Sustainability of TOTUM•448 beneficial effects following interruption of supplementation in a hamster model of diet-induced NASH

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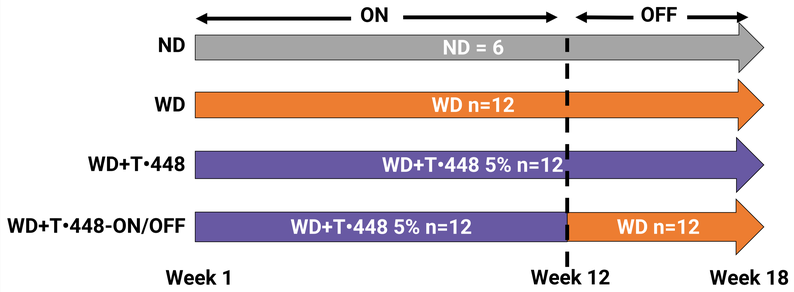
1 – Background and aim

TOTUM•448 is a patented combination of 5 plant extracts and choline. We previously showed that 12 weeks of supplementation with TOTUM•448 improved steatosis, inflammation, and fibrosis markers in hamsters with diet-induced metabolic dysfunction-associated steatohepatitis (MASH, formally non-alcoholic steatohepatitis, NASH). In this work, we aimed at assessing the sustainability of TOTUM•448 associated beneficial effects on MASH by interrupting treatment after a 12-week supplementation period, in western-diet (WD)-fed hamsters.

2 – Methods

Male golden Syrian hamsters were fed a WD (D99122211, Research Diets, USA. 45% kcal from fat, 17% kcal from fructose, 1.2% cholesterol w/w) supplemented or not with TOTUM•448 (5%, WD+T-448) for 18 weeks. A third group of hamsters was fed WD+T-448 for 12 weeks before switching to non-supplemented WD for 6 more weeks (WD+T•448-ON/OFF) and a fourth group of hamsters fed a normal diet (ND, D09100304, Research Diets) was used as control. Food intake, body weight and body composition were monitored continuously. MASH was evaluated using circulating and hepatic biochemical and histological markers of steatosis, inflammation, and fibrosis.



3 – Results

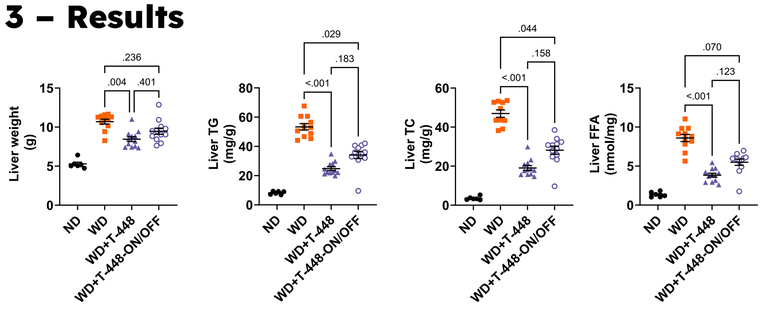


Figure 1: Liver weight and liver lipids

After 18 weeks, liver weight was decreased in TOTUM•448 supplemented animals and liver triglyceride (TG), total cholesterol (TC) and free fatty acid (FFA) levels were reduced compared to WD. These beneficial effects persisted in WD+T•448-ON/OFF despite interruption of supplementation for the last 6 weeks, though being less pronounced.

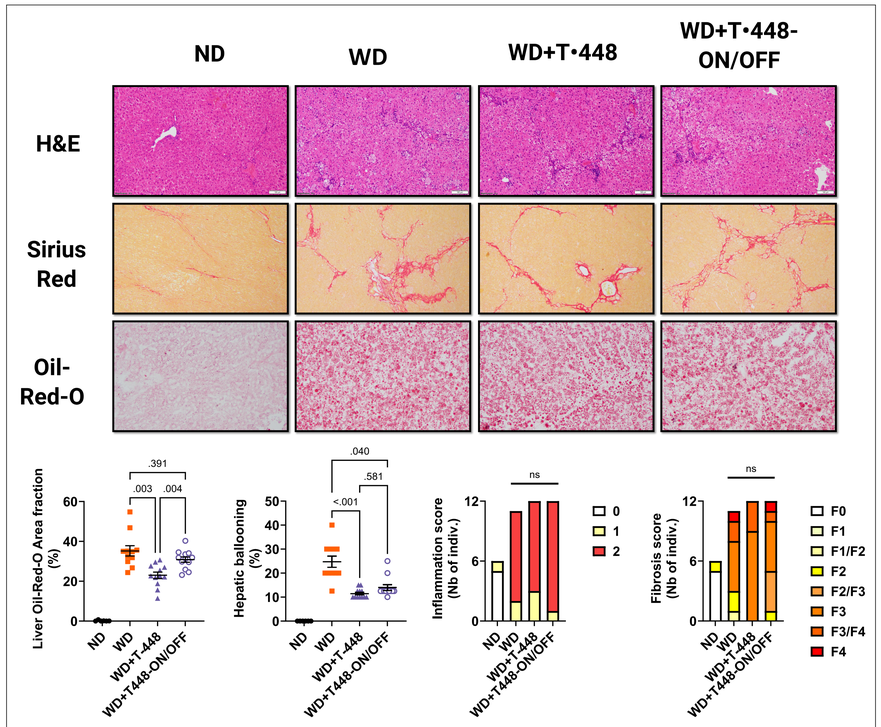


Figure 2: Histological analyses of liver sections

Improvement of steatosis was confirmed in WD+T•448 *vs*. WD by analysis of area fraction of Oil-red-O-stained liver sections. Interruption of supplementation in WD+T•448-ON/OFF resulted in significantly higher stained area, compared to WD+T•448. Hepatic ballooning was significantly reduced by TOTUM•448 and this effect persisted in group WD+T•448-ON/OFF. No differences were noted among groups in inflammation and fibrosis score.

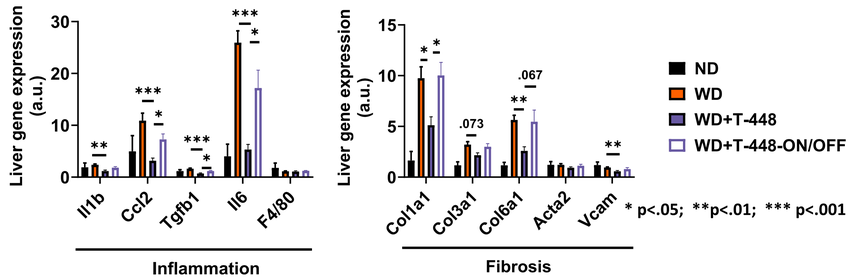


Figure 3: Liver gene markers of inflammation and fibrosis

Expression of Il1b, Ccl2, Tgfb1 and Il6 was significantly decreased in WD+T•448, *vs*. WD, suggesting reduction of inflammation in the liver. Similarly, we observed a decrease in the expression of Col1a1, Col3a1 (p=.073), Col6a1 and Vcam following TOTUM•448 supplementation, suggesting prevention of fibrosis. Interruption of supplementation for 6 weeks in WD+T-448-ON/OFF resulted in a dramatic increase in the expression of inflammation and fibrosis gene markers.

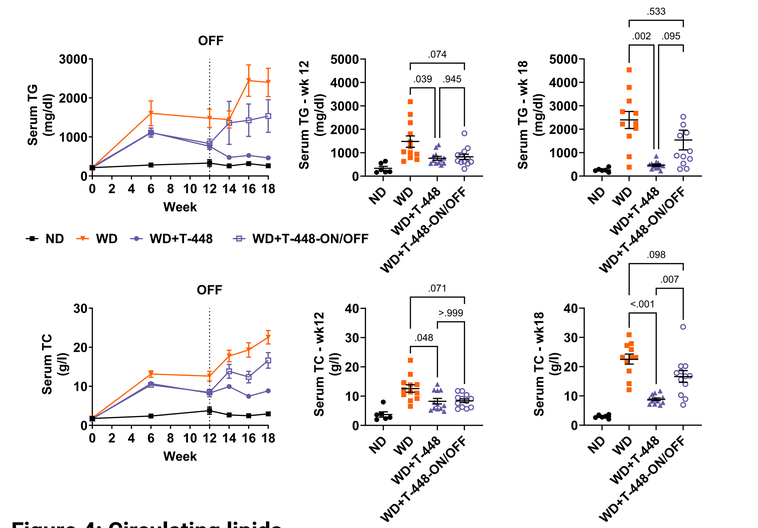


Figure 4: Circulating lipids

Improvement of serum triglycerides (TG) and total cholesterol (TC) was visible after 12 weeks of supplementation with TOTUM•448 in WD+T•448 and WD+T•448-ON/OFF, compared to WD. Ceasing of supplementation for 6 weeks led to an increase in both circulating lipids in WD+T•448-ON/OFF, although some benefits were conserved *vs*. WD at wk 18.

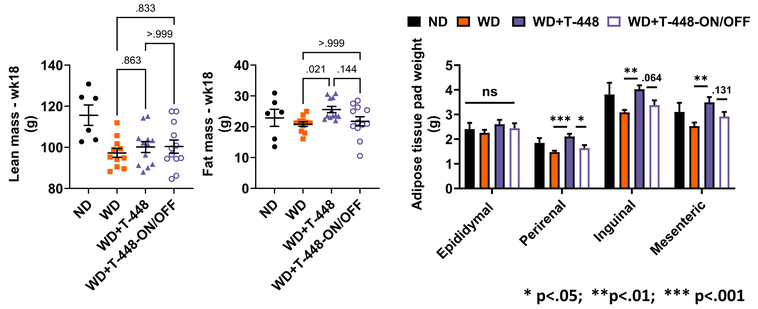


Figure 5: Body composition

At the end of the study (18 weeks), hamsters in WD+T•448 displayed significantly higher fat mass *vs*. WD. Consequently, perirenal, inguinal and mesenteric fat pad weights were increased, suggesting increased lipid storage capacity. Interruption of TOTUM•448 supplementation led to a decrease in adipose fat pads weights.

4 – Conclusion

Supplementation with TOTUM•448 for 18 weeks in WD-fed hamsters resulted in major improvements in several hallmarks of MASH development, including reduction of liver lipid content (steatosis), ballooning, and inflammation and fibrosis gene markers, along with circulating TG and TC. Interruption of supplementation for the last 6 weeks of study in group WD+T•448-ON/OFF led to an intermediate profile, with most benefits conserved, to a lesser degree. The improvement of lipid storage capacity may suggest reduced lipolysis in adipose tissue, which could constitute a first explanatory hypothesis as for TOTUM•448 mechanism of action.

Conflict of interest statement: VC, YFO, DR, MV, CL, FLJ, CBR, are Valbiotis employees. SP is Valbiotis CEO, PS is Valbiotis CSO. TM is a member of Valbiotis' scientific and medical board.