# In vivo MR approaches to validate the capacity of a new vanadium compound as a promising anti-diabetic drug

**A. M. Metelo[1], R. Pérez-Carro[1], M. M. Castro[2], and P. López-Larrubia[1]**
[1]Instituto de Investigaciones Biomédicas "Alberto Sols", CSIC/UAM, Madrid, Madrid, Spain, [2]Dept. Life Sciences, Faculty of Science and Technology, University of Coimbra, Coimbra, Coimbra, Portugal

## Objectives:

Diabetes *mellitus* (DM) is a known metabolic disorder characteristic of developed countries and with high prevalence in children and adolescent. Recently, World Health Organization had indicated that DM is the world's fastest-growing disease, being responsible for almost 3 million deaths worldwide per year[1]. Type 1 and type 2 DM are both associated with a continuous hyperglycemic condition that contributes to the development of other dangerous pathologies such as obesity, hypertension and cardiovascular diseases[2]. At the moment, some anti-diabetic treatments are available, since synthetic drugs to insulin injections; but the painful, discomfort and the lack of efficiency of these strategies ask for a drug that solves this current problem[3]. In recent years, different studies have emerged about the anti-diabetic properties of vanadium compounds. The potentiality of these agents encouraged their use in human clinical trials; however the toxicological effects compromise their success[4]. Recently, a more efficient insulin-mimetic drug, Bis-[3-hydroxy-1,2-dimethyl-4-pyridinonato] oxovanadium (IV), also called $VO(dmpp)_2$, has been studied[5]. The efficacy obtained with this compound *in vitro* encouraged further work, paving the way to implement this insulin-mimetic compound in clinical trials. Therefore, an *in vivo* study was conducted using obese Zucker rats as an animal model of obesity and insulin resistance. The aim of this study is to elucidate the effect of $VO(dmpp)_2$ on impaired lipid and glucose metabolism of pre-diabetic obese Zucker rats[6] by using *in vivo* Magnetic Resonance and others biological techniques.

## Methods:

Lean Zucker (fa/+) and obese Zucker (fa/fa) rats (7 weeks-old) were used to unveil the insulin-mimetic properties of $VO(dmpp)_2$ *in vivo*. During four weeks, the animals were daily weighted and intraperitoneally injected with $VO(dmpp)_2$ compound (15 mg/Kg body weight) or serum, being divided in four groups: $VO(dmpp)_2$-treated lean rats (n=6), lean non-treated rats (n=8), $VO(dmpp)_2$-treated obese rats (n=7) and obese non-treated rats (n=7). Once a week, the animals were assessed in terms of hepatic triglyceride (HTG) content determined by $^1$H MRS and studied by *in vivo* MRI. On the last day of the experiment, a glucose tolerance test was performed and blood was withdrawn from the tail of overnight fasted animals to determine possible toxicological effects.

## Results:

After the $VO(dmpp)_2$ treatment, different parameters indicative of obesity, insulin resistance and pre-diabetes were reverted in obese Zucker rats such as, gain of body weight (Figure 1), subcutaneous fat width (Figure 2), glucose intolerance profile and high HTG content (Figure 3). The lack of renal toxicity in obese Zucker rats treated with $VO(dmpp)_2$ showed the efficiency of this potential drug to revert obesity symptoms in non-toxic concentrations.

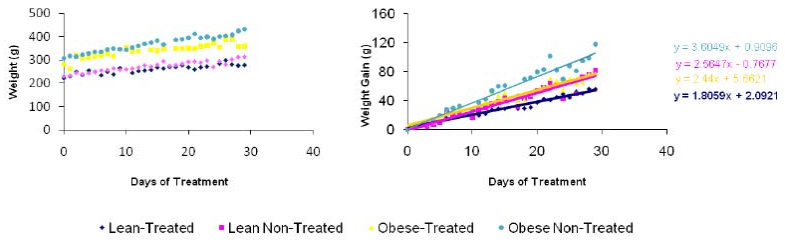


**Figure 1 – Gain of body weight of Zucker rats submitted to different treatments during four weeks:** the lean-treated rats (dark blue), the lean non-treated rats (pink), the obese vanadium-treated rats (yellow) and the obese non-treated rats (light blue). The trendline was drawn to each group of animals and the respective equations are shown. Data are present as mean values. Initially, vanadium-treated and non-treated obese Zucker rats had the same weight values; however after $VO(dmpp)_2$ treatment vanadium-treated obese Zucker rats had a gain of body weight lower than non-treated obese Zucker rats.

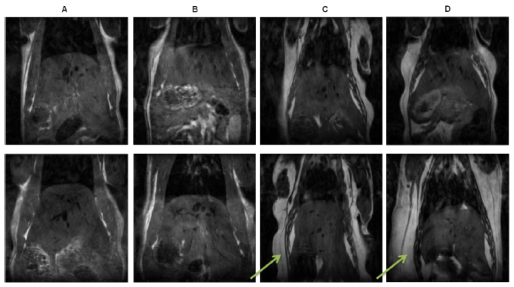


**Figure 2 - Coronal T1-weighted images of the liver of four different Zucker rats.** T1-weighted images of the liver of a non-treated lean rat (column **A**), $VO(dmpp)_2$-treated lean rat (column **B**), $VO(dmpp)_2$-treated obese rat (column **C**) and non-treated obese rat (column **D**), in the first day (top) and in the last day of the study (bottom). The difference between the subcutaneous fat width of obese-treated and obese non-treated rat is shown by the arrows. During the treatment with $VO(dmpp)_2$, the thickness of subcutaneous fat is minimized in obese-treated animals, in contrast with obese non-treated rats. Indeed, this remarkable difference is a consequence of $VO(dmpp)_2$ treatment, qualitatively proving the action of this compound on the impaired lipid metabolism of obese Zucker rats.

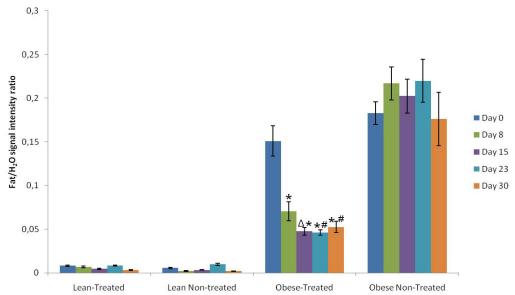


**Figure 3 - Quantification of HTG in lean and obese Zucker rats during the four weeks of $VO(dmpp)_2$ treatment.** The quantification of the HTG content was done by the ratio between the peak areas of $^1$H fat signal and $^1$H water signal, obtained by $^1$H MRS technique. Obese Zucker rats presented high levels of HTG since the first day of the study. However, after the first week of $VO(dmpp)_2$ treatment, the HTG content remarkably decreased in the obese-treated animals, whereas HTG in non-treated obese rats remained high. In the last day of the treatment, the significantly difference between $Fat/H_2O$ ratio of obese-treated and obese non-treated Zucker rats demonstrated the action of $VO(dmpp)_2$ on revert the high HTG profile of obese Zucker rats. Data are shown as mean values ± SEM. Paired bilateral *t* test was used in statistical analysis where $P<0.05$ was considered to be significant. * $P<0.0005$ relative to the respective day of obese non-treated Zucker animals. Δ $P<0.005$ relative to the day 0 of obese-treated Zucker rats. # $P<0.05$ relative to the day 0 of obese-treated Zucker rats.

## Conclusion:

In summary, this study proved that $VO(dmpp)_2$ compound acts on glucose and lipid metabolism of the obese Zucker rat, reverting its pre-diabetic profile[3] within a non-toxic range. Consequently, $VO(dmpp)_2$ becomes a strong candidate to enter in future clinical trials to be used against steatosis, obesity or Type 2 DM.

**References:** 1) Wild S *et al*. *Diabetes Care*. 2004, 27(5):1047-1053. 2) Hsueh W *et al. Postgrad Med.* 2010, 122(4):129-43. 3) Sakurai H *et al. Chemical Society Reviews*, 2008, 37(11):2383-2392. 4) Thompson K *et al*. *Journal of Inorganic Biochemistry*. 2009, 103(4):554-558. 5) Passadouro M *et al*. *Journal of Inorganic Biochemistry*. 2010, 104(9):987-992. 6) Augstein P & Salzsieder E. *Methods in Molecular Biolog.* 2009, 560:159-189.