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$VO(dmpp)_2$ reverts pre-diabetic features in fatty Zucker rats: MRI/MRS techniques as non-invasive powerful tools in drug development

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1. Purpose

Diabetes mellitus (DM) is the world's fastest-growing disease being responsible for almost 3 million deaths per year [1]. In the recent years, vanadium compounds have attracted a lot of interest due to their potential therapeutic application, particularly in the treatment of DM [2]. Many V(IV) and V(V) complexes have been synthesized to improve specific properties such as hydrolytic stability, water solubility, neutral charge and/or lipophilicity, and their toxicity and insulin mimetic action have been evaluated.

In this work we report an in vivo study with a pre-diabetic animal model - Zucker fatty (fa/fa) rats - treated during four weeks with the compound $VO(dmpp)_2$ (Fig. 1) [3].

This vanadium compound has previously shown promising insulin-mimetic properties through ex vivo studies in rat adipocytes [4]. In this work, the gain of body weight was daily determined and a glucose tolerance test was performed at the end of the study. Hepatic and subcutaneous lipid content were assessed by Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS). The obtained results show that gain of body weight (Fig. 2), hepatic triglycerides content (Fig. 3) and subcutaneous fat width (Fig. 4) were significantly lower in obese-treated Zucker rats than in obese non-treated Zucker rats. In addition, the typical glucose intolerance profile of fatty rats was reversed by the action of $VO(dmpp)_2$ (Fig. 5).

All these results corroborate with previous ex vivo data [4], showing that $VO(dmpp)_2$ is able to restore normal glucose and lipid metabolism in Zucker fatty rats, by effectively reverting some of their pathological pre-diabetic indexes.

Molecular structure of VO(dmpp)2

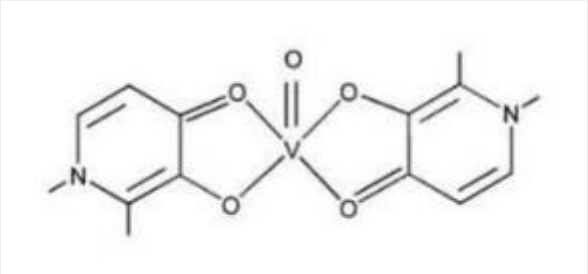

Figure 1- Schematic representation of the molecular structure of the V(IV) complex $V^{IV}O(dmpp)_2$ - bis-[3-hydroxy-1,2-dimethyl-4-pyridinonate] oxovanadium (IV).

2. Material and Methods

In vivo studies were conducted using 7 weeks-old Zucker lean (fa/+) (controls) and Zucker fatty (fa/fa) rats (a pre-diabetic animal model characterized by normoglycaemia, hyperinsulinaemia, hyperlipoproteinaemia, hyperphagia, hepatic and peripheral insulin resistance and obesity [3]). The animals were divided in four different groups: $VO(dmpp)_2$-treated lean (n=8), non-treated

lean(n=8), $VO(dmpp)_2$-treated obese (n=8) and non-treated obese (n=8).

During four weeks, the lean-treated and obese-treated animals were daily intraperitoneally (i.p.) injected with a solution containing the V(IV) species resulting from the dissolution of $V^{IV}O(dmpp)_2$ in physiological serum under aerobic conditions (15mg/Kg body weight). Lean and obese non-treated animals were injected with the same volume of physiological serum.

Once a week (on days 1, 8, 15, 23 and 30), the animals were assessed in terms of hepatic triglyceride (HTG) content determined by ^1H MRS. In vivo MRI and ^1H MRS studies were performed on a 7 T Bruker Pharmascan using a 60mm inner diameter volume coil for radiofrequency transmitting and signal receiving.

On day 31, a glucose tolerance test was performed. After an i.p. injection of glucose (glucose load: 1.5mg/g body weight), at the minute 0, blood glycemia was assessed during the following 120 minutes, in pre-determined intervals (0, 15, 30, 60 and 120 minutes).

3. Results

1. Gain of rat body weight

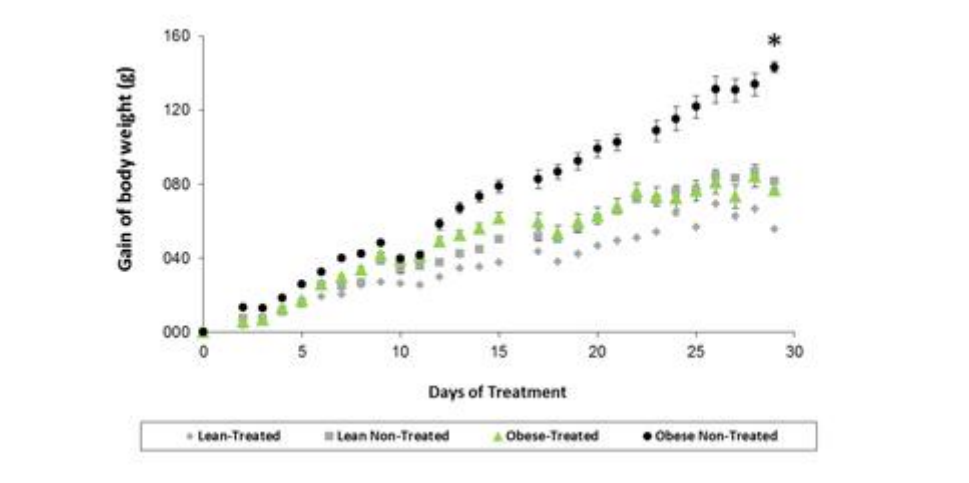

Figure 2 – Graphical representation of gain of body weight of lean and obese Zucker rats during $V^{IV}O(dmpp)_2$ treatment: $VO(dmpp)_2$-treated lean rats (grey losanges, n=8), non-treated lean rats (grey squares, n=8), $VO(dmpp)_2$-treated obese rats (green triangles, n=8) and non-treated obese rats (black circles, n=8). Gain of body weight was calculated by normalizing daily weight values according to weight values at Day 0. 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. * $P < 0.05$; treated-obese vs. non-treated obese at Day 30.

2. Assessment of Hepatic Triglycerides Content by MRI / MRS

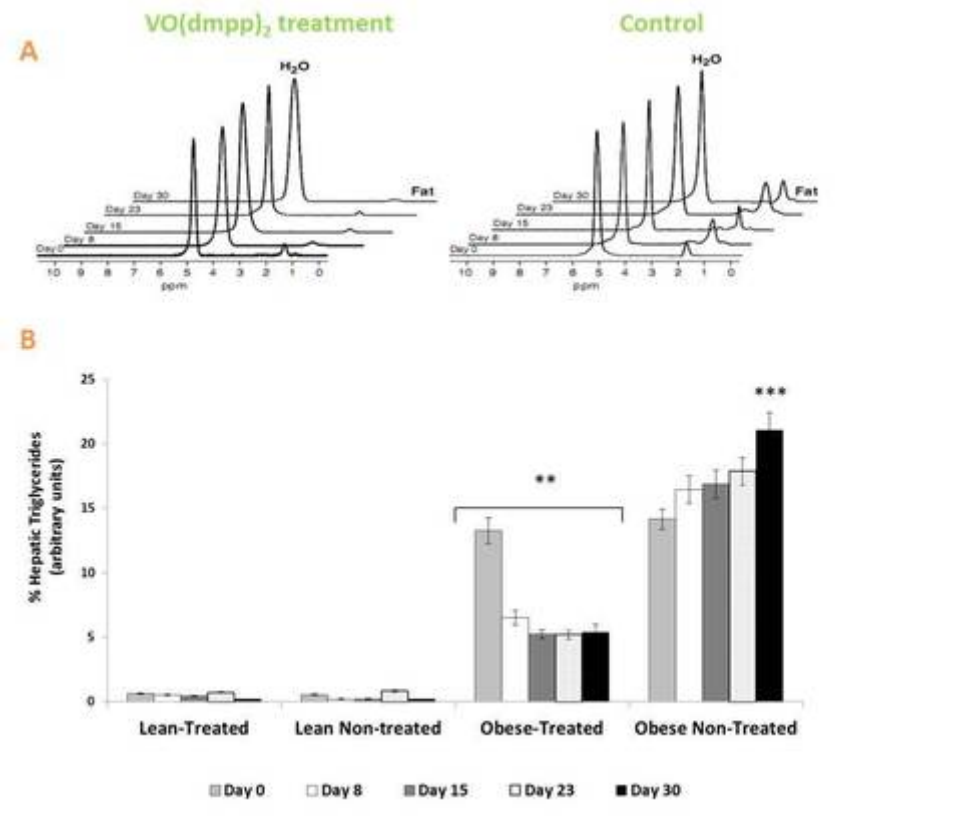

Figure 3 – Assessment and quantification of Hepatic Triglycerides Content (%) by MRS. Once a week, during four weeks, the HTG content was measured in each group of animals. **(A)** Representative ^{1}H MRS spectra acquired in the liver, along the different weeks of the treatment, are shown for $VO(dmpp)_2$-treated fatty Zucker rats and non-treated fatty Zucker rats. PRESS sequence was used to acquire the spectra in all cases. Water signal appears at 4.7 ppm whereas fat signal constituted by methylene $(CH_2)_n$ and methyl (CH_3) groups is observed between 1.2-1.5 ppm. **(B)** HTG content was quantified by ^{1}H MRS. ^{1}H MR spectra were acquired from four different ROIs (Region Of Interest) in the liver of each animal. The intensity of the water and fat signals was used to calculate the HTG percentage in arbitrary units through the formula: $[^{1}H\ ((CH_2)_n + CH_3)\ \text{peak area}] / [^{1}H\ (H_2O)\ \text{peak area}] \times 100$. This procedure was performed during four weeks, for $VO(dmpp)_2$-treated lean rats (n=8), non-treated lean rats (n=8), $VO(dmpp)_2$-treated obese rats (n=8) and non-treated obese Zucker rats (n=8). The increase in liver fat content is reflected as an increase in the intensity of the fat ^{1}H MRS signal and thus an increase in the value of HTG percentage. Data are shown as mean values ± SEM. Paired bilateral t test and one-way ANOVA were used in statistical analysis to compare two or more groups respectively, where $P < 0.05$ was considered to be significant. ** $P < 0.005$; Obese-treated rats in Day 0 vs. Day 8 vs. Day 15 vs. Day 23 vs. Day 30. *** $P < 0.0005$; Obese-treated vs. Obese non-treated at Day 30.

3. Subcutaneous adipose tissue thickness as detected by MRI

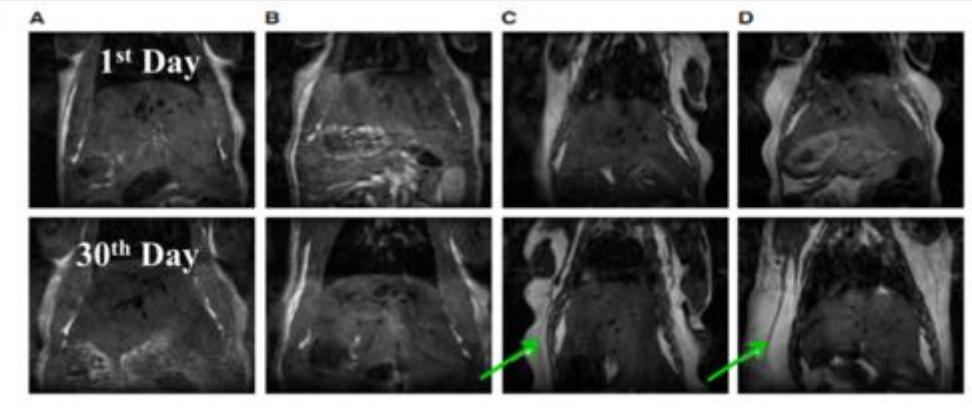

Figure 4 – Coronal T1W images of the liver of four different rats acquired with MSME sequence: non-treated lean Zucker rat **(A)**, $VO(dmpp)_2$-treated lean Zucker rat **(B)**, $VO(dmpp)_2$-treated obese Zucker **(C)** non-treated obese Zucker rat **(D)**, in the 1st (top) and in the 30th day of the study (bottom). The difference between the subcutaneous fat width of obese-treated and obese non-treated rat is shown by the arrows.

4. Glucose Tolerance Test

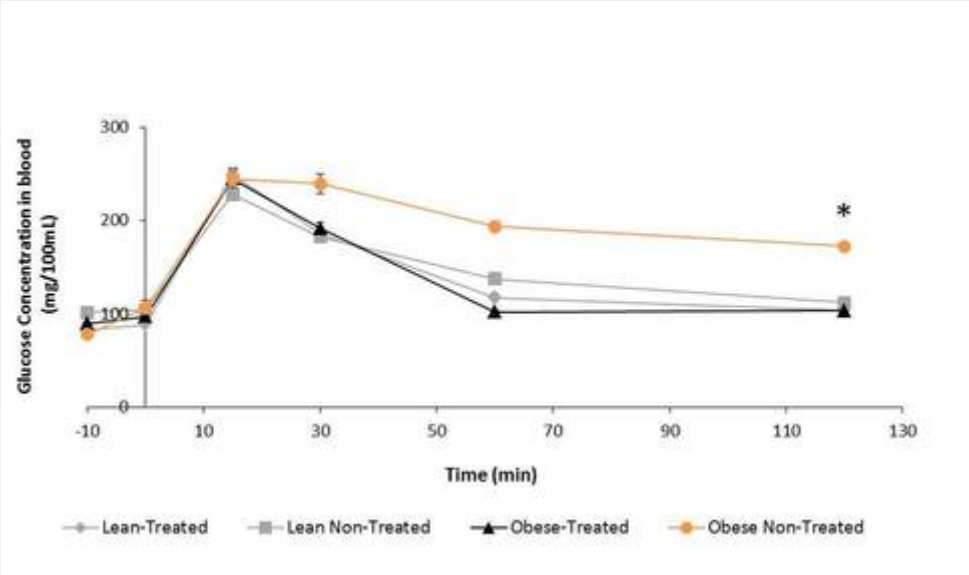

Figure 5 – Glucose Tolerance Test. The glucose measurements (g/100mL) were performed at different time points in the four groups of animals: $VO(dmpp)_2$-treated lean (n=8), non-treated lean

(n=8), $VO(dmpp)_2$-treated obese (n=8) and non-treated obese (n=8) Zucker rats. Glucose load was injected at 0 min. 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 glucose concentration of obese non-treated Zucker rats at the minute -10.

4. Conclusion

After 4 weeks of $V^{IV}O(dmpp)_2$ treatment, different parameters indicative of insulin resistance, obesity and pre-diabetic state were reverted in fatty (fa/fa) Zucker rats, such as gain of body weight, HTG high content, subcutaneous fat width and glucose intolerance profile.

This study demonstrates the in vivo activity of $V^{IV}O(dmpp)_2$. This compound restores glucose and lipid metabolism in fatty Zucker rats, reverting their metabolic profile of insulin resistance and pre-diabetic condition.

Current studies are being conducted to confirm the insulin-mimetic properties of this vanadium compound, which seems to be a promising drug to be further tested in different animal models.

References

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[2] Thompson K et al. Journal of Inorganic Biochemistry. 2009, 103(4):554-558.

[3] Augstein P & Salzsieder E. Methods in Molecular Biolog. 2009, 560:159-189.

[4] Passadouro M et al. Journal of Inorganic Biochemistry. 2010, 104(9):987-992.

5. Mediafiles

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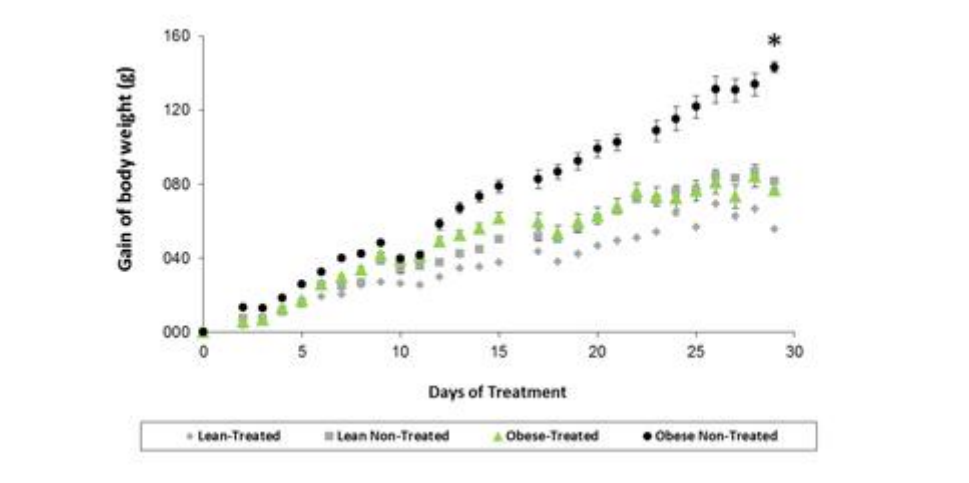

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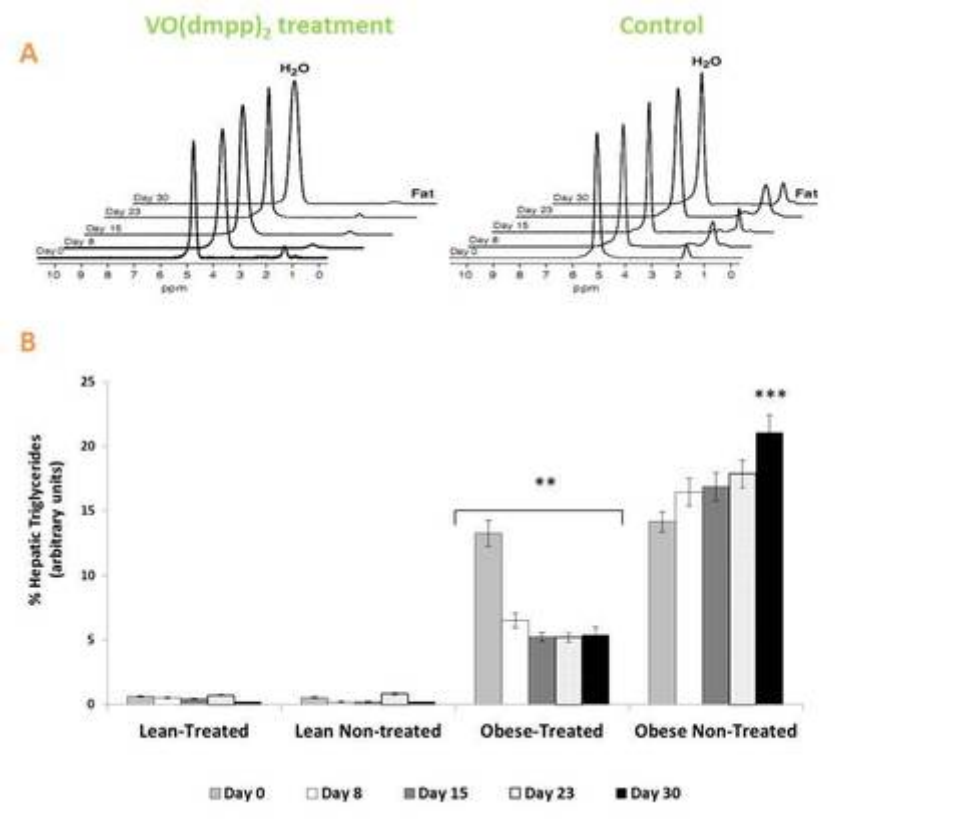

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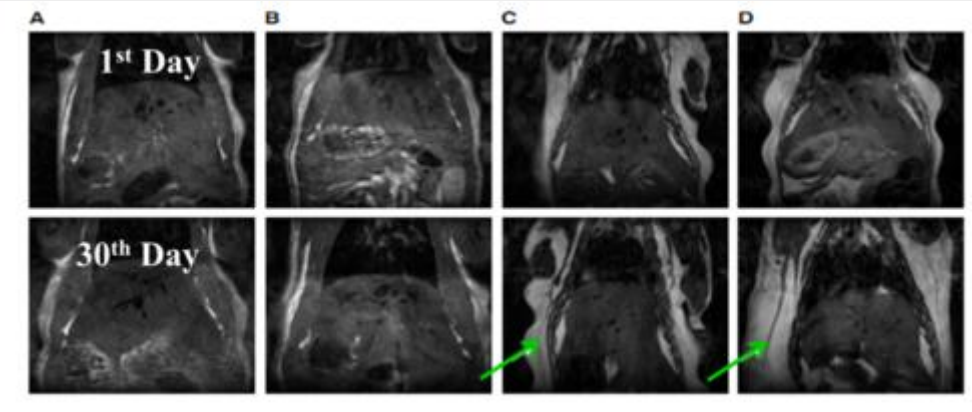

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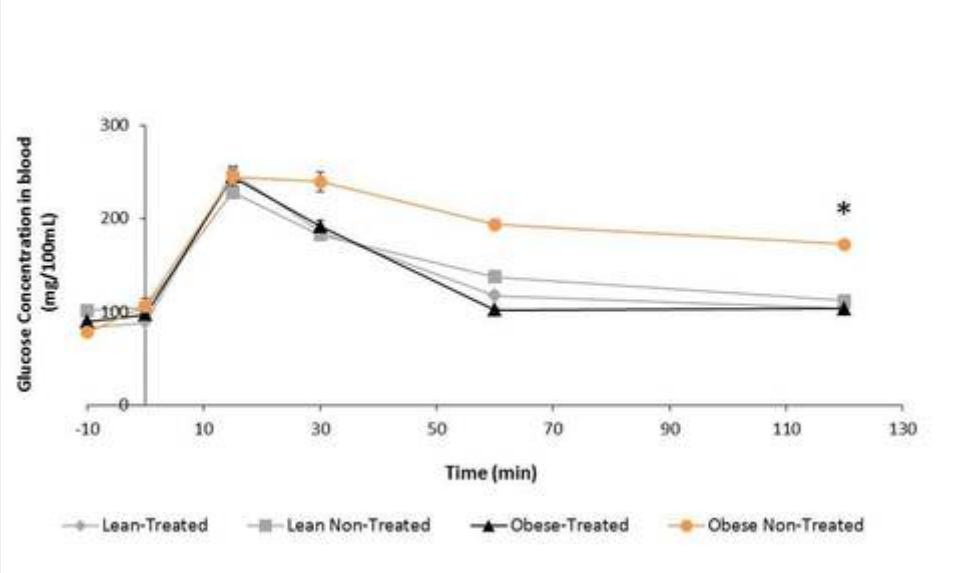

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