PRESENTATION #623

TOTUM-63: A NEW PROMISING APPROACH FOR THE MANAGEMENT OF OBESITY AND RISK REDUCTION FOR DEVELOPING TYPE 2 DIABETES

V. Chavanelle[1], Y.F. Otero[1], D. Ripoche[1], C. Langhi[1], M. Bargetto[1], F. Le Joubioux[1], B. Guigas[2], N. Boisseau[3], T. Maugard[4], G. Pickering[5], M. Cazaubiel[1], S.L. Peltier[1], P. Sirvent[1]

[1]Valbiotis, Perigny, France, [2]Department of Parasitology, Leiden University Medical Center, Leiden, The Netherlands, [3]Université Clermont Auvergne, AME2P, Clermont-Ferrand, France,
[4]La Rochelle Université - LIENSs UMR CNRS 7266, La Rochelle, France, [5]Centre Hospitalier Universitaire de Clermont-Ferrand, Clermont-Ferrand, France

BACKGROUND AND AIMS

Global prevalence of diabetes was estimated at 463 million people in 2019 and is projected to reach 700 million by 2045[1]. We have developed TOTUM-63 (T63), a plant-based active substance, to reduce the risk of developing type 2 diabetes (T2D). We assessed the effects of T63 in HFD-induced obese mice and in a phase I/II open clinical trial conducted in overweight individuals.

MATERIALS AND METHODS

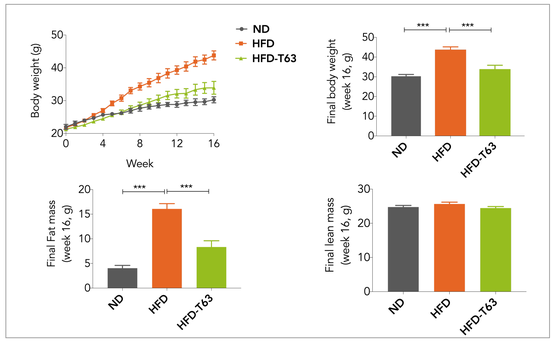
Male C57BL6 mice were fed a High-Fat-Diet (HFD) with or without T63 supplementation (2.7%) for 10, 12 or 16 weeks. A first Phase I/II open clinical trial was conducted in 14 overweight (BMI: 27.7±1.9 kg/cm^2) male individuals. It included an initial period of supplementation with 2.5g/day for 4 weeks followed by a 2-week wash-out period, then 4 weeks with 5g/day supplementation. Usual safety parameters were assessed at all visits. Glycemia and insulinemia were monitored for 2h after a carbohydrate tolerance test before and after the 5g/day supplementation period.

RESULTS

HFD-fed mice study:

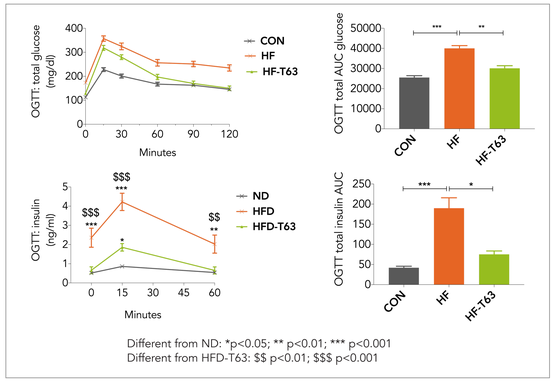
Effect on body weight and body composition

In HFD-fed mice, T63 supplemented animals were protected against excessive body weight gain (after 16 weeks: -23%, p<0.001) and fat mass (-48%, p<0.001). Lean mass was not significantly different between groups.



Response to an OGTT

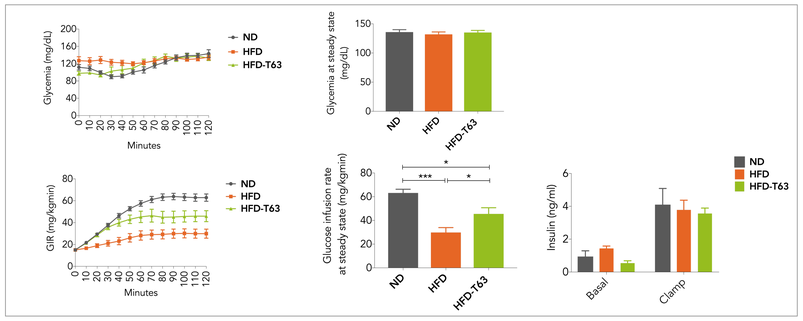
OGTT (2.3 g glucose/kg lean mass) was performed after 16 weeks of experiment in fasted (6h) mice. Glucose AUC was reduced in HFD-T63 (-25% *vs.* HFD, p<0.01) and insulin levels were lower before glucose gavage (T0 HFD-T63 *vs.* HFD, p<0.001), 15 min (T15, p<0.001) and 60 min (T60, p<0.01) after gavage. Subsequently, insulin AUC was reduced by T63 supplementation (-61% *vs.* HFD, p<0.05). Taken together, these data suggest an improvement of systemic glucose tolerance in T63 supplemented mice.



Different from ND: *p<0.05; ** p<0.01; *** p<0.001
Different from HFD-T63: $$ p<0.01; $$$ p<0.001

[1]Saeedi P. et al Diabetes Res Clin Pract. 2019;
[2]Chao A, Scan J of stat, 1984;
[3]Shannon A, The Bell Syst Tech J, 1948.

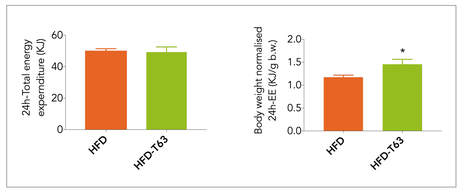
Response to an euglycemic hyperinsulinemic clamp



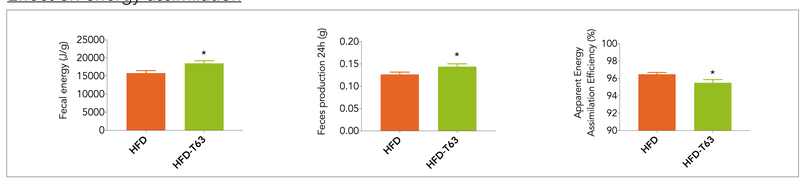
Euglycemic hyperinsulinemic clamp was performed after 12 weeks of experiment. Glucose steady state was achieved in all groups around 135 mg.dl $^{-1}$ in average. Glucose infusion rate (GIR) at steady state was increased in HFD-T63, *vs.* HFD (+53%, p<0.05) suggesting improvement of insulin sensitivity in this group. Insulin levels were not significantly different between groups.

Effect on energy expenditure

24-h energy expenditure was estimated in groups HFD and HFD-T63 by indirect calorimetry. No difference was observed in total energy expenditure. When normalized by body weight, energy expenditure was higher in HFD-T63 (+25%, p<0.05). TOTUM-63 may have preserved energy expenditure despite body weight loss. This mechanism could have contributed to the effect observed on body weight, in HFD-T63.



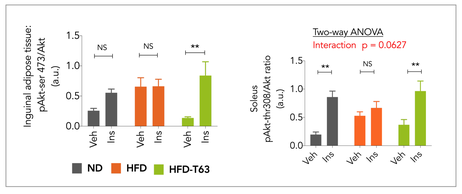
Effect on energy assimilation



Fecal energy density was assessed by bomb calorimetry in groups HFD and HFD-T63.
T63 supplemented mice had higher fecal energy density (+17%, p<0.05). Daily feces production was also increased in HFD-T63 (+13%, p<0.05). Subsequently, apparent energy assimilation efficiency was reduced in HFD-T63 (-1%, p<0.05). This mechanism may have participated to the effects on body weight observed in HFD-T63.

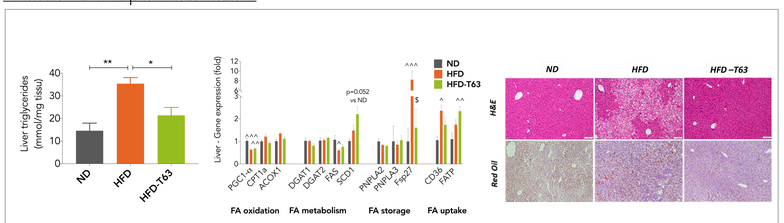
Effect on the insulin pathway

Insulin (Ins, 0.75 U/kg lean mass) or an equivalent volume of vehicle (Veh, NaCl 0.9%) was administered intraperitoneally to the mice 10 min before sacrifice.
Insulin stimulated response on Akt phosphorylation ratio was blunted in HFD in inguinal fat and soleus muscle.



Interestingly, supplementation with T63 restored the ability of insulin to stimulate Akt phosphorylation in these organs (statistical trend only for soleus muscle). No detrimental effects of HFD on Akt phosphorylation was observed in liver, epididymal fat and gastrocnemius muscle (data not shown).

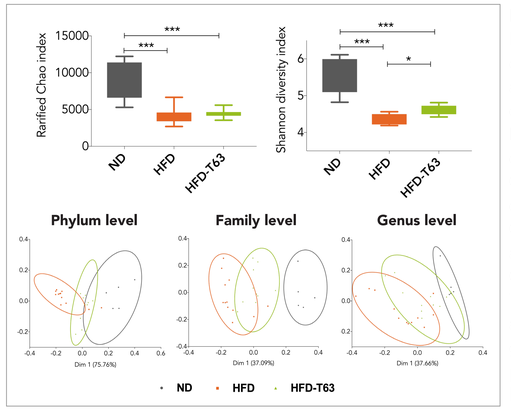
Effect on liver lipid metabolism



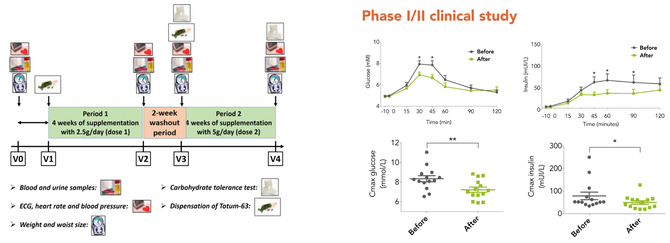
Different from ND: *p<0.05; ** p<0.01; *** p<0.001, Different from HFD: # p<0.051; ## p<0.01

Hepatic triglyceride content was reduced in HF-TOTUM-63 group, compared to HF (-40%, p<0.001). H&E and Red Oil-stained sections confirmed reduced steatosis in T63 supplemented animals. The expression of several genes associated with fatty acid (FA) oxidation, FA metabolism, lipid droplets formation and FA uptake were assessed by PCR. Only Fsp27, a gene associated with lipid droplet growth, was reduced by T63 supplementation, compared to HFD (p<0.05). These results suggest that the beneficial effects of T63 on hepatic lipids may be due to reduced lipid flux into the liver rather than a direct effect on target genes.

Effect on HFD-induced dysbiosis



Metagenomic analyses of microbiota was assessed in frozen feces on a fragment of a sequence amplified by PCR. Richness and diversity were estimated by Rarefied Chao and Shannon indexes, respectively[2,3]. Principal Coordinates Analysis (PCoA) were drawn using dissimilarity Bray-Curtis matrix based on relative abundances at phylum, family and Genus level. Cecal microbiota of T63 supplemented mice displayed an intermediate profile between groups ND and HFD. T63 may partly restore HFD-induced microbiota dysbiosis.



The response to a carbohydrate tolerance test performed before (V3) and after (V4) a 4-week supplementation period with T63 (5g/d) was improved in overweight individuals. Specifically, glucose and insulin peaks (Cmax) were reduced following carbohydrate ingestion, after supplementation (-13%, p<0.01 and -37%, p<0.05, respectively). These results suggest that T63 may be efficient for improving glucose tolerance in humans. Safety (biological, hemodynamic and anthropometric) parameters were also assessed (data not shown). T63 was considered a well-tolerated product. No adverse event related to T63 supplementation was observed.

CONCLUSION

In HFD-fed mice, TOTUM-63 protected supplemented mice from excessive weight and fat mass gain. This effect could be mediated by preserved energy expenditure despite body weight loss and lower nutrient absorption in the digestive tract. In the liver, steatosis was improved in supplemented mice. Systemic glucose tolerance and insulin sensitivity were improved in T63 supplemented mice and insulin pathway may have been restored in 2 major insulin-sensitive organs involved in glucose uptake: inguinal fat and soleus muscle. In the gut, HFD-induced dysbiosis was partially restored by T63 supplementation. This effect could have contributed to the effects on general adiposity, glucose homeostasis and insulin sensitivity. Finally, preliminary data on overweight individuals have shown a good tolerance and signs of glucose tolerance improvement with T63 supplementation.
In conclusion, T63 appears as an efficient strategy to tackle the obesity and T2D global pandemic, potentially via a pleiotropic action on various organs. Further studies are presently conducted to elucidate the mechanisms of action in different preclinical models and in a new clinical trial.