**Effects of pioglitazone on visceral fat metabolic activity in impaired glucose tolerance or type 2 diabetes mellitus**

**Short title;** Pioglitazone decreases visceral fat metabolism

Norihiro Kodama, Nobuhiro Tahara, Atsuko Tahara, Akihiro Honda, Yoshikazu Nitta, Minori Mizoguchi, Hayato Kaida, Masatoshi Ishibashi, Toshi Abe, Hisao Ikeda, Jagat Narula, Yoshihiro Fukumoto, Sho-ichi Yamagishi, and Tsutomu Imaizumi

Department of Medicine, Division of Cardio-Vascular Medicine (N.K., N.T., A.T., A.H., Y.N., M.M., H.I., Y.F., T.I.), Division of Nuclear Medicine, PET Center and Department of Radiology (H.K., M.I., T.A.), Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications (S-I.Y.), Kurume University School of Medicine, Kurume 830-0011, Japan, and Zena and Michael A. Wiener Cardiovascular Institute (J.N.), Mount Sinai School of Medicine, New York, New York 10029, USA

**Address all correspondence and requests for reprints to:** Dr. Nobuhiro Tahara, Department of Medicine, Division of Cardio-Vascular Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, 830-0011, Japan. Tel: 81 942 31 7580, Fax: 81 942 31 7707, E-mail: ntahara@med.kurume-u.ac.jp.

**Disclosure Summary:** The authors have nothing to disclose.

**Number of text words;** 2,996

**Number of tables and figures;** 3 tables and 3 figures

**Clinical Trial Registration—**URL: http://clinicaltrials.gov. Unique identifier: NCT00722631.

**Grant;** This study was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Tokyo, Japan (to N.T., S-I.Y. and T.I.).

**Keywords;** visceral fat metabolism • FDG-PET/CT • pioglitazone • adiponectin • HDL cholesterol

**Structured Abstract**

**Context:** Excess visceral fat is associated with chronic systemic inflammation and cardiovascular complications. Pioglitazone has been reported to variably influence visceral fat volume, but its effect on metabolic activity of the visceral fat remains uncharacterized.

**Objective:** The aim of this study was to assess the effects of pioglitazone on glucose metabolism of fat tissue by using 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) and computed tomography (CT) imaging.

**Design, Setting, and Participants:** FDG-PET and computed tomography imaging were performed in 56 patients with impaired glucose tolerance (IGT) or type 2 diabetes mellitus (T2DM); lipid and glycemic profiles and inflammatory biomarkers were obtained in all patients. These patients were randomized to treatment with either pioglitazone or glimepiride for 16 weeks.

**Main Outcome Measures:** The metabolic activity of the visceral fat tissues as assessed by FDG uptake was expressed as a target-to-background ratio (TBR) of blood-normalized standardized uptake value.

**Results:** The study was completed in 32 pioglitazone-treated and 21 glimepiride-treated patients (40 males and 13 females; mean age, 67.7±8.1 years; body mass index, 25.0±3.6 kg/$m^2$; HbA1c, 6.78±0.70 %). Both treatments were well-tolerated and comparably improved glycemic control. At baseline, visceral fat exhibited a higher TBR value than subcutaneous fat (0.55±0.14 vs 0.30±0.07, $P$<0.001). Pioglitazone significantly decreased the visceral fat volume (130.5±53.0 to 122.1±51.0 $cm^2$, $P$=0.013) and TBR values (0.57±0.16 to

0.50±0.11, *P*=0.007); glimepiride did not influence visceral fat volume or TBR values. Neither pioglitazone nor glimepiride treatment showed any effect on the volume or TBR values of subcutaneous fat. After 16-week treatment with pioglitazone reduction in visceral fat TBR was correlated to increase in HDL cholesterol levels.

**Conclusions:** Our study indicated that pioglitazone decreased the visceral fat volume and its metabolic activity in patients with IGT or T2DM. The beneficial effects of pioglitazone on visceral fat may be independent of its glucose-lowering effect.

**Abbreviations:** IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; CT, computed tomography; FDG, 18F-fluorodeoxyglucose; PET, positron emission tomography; PPAR, peroxisome proliferator-activated receptor; FPG, fasting plasma glucose; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c, glycated hemoglobin; hsCRP, high-sensitivity C-reactive protein; ROI, region of interest; SUV, standardized uptake value; TBR, target-to-background ratio.

Obesity has reached epidemic proportions globally and has become a major public health concern (1-3). The visceral obesity is closely associated with chronic inflammation and disturbed adipocytokine profiles, and consequently contributes to the risk of a variety of metabolic disorders including hypertension, dyslipidemia, insulin resistance, and hyperglycemia (4,5). The visceral obesity is considered a major risk factor for impaired glucose tolerance (IGT), type 2 diabetes mellitus (T2DM) and cardiovascular disease (6-8).

Fat tissue volume has been traditionally measured by computed tomography (CT) imaging (9,10), and combined imaging with 18F-fluorodeoxyglucose (FDG)-positron emission tomography (PET) allows simultaneous assessment of the metabolic activity of adipose tissue (11). An increased visceral fat volume is associated with both hyperplasia and hypertrophy of adipocytes, expression of inflammatory biomarkers (4,12), and higher metabolic rates and oxygen consumption. Visceral fat tissues have demonstrated a higher FDG-verified metabolic activity compared with subcutaneous fat tissues (11).

Pioglitazone, a peroxisome proliferator-activated receptor (PPAR)-gamma agonist, is a commonly used oral hypoglycemic agent for the treatment of T2DM (13). It improves insulin resistance and ameliorates hyperglycemia in diabetes; pioglitazone also demonstrates an anti-oxidative and anti-inflammatory property, considered a pleiotropic action in humans (14-16). Since PPAR-gamma is a key transcriptional factor that induces the differentiation from a pre-adipocyte to matured adipocyte and stimulates the induction of enzymes involved in lipogenesis (17-19), it is possible that pioglitazone may increase subcutaneous fat volume (20-22). There still remains a controversy about the effects of

pioglitazone on visceral fat volume (20-23) and metabolic activity. In this study, using FDG-PET and CT imaging, we examined the effects of pioglitazone on volume and metabolic activity of visceral and subcutaneous fat tissues of patients with IGT or T2DM. The influence of pioglitazone was compared with that of glimepiride, another oral hypoglycemic agent.

## Subjects and Methods

### Study design and patients

This present study comprised a 16-week prospective, randomized, open-label, comparator-controlled, single-center intervention design. IGT was diagnosed by 75 gram oral glucose tolerance test and T2DM was defined as recommended by the current diagnostic criteria of the American Diabetes Association (24). Fifty eight patients with IGT or T2DM who underwent whole-body PET and CT scan were prospectively enrolled for evaluation fat volume and FDG activity in the abdominal fat tissues. We excluded any patients with uncontrolled diabetes (fasting plasma glucose [FPG] $\geq$200 mg/dL), treatment regimen including pioglitazone or insulin, significant hepatic disorders (aminotransferase $>$2.5-fold the specific normal value), left ventricular dysfunction (left ventricular ejection fraction$<$ 40%) or heart failure (New York Heart Association functional class $\geq$II), symptomatic coronary artery disease or symptomatic stroke within past 6 months at enrollment, vasculitis, collagen disease, pneumonia, or malignancy. All patients were enrolled after their first screening visit; two patients met the exclusion criteria. Of 56 patients, 34 were allocated to pioglitazone arm and 22 to glimepiride arm. We could not obtain the informed consent from some patients

assigned to glimepiride group. This is a reason why there was a 1.5:1 ratio between the 2 groups. All study measurements were obtained at study entry and at the end of the observation period after 16 weeks. The disposition of patients in the study is shown in Figure 1. Two patients from pioglitazone arm and 1 from glimepiride arm withdrew before the follow-up evaluation at 16 weeks. Written informed consent was obtained from all patients. The research protocol was reviewed and approved by the Ethical Committee for Clinical Research of Kurume University approved this study. The trial was duly registered with Clinical Trial Registration (NCT00722631).

**Pharmacological treatment**

The consecutive eligible patients who had never received pioglitazone were randomized to receive either pioglitazone 15 mg daily in the morning or glimepiride 0.5 to 1 mg daily, as an active comparator. In the glimepiride group, when FPG level was ≥150 mg/dL, we chose the starting dose of 1 mg glimepiride. If patients were already being treated for hyperglycemia and were not optimally controlled, glimepiride or pioglitazone was added. Pioglitazone or glimepiride dose was titrated for optimal glycemic control for 16 weeks. The optimal glycemic control was defined as FPG level of ≤110 mg/dL. Any medications for hypertension, diabetes, dyslipidemia, or antiplatelet remained unchanged during the course of the study period.

**Data collection**

Presence of smoking habit, medical history, use of medication, and family history

of cardiovascular disease were assessed by a questionnaire. Smoking status was classified as current smoking or not smoking. Weight and height were measured to calculate body mass index (BMI). Waist circumference was measured at the umbilical level in the late exhalation phase. BMI and waist circumference were measured as an index of obesity. Blood pressure was measured in the sitting position using an upright standard sphygmomanometer. Vigorous physical activity and smoking were avoided for at least 30 minutes before resting blood pressure and heart rate measurements.

At baseline and 16 weeks after the treatments, blood was obtained from all the patients in the morning after an overnight fast of more than 12 hours for determinations of lipid profiles {low-density lipoprotein (LDL) cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol}, FPG and serum immunoreactive insulin, glycated hemoglobin (HbA1c), estimated glomerular filtration rate, high-sensitivity C-reactive protein (hsCRP), and total adiponectin. These blood chemistry variables were measured by standard methods at a commercial laboratory (The Kyodo Igaku Laboratory, Fukuoka, Japan) as described previously (25). The value for HbA1c (%) is estimated as a National Glycohemoglobin Standardization Program equivalent value (%) calculated by the formula HbA1c (National Glycohemoglobin Standardization Program) (%) = 1.02 × HbA1c (Japan Diabetes Society) (%) + 0.25% (26).

**Adipose tissue imaging**

Adipose tissue imaging was performed by a hybrid PET-CT after at least 12-hour of fasting. In brief, the enrollees received an intravenous administration of FDG

(4.2 MBq [0.12 mCi]/kg body weight) through the antecubital vein. One hour after the FDG injection, 3-dimensional whole-body PET imaging was carried out using an integrated full-ring PET and 16-slice multidetector computed tomography (CT) scanner (Gemini-GXL 16, Philips Medical Systems, Inc., Cleveland, Ohio). The non-contrast CT data were used for attenuation correction and localization. After both the transmission and emission images were obtained, images were reconstructed using the 3-dimensional line-of-response row-action maximum likelihood algorithm (3D-LOR-RAMLA, Philips, Eindhoven, the Netherlands).

**Abdominal fat distribution**

Abdominal fat distribution was estimated by calculating subcutaneous and visceral fat areas using a standardized method with CT scan and Fat Scan software (N2 System Corp, Osaka, Japan) (9) (Figure 2A). In brief, a region of interest (ROI) of the fat layer was defined by tracing its contour on each scan, and the attenuation range of CT numbers in Hounsfield units for fat tissue was calculated. The fat area was determined as the average of areas at the umbilical level and the additional 10 levels separated by 4 mm in length in top and bottom from the umbilical level obtained from consecutive CT images (Figure 2B). The investigators who performed the measurements of fat tissue were blinded to the patients' characteristics.

**Metabolic activity in the fat tissues**

Metabolic activity in the fat tissues was assessed by FDG-PET and CT scan. National Institutes of Health ImageJ was used for image analysis. We created a

“fat mask image” from the original CT image and a “re-sampled PET image” from the original PET image set by corresponding to fat volume slices in the patients, and then combined them to accomplish a “PET fat mask image” on a workstation as described previously (11) (Figure 2C). We drew ROIs in each contour of visceral and subcutaneous fat tissues in consecutive levels and evaluated as follows. The intensity of FDG uptake was quantified by measuring the standardized uptake value (SUV) corrected for body weight, injected FDG dose, and ROI volume. Subsequently, the FDG uptake on the fat tissue was corrected for blood activity by dividing by venous blood SUV to produce a blood-corrected glucose metabolism; as known target-to-background ratio (TBR). We then averaged TBR values from 11 levels to obtain a TBR score in both subcutaneous and visceral fat tissue regions. Two blinded radiologists measured the volume and FDG uptake on the fat tissues. The intraobserver or interobserver variability of these measurements was less than 5%.

**Statistical analysis**

Data were analyzed by with per protocol results rather than intention-to-treat. Data were described presented as mean values ± standard deviation or medians with the interquartile range. We performed the Shapiro-Wilk test to evaluate the assumption of normality. Statistical analysis was performed by means of appropriate parametric and nonparametric methods. Treatment groups were compared at baseline by using an unpaired t-test for continuous variables and $\chi^2$ for categorical variables. First, 2-tailed paired t-test was performed for comparisons between the baseline and post-treatment. Second, the changes

from baseline were compared by 2-tailed unpaired t-test between the two groups. Statistical significance was defined as $P < 0.05$. All statistical analyses were performed with the use of the SPSS system (SPSS Inc., Chicago, IL).

## Results

### Study design

Of 58 enrolled patients, 2 patients were excluded due to cancer diagnosis before the randomization. Finally, 56 patients were randomly assigned to receive either pioglitazone (N=34) or glimepiride (N=22). But, 3 patients dropped out of the study because of refusal of assessment of blood chemistry or drug treatment. The study was completed by 53 patients (Figure 1). A summary of the baseline characteristics of the patients in each group are presented in Table 1. As shown in Table 1, baseline characteristics were well-matched between the two groups with respect to gender distribution, age, BMI, waist circumference, blood pressure, renal function, lipids, glycemic or inflammatory status, and plasma adiponectin concentrations at baseline. Percentage of patients who were taking medications for diabetes or hypertension, statins or aspirin was similar between the two groups, and doses of these drugs were not changed during the intervention periods.

### Effects of pioglitazone and glimepiride

Both treatments were well-tolerated. The average daily dose of glimepiride was $1.3 \pm 1.1$ mg daily, and that of pioglitazone $16.4 \pm 4.3$ mg daily. No treatments-related adverse side effects such as hospitalization for heart failure

or severe hypoglycemia were observed in the present study. Table 2 shows the clinical variables in both treatment groups at the baseline and end of the study. Glycemic control evaluated by FPG or HbA1c was similar between the two groups. Fasting insulin concentrations remained unchanged in the both groups. There were no significant changes of blood pressure, LDL cholesterol or triglycerides during the study periods in either group. HDL cholesterol was significantly increased in the pioglitazone group ($P = 0.002$), but not in the glimepiride group ($P = 0.150$). Pioglitazone significantly decreased serum level of hsCRP ($P < 0.001$), whereas it significantly increased in glimepiride group ($P = 0.018$). Also, treatment with pioglitazone was associated with significant increase in plasma adiponectin concentrations ($P < 0.001$), but glimepiride did not affect the adiponectin level ($P = 0.366$).

**Treatment effect on visceral fat volume**

Although there was no significant difference in change of waist circumference from baseline between the two groups, pioglitazone ($P = 0.016$), but not glimepiride ($P = 0.469$), modestly increased waist circumference, whereas either treatment did not affect body weight. In the pioglitazone group, the change in waist circumference was inversely associated with that in glycemic control (FPG, $r = -0.46$, $P = 0.008$; HbA1c, $r = -0.47$, $P = 0.006$), but not that in visceral fat ($r = -0.25$, $P = 0.168$) or subcutaneous fat volume ($r = -0.04$, $P = 0.815$). There was no significant change in subcutaneous fat volume after either treatment (Table 3). However, a significant decrease in visceral fat volume was only observed by the treatment with pioglitazone ($P = 0.013$; Table 3). Representative cases after both

treatments are shown in Figure 3. The decrease in the visceral fat volume was correlated with the increase in plasma adiponectin concentration using both groups, but not pioglitazone group only ($r = -0.20$, $P = 0.298$ in the pioglitazone group; $r = -0.30$).

**Treatment effect on metabolic activity of adipose tissues**

There was no significant difference of TBR values in the subcutaneous or visceral fat between the two groups at baseline. The visceral fat tissues exhibited a substantially higher metabolic activity than subcutaneous fat tissues ($P < 0.001$); the result was consistent with that of the previous report (11). For the visceral fat tissues, pioglitazone significantly decreased the metabolic activity ($P = 0.007$), but glimepiride did not ($P = 0.145$; Table 3). Either treatment did not affect the metabolic activity in the subcutaneous fat tissues (Table 3). Although pioglitazone significantly decreased the visceral fat volume, there was no significant association between reduction of visceral fat volume and suppression of its metabolic activity ($r = -0.01$, $P = 0.958$). The decrease in metabolic activity in the visceral fat tissues was significantly associated with the elevation of HDL cholesterol ($r = -0.59$, $P < 0.001$ in the pioglitazone group; $r = -0.153$; $P = 0.508$ in the glimepiride group), but not with the changes in waist circumference, FPG, HbA1c, or adiponectin concentrations.

**Discussion**

It is well established that visceral adiposity is associated with insulin resistance and that thiazolidinediones improve diabetes control by lowering insulin

resistance (27). Therefore, it is conceivable that pioglitazone may decrease visceral fat volume with the mechanism by which it improves diabetic control. However, numerous clinical studies have yielded inconsistent results about the effects of pioglitazone on abdominal fat volume (20-23). Further, the relationship between visceral fat volume and its metabolic activity is unclear, and effects of pioglitazone on metabolic activity of adipose tissues are largely unknown. Therefore, in this study, we compared the effects of pioglitazone and glimepiride on visceral fat volume and its metabolic activity in patients with IGT or T2DM. The treatment with pioglitazone for 16 weeks, but not glimepiride substantially reduced the visceral fat volume and its metabolic activity. On the other hand, neither treatment affected the subcutaneous fat volume or activity. Since there was no significant difference in glycemic control and lipid profile between the two treatment groups, the beneficial effects of pioglitazone on visceral adipose tissues may be independent on its glucose-lowering property or possibly its pleiotropic action.

Visceral adipocytes are recognized as an endocrine organ, which produce various pro-inflammatory cytokines such as tumor necrosis factor-alpha (28-30). In addition, recently, cytokine-induced macrophage infiltration into the adipose tissues has been shown to further augment the inflammatory milieu (31). Therefore, visceral adiposity and enhanced inflammatory reactions could induce a vicious cycle to exaggerate insulin resistance and augment the risk of cardiovascular disease (32,33). In the study, we found that pioglitazone significantly decreased the metabolic activity in the visceral fat tissues, which inversely correlated with increase in HDL cholesterol levels. Lack of any

relationship between the decrease in visceral fat and decrease in its metabolic activity in the pioglitazone group has suggested that visceral fat volume and its metabolic activity may be differently regulated.

Waist circumference in the pioglitazone group is slightly increased even though pioglitazone treatment decreased visceral fat volume without any significant changes in subcutaneous fat. So, waist circumference may not necessarily reflect changes in visceral adiposity. In this study, reduction of metabolic activity in the visceral fat tissues was significantly associated with the elevation of HDL cholesterol in the pioglitazone group, whereas decrease in visceral fat volume was significantly correlated with the increase in plasma adiponectin concentration using both groups only. The lack of association in the pioglitazone group is probably related to the small number of study group.

We, along with others, have found that FDG-PET is a reliable and sensitive method for quantifying vascular inflammation and identifying vulnerable plaques within an area of atherosclerosis (34,35). Further, we have shown that vascular FDG uptake activity is significantly higher in proportion to the accumulation to the number of the components of the metabolic syndrome and negatively correlated with HDL-cholesterol levels (35). Our present findings suggest that pioglitazone could have insulin-sensitizing and anti-atherogenic properties in humans partly via its anti-inflammatory actions on visceral adipose tissues. In this study, although pioglitazone treatment significantly decreased the visceral fat volume, there was no association between the reduction of fat volume and suppression of the metabolic activity in adipose tissues. Moreover, the change in the visceral fat volume after treatments was inversely correlated

with that in plasma adiponectin concentrations. Size of fat cells in adipose tissues is associated with insulin sensitivity; adipocyte size is positively correlated with pro-inflammatory adipocytokines levels, while inversely associated with adiponectin levels (36). Pioglitazone has been reported to induce differentiation of pre-adipocytes into adipocytes, thereby increasing the number of small adipocytes because of both the appearance of the new adipocytes and the shrinkage and/or disappearance of existing mature adipocytes (37,38). Therefore, there could exist two distinct insulin-sensitizing mechanisms for piogitazone in visceral fat tissues including the reduction of fat cell size (possibly associated with the elevation of adiponectin), and the suppression of inflammatory reactions (possibly linked to increase in HDL cholesterol levels).

**Study Limitation**

The small sample size may limit the findings of the present study. We can not confirm that FDG uptake represents a true reduction in inflammatory activity in adipose tissue due to our inability in obtaining the fat tissue biopsy. It is also not known as to which type of cells would reflect the FDG uptake in adipose tissues. Although the primary endpoints failed to reach statistical significance in the PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events) study, pioglitazone significantly reduced the composite of all-cause mortality, non-fatal myocardial infarction, and stroke in patients with type 2 diabetes who have a high risk of macrovascular events (15). Furthermore, in a subgroup analysis from PROactive, pioglitazone significantly reduced the risk of recurrent

stroke and myocardial infarction in high-risk patients with type 2 diabetes (39,40). Longitudinal study is needed to examine whether unique and glucose-lowering independent effects of pioglitazone on visceral adipose tissues could indeed contribute to the risk reduction of cardiovascular disease.

In conclusion, we observed that pioglitazone treatment may lead to decrease visceral fat volume and its metabolic activity in IGT or T2DM patients. Its glycemic control independent effect on visceral adipose tissues may partly explain the beneficial effect of pioglitazone on cardiometabolic disorders.

**Acknowledgments**

The authors thank Kouichi Nitta (Hitachi-medical co.) and radiation technologists at Kurume University Hospital for their excellent technical assistance. They also thank Kazumi Hirakawa, Naoko Tanaka, Yuri Nishino, Mami Nakayama, Miho Nakao-Kogure, Miyuki Nishikata, Makiko Kiyohiro, and Kimiko Kimura for their efforts.

**References**

1. **Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL.** The global obesity pandemic: shaped by global drivers and local environments. *Lancet.* 2011;378:804-814.

2. **Yanovski SZ, Yanovzski JA; World Health Organization.** Obesity and overweight. Fact sheet N°311. Updated May 2012; Available at: http://www.who.int/mediacentre/ factsheets/fs311/en/index.html.

3. **Novak NL, Brownell KD.** Role of policy and government in the obesity epidemic. *Circulation.* 2012;126:2345-2352.

4. **Ouchi N, Parker JL, Lugus JJ, Walsh K.** Adipokines in inflammation and metabolic disease. *Nat Rev Immunol.* 2011;11:85–97.

5. **Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L; INTERHEART Study Investigators.** Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet.* 2004;364:937–952.

6. **Larsson B, Bjorntorp P, Tibblin G.** The health consequences of moderate obesity. *Int J Obes.* 1981;5:97–116.

7. **Hubert HB, Feinleib M, McNamara PM, Castelli WP.** Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation.* 1983;67:968–977.

8. **Rexrode KM, Hennekens CH, Willett WC, Colditz GA, Stampfer MJ, Rich-Edwards JW, Speizer FE, Manson JE.** A prospective study of body mass index, weight change, and risk of stroke in women. *JAMA.* 1997;277:1539–1545.

9. **Yoshizumi T, Nakamura T, Yamane M, Islam AH, Menju M, Yamasaki K, Arai T, Kotani K, Funahashi T, Yamashita S, Matsuzawa Y.** Abdominal fat: standardized technique for measurement at CT. *Radiology.* 1999;211:283–286.

10. **Lee S, Janssen I, Ross R.** Interindividual variation in abdominal subcutaneous and visceral adipose tissue: influence of measurement site. *J Appl Physiol.* 2004;97:948 –954.

11. **Christen T, Sheikine Y, Rocha VZ, Hurwitz S, Goldfine AB, Di Carli M, Libby P.** Increased glucose uptake in visceral versus subcutaneous adipose tissue revealed by PET imaging. *JACC Cardiovasc Imaging.* 2010;3:843-851.

12. **Skurk T, Alberti-Huber C, Herder C, Hauner H.** Relationship between adipocyte size and adipokine expression and secretion. *J Clin Endocrinol Metab.* 2007;92:1023-1033.

13. **Miyazaki Y, Mahankali A, Matsuda M, Glass L, Mahankali S, Ferrannini E, Cusi K, Mandarino LJ, DeFronzo RA.** Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. *Diabetes Care.* 2001;24:710–719.

14. **Satoh N, Ogawa Y, Usui T, Tagami T, Kono S, Uesugi H, Sugiyama H, Sugawara A, Yamada K, Shimatsu A, Kuzuya H, Nakao K.** Antiatherogenic effect of pioglitazone in type 2 diabetic patients irrespective of the responsiveness to its antidiabetic effect. *Diabetes Care.* 2003;26:2493–2499.

15. **Dormandy JA, Charbonnel B, Eckland DJ, Erdmann E, Massi-Benedetti M, Moules IK, Skene AM, Tan MH, Lefèbvre PJ, Murray GD, Standl E, Wilcox RG, Wilhelmsen L, Betteridge J, Birkeland K, Golay A, Heine RJ, Korányi L, Laakso M, Mokán M, Norkus A, Pirags V, Podar T, Scheen A, Scherbaum W,**

**Schernthaner G, Schmitz O, Skrha J, Smith U, Taton J; PROactive investigators.** Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet.* 2005;366:1279–1289.

16. **Pfützner A, Marx N, Lübben G, Langenfeld M, Walcher D, Konrad T, Forst T.** Improvement of cardiovascular risk markers by pioglitazone is independent from glycemic control: results from the pioneer study. *J Am Coll Cardiol.* 2005;45:1925-1931.

17. **Hallakou S, Doaré L, Foufelle F, Kergoat M, Guerre-Millo M, Berthault MF, Dugail I, Morin J, Auwerx J, Ferré P.** Pioglitazone induces in vivo adipocyte differentiation in the obese Zucker fa/fa rat. *Diabetes.* 1997;46:1393–1399.

18. **Spiegelman BM.** PPAR-γ: adipogenic regulator and thiazolidinedione receptor. *Diabetes.* 1998;47:507–514.

19. **Lambe KG, Tugwood JD.** A human peroxisome-proliferator-activated receptor γ is activated by inducers of adipogenesis, including thiazolidinedione drugs. *Eur J Biochem.* 1996;239:1–7.

20. **Miyazaki Y, Mahankali A, Matsuda M, Mahankali S, Hardies J, Cusi K, Mandarino LJ, DeFronzo RA.** Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. *J Clin Endocrinol Metab.* 2002;87:2784-2791.

21. **Hirose H, Kawai T, Yamamoto Y, Taniyama M, Tomita M, Matsubara K, Okazaki Y, Ishii T, Oguma Y, Takei I, Saruta T.** Effects of pioglitazone on

metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. *Metabolism.* 2002;51:314-317.

22. **Jonker JT, Lamb HJ, van der Meer RW, Rijzewijk LJ, Menting LJ, Diamant M, Bax JJ, de Roos A, Romijn JA, Smit JW.** Pioglitazone compared with metformin increases pericardial fat volume in patients with type 2 diabetes mellitus. *J Clin Endocrinol Metab.* 2010;95:456-460.

23. **Basu A, Jensen MD, McCann F, Mukhopadhyay D, Joyner MJ, Rizza RA.** Effects of pioglitazone versus glipizide on body fat distribution, body water content, and hemodynamics in type 2 diabetes. Diabetes Care 2006;29:510-4.

24. **American Diabetes Association.** Standards of medical care in diabetes--2012. *Diabetes Care.* 2012;35 Suppl 1:S11-63.

25. **Tahara N, Yamagishi S, Tahara A, Ishibashi M, Hayabuchi N, Takeuchi M, Imaizumi T.** Adiponectin is inversely associated with ratio of serum levels of AGEs to sRAGE and vascular inflammation. *Int J Cardiol.* 2012;158:461-462.

26. **Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I, Hoshino T, John WG, Kobold U, Little R, Mosca A, Mauri P, Paroni R, Susanto F, Takei I, Thienpont L, Umemoto M, Wiedmeyer HM; IFCC Working Group on HbA1c Standardization.** IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem.* 2004;50:166-174.

27. **Saltiel AR, Olefsky JM.** Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes.* 1996;45:1661-1669.

28. **Galic S, Oakhill JS, Steinberg GR.** Adipose tissue as an endocrine organ.

*Mol Cell Endocrinol.* 2010;316:129-139.

29. **Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K.** cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). *Biochem Biophys Res Commun.* 1996;221:286–289.

30. **Trayhurn P, Beattie JH.** Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *Proc Nutr Soc.* 2001;60:329–339.

31. **Gustafson B, Hammarstedt A, Andersson CX, Smith U.** Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. *Arterioscler Thromb Vasc Biol.* 2007;27:2276-2283.

32. **Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr.** Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest.* 2003;112:1796-1808.

33. **Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H.** Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest.* 2003;112:1821-1830.

34. **Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, Yates D, LaMuraglia GM, Furie K, Houser S, Gewirtz H, Muller JE, Brady TJ, Fischman AJ.** In vivo 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. *J Am Coll Cardiol.* 2006;48:1818-1824.

35. **Tahara N, Kai H, Yamagishi S, Mizoguchi M, Nakaura H, Ishibashi M, Kaida H, Baba K, Hayabuchi N, Imaizumi T.** Vascular inflammation evaluated

by [18F]-fluorodeoxyglucose positron emission tomography is associated with the metabolic syndrome. *J Am Coll Cardiol.* 2007;49:1533-1539.

36. **Bahceci M, Gokalp D, Bahceci S, Tuzcu A, Atmaca S, Arikan S.** The correlation between adiposity and adiponectin, tumor necrosis factor alpha, interleukin-6 and high sensitivity C-reactive protein levels. Is adipocyte size associated with inflammation in adults? *J Endocrinol Invest.* 2007;30:210-214.

37. **de Souza CJ, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, Burkey BF.** Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes.* 2001;50:1863-1871.

38. **Takahashi M, Kamei Y, Ezaki O.** Mest/Peg1 imprinted gene enlarges adipocytes and is a marker of adipocyte size. *Am J Physiol Endocrinol Metab.* 2005;288:E117-124.

39. **Wilcox R, Bousser MG, Betteridge DJ, Schernthaner G, Pirags V, Kupfer S, Dormandy J; PROactive Investigators.** Effects of pioglitazone in patients with type 2 diabetes with or without previous stroke: results from PROactive (PROspective pioglitAzone Clinical Trial In macroVascular Events 04). *Stroke.* 2007;38:865-873.

40. **Erdmann E, Dormandy JA, Charbonnel B, Massi-Benedetti M, Moules IK, Skene AM; PROactive Investigators.** The effect of pioglitazone on recurrent myocardial infarction in 2,445 patients with type 2 diabetes and previous myocardial infarction: results from the PROactive (PROactive 05) Study. *J Am Coll Cardiol.* 2007;49:1772-1780.

## Figure legends

### Figure 1. Disposition of patients.

Eligible 56 patients were randomly assigned to receive either pioglitazone or glimepiride as an active comparator for 16 weeks. The study was fully completed by 32 patients in the pioglitazone group and 21 in the glimepiride group.

IGT, impaired glucose tolerance; T2DM, type 2 diabetes mellitus; FDG-PET, 18F-fluorodeoxyglucose-positron emission tomography; CT, computed tomography.

### Figure 2. Analyses of abdominal fat distribution.

Abdominal fat distribution was estimated using CT imaging and Fat Scan software. Magenta area indicates subcutaneous fat, while red area shows visceral fat (A). The fat area was determined as the average of areas at the umbilical level and the additional 10 levels separated by 4 mm in length in top and bottom from the umbilical level obtained from consecutive CT images (B). We created a “fat mask image” from the original CT image and a “re-sampled PET image” from the original PET image set by corresponding to fat volume slices in the patients, and then combined them to accomplish a “PET fat mask image” on a workstation (C).

### Figure 3. Treatment effects on abdominal fat volume.

Representative computed tomography at baseline (left) and after 16-week treatment (right) with pioglitazone (top) or glimepiride (bottom). Note the reduction in fat volume in the visceral fat tissues by pioglitazone treatment.

**Table caption**

**Table 1. Patient characteristics.**

LDL indicates low-density lipoprotein; HDL, high-density lipoprotein; CRP, C-reactive protein.

Values are n (%), mean ± SD, or *median (interquartile range).

**Table 2. Clinical data at baseline and after 16-week treatment with pioglitazone or glimepiride.**

Values are mean ± SD or *median (interquartile range).

**Table 3. Changes in adipose tissue parameters after 16-week treatment with pioglitazone or glimepiride.**

Figure 1

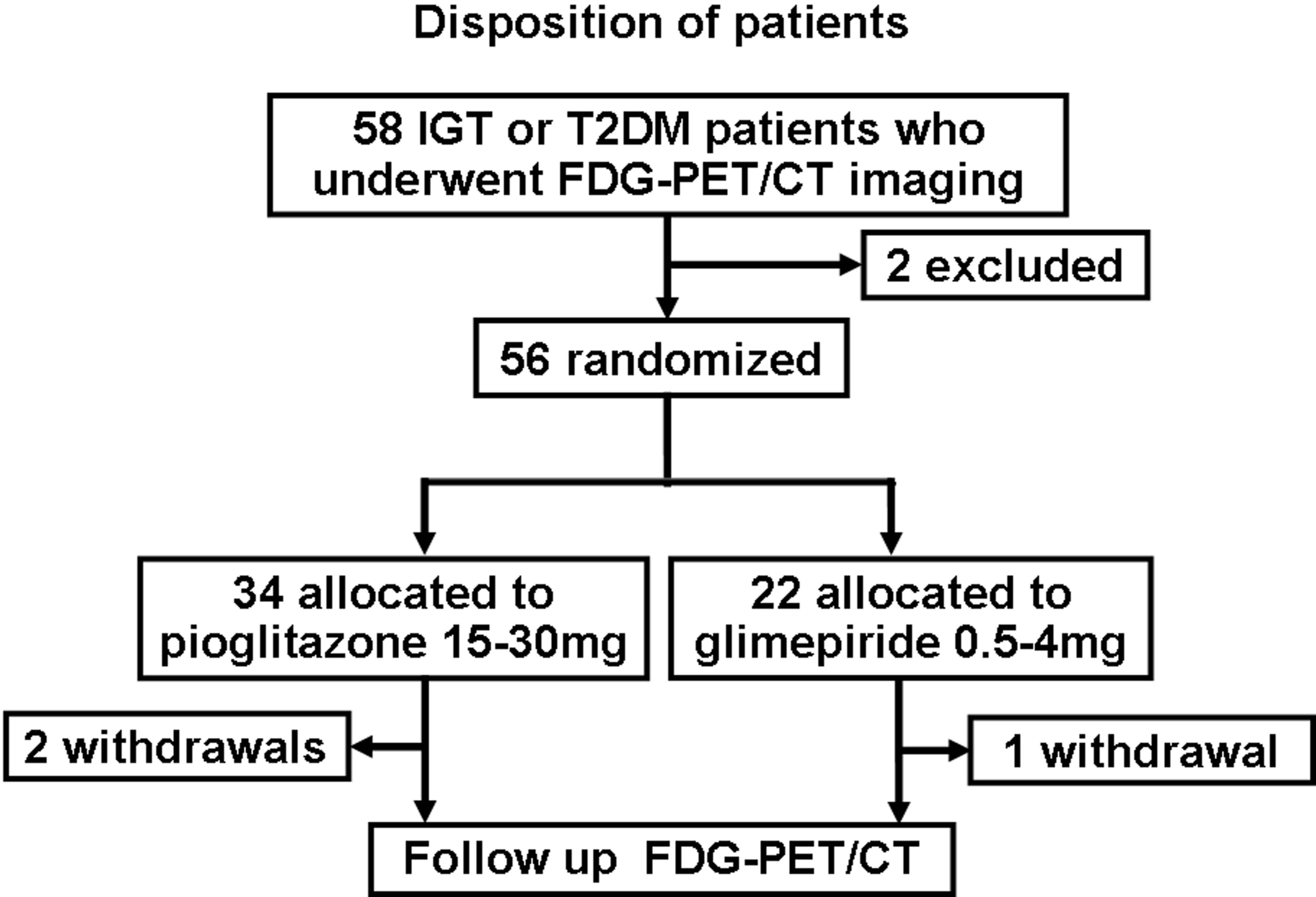
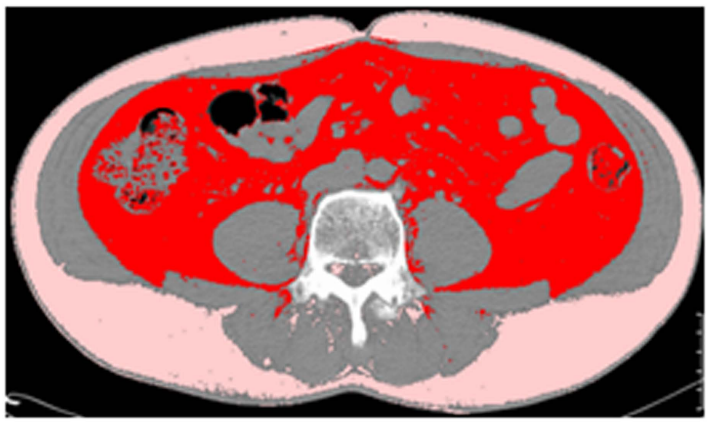

Disposition of patients
58 IGT or T2DM patients who underwent FDG-PET/CT imaging
2 excluded
56 randomized
34 allocated to pioglitazone 15-30mg
22 allocated to glimepiride 0.5-4mg
2 withdrawals
1 withdrawal
Follow up FDG-PET/CT

Figure 2

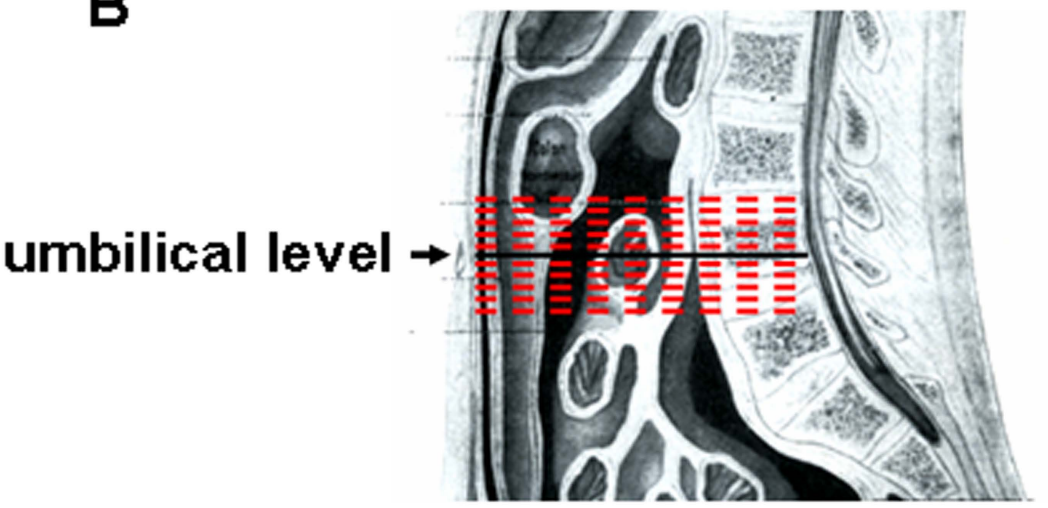
# Analyses of abdominal fat distribution

A

Fat CT image

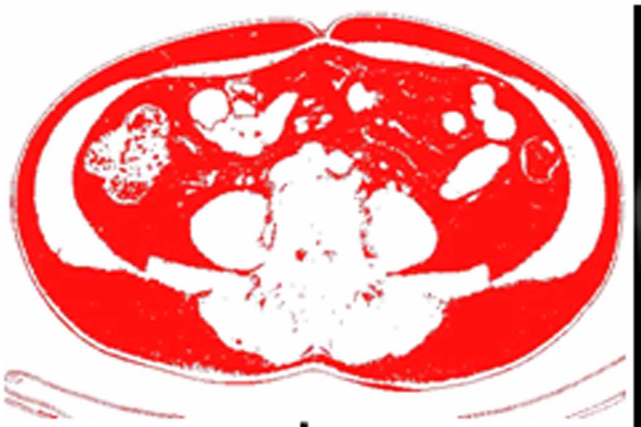


B

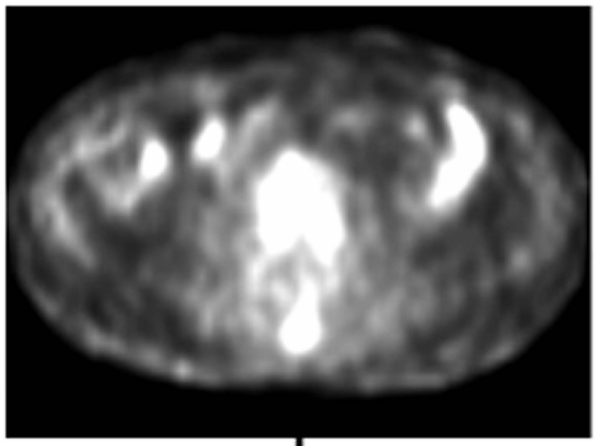


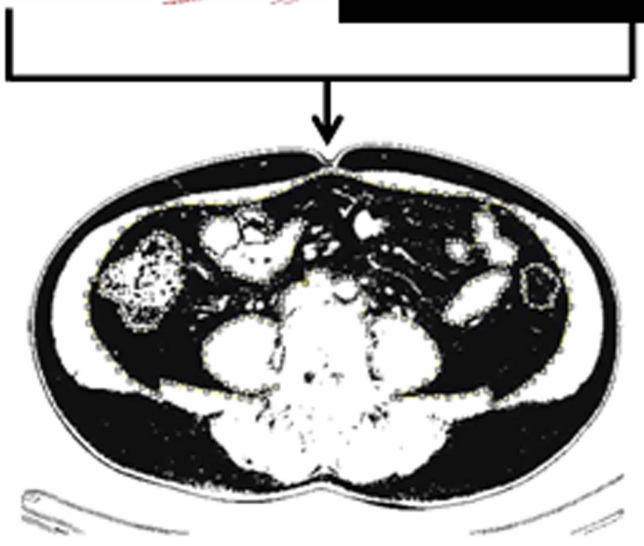

C

Fat mask image



Resampled PET

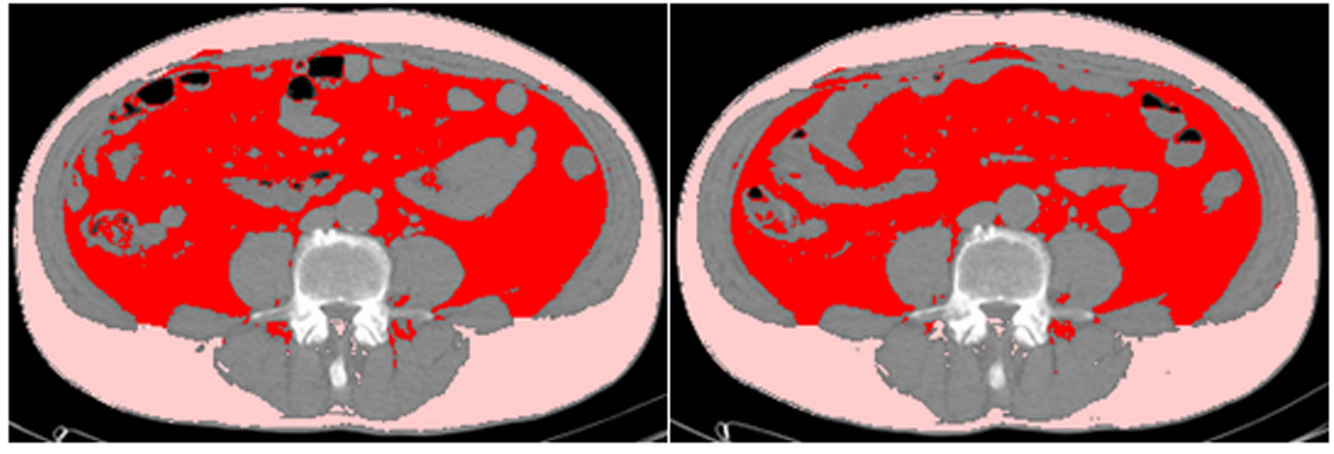
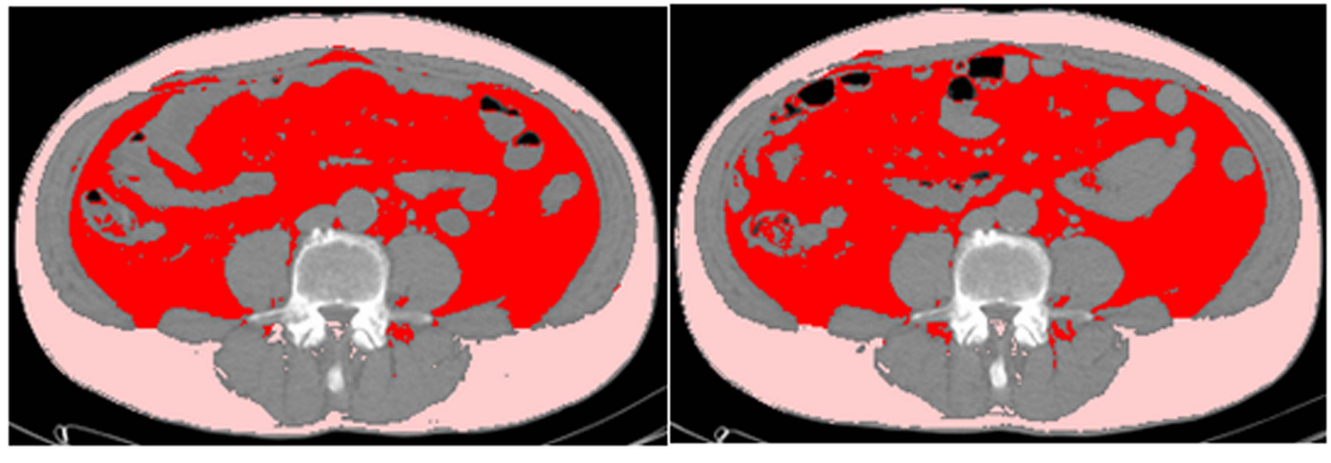




PET fat mask

Figure 3

## Treatment effect on abdominal fat volume

| | Baseline | Post treatment |
|---|---|---|
| **Pioglitazone** |  | |
| Subcutaneous fat area: | 132 $cm^2$ | 146 $cm^2$ |
| Visceral fat area: | 140 $cm^2$ | 113 $cm^2$ |
| | **Baseline** | **Post treatment** |
| **Glimepiride** |  | |
| Subcutaneous fat area: | 142 $cm^2$ | 157 $cm^2$ |
| Visceral fat area: | 188 $cm^2$ | 210 $cm^2$ |

| Parameters | Pioglitazone (n=32) | Glimepiride (n=21) | P value |
|---|---|---|---|
| Male, n (%) | 23 (71.9) | 17 (81.0) | 0.671 |
| Age (years) | 68.4 (7.3) | 66.7 (9.1) | 0.460 |
| Body mass index (kg/m$^2$) | 25.2 (3.5) | 24.7 (3.7) | 0.594 |
| Waist circumference (cm) | 89.6 (10.0) | 89.1 (10.8) | 0.844 |
| Systolic blood pressure (mmHg) | 129.9 (12.2) | 123.1 (13.5) | 0.068 |
| Diastolic blood pressure (mmHg) | 70.7 (9.1) | 69.1 (9.5) | 0.553 |
| LDL cholesterol (mg/dL) | 109.8 (25.8) | 116.2 (24.5) | 0.381 |
| HDL cholesterol (mg/dL) | 48.4 (12.2) | 51.5 (13.2) | 0.388 |
| Triglycerides* (mg/dL) | 113.0 [86.0-159.8] | 121.0 [78.8-165.0] | 0.682 |
| Estimated GFR (mL/min) | 68.2 (15.6) | 69.1 (17.9) | 0.851 |
| Fasting plasma glucose* (mg/dL) | 122.5 [110.0-146.3] | 131.0 [121.0-146.0] | 0.555 |
| Fasting plasma insulin* (μU/mL) | 7.15 [5.03-11.35] | 4.70 [3.55-8.50] | 0.100 |
| Hemoglobin A1c (%) | 6.72 (0.72) | 6.87 (0.64) | 0.454 |
| High-sensitivity CRP* (mg/dL) | 0.74 [0.33-1.94] | 0.51 [0.34-1.51] | 0.994 |
| Adiponectin* (μg/mL) | 4.56 [2.32-6.33] | 5.01 [2.64-7.67] | 0.855 |
| Current smoking, n(%) | 5 (15.6) | 4 (19.0) | 0.961 |
| Coronary artery disease, n(%) | 17 (53.1) | 12 (57.1) | 0.774 |
| Cerebral vascular disease, n(%) | 4 (12.5) | 6 (28.6) | 0.270 |
| Medications, n(%) | | | |
| For diabetes | 13 (40.6) | 12 (57.1) | 0.234 |
| For hypertension | 30 (93.8) | 17 (81.0) | 0.320 |
| Statins | 18 (56.3) | 9 (42.9) | 0.340 |
| Aspirin | 17 (53.1) | 15 (71.4) | 0.183 |

| Parameters | Pioglitazone | Glimepiride | P value between groups |
|---|---|---|---|
| **Systolic blood pressure (mmHg)** | | | |
| Baseline | 129.9 (12.2) | 123.1 (13.5) | 0.068 |
| Post treatment | 126.7 (14.6) | 124.0 (14.4) | 0.519 |
| P value vs baseline | 0.136 | 0.733 | |
| **Diastolic blood pressure (mmHg)** | | | |
| Baseline | 70.7 (9.1) | 69.1 (9.5) | 0.553 |
| Post treatment | 71.6 (9.7) | 68.3 (10.0) | 0.245 |
| P value vs baseline | 0.623 | 0.459 | |
| **LDL cholesterol (mg/dL)** | | | |
| Baseline | 109.8 (25.8) | 116.2 (24.5) | 0.381 |
| Post treatment | 109.6 (26.2) | 117.8 (20.2) | 0.236 |
| P value vs baseline | 0.952 | 0.705 | |
| **HDL cholesterol (mg/dL)** | | | |
| Baseline | 48.4 (12.2) | 51.5 (13.2) | 0.388 |
| Post treatment | 53.8 (14.1) | 53.7 (12.6) | 0.972 |
| P value vs baseline | 0.002 | 0.150 | |
| **Triglycerides* (mg/dL)** | | | |
| Baseline | 113.0 [86.0-159.8] | 121.0 [78.0-165.0] | 0.682 |
| Post treatment | 119.5 [82.3-149.8] | 123.0 [83.0-208.5] | 0.785 |
| P value vs baseline | 0.787 | 0.186 | |
| **Fasting plasma glucose* (mg/dL)** | | | |
| Baseline | 122.5 [110.0-146.3] | 131.0 [121.0-146.0] | 0.555 |
| Post treatment | 114.5 [102.8-123.3] | 119.0 [111.5-131.0] | 0.375 |
| P value vs baseline | 0.003 | <0.001 | |
| **Fasting plasma insulin* (µU/mL)** | | | |
| Baseline | 7.15 [5.03-11.35] | 4.70 [3.55-8.50] | 0.100 |
| Post treatment | 6.55 [4.20-9.68] | 5.50 [3.80-7.25] | 0.519 |
| P value vs baseline | 0.057 | 0.808 | |
| **Hemoglobin A1c (%)** | | | |
| Baseline | 6.72 (0.72) | 6.87 (0.64) | 0.454 |
| Post treatment | 6.36 (0.77) | 6.50 (0.52) | 0.462 |
| P value vs baseline | 0.004 | <0.001 | |
| **High-sensitivity CRP* (mg/L)** | | | |
| Baseline | 0.74 [0.33-1.94] | 0.51 [0.34-1.51] | 0.306 |
| Post treatment | 0.50 [0.27-0.94] | 1.04 [0.44-2.10] | 0.010 |
| P value vs baseline | <0.001 | 0.018 | |
| **Adiponectin* (µg/mL)** | | | |
| Baseline | 4.56 [2.32-6.33] | 5.01 [2.64-7.67] | 0.855 |
| Post treatment | 11.33 [6.20-19.32] | 5.63 [2.76-8.41] | 0.002 |
| P value vs baseline | <0.001 | 0.366 | |

| Parameters | Pioglitazone | Glimepiride | P value between groups |
|---|---|---|---|
| **Weight (kg)** | | | |
| **Baseline** | **65.5 (12.3)** | **65.6 (11.0)** | **0.976** |
| **Post treatment** | **66.1 (12.4)** | **66.1 (10.6)** | **0.989** |
| **P value vs baseline** | **0.126** | **0.153** | |
| **Waist circumference (cm)** | | | |
| **Baseline** | **89.6 (10.0)** | **89.1 (10.8)** | **0.844** |
| **Post treatment** | **90.9 (9.5)** | **89.5 (10.2)** | **0.603** |
| **P value vs baseline** | **0.016** | **0.469** | |
| **Subcutaneous fat volume ($cm^2$)** | | | |
| **Baseline** | **141.7 (49.1)** | **139.9 (59.9)** | **0.910** |
| **Post treatment** | **146.5 (49.6)** | **134.5 (52.0)** | **0.411** |
| **P value vs baseline** | **0.318** | **0.301** | |
| **Visceral fat volume ($cm^2$)** | | | |
| **Baseline** | **130.5 (53.0)** | **119.8 (54.0)** | **0.486** |
| **Post treatment** | **122.1 (51.0)** | **122.5 (51.7)** | **0.978** |
| **P value vs baseline** | **0.013** | **0.506** | |
| **Subcutaneous fat activity** | | | |
| **Baseline** | **0.30 (0.07)** | **0.30 (0.06)** | **0.951** |
| **Post treatment** | **0.29 (0.06)** | **0.32 (0.06)** | **0.085** |
| **P value vs baseline** | **0.326** | **0.119** | |
| **Visceral fat activity** | | | |
| **Baseline** | **0.57 (0.16)** | **0.54 (0.11)** | **0.455** |
| **Post treatment** | **0.50 (0.11)** | **0.58 (0.09)** | **0.015** |
| **P value vs baseline** | **0.007** | **0.145** | |