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ORIGINAL ARTICLE

Effect of pioglitazone treatment on brown adipose tissue volume and activity and hypothalamic gliosis in patients with type 2 diabetes mellitus: a proof-of-concept study

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Abstract

Aims This study aimed to evaluate the effect of pioglitazone on brown adipose tissue function and hypothalamic gliosis in humans. Brown adipose tissue and the hypothalamus are regarded as important potential pharmacological targets to metabolic diseases, and defining the impact of current therapies on their structure and/or function could provide therapeutic advance in this field.

Methods Six patients with type 2 diabetes were treated for 24 weeks with pioglitazone 30 mg/day as an add-on therapy. Brown adipose tissue glucose uptake and volume were determined using ^{18}F-FDG PET/CT scans; hypothalamic gliosis was determined using MRI scans; blood was collected for hormone and biochemistry measurements. All tests were performed at inclusion and six months after pioglitazone introduction.

Results Pioglitazone treatment led to a significant 3% body mass increase. There were neither changes in cold-induced brown adipose tissue glucose uptake and volume nor changes in hypothalamic gliosis.

Conclusions This is a proof-of-concept study that provides clinical evidence for a lack of action of a thiazolidinedione, pioglitazone, to promote homogeneous and measurable changes in brown adipose tissue volume and also in hypothalamic gliosis after 6 months of treatment.

Keywords Insulin resistance · Glucose intolerance · Obesity · Hypothalamus · Brown adipose tissue

Introduction

Brown adipose tissue (BAT) has emerged as a potential therapeutic target in the management of diabetes because of its potential role in whole-body glucose homeostasis [1]. In response to cold, excess feeding or adrenergic stimuli, BAT promotes dissipation of chemical energy through proton leak driven mostly by uncoupling protein

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1 (UCP1) [2]. The thermogenic activity of BAT is primarily under the control of sympathetic inputs from the hypothalamus, and a number of studies have used distinct approaches to modulate sympathetic tonus in order to modify BAT activity [2]. Currently, the most reliable method to evaluate BAT activity is the determination of cold-induced ([^{18}F]-FDG) uptake via positron emission tomography/computed tomography (PET/CT) [3]. Cold-induced BAT activity and hypothalamic function/structure are beneficially modified in obese subjects without diabetes undergoing body mass reduction as a result of bariatric surgery [4, 5]. However, in obese subjects with diabetes, bariatric surgery followed by body mass reduction was not sufficient to significantly improve BAT function [5], which may be also compatible with inflammatory mechanisms acting on target tissues of insulin resistance [6]. Here, we designed and tested a proof-of-concept study to evaluate whether a pharmacological approach, pioglitazone, could improve BAT function and reduce hypothalamic gliosis in a group of patients with diabetes and overweight/class I obesity.

Methods

Research design

This is an open-label study, which evaluated eight subjects with type 2 diabetes mellitus (according to American Diabetes Association criteria) [7] and BMI ranging from 25 to 35 kg/m^2 that were selected at the University of Campinas Clinics Hospital to receive pioglitazone 30 mg for 24 weeks as add-on therapy to standard treatment with oral anti-diabetic drugs. Additionally, subjects were submitted to anthropometric evaluation and lean body mass (LBM) was measured using Janmahasatian formulation [8]. Exclusion criteria were habitual tobacco use, habitual excessive alcohol use, pregnancy, plasma glucose > 8.3 mM on examination day, heavy daily exercise, cancer, use of drugs, benzodiazepines, β-adrenergic receptor agonists, beta blockers according to Brown Adipose Reporting Criteria in Imaging STudies (BARCIST 1.0) [3] or yet serotonin reuptake inhibitors, melatonin and PET/CT within the last year. The study was approved by the local medical ethical committee of the University of Campinas (Ethics Committee approval CAAE: 32930314.8.0000.5404). Written informed consent was obtained before inclusion. Six patients completed the study and were included in the final analysis. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

PET/CT scanning protocol and analysis

The cold exposure protocol and the PET/CT scanning protocol were performed as previously reported [4]. We followed the recommendations for FDG-PET/CT human experiments of BAT [3]. Briefly, 60 min prior to injection individuals were exposed to 19 °C wearing light clothing (0.49 *clo*) until clinically detectable shivering. After any incidence of observable shivering, the room temperature was gradually increased up to 22 °C or until the individual stops shivering. The volunteer was maintained at this temperature for about 60 min (with 55–70 min range) before the injection and then another 60 min until the scan. The volunteers had median glycemic values of 10.5 (0.8) mM before thiazolidinedione (TZD) treatment and 10.5 (2.7) mM after TZD treatment just before FDG injection, as recommended to be below 11 mM. The images were acquired using a Siemens PET/CT Biograph mCT 40 (Siemens Medical Solutions, Chicago, IL) and Syngo Multimodality VB10B WinNT 4.0 Station after an intravenous injection of 4.0 MBq/kg of ^{18}F-FDG and a period of 1-h exposure in the cold. The CT study was performed as a low-dose protocol. We used CARIMAS 2.8 software (Turku PET Centre, Turku, Finland) to analyze PET/CT images and estimate brown adipose tissue activity and volume. For the BAT activity, standardized uptake values (SUVs) were calculated by drawing the regions of interest (ROIs) on the fused image of the static [^{18}F] FDG-PET image and the CT image (as an anatomical reference). SUV is defined as the normalized measure of signal intensity, being calculated as the ratio of the radioactivity concentration in a defined region by the operator (MBq/mL) to the injected radiotracer dose. This measure is normalized to some index of body mass (MBq/g), as body weight [3]. $SUV_{bw/mean}$ is defined as average SUV_{bw} in any volume of BAT. For the estimation of BAT volume in the cervico-upper thoracic region (including cervical, supraclavicular and axillary adipose tissues), the fused PET-CT image was used. The volumes of interest (VOI) of potential BAT sites were initially drawn manually, and a threshold of CT voxels between −50 and −250 Hounsfield Units was used (Supplemental Figure 1). Also, all VOIs underwent a second thresholding of voxels with $SUV_{bw/mean}$ higher than 1.5 were counted. In the end, the software reconstructs all the identified active metabolically fat in this fat depot (cervical/supraclavicular) in an analysis mask, sums all VOIs to obtain the total metabolic volume of BAT. Volume multiplied by $SUV_{bw/mean}$ generates an estimate of total BAT activity (measured in g/ml) in all fat depot.

Magnetic resonance image (MRI)

Scans were obtained on a 3-Tesla Philips Achieva MR scanner as reported in Sewaybricker et al. [9] (version 3.2, Philips Medical Systems, the Netherlands). Briefly, we identified the

rostral arcuate nucleus in the coronal slice posterior to the optic chiasm. Within the same slice, we designed the ROIs in the arcuate nucleus, putamen and amygdala on high-resolution coronal images and transferred to the T2 parametric map in order to convert the brightness seen on T2-weighted image into relaxation times as parameter of gliosis. We used OsiriX Imaging Software version 8.0.2.

Biochemical analysis

Fasting plasma glucose, insulin, LDL cholesterol, HDL cholesterol, HbA1c, total cholesterol and triglycerides were collected and analyzed in the central laboratory of the Hospital de Clínicas da Universidade Estadual de Campinas. These biochemical parameters were determined at baseline and at the end of the study. Additionally, none of them was used as a primary outcome.

Statistical analysis

BAT data and biochemical data were analyzed by Wilcoxon matched-pairs signed-rank two-tailed test. For MRI analysis, multiple *t* tests corrected for multiple comparisons using Holm–Sidak method were applied.

Results

We investigated whether pioglitazone treatment increases cold-induced BAT activity in diabetic subjects who had received pioglitazone for 6 months. At baseline, the median (interquartile range) age of the patients was 46.9 years (20.0), the median weight was 76.9 (16.6), and the median BMI was 28.1 (4.2). The daily administration of 30 mg pioglitazone was well tolerated. No episodes of hypoglycemia were reported. After 24 weeks, patients had gained a mean of 3.40 ± 4.02% (2.58 ± 3.28 kg) of their body weight. There were no other major changes in metabolic parameters, besides a decrease of HbA1c of 0.4% (Table 1).

We found that in adult human individuals supraclavicular/cervical BAT activity (Fig. 1a) was unchanged after pioglitazone treatment (0.34 ± 0.13 g/mL vs 0.36 ± 0.05 g/mL, two-tailed *p* value 0.56). Similarly, BAT SC/cervical volume (Fig. 1b) was also unchanged after pioglitazone treatment (0.78 ± 5.13 mL vs 1.17 ± 2.95, two-tailed *p* value 0.84). In addition, the illustrative results are heterogeneous, since in some patients the BAT volume increased (Fig. 2a–d), while in others it decreased (Fig. 2e–h), as shown in Fig. 2.

Still, although it had been well demonstrated in rodents that sympathetic control of BAT activity can be modified as there are hypothalamic modifications, human data about such association are lacking. Here, we demonstrated that there were no significant changes in T2 relaxation times (Fig. 1c), indicative of local gliosis, by pioglitazone treatment within the arcuate nucleus (105.30 ± 4.81 ms vs 101.81 ± 3.54 ms, two-tailed *p* value 0.57), neither within the reference regions amygdala (81.69 ± 0.94 ms vs 79.65 ± 0.68 ms, two-tailed *p* value 0.11) and putamen (58.26 ± 1.39 ms vs 57.57 ± 1.52 ms, two-tailed *p* value 0.75).

Discussion

In this study, we evaluated cold-induced BAT glucose uptake and hypothalamic gliosis in patients with type 2 diabetes, as a proof of concept for the putative actions of pioglitazone to regulate tissues that are involved in whole-body energy homeostasis. We provided initial clinical evidence for a lack of action of a TZD, pioglitazone, to modify brown adipose tissue volume and glucose uptake, as well as hypothalamic gliosis. There were no homogeneous changes (e.g., in some patients BAT increases, in other patients decreased) in the parameters studied, in 6 patients, after 6 months. That does not mean that pioglitazone may not induce significant changes in longer duration and larger studies, because this is a small study of rather short duration (6 months).

Increasingly the understanding of the pathophysiology of obesity becomes complex and the role of adipose tissue in metabolic diseases as well, particularly the association of body composition with metabolic complications [10, 11] or even the role of new and different fat depots in the pathophysiology of obesity [12]. Still, there is an association between particular fat depots and structural complications of obesity still poorly understood, such as cardiac morphological changes [13].

Thus, we showed that SC/cervical BAT activity/volume as well as hypothalamic gliosis was unchanged following pharmacological therapy. Concerning BAT, our results are in line with another recent study that reported a lack of effect for pioglitazone to induce BAT glucose uptake in patients [14]. However, it differs from experimental data, showing that in cultured cells and in mice, TZD promotes browning [15]. The preservation of appropriate sympathetic outflow could be one of the reasons to explain the differences between the human and experimental studies [14, 15], as even subclinical diabetic neuropathy could impair BAT innervation [16]. In addition, PPAR-γ-driven BAT differentiation requires intact innervation [15].

Fat depot-specific sensitivity to PPAR-γ agonists could be yet another reason behind the negative finding in this study. PPAR-γ is more expressed in subcutaneous fat than in omental depot, which explains the predominance of subcutaneous fat increase in subjects treated with pioglitazone [17]. Unfortunately, there are no data comparing the actions of PPAR-γ agonists in BAT versus other fat types or depots, placing this

Table 1 Unadjusted baseline and 6-month follow-up for clinical variables

	Before pioglitazone $N=6$	After pioglitazone $N=6$	p value
Age (year)	46.9 (12.8)	47.7 (13.0)	
Female sex (%)	83.3	83.3	
Weight (kg) Median (interquartile range)	76.9 (16.6)	80.3 (21.2)*	*0.039*
Change in body weight			
% of body weight (mean ± SD)		3.40 (4.0)	
Kilograms of body weight		2.58 (3.3)	
BMI (kg/m^2) Median (interquartile range)	28.1 (4.2)	30.6 (4.0)*	*0.039*
LBM (kg) Median (interquartile range)	46.7 (13.4)	47.9 (13.4)	0.640
Glucose (mg/dL) Median (interquartile range)	110.5 (47.2)	107.0 (94.7)	0.906
Insulin (mUI/mL) Median (interquartile range)	14.9 (8.7)	12.8 (6.4)	0.438
LDL cholesterol (mg/dL) Median (interquartile range)	130 (47.0)	104.5 (63.3)	0.397
HDL cholesterol (mg/dL) Median (interquartile range)	43.0 (23.0)	39.0 (13.0)	0.438
Triglycerides (mg/dL) Median (interquartile range)	160.0 (84.5)	173.5 (170.0)	0.412
Total cholesterol (mg/dL) Median (interquartile range)	218 (74.5)	175 (81.0)	0.430
HbA1c (%) Median (interquartile range)	6.7 (2.13)	6.3 (2.6)	0.844

The values are represented as median (interquartile range). The sample size does not include data from patients who lost to follow-up. The lipid sample had only five patients. We performed Wilcoxon matched-pairs signed-rank test. No adjustments were performed for multiple comparisons. To convert glucose to millimoles per liter, multiply by 0.01129. To convert cholesterol to millimoles per liter, multiply by 0.02586. To convert triglycerides to millimoles per liter, multiply by 0.01129

CI confidence interval, *LDL* low-density lipoprotein, *HDL* high-density lipoprotein, *LBM* lean body mass (Janmahasatian formula)

*$p < 0.05$

possibility as hypothetical. Nevertheless, in our cohort, there was no significant change in BAT volume, although there was a significant weight gain, providing additional strength to the hypothesis that there is a distinct and depot-specific regulation of adipogenesis in vivo, which can be controlled also by pioglitazone. Conversely, there is little biological rationale for advocating that PPAR-γ agonists could inhibit BAT function, since pioglitazone primarily acts to improve glucose metabolism [18]. In fact, as one of the most important aspects of BAT function is to increase glucose clearance, PPAR-γ agonism should further stimulate BAT activity [19, 20].

It has been shown that rosiglitazone-stimulated peroxisome proliferation in hypothalamic POMC neurons decreased reactive oxygen species (ROS) and increased food intake in diet-induced obese mice [21]. Conversely, suppression of peroxisome in the hypothalamus by PPAR-γ antagonist induced increased ROS in POMC neurons, thereby increasing caloric intake [21]. Thus, impaired hypothalamic production of ROS is linked to the PPAR-γ nutritional stress metabolic sensor which modulates POMC neuron function [21]. With these experimental concepts in mind, we asked whether in humans, a PPAR-γ agonist could provide structural improvement to the hypothalamus. For that, we employed an MRI method that has been optimized and used to demonstrate hypothalamic gliosis in different cohorts of adults and one cohort of children with obesity [22–24]. Differently of the results obtained when body mass was reduced [24], here, pioglitazone treatment for 6 months was insufficient to modify gliosis. This could reinforce the concept that reduction in caloric intake and reduction in body mass are the main drivers of hypothalamic gliosis, and only by modifying these parameters, the structure of the hypothalamus could be improved.

In conclusion, we demonstrated that cold-induced BAT activity and volume and hypothalamic gliosis were not

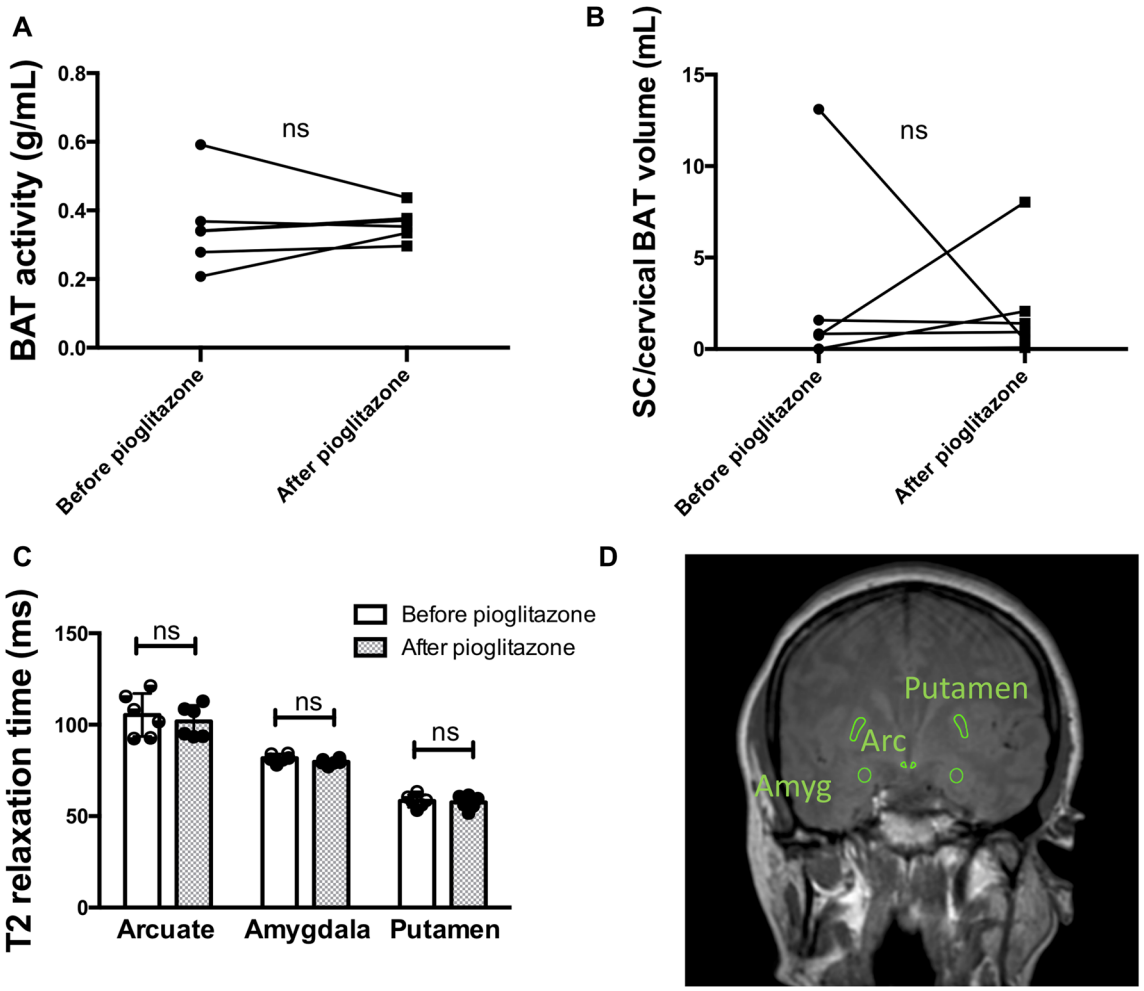

Fig. 1 SC/cervical BAT activity (**a**) and volume (**b**), measured by PET-CT after cold exposure, before and after pioglitazone treatment. T2 relaxation time (**c**) measured by T2-weighted MRI before and after pioglitazone treatment. Wilcoxon matched-pairs signed-rank test: **a** $p=0.563$; **b** $p=0.843$. Multiple *t* tests corrected for multiple comparisons using the Holm–Sidak method: **c** $p=0.573$ (Arc), $p=0.110$ (amygdala), $p=0.746$ (putamen); **d** illustrative image of the regions where the measurements were taken in MRI

significantly modified after TZD treatment with 30 mg/day for 6 months. Further research on TZDs in a randomized and of longer duration clinical trial approach would be required to shed light on the relevance and validity of using it as a therapeutic option targeting the BAT and hypothalamic gliosis, possibly with lower doses of pioglitazone (15 mg/daily), to minimize weight gain in an already overweight/obese patient's population.

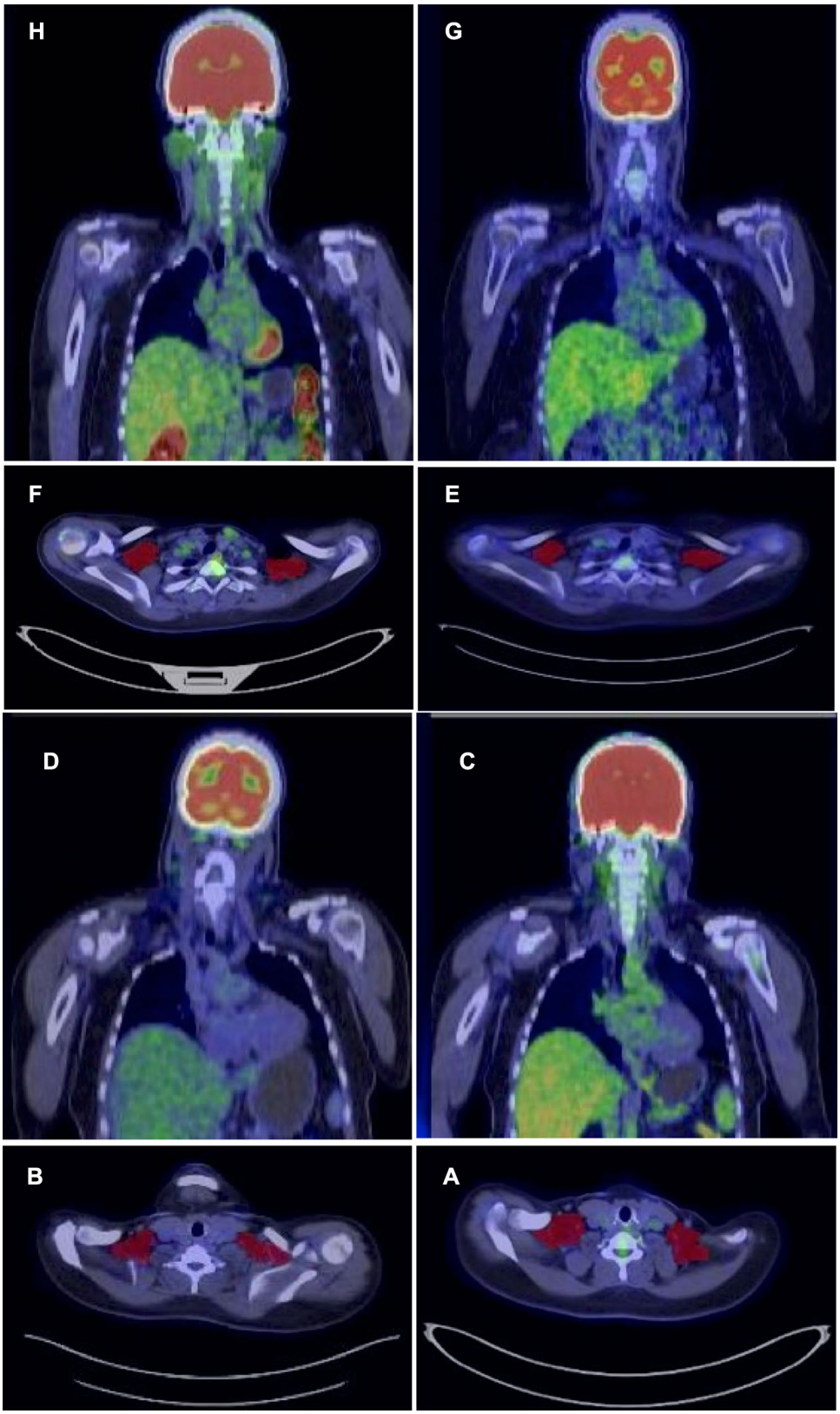

Fig. 2 Coronal (**a**, **b**, **e**, **f**) and axial (**c**, **d**, **g**, **h**) PET scan images from representative patients 1 (increasing [^{18}F]-FDG uptake—**a**–**d**) and 2 (decreasing [^{18}F] FDG uptake—**e**–**h**) before (**a**, **c**, **e**, **g**) and after (**b**, **d**, **f**, **h**) pioglitazone treatment

Author's contributions JCLJ drafted the first version of the manuscript. JCLJ, FF and LV wrote the manuscript. JCLJ, FF and LV edited and reviewed the manuscript. JCLJ, RMC and MMP performed the statistical analyses, reviewed/edited the manuscript and researched the data. JCLJ and MMP performed PET/CT analysis. CDR performed PET/CT scanning. FC performed MRI scanning and edited partially the manuscript. SVSL performed MRI analysis. LV, SR and BR conceived the design of the study. JCLJ, SR and BR recruited and assisted the patients.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standard The study was approved by the local medical ethical committee of the University of Campinas (Ethics Committee approval CAAE: 32930314.8.0000.5404).

Informed consent All patients being enrolled into this registry provided written informed consent.

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