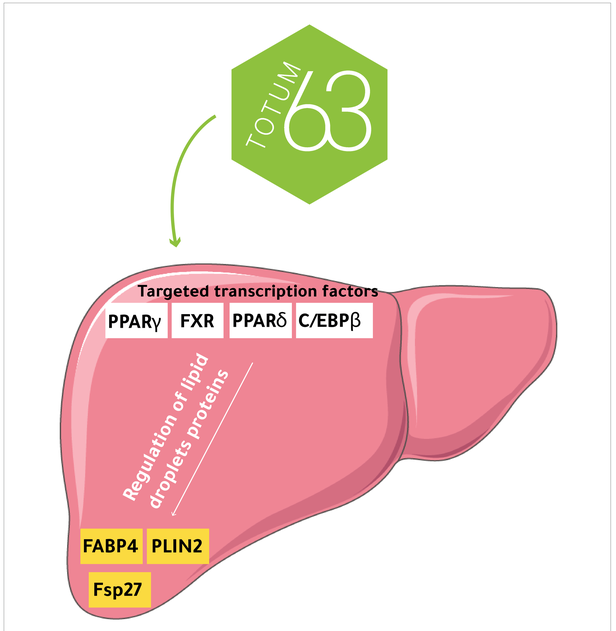

Figure 6: Summary of the potential mechanisms involved in the effects of TOTUM-63 on hepatic steatosis. TOTUM-63 regulates the expression of transcription factors known to play a key role in hepatic lipid metabolism. These transcription factors may explain the down-regulation of several genes involved in hepatic lipid storage following TOTUM-63 supplementation.