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Effects of bed-time insulin versus pioglitazone on abdominal fat accumulation, inflammation and gene expression in adipose tissue in patients with type 2 diabetes☆

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ABSTRACT

Aims/hypothesis: Intra-abdominal fat (IAF) and inflammatory markers are correlated with cardio-vascular risk. We compared the impact of bed-time insulin versus pioglitazone treatment on these parameters in type 2 diabetic (T2D) patients.

Methods: Twenty-eight T2D patients poorly controlled with metformin and sulfonylurea were randomized to receive add-on therapy with pioglitazone or bed-time NPH insulin. IAF and subcutaneous fat (SCF) content, systemic low-grade inflammation level and expression of inflammation related genes in SCF, were measured before and after 24 weeks of treatment.

Results: Insulin and pioglitazone resulted in a significant decrease in HbA1c (−1.6% and −1.2%, respectively) and a significant increase in total body fat mass (1 ± 2.3 and 3.3 ± 2.7 kg, respectively). There was no change in IAF content after both treatments whereas significant increase in SCF content was only seen after pioglitazone treatment ($p < 0.05$ versus insulin). hsCRP level decreased after pioglitazone and ferritin level decreased after insulin treatment. No change in mRNA expression of inflammation related genes was found after either treatment.

Conclusion/interpretation: This suggests that a 24-week treatment with pioglitazone or bed-time insulin has a similar impact on intra-abdominal fat mass and systemic low-grade inflammation.

☆ This study has been registered with ClinicalTrials.gov, number NCT00159211.

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Abbreviations: hsCRP, high sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; IAF, intra-abdominal fat; SCF, subcutaneous fat; T2D, type 2 diabetes.

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1. Introduction

Intra-abdominal fat and low-grade inflammation are both associated with cardio-vascular risk [1–5]. Peroxisome proliferator-activated receptor (PPAR)-γ agonists (thiazolidinediones) and bed-time insulin are widely used as anti-diabetic agents in type 2 diabetes. In association with sulfonylurea and metformin they similarly improve glycemic control but are both associated with significant weight gain [6]. Their impact on abdominal fat accumulation and on the modification of inflammatory markers has never been compared.

Waist circumference, a clinical trait associated with intra-abdominal adipose tissue mass, significantly correlates with insulin resistance parameters [7] and cardio-vascular risks [1,2]. Fat mass gain induced by PPAR-γ agonists is a common phenomenon in diabetic patients [8,9]. However the effect of PPAR-γ agonists appears to impact differently on fat depot types. Pioglitazone or rosiglitazone were shown to have no effect [9,10] or to slightly decrease [8,11] intra-abdominal adipose tissue mass, whereas they lead to an increase in subcutaneous adipose tissue mass [9,10]. Weight gain during insulin therapy in type 2 diabetes is also commonly observed but is related to the increase in both fat and fat free mass [12,13]. The only published data about the impact of insulin therapy on abdominal fat distribution were obtained in absence of a significant fat mass gain [14] and no significant change was reported. Relying on previous observations made in patients with insulinoma [15], it cannot be excluded that insulin therapy could increase visceral fat deposition in type 2 diabetic patients.

Moreover insulin resistance and cardio-vascular risks are associated with increased systemic levels of inflammation mediators such as tumor necrosis factor (TNF)-α, interleukin-6 (IL-6), C-reactive protein (CRP) and serum amyloid A (SAA) [1–5,16]. These inflammatory factors, eventually produced by the adipose tissue [17,18], could constitute a pathophysiological link between obesity, insulin resistance and cardio-vascular diseases [19–21].

While improving insulin sensitivity, PPAR-γ agonists modulate both adipose tissue and systemic inflammation in rodent and humans. A down-regulation of macrophage specific-genes in mice adipose tissue [22] or a decreased IL-6 expression in human adipose tissue [23] have been described upon chronic rosiglitazone therapy. PPAR-γ agonists reduce systemic CRP level in humans [24,25].

Intriguingly, whereas insulin therapy improves hepatic insulin sensitivity, its association with an improvement in inflammation markers is debated [24–26]. Whether insulin therapy is able to regulate inflammation related genes expression in adipose tissue in human is still unclear.

In the present randomized study, we wished to directly compare effects of pioglitazone to those of bed-time insulin treatment, on abdominal fat distribution, systemic markers of inflammation and expression of subcutaneous adipose tissue inflammation related genes, in type 2 diabetic patients poorly controlled with sulfonylurea and metformin.

2. Research design and methods

This study was an investigator-initiated study, financially supported by public funds from Assistance Publique des Hopitaux de Paris.

The enrolled subjects were type 2 diabetic men or women (BMI $\geq$ 26 kg/m^2) aged 18–80 years, with an HbA1c between 7.5% and 9.5%, and treated with maximal tolerated and stable doses of sulfonylurea and metformin for $\geq$6 months. Exclusion criteria included prior use of insulin or glitazone, use of other affecting glycemic control agents, ASAT or ALAT >2.5-fold above the upper limit of normal level, glomerular filtration rate <60 ml/min, heart failure $\geq$grade 2, hemoglobin <10 g/dl, and inability to provide informed consent.

Volunteers were recruited in the diabetes department of Pitie-Salpetriere Hospital in Paris, France, from May 2005 to October 2006. The nature and potential risks of the study were explained to all subjects before obtaining their written informed consent. The experimental protocol was approved by the ethical committee of the Pitie-Salpetriere Hospital in Paris, France.

Patients were randomized to receive either pioglitazone (30 mg/day) (Actos; TAKEDA) or human NPH insulin (0.2 IU/kg/day) (Umuline NPH; LILLY) at bedtime for 24 weeks, while sulfonylurea and metformin were continued. Randomization was made by reference to a statistical series whose details were unknown to any of the investigators and were contained in a set of sealed envelops. The target fasting plasma glucose (FPG) was <110 mg/dl (<6.1 mmol/l). Insulin treated patients were contacted weekly by phone to discuss dosage changes. At the follow up visits (2 and 4 months) pioglitazone dose was increased from 30 to 45 mg/day if HbA1c had not decrease by at least 1%. In presence of significant lower limb oedema, the investigator could decrease the pioglitazone dose to 15 mg/day.

All the following data were measured after an overnight fast twice during the trial: at the enrollment visit and after the 24-week treatment phase.

A single-cut computed tomography scan of the abdomen was performed at L4–L5 level to assess visceral fat and subcutaneous fat contents as previously described [27]. Assessment was made by a treatment-blind reader. Body composition (total fat mass and fat free mass) was determined using a single-frequence bioelectrical impedance device (TBF-310, TANITA, Tokyo, Japan). An abdominal subcutaneous fat specimen (~1 g) was obtained by needle aspiration under local anaesthesia after an overnight fast. Biopsies were immediately snap frozen and stored at −80 °C until analysis.

Serum levels of adiponectin and IL-6 were determined by enzyme-linked immunosorbent assay (Quantikine; R&D Systems, Oxford, UK). The sensitivity of these assays was 0.7 pg/ml for IL-6 and 24.6 ng/ml for adiponectin. High sensitivity CRP and ferritin were assessed by immuno-nephelometry on Behring Nephelometer 2 (Dade-Behring, La Défense, France). FFA were assessed by colorimetry (Wako Chemical, Neuus, Germany).

2.1. RNA extraction and gene expression study

Total RNA was extracted using the RNeasy total RNA Mini kit (Qiagen, Courtaboeuf, France), according to the manufacturer's protocol. The concentration of total RNA was deter-