Effect of pioglitazone treatment on liver fat and visceral fat in patients with congenital adrenal hyperplasia

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Introduction

Visceral fat in the abdomen and in particular intracellular fat in the liver are strongly correlated with insulin resistance and play an important role in the etiology of type 2 diabetes. Patients with Congenital Adrenal Hyperplasia (CAH) receive a lifelong (over)supplementation with glucocorticoids, which will almost certainly induce abnormal insulin sensitivity. However, this has never been studied in this specific group of patients. Pioglitazone is a medication that improves insulin sensitivity and it is hypothesized that it works via its effect on abdominal and liver fat, by shifting fat from the liver/intra-abdominal compartment to the subcutaneous compartment. The **aim** of this study was to assess the effects of pioglitazone on abdominal and liver fat in patients with CAH by magnetic resonance imaging and spectroscopy.

Methods

Subjects and study design: We included twelve CAH patients (5M/7F, age: 35.6 ± 8.9 (SD), BMI: 26.7 ± 4.7 kg/m^2) in a double blind, randomized, placebo-controlled cross-over study. The subjects received either 45 mg pioglitazone once a day for 16 weeks, followed by a placebo for 16 weeks or vice versa. At the end of each period of 16 weeks MR experiments were performed. Approval of the local ethics committee was obtained and the volunteers gave written informed consent.

Methods: After an overnight fasting period hepatic lipid content and abdominal fat distribution were determined by ^{1}H MRS and MRI, respectively. The combined MR examination was performed on a clinical 3T whole body MR system (Siemens Magnetom Tim Trio) using the bodycoil for excitation and two phased-array surface coils positioned at the liver and the abdomen for signal reception with a special cushion placed between the patient and the surface coils to reduce B1 field inhomogeneities.

Hepatic lipid content: After the acquisition of localizers in three orthogonal directions during breath holding, single voxel proton MR spectra were acquired from a volume of 30x30x30mm^3 positioned in the center of a liver lobe avoiding large vessel structures. A STEAM [1] localization sequence without water suppression was used for data acquisition. To minimize relaxation effects on signal intensity long repetition time (TR = 3s) and short echo time (TE = 20 ms) were used. Six averages were obtained during breath holding for 15 seconds. Generally, the MRS measurement was performed in duplo. Post-processing consisted of time-domain fitting of the water signal resonating at 4.7 ppm and the methylene lipid peak at 1.3 ppm using the AMARES routine in jMRUI [2]. To calculate the hepatic fat percentage the lipid signal intensity was multiplied with 100 and the result was divided by the sum of the methylene lipid and water signal intensities.

Abdominal fat distribution: A series of T1-weighted FLASH 2D axial MR images were acquired from a region extending from 4 cm above to 4 cm below the fourth and fifth lumbar interspace (16 slices, matrix size 192/256, field of view 300-353 / 400-470 mm2, slice thickness 5 mm, breath-hold, TR/TE 80 ms/2.46 ms). The images were analyzed based upon pixel intensities with high signal intensity corresponding to adipose tissue. On each image the subcutaneous fat area was semi-automatically segmented and analyzed using the dedicated software program HIPPO FAT [3]. The visceral adipose tissue was analyzed using the public domain software ImageJ 1.35s [4]. After a manual delineation of the edge of the visceral tissues the outer region was removed and the pixels in the inner region were converted into a binary image. The threshold level was automatically determined at the intensity corresponding to the nadir of the histogram of the pixel intensities of the selection. Both for total subcutaneous and total visceral fat the volumes determined on each of the 16 images were summed.

Statistical analysis: The results were tested for significant difference using a paired two-tailed Student's t-tests. Data are expressed as mean±SEM, unless otherwise indicated.

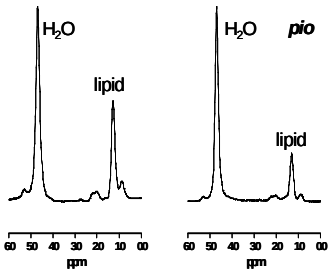

Fig. 1

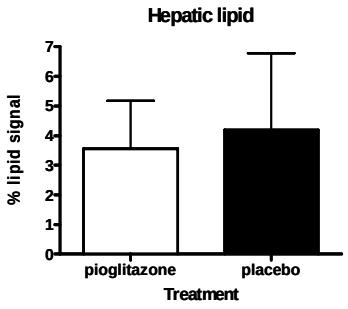

Fig. 2

Results

As no time effect was observed in the results, all data were evaluated in two groups: treatment with pioglitazone (*pio*) or placebo (*plac*). Figure 1 shows the ^{1}H MRS spectra of the patient with the highest hepatic lipid content without and with pioglitazone treatment, with 33 and 19% hepatic lipid, respectively. However, the spectra of most subjects showed rather low hepatic lipid percentages with values below 3.5% during both treatments. Although the mean value of 3.6±1.6% hepatic lipid for *pio* was lower than 4.2±2.6% hepatic lipid for *plac* (Fig. 2), no significant effect of pioglitazone treatment was found.

Also for the amounts of visceral fat (0.58±0.07 L for *pio* and 0.58±0.09 L for *plac*) and subcutaneous fat (2.00±0.75 L for *pio* and 1.98±0.83 L for *plac*) no significant effect of pioglitazone treatment was observed.

Discussion and conclusion

In contrary to the positive effect of pioglitazone on fat distribution in patients with type 2 diabetes mellitus [5], a 16-week pioglitazone treatment had no significant effect on hepatic lipid content and abdominal fat distribution in CAH patients in this study. Although we expected elevated hepatic lipid content in this patient group as a result of oversupplementation with glucocoticoids, not a single subject showed a hepatic lipid percentage higher than observed for healthy volunteers [6]. In addition, the CAH patients in our study represented a relatively healthy, young and lean group of subjects. *In conclusion*: Although pioglitazone may improve hepatic lipid content in an individual subject with CAH (Fig. 1), no effects of pioglitazone treatment on hepatic lipid content or abdominal fat were observed in this patient group as a whole.

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