Pharmacological effects of $VO(dmpp)_2$ as assessed by *in vivo* Magnetic Resonance Imaging and Spectroscopy

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Abstract:

Type 2 diabetes *mellitus* has been associated with obesity, metabolic syndrome, cardiovascular diseases and cancer. Attempts have been made for early diagnosis and finding effective drugs to prevent severe consequences and ameliorate the symptoms of this disorder. In this work, the pharmacological properties of $VO(dmpp)_2$, [bis(1,2-dimethyl-3-hidroxy-4-pyridinonato)oxovanadium(IV)], were evaluated *in vivo* using Magnetic Resonance techniques. During four weeks fatty Zucker rats were subjected to a daily dose of $VO(dmpp)_2$ (44 μmol/kg) and their metabolic profile was followed by measuring different parameters: rat body weight, subcutaneous fat width and hepatic triglycerides content. The glucose tolerance test was performed at the end of the experiment. After four weeks of treatment, obese treated rats presented a weight significantly lower than obese non-treated animals (359.0 ± 11.1 *vs.* 433.5 ± 6.2 g), a thinner subcutaneous fat width qualitatively observed by Magnetic Resonance Imaging and a remarkable decrease in hepatic triglycerides content (5.41 ± 0.59 *vs.* 21.03 ± 1.40 %), as determined by Magnetic Resonance Spectroscopy. Additionally, the glucose intolerant profile characteristic of fatty Zucker rats was reversed in treated animals. These results support the previous published *ex vivo* studies, reinforcing the $VO(dmpp)_2$ therapeutic action as an effective anti-diabetic drug with particular effects on lipid metabolism.

Keywords: [bis(1,2-dimethyl-3-hidroxy-4-pyridinonato)] oxovanadium (IV) or $VO(dmpp)_2$, type 2 Diabetes *mellitus*, Zucker rats, hepatic triglycerides, magnetic resonance imaging, magnetic resonance spectroscopy

Abbreviations:

BMOV – bis(maltolato) oxovanadium (IV)
HTG – hepatic triglycerides
IR – Insulin resistance
MR – Magnetic Resonance
MRI – Magnetic Resonance Imaging
MRS – Magnetic Resonance Spectroscopy
T_1W – T_1-weighted

VC – vanadium compound

$VO(dmpp)_2$ - bis(1,2-dimethyl-3-hidroxy-4-pyridinonato) oxovanadium (IV)

1. Introduction

Diabetes *mellitus* is the world's fastest-growing disease responsible for almost 3 million deaths per year [1]. Due to its severe consequences such as hypertension, obesity, cardiovascular and ocular diseases, which compromise life style [2], this disorder has a great social and economical impact, being a main concern worldwide, especially in developed countries. In particular type 2 diabetes, the most current type of diabetes, is characterized by insulin resistance (IR) and it is associated with deregulation of glucose and lipid metabolism [3]. It has also been correlated with obesity, since obesity represents the major risk factor for IR and diabetes according to the World Health Organization.

Several drugs have been used in the treatment of type 2 diabetes, such as sulphonylureas, biguanides, alpha-glucosidase inhibitors and thiazolidinediones [4]. However, their therapeutic action is accompanied by undesired side effects which compromise the quality of life of the patients. Therefore, intensive research has been carried out to develop more effective drugs and a diagnostic tool for early detection of this disease.

At the moment several vanadium compounds (VCs) such as bis(maltolato) oxovanadium (IV) (BMOV) and bis(ethylmaltolato) oxovanadium (IV) (BEOV) [5-7], bis(picolinato) oxovanadium (IV) [8-10], bis(allixinato) oxovanadium (IV) [11-13], and bis-(-3-hidroxy-1,2-dimethyl-4-pyridinonato] oxovanadium (IV) ($VO(dmpp)_2$) [14-16] have shown promising anti-diabetic activity. Actually, BEOV passed phase I and II in human trials [6]. To understand the mechanism of action of these compounds at the molecular level, has been a main goal in the last years, and several studies have been performed to know their role on the insulin signaling cascade and on glucose and lipid metabolism [17]. The effect of VCs on the phosphorylation of key proteins of the insulin cascade has been demonstrated [6], particularly kinases and phosphatases seem to be the main targets, being activated and/or inhibited by these compounds. Literature data have shown an activation of Akt protein and an increase in GLUT4 translocation to the plasma membrane of adipocytes by bis(allixinato) oxovanadium (IV) [18]. This activation occurs in a PI3K-dependent manner together with an enhancement of tyrosine phosphorylation of Insulin Receptor β and Insulin Receptor Substrate. Accordingly, VCs seem to activate the insulin cascade and to interfere in the signaling processes connected to glucose and lipid metabolic pathways, which are usually deregulated in diabetes *mellitus* [19]. Actually, it has been shown

that VCs stimulate glucose uptake [20], glycolysis [21], glycogenesis [22] and lipogenesis [23], and inhibit gluconeogenesis [24], glycogenolysis [25] and lipolysis [26].

The vanadium compound - $V^{IV}O(dmpp)_2$ –containing the ligand 1,2-dimethyl-3-hydroxy-4–pyridinone, has been extensively studied. The structure of the different species formed in aqueous solution, under aerobic conditions, was characterized and the respective formation constants were determined [14, 27]. The cytotoxicity of $VO(dmpp)_2$ was evaluated by *in vitro* studies using fibroblast SV 3T3, human skin fibroblast F26, human fibroblast and 3T3-L1 cell lines [28, 29]. Preliminary indication of the anti-diabetic properties of the $VO(dmpp)_2$ was obtained by e*x vivo* studies with human erythrocytes, where it was shown that this compound increases glucose uptake in non-toxic concentrations [14, 15, 30]. This positive effect prompted us to investigate further the capacity of this compound to regulate glucose and lipid metabolism using a more pertinent cellular model. Actually, $VO(dmpp)_2$ acts as an insulin-mimetic compound, increasing glucose uptake and decreasing free fatty acids release in primary rat adipocytes [16]. It was also shown that $VO(dmpp)_2$ acts at the molecular level on the insulin cascade by activating some key proteins of this signalling pathway as Akt [16], similarly to what is described in literature for other VCs [18]. The promising anti-diabetic properties presented by this compound, justify the *in vivo* studies carried out in this work with the well known pre-diabetic animal model – fa/fa Zucker rats.

Magnetic Resonance (MR) techniques have been largely used in clinical studies to detect and quantify the hepatic triglycerides content [31], a biological marker of obesity and pre-diabetic state. Magnetic Resonance Imaging (MRI) and Spectroscopy (MRS) were successfully used in this work, giving accurate and non-invasive *in vivo* information about the effect of a VC on lipid metabolism. These techniques showed to be an important tool to better understand the pharmacological action of $VO(dmpp)_2$.

2. Methods

2.1 Animals

Zucker lean and Zucker fatty rats, 7 weeks-old, were obtained from Charles River Laboratories International Inc., France. All animals were housed in a 12 h light-dark cycle room

(08:00-20:00 h light) under constant temperature (22-25 ºC) and with *ad libitum* access to food and water. All the experiments were performed accordingly to the European Council Directives on Animal Care and to the local Institutional Animal Care Committee.

2.2 Experimental design

Zucker fatty rat (fa/fa) is a pre-diabetic animal model characterized by normoglycaemia, hyperinsulinaemia, hyperlipoproteinaemia, hyperphagia, hepatic and peripheral insulin resistance and obesity [32]. Lean Zucker rats (fa/+) are commonly used as the control model of Zucker fatty rats. The animals were divided into treated and non-treated groups: treated lean (n = 8), non-treated lean (n = 8), treated obese (n = 8) and non-treated obese (n = 8). A standard laboratory rodent diet with a constant formula Diet A04 (PanLab, Spain) was used throughout the study.

During four weeks, treated animals received daily 44 μmol/kg (animal body weight (b.w.)) of $VO(dmpp)_2$ (in saline solution) by intraperitoneal (i.p.) administration [33, 34]. Untreated animals received the same volume of saline serum (Vitulia Laboratorios ERN S.A., Spain). The body weight was assessed every day for each animal using a digital scale Letica® LE-2000. *In vivo* MRI studies were performed once a week (on days 1, 8, 15, 23 and 30) and each animal was assessed in terms of HTG content as determined by ^{1}H MRS. At day 31, the glucose tolerance test was performed on overnight (o.n) fasted animals, after an i.p. injection of glucose (1.5 mg/g b.w.). During the following 120 minutes, blood glycaemia was measured with a standard glucometer One Touch UltraEasy (Johnson & Johnson Lda – LifeScan, Portugal) at pre-determined intervals (15, 30, 60 and 120 minutes).

2.3 Preparation of $VO(dmpp)_2$ solution

The compound bis(1,2-dimethyl-3-hydroxy-4-pyridonate)oxovanadium (IV), $VO(dmpp)_2$, was synthesized according to a published procedure [27]. Its purity was confirmed by elemental analysis and spectroscopic data. A $VO(dmpp)_2$ solution (with a concentration of 3mM) was prepared in a saline solution (155 mM NaCl) at pH 7.4. This solution was filtered using a 0.2 μm membrane and stored at 4ºC.

2.4 ^{1}H Magnetic Resonance Imaging and Spectroscopy

The Magnetic Resonance Imaging (MRI) experiments were performed on a Bruker Pharmascan system (Bruker Medical Gmbh, Ettlingen, Germany) using a 7.0-T horizontal-bore superconducting magnet, equipped with a ^{1}H selective birdcage resonator of 60 mm and a Bruker gradient insert with 90 mm of diameter (maximum intensity 360 mT/m). All data were acquired using a Hewlett-Packard console running Paravision 4.0 software (Bruker Medical Gmbh, Ettlingen, Germany) operating on a Linux platform. Anesthesia was initiated by inhalation, in an induction box, of oxygen (1 l/min) containing 3 % of isofluorane (Veterinaria Esteve, Spain), and maintained throughout the experiment at 1-1.5 % of isofluorane in O_2 (v/v). Animals were placed in a heated probe, which maintained the core body temperature at approximately 37 ºC. The physiological state of the animal was monitored through the respiratory rate using a Biotrig physiological monitor (Bruker, Germany). Sagittal and coronal T_1-weighted (T_1W) images of the liver were obtained using a spin-echo sequence with FOV = 5x5 cm for lean rats and FOV = 6x6 cm for fatty rats, matrix acquisition = 256x256, TR/TE = 500/10.6, 2 averages, 2 mm slice thickness and a total acquisition time of 3 min 12 s. ImageJ 1.43u (Rasband, WS, U. S. National Institute of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/) was the software used to analyse T_1W images.

Single-voxel volume-selected ^{1}H MR spectra were acquired by selecting cubic voxels (4x4x4 mm) in different regions of interest (ROI) in the liver image of each animal. MRS data were obtained by using a point-resolved spectroscopy (PRESS) sequence with TR/TE = 1200/20, 128 average scans without water suppression and a total acquisition time of 2 min 43 s [35]. Spectra were analysed with the NMR data processing program, MestReNova 6.1 (MestreLab Research, Spain), to determine the area of the signals assigned to water (4.7 ppm) and to the methylene $(CH_2)_n$ and methyl (CH_3) groups (1.2-1.5 ppm) of lipids. The lipid signal corresponding to the methyl and methylene proton resonances was quantified using the water signal as reference [35, 36]. For each rat, hepatic fat was calculated as an average of the results obtained from the analysis of four different voxels and expressed as a percentage using the following equation:

$$\text{HTG content (\%)} = [(^{1}H\ (CH_2)_n + (CH_3)_{peak\ area})/(^{1}H\ (H_2O)_{peak\ area})] \times 100 \quad (1)$$

2.5 Statistical analysis

Data are presented as mean values ± SEM (standard error of the mean). Statistical differences were determined using paired bilateral *t* test and one-way ANOVA where $P < 0.05$ was considered to be significant in both tests.

3. Results and Discussion

In vivo experiments were conducted using the Zucker fatty rat, a known animal model of obesity, insulin resistance and pre-diabetes. Its genetic deficiency in the leptin receptor promotes the development of different pathological features such as obesity, steatosis, hyperinsulinaemia, hyperlipoproteinaemia, hyperphagia and a glucose intolerant profile [32, 37]. This animal model was carefully chosen according to its pre-diabetic features, being very suitable to assess the pharmacological properties of the $VO(dmpp)_2$ compound under study.

3.1 $VO(dmpp)_2$ treatment reverts characteristic gain of body weight in fa/fa Zucker rats

The gain of b.w. is an important index to evaluate the metabolic conditions of the animals considering that within the same phenotype slight differences can occur in this parameter. Thus, the b.w. was daily calculated for each animal and Fig. 1 shows the trend lines of the gain of b.w. for the four groups of animals throughout the study.

In the first day, mean b.w. values for lean and obese Zucker rats were, respectively, 213.7 ± 1.7 and 274.4 ± 2.7 g. After 30 days of $VO(dmpp)_2$ treatment, the difference between treated and non-treated animals was evident. Mean b.w. values for treated- and non-treated lean rats were respectively 277.7 ± 7.4 *vs.* 312.5 ± 5.2 g, while for obese rats the values were 359.0 ± 11.0 *vs.* 433.5 ± 6.2 g (statistically different, $P < 0.05$).

<Figure 1>

As expected, obese Zucker rats (fa/fa) always presented b.w. values higher than lean Zucker rats (fa/+), since lean rats are control animals without the genetic deficiency in the leptin receptor [32]. After four weeks of $VO(dmpp)_2$ treatment, obese treated rats showed a gain of b.w. lower than the non-treated obese group (84.5 ± 5.9 *vs.* 133.9 ± 6.9 g), and similar to the non-treated lean group (86.7 ± 2.5 g). The compound had still an effect on the lean treated rats as they

presented a gain of b.w. lower than non-treated ones (66.9 ± 2.3 *vs.* 86.7 ± 2.5 g). These results show that $VO(dmpp)_2$ modulates the normal increase in b.w. of fa/fa Zucker rats, even under a normal diet, being a good indication of its positive effect on lipid metabolism and obesity development, in agreement with previously published data from studies with primary rat adipocytes [18].

3.2 Subcutaneous fat width decreases after $VO(dmpp)_2$ treatment

MRI was used to investigate the effect of $VO(dmpp)_2$ on lipid metabolism of Zucker rats. Coronal T_1W images of rat abdominal region were acquired and the subcutaneous fat width was analysed qualitatively in lean and obese rats with and without $VO(dmpp)_2$ treatment. Comparable coronal slices were selected in the 1^{st} and in the 30^{th} day of the experiment, for the different groups, as it is shown in Fig. 2.

<Figure 2>

In the first day of the study, the width of subcutaneous fat tissue was thicker in the obese groups (Fig. 2 C and D– top) than in the lean groups (Fig. 2 A and B – top). Throughout the study, lean animals presented a similar subcutaneous fat layer width, which increased according to the age of the animal and independently of the $VO(dmpp)_2$ treatment. However, the same did not occur for obese rats. After $VO(dmpp)_2$ treatment, the difference between treated and non-treated obese rats was qualitatively evident (Fig. 2C-bottom *vs.* Fig. 2D - bottom), demonstrating the *in vivo* effect of $VO(dmpp)_2$ on lipid metabolism [16], and corroborating the results of gain of body weight.

3.3 Hepatic Triglyceride Content decreases in obese Zucker rats treated with $VO(dmpp)_2$

Triglycerides can accumulate in the liver as a consequence of metabolic impairment and, over the past two decades, this biological parameter has been correlated with insulin resistance and type 2 diabetes [19, 31, 38, 39]. Single-voxel ^1H MRS has been the method mainly used in

clinical studies to quantify HTG levels [19], being a useful tool for early detection of pathological indexes.

Thus, in this study MRS was used to quantify the HTG content in the four groups of animals. Every week and during the time course of the experiment, T_1W images of the liver of each rat were obtained and ^1H MR spectra were acquired from four different regions of interest. Fig. 3 illustrates some of the results obtained for treated and untreated obese Zucker rats.

<Figure 3>

The effect of $VO(dmpp)_2$ on the HTG levels of obese rats is clearly shown after eight days of treatment. The administration of the compound promoted a significant decrease in their HTG content, as it is shown by a decrease in the intensity of the lipid signal in the ^1H MR spectra (CH_3- and -CH_2- protons at 1.3-1.5 ppm) (Fig. 3A). The same did not occur in non-treated obese rats (Fig. 3B), which maintained the high HTG levels throughout the experiment. This biological parameter was quantitatively analysed by determining the area of the ^1H MR signal of the fat in all animals along the study and the values were normalized relatively to the water signal intensity. Data are expressed as a percentage (as determined by Eq. 1 presented in Materials and Methods), and are shown in the graphical representation of Fig. 4.

<Figure 4>

At the beginning of the study, the HTG content in lean and obese Zucker rats was 0.59 ± 0.03% (mean value) and 13.68 ± 0.45 % (mean value), respectively. This value did not significantly change in treated and non-treated lean animals and, at day 30, their mean values were 0.19 ± 0.03 % and 0.20 ± 0.02 %, respectively.

In contrast, after eight days of $VO(dmpp)_2$ treatment, the HTG content in treated obese animals significantly decreased from 13.68 ± 0.45 % to 6.51 ± 0.55 % ($P < 0.005$). This difference remained consistent and statistically significant until the end of the study ($P < 0.005$). Non-treated obese animals, on the other hand, showed an expected gradual increase of the HTG (without statistical difference among the different days). At the end of the experiment, HTG

levels in treated and non-treated obese groups were respectively 5.41 ± 0.59 % *vs.* 21.03 ± 1.40 % ($P < 0.0005$).

^{1}H MR spectra of the voxels in the liver (Fig. 3) and the quantification of the respective CH_2-CH_3 signals assigned to lipids (Fig. 4) clearly show the role of $VO(dmpp)_2$ in reverting the lipid metabolic impairment. Lean animals maintained low HTG values throughout the experiment, independently of $VO(dmpp)_2$ treatment, while obese rats showed high HTG values since the beginning of the study. The crescent HTG content in non-treated obese animals is a consequence of their obesity, insulin resistance and pre-diabetic conditions, our results being in agreement with the literature data [40]. However, the group of treated obese rats immediately respond to $VO(dmpp)_2$ therapy and after one week of treatment a decrease of 52% in their HTG levels was observed. These data show a direct and fast effect of this compound, which promotes important pharmacological effects within the first 8 days of treatment. This positive outcome was maintained during the treatment and, at the end of the study, a notorious decrease of 74% of the initial HTG content was detected in $VO(dmpp)_2$ treated obese animals.

These data provide clear evidence of the action of $VO(dmpp)_2$ on lipid metabolism, attenuating the pathological tendency of obese Zucker rats to accumulate triglycerides in the liver. Treated obese rats did not achieve HTG values similar to the controls (lean animals) but the difference between $VO(dmpp)_2$-treated and non-treated obese rats is statistically significant. The overall results presented here indicate that $VO(dmpp)_2$ can be a promising drug against steatosis and obesity since it is able to decrease the gain of b.w., the high HTG levels and the thickness of subcutaneous fat layer of obese Zucker rats. Actually, as stated in the literature, the majority of the anti-steatosis drugs currently used in the clinic did not achieve *in vivo* results similar to those obtained with $VO(dmpp)_2$ [40-42]. These findings point out $VO(dmpp)_2$ as a potential drug to be used against steatosis and obesity.

Although the molecular mechanism behind this therapeutic effect is not clearly understood, it is known that $VO(dmpp)_2$ activates the insulin signalling cascade by Akt phosphorylation [16]. This activation and other possible $VO(dmpp)_2$ interactions can interfere in different pathways of lipid and glucose metabolism, leading to a decrease in triglycerides accumulation in the liver of fatty Zucker rats.

3.4 $VO(dmpp)_2$ reverts the profile of glucose intolerance of obese Zucker rats

The action of $VO(dmpp)_2$ on the glucose metabolism of Zucker rats was also investigated through a glucose tolerance test, which is currently used to evaluate clinical pre-diabetic and diabetic conditions [43, 44]. The glucose tolerance test evaluates the metabolic profile of the animal by measuring blood glucose levels for a period of time after the administration of a glucose load. In insulin sensitive animals, blood glucose concentration will drop after a certain period of time, restoring the normal glucose values. However, in conditions of impaired glucose metabolism, IR or type 2 diabetes, insulin signalling does not function properly and blood glucose concentration remains high for a longer period of time [45-47].

To evaluate the effect of $VO(dmpp)_2$ on the glucose metabolic profile of lean and obese rats, a glucose tolerance test was performed at the end of the treatment (on the 31st day) and the results are illustrated in Fig. 5.

<Figure 5>

At the beginning of the experiment, overnight fasted lean and obese animals had similar blood glucose values, as shown by the initial point of the graph in Fig. 5 (t=0 min). These data are in agreement with their normoglycaemic state as previously described [32].

At minute 0, a glucose load (1.5 mg/g b.w.) was injected in each animal, leading to a rapid increase in their blood glucose concentration. After 15 minutes, all the animals have responded to the stimulus, presenting high glucose levels (with values not significantly different among them). The blood glucose concentration increased in all groups and it gradually decreased during the following 60 minutes except for non-treated obese rats (117.7 ± 3.9, 137.5 ± 2.7 and 102.3 ± 2.1 mg/100mL, respectively for treated and non-treated lean and for treated obese animals). These values are not significantly different from the respective values at minute 0, which mean that lean and obese treated animals have recovered from the glucose load.

In contrast, blood glucose concentration in non-treated obese rats remained high for at least 120 minutes (172.4 ± 1.3 mg/100mL; $P < 0.0005$), and significantly different from all the other groups at this time point, as expected considering their typical glucose intolerant and insulin resistant profile [48].

According to these data, $VO(dmpp)_2$ ameliorates insulin resistance in obese Zucker rats since the behaviour of these animals, concerning the recovery from the glucose load, is similar to the control group (Fig. 5). These results are a consequence of the *in vivo* effect of $VO(dmpp)_2$ on glucose metabolism, reinforcing *ex vivo* data previously described [16] and demonstrating that one month of $VO(dmpp)_2$ treatment is enough to revert the pre-diabetic features of obese Zucker rats.

4. Conclusions

This work clearly demonstrates the *in vivo* effect of $VO(dmpp)_2$ on glucose and lipid metabolism using an obese pre-diabetic animal model.

$VO(dmpp)_2$ is able to restore normal glucose and lipid metabolism in Zucker fatty rats, by effectively reverting some of their pathological pre-diabetic indexes such as gain of body weight, subcutaneous fat thickness, high HTG content and insulin resistance.

These are important data to reinforce the promising anti-diabetic capacity of this vanadium compound, as previously shown by *ex vivo* studies. Further investigation is needed to consolidate the *in vivo* therapeutic properties of this vanadium compound and for a better understanding of its mechanism of action at the molecular level.

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A.M. obtained data and wrote the manuscript. R.C. obtained data. M.C. designed the experiments, contributed to discussion and reviewed/edited the manuscript. P.L. designed the experiments, supervised the MRI/MRS experiments, contributed to discussion and reviewed/edited the manuscript.

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Figure Legends

Figure 1 –Graphical representation of gain of body weight of lean and obese Zucker rats submitted to $VO(dmpp)_2$ **treatment during four weeks:** vanadium-treated lean rats (grey losanges, n=8), non-treated lean rats (grey squares, n=8), vanadium-treated obese rats (black 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 and trendlines were drawn for each group of animals. Paired bilateral *t* test was used in statistical analysis where $P < 0.05$ was considered to be significant. ***** $P < 0.05$; obese-treated *vs.* obese non-treated at Day 30.

Figure 2 – Coronal T_1W images of the abdominal region of four different groups of rats: $VO(dmpp)_2$-treated **(A)** and non-treated **(B)** lean Zucker rats; $VO(dmpp)_2$-treated **(C)** and non-treated **(D)** obese Zucker rats, in the first day (top) and in the last day (bottom) of the study. **A** and **B** images were acquired using a FOV of 5, whereas **C** and **D** were acquired with FOV of 6. Slice selection was made in order to obtain comparable coronal slices between different groups and different days. White arrows are picked in the image to show the difference between treated and non-treated obese rats in the 30^{th} day of the experiment.

Figure 3 – Evolution of hepatic lipid content in $VO(dmpp)_2$-treated and non-treated obese Zucker rats as assessed by ^{1}H MRS. T1-weighted coronal and sagittal images, centered in the liver, were acquired using MSME sequence. Both images were used to select the 4x4x4 cubic voxel in the region of interest (ROI), where a ^{1}H MRS spectrum was obtained every week. Representative ^{1}H MR spectra are shown for $VO(dmpp)_2$-treated (**A**) and non-treated (**B**) obese Zucker rats. PRESS sequence was used to acquire the spectra in all cases. The water signal appears at 4.7 ppm and the one assigned to the methylene $(CH_2)_n$ and methyl (CH_3) groups of triglycerides is observed at 1.2-1.5 ppm.

Figure 4 – Graphical representation of HTG content (%) in the four groups of rats during the study. 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: $[^1H\ ((CH_2)_n + CH_3)_{\text{peak area}}] / [^1H\ (H_2O)_{\text{peak area}}] \times 100$. 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 the intensity of the fat ^{1}H NMR signal reflects the increase in liver fat content and thus a higher 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 at 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.

Figure 5 – Glucose Tolerance Test for lean and obese Zucker rats at the end of the study. Each group of animals (n=8) was submitted to a glucose tolerance test at Day 31. Each animal received an i.p. glucose load (1.5 mg/g b.w.) at minute 0 and glucose levels were measured at different time points during 120 minutes. Data are shown as mean values ± SEM. One-way ANOVA was used in statistical analysis where $P < 0.05$ was considered to be significant. ******* $P < 0.0005$, obese non-treated animals *vs.* other groups, at minute 120.

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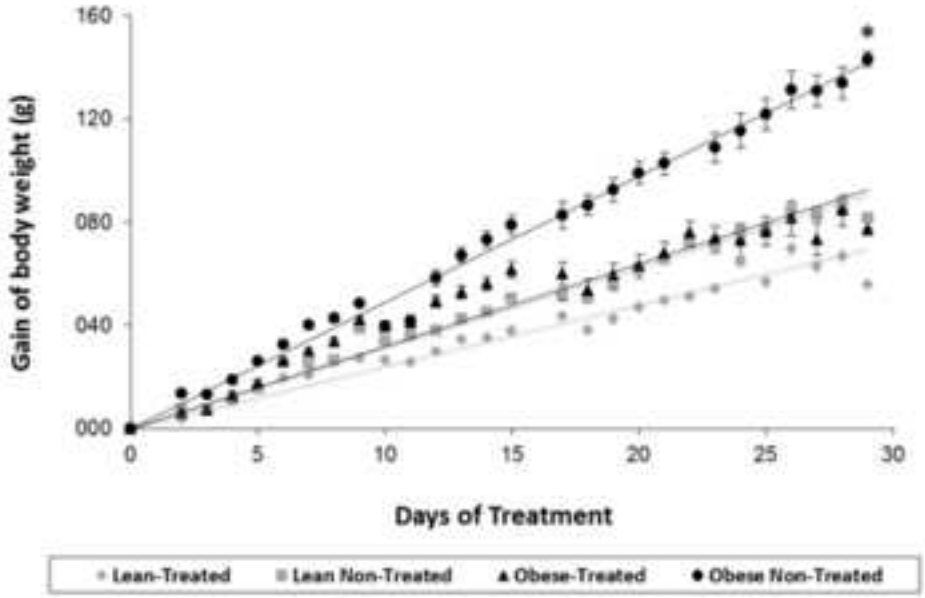

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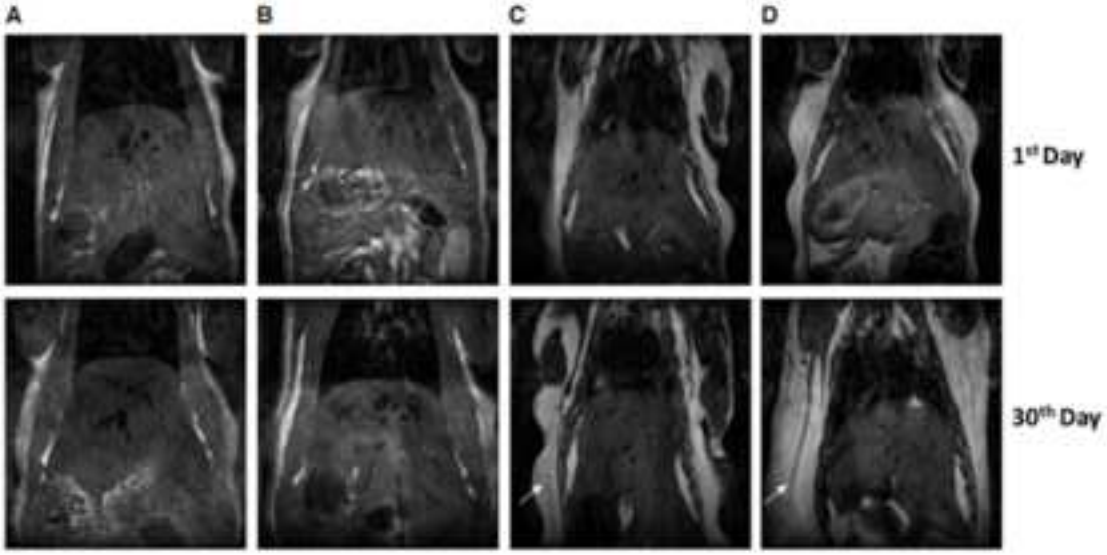

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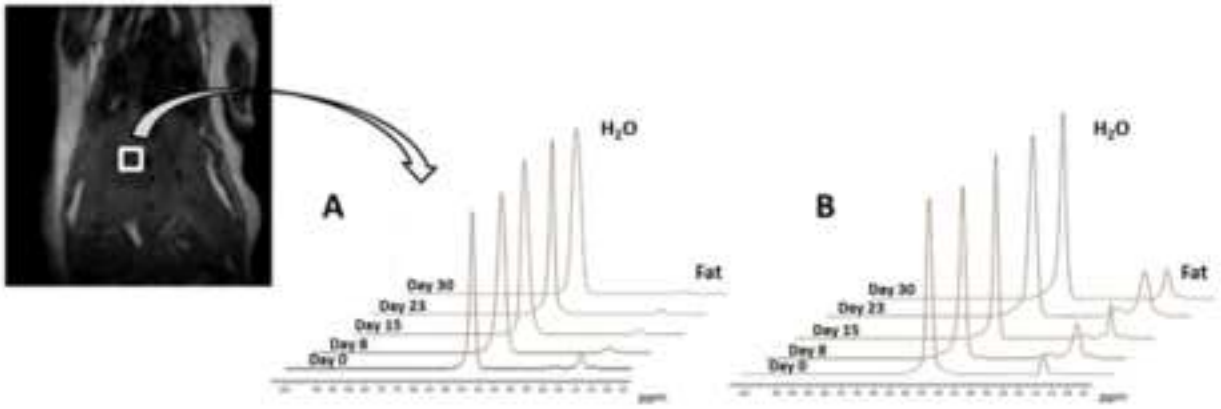

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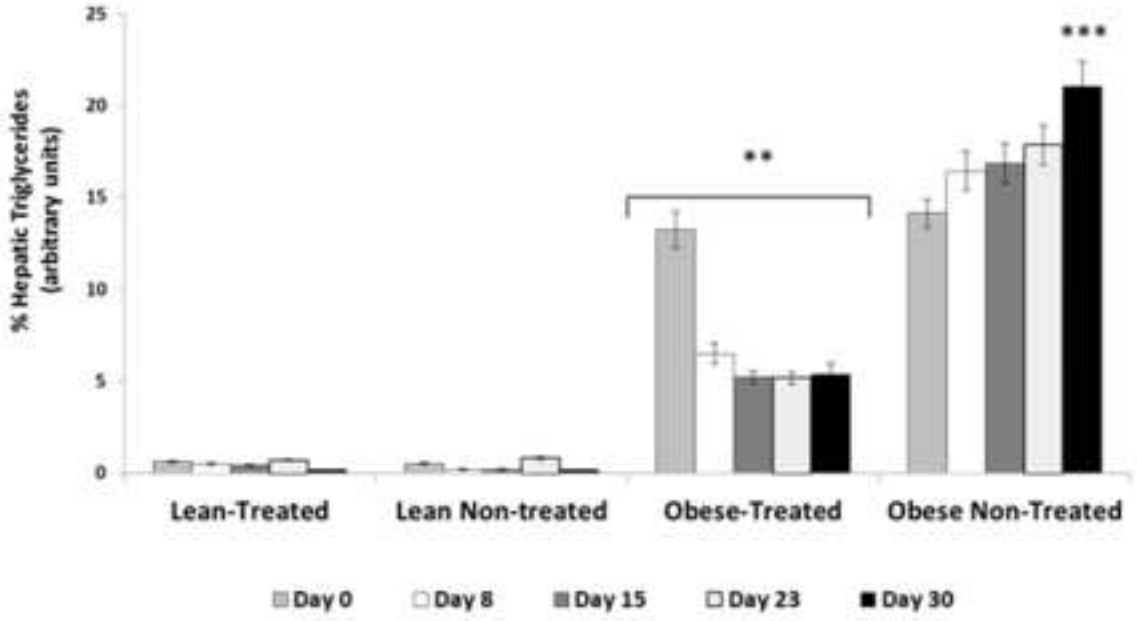

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