UNIVERSITÀ DEGLI STUDI DI PADOVA

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"LONG-TERM EFFECTS OF PIOGLITAZONE VS GLIMEPIRIDE ON LIPOPROTEINS AND GLYCO-OXIDATION IN PATIENTS WITH TYPE 2 DIABETES"

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INDICE

ABSTRACT

Background and aim

Cardiovascular complications are the first cause of mortality and morbidity in type 2 diabetic patients. Among antidiabetic drugs, those who have shown cardiovascular benefits have ancillary activities that simultaneously control several risk factors. In the PROACTIVE trial, pioglitazone determined a 16% reduction of death for all causes, non-fatal myocardial infarction, non-fatal stroke, compared to placebo. The aim of the study is to investigate the effects of 5 years treatment with pioglitazone/metformin compared to 5 years treatment with glimepiride/metformin on diabetic dyslipidemia, both quantitatively and qualitatively, and on glyco-oxidation processes.

Methods

96 diabetic patients, treated with metformin (2g/day) for at least 2 months, were randomized to treatment with pioglitazone or glimepiride. Patients were followed for 5 years: body mass index (BMI), waist circumference (CV), blood pressure, HbA1c; total cholesterol (CT), high density lipoproteins (HDL), low density lipoproteins (LDL), triglycerides (TG), advanced glycation end products (AGE), oxidized LDL (oxLDL) were determined at baseline and after 5 years of treatment.

Results

Treatment with pioglitazone resulted in significant increase in HDL (47.1±11.7 vs 51.3±15.7 mg/dl; p=0.02), non-significant reduction in CT, LDL and TG. Glimepiride treatment resulted in a significant reduction in HDL (48.6±14.1 vs 45.8±12.7 mg/dl; p=0.03), a non-significant reduction in CT, LDL and TG. Only the variation of HDL and oxLDL were significantly different between the two groups ($\Delta HDL_{Pioglitazone}$= +4.2±10.5 mg/dl vs $\Delta HDL_{Glimepride}$= -2.9±7.7 mg/dl; p=0.002); ($\Delta oxLDL_{Pioglitazone}$= -2.1±9.8 U/l vs $\Delta oxLDL_{Glimepride}$= +3.6±11.4 U/l; p=0.01).

AGEs reduction, significant for both treatments, is not significantly different between the two groups. HbA1c reduction was significant only in patients treated with glimepiride (7.7±0.4 vs 7.1±0.8 %; p <0001) and was not correlated with AGEs variation (r=1.22; p=0.59).

Conclusions

Long-term treatment with pioglitazone significantly improves lipid profile of type 2 diabetic patients, increasing HDL levels and reducing oxLDL levels. In addition, it reduces AGEs formations. The inhibition of glyco-oxidative processes is one of the mechanisms that may explain the drug ability to prevent cardiovascular events.

INTRODUCTION

Cardiovascular disease is the most common cause of morbidity and mortality in patients with diabetes (1). It is known that diabetes, obesity, hypertension, dyslipidemia and smoking habit, account for over 80% of the risk of myocardial infarction (2). On the other hand it is known that many times diabetes isn't an isolated disease, but it is within the framework of metabolic syndrome: the simultaneous presence of obesity, hypertension, dyslipidemia, insulin resistance and hyperglycemia (3). Thus diabetic patients have a higher risk of atherosclerosis than the general population which lead to diffuse vascular damage and multi-organ dysfunction. In the long term, diabetic patients risk both micro- and macrovascular complications (4).

The findings of the Diabetes Control and Complications Trial (DCCT) and the UK Prospective Diabetes Study (UKPDS) have confirmed that hyperglycemic injury is the primary causal factor behind vascular damage, specially microvascular damage (5, 6).

Hyperglycemia promotes vascular damage through at least five main mechanisms: an increased flux of glucose and other sugars through the polyol pathway; an increased intracellular advanced glycation end products (AGE) formation; interaction between AGEs and their receptors (termed RAGEs) leading to intracellular signaling, which disrupts cell function (7); a persistent activation of protein kinase C (PKC) isoforms (8); and an increased hexosamine pathway activity (9). Among all, advanced AGE excess is one of the most important mechanism.

Another important factor that promotes vascular damage in terms of atherosclerosis in diabetic patients is the quantitative and qualitative alterations of the lipid profile. Below we analyze these factors in detail.

ADVANCED GLYCATION END PRODUCTS AND CARDIOVASCULAR COMPLICATIONS

The most important process responsible for AGE accumulation in diabetic patients is the non-enzymatic glycation reaction, or Maillard reaction. This can be globally seen as a three-step process, the final stage of which comprises a complex series of oxidation, dehydration and cyclization reactions, which give rise to so-called endogenous AGEs, i.e. thermodynamically unstable compounds that typically accumulate on proteins with a long half-life, though they have recently been shown to form on proteins with a short half-life too, such as plasma proteins, lipoproteins, and intracellular proteins (10). Much attention has recently been paid to the so-called exogenous AGEs, harmful products of "browning" (or the Maillard reaction) in various foods. Together with endogenous AGEs, these compounds form the majority of glycation-free adducts, the greater proportion of circulating AGEs in diabetic and non-diabetic subjects. Among the various food processing methods, heating, sterilizing, and microwaves contribute to the generation of exogenous AGEs, all of which tend to accelerate the nonenzymatic addition of non-reducing sugars to free NH2 groups of proteins and lipids (11).

The main mechanisms behind the tissue damage caused by AGEs are intracellular glycation, cross-link formation and interaction with RAGEs. The intracellular accumulation of AGEs alters cytoplasmic and nuclear factors, including the proteins involved in regulating gene transcription (12). Another important mechanism is the formation of stable abnormal cross-links on collagen, which causes chemical and physical changes in the collagen’s structure and consequent functional changes typical of chronic diabetic complications, such as basement membrane thickening with a resistance to proteolytic digestion (13) and arterial stiffening. Finally, the AGE-RAGE interaction leads to cellular signaling, including nuclear factor-kB (NF-kB) activation, increased cytokine and adhesion molecule expression, induction of oxidative stress (14).

An excessive AGE formation has been implicated in the newly disclosed biochemical pathways involved in the microvascular pathobiology of type 2 diabetes, confirming its central role in the progression of microvascular complications (diabetic neuropathy, retinopathy and nephropathy). Moreover, AGEs play an important part in the development of cardiovascular complications in patients with type 2 diabetes. From the first observations of high AGE levels in atherosclerotic coronary plaque in selected type 2 diabetic patients (15) to Kiuchi et al.'s demonstration that serum AGE concentrations increase consistently with the severity of coronary atherosclerosis in type 2 diabetic patients with obstructive coronary artery disease (16), the hypothesis that AGE concentrations may be a risk marker in type 2 diabetic patients with coronary atherosclerosis has taken shape and has been put to the test. The results of many studies show a strong correlation between glycoxidation markers and the onset of complications, in terms of cardiovascular events or vascular damage in type 2 diabetes (17-19).

RAGE-ligand axis has an important player in modulating several steps of atherogenesis in fact AGEs induced oxidative stress through interaction with RAGEs. The binding of AGEs to RAGE of monocytes/macrophages induces the production of cytokines (interleukin 1B, TNF-alpha, IGF-1, PDGF) and growth factors, with a consequent increase in the synthesis of type IV collagen, a greater proliferation of vessel smooth muscle cells, and a stimulation of macrophage chemotaxis. In addition, through a mechanism of oxidative stress, AGE-RAGE binding on endothelial cells induces the transcription factor NF-kB, which in turn increases the expression of the vascular cellular adhesion molecule (VCAM-1). The resulting VCAM-1 overexpression increases the adhesivity of monocytes to endothelial cells, and vascular permeability, speeding up the trans-endothelial passage of AGE-modified proteins. At last AGE-RAGE binding has been shown to specifically reduce endothelial nititc oxide (NO) activity and NO production through a series of complex interactions with multiple enzymes (20). The role of AGE-RAGE binding is therefore both

to kick off the atherosclerotic process, both to maintain the inflammatory state in the progression of atherosclerotic plaque.

It is known that microvascular changes occurring in the first stage of diabetic disease could be reversible if the patient's hyperglycemia is corrected promptly; if it is not, the resulting macrovascular changes are irreversible (21).

It could be hypothesized that in the early years of the disease there is a linear relationship between hyperglycemia, increased oxidative stress and excessive AGE formation, with all three factors linked by a causal association; later on, persistent respiratory chain protein glycation and DNA damage in the mitochondria generate a hyperglycemia-independent vicious cycle (22), in which oxidative stress is self-supporting and AGEs "feed" this process (Figure 1).

The main downstream effects of this physiopathological scenario include changes in the composition and structure of the extracellular matrix, mediated by inflammatory processes induced by receptor binding of AGEs or oxidative stress. Subsequent fibrosis and the extension of the extracellular structures interfere with capillary blood flow, reducing capillary density in particular (21). These structural changes reduce the flow reserve and this can directly affect upstream arteries, causing endothelial dysfunction (overtime) and then atherosclerosis and cardiovascular disease.

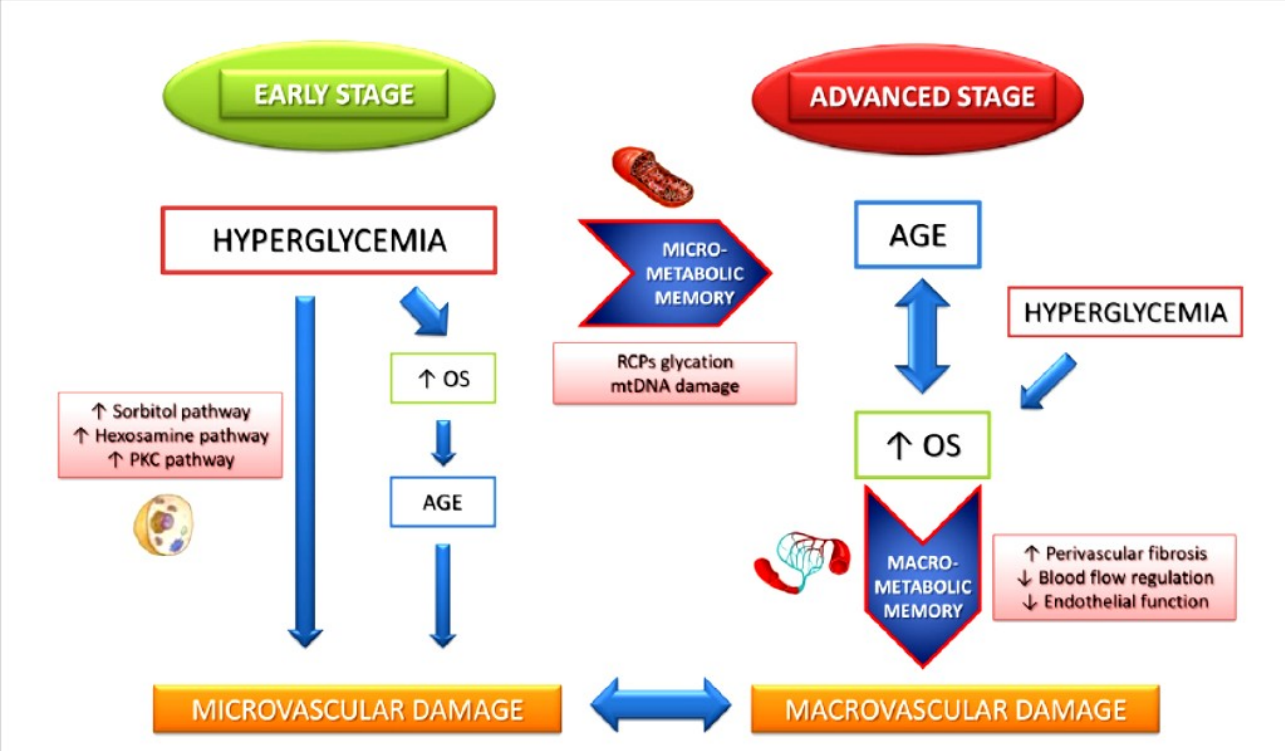

Figure 1. Schematic overview of the different etiological contributions of hyperglycemia, AGEs and oxidative stress in different phases of diabetes mellitus (23).

DIABETIC DYSLIPIDEMIA AND CARDIOVASCULAR COMPLICATIONS

In type 2 diabetic patients the alterations of the lipid profile play an important role in the etiopathogenesis and progression of atherosclerosis. These alterations are both quantitative and qualitative: increased triglyceride (TG) levels; qualitative changes in low density lipoproteins (LDL); both quantitative and qualitative alterations of high density lipoproteins (HDL) (24).

In diabetic patients, TG levels are significantly higher than in the non-diabetic population and represent an independent risk factor for cardiovascular complications (25). Insulin resistance, typical of type 2 diabetic patients, leads to an increase in lipolysis and the release of free fatty acids (FFA) from adipose tissue and a lower uptake of these by skeletal muscle. Therefore the liver concentrations of FFA and the synthesis of very low density lipoprotein (VLDL), lipoproteins

whose main function is the transport of triglycerides, increase (26, 27). The result of these mechanisms is the increase of TG levels.

As regards LDL cholesterol, in the diabetic patients their levels are normal or only slightly increased, although it is formed by the catabolism of the VLDL which are significantly increased, because the catabolism of the VLDL is reduced (28). The alterations of LDL cholesterol are qualitative, alterations in density and dimensions. In fact diabetic patients have higher levels of small and dense LDL than non diabetic subjects.

This alteration is due to the presence of high levels of circulating VLDL and the consequent activation of the cholesteryl ester transfer protein (CETP), which transfers triglycerides from VLDL to HDL and LDL, in exchange for cholesterol esters. the ester content of LDL cholesterol decreases and increases the triglyceride content, which makes it more susceptible to lipolytic action of hepatic lipase: the result is the formation of smaller and denser LDL (29). Small and dense LDLs have greater atherogenic potential, mainly because such lipoproteins are substrates of glycation and oxidation processes, as well as having facilitated penetration through the endothelium and favoring foam cell formation (30, 31). In fact, oxidized LDLs (oxLDL) have less affinity for LDL receptor and have a preferential internalization pathway in endothelial macrophages (32). Moreover, the oxidized LDL induces the synthesis of some endothelial cytokines, in particular the intercellular adhesion molecules 1 (ICAM-1), and macrophages (TNFα and IL-1), with a pro-inflammatory effect that favors atherosclerosis (29, 32).

At last, diabetic patients have quantitative and qualitative alterations of HDL cholesterol. HDL levels in diabetic patients are lower than non diabetic patients (25). In fact, the increase of VLDL determines a greater activity of the CETP enzyme, which removes cholesterol esters from HDL, enriching them in triglycerides and making them more susceptible to catabolism by the hepatic lipase (24). These mechanisms lead to a quantitative reduction of HDL levels in diabetic patients;

however, it is now evident that cardiovascular risk is significantly associated with qualitative as well as quantitative alteration.

The main qualitative alterations of HDL are their glycation and oxidation; both of these processes determine a reduction in the ability of reverse cholesterol transport. A recent published study showed that patients with type 2 diabetes and patients with early myocardial infarction have elevated levels of oxidized ApoA1 (oxApoA1) regardless of HDL cholesterol levels (33). The oxidation of ApoA1 reduces the antioxidant potential of HDL and alters its ability to bond with ABCA1 transporters, thus compromising the reverse transport of cholesterol (34).

ANTIHYPERGLYCAEMIC DRUGS AND CARDIOVASCULAR COMPLICATIONS: THIAZOLIDINEDIONES AND SULPHONYLUREAS

It is known that hyperglycemia is one of the factors that independently predicts the risk of cardiovascular events and that it is primary achieve a good glycaemic control early to prevent cardiovascular events. The results of UKPDS showed that good glycaemic control established at the time of diagnosis can reduce the incidence of cardiovascular events (35). However, it is necessary to analyze how to obtain good glycaemic control because the ACCORD, ADVANCE and VADT studies have suggest that it is essential to choose the right treatment for the type of patient to be treated to obtain a reduction in cardiovascular risk (36-38).

In literature there are several studies that have investigate the effect of different antidiabetic drugs on cardiovascular risk. In fact after the withdrawal of rosiglitazone, the Food and Drug Administration (FDA) has established that for the approval of new antidiabetic drugs it is necessary to ensure their cardiovascular safety by performing randomized placebo-controlled trials.

In particular, the results of the studies suggest that pioglitazone could reduce the cardiovascular risk of type 2 diabetic patients while sulphonylureas (as glimepiride) seem to have adverse cardiovascular effects.

Pioglitazone is a peroxisome proliferator-activated receptor γ (PPARγ) agonist, a cellular transcription factor involved in the regulation of glucose and lipid homeostasis. The hypoglycaemic effect is due to an increase in insulin sensitivity of the liver, skeletal muscle, adipose tissue (39).

Pioglitazone has a demonstrated efficacy in lowering TG and raising HDL-C levels, superior to that of metformin or sulfonylurea, in type 2 diabetic patients (40). Moreover, despite its neutral effect on LDL cholesterol, treatment with pioglitazone reduces the number of small dense LDL (41). These quantitative effects on the lipid profile were confirmed by long-term studies (42), regardless of an individual's glycemic control or any concomitant use of statins.

Pioglitazone has also been associated with an anti-inflammatory and antioxidant activity in patients with type 2 diabetes (43), particularly as regards AGE (44).

As summarized in Figure 2, pioglitazone has effects on both lipoproteins and glyco-oxidation processes and therefore a protective cardiovascular effect of the drug can be hypothesized.

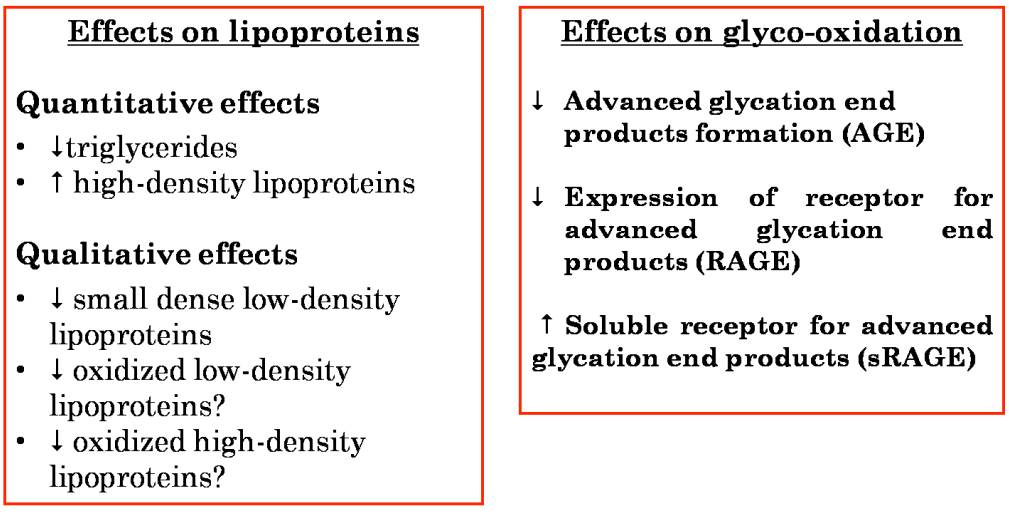

Figure 2. Effects of pioglitazone on lipoproteins and glyco-oxidation processes.

The PROspective pioglitAzone Clinical Trial In macroVascular Events (PROACTIVE), a prospective, randomised controlled trial in 5238 patients with type 2 diabetes who had evidence of macrovascular disease, investigators studied the effect of pioglitazone on cardiovascular risk (42). In particular the primary outcome was: all-cause mortality, non-fatal myocardial infarction (including silent myocardial infarction), stroke, acute coronary syndrome, endovascular or surgical intervention on the coronary or leg arteries, or amputation above the ankle. The main secondary endpoint was the composite of all-cause mortality, non-fatal myocardial infarction, and stroke. The results showed that treatment with pioglitazone reduced significantly the secondary endpoint by 16%.

On the other hand, sulphonylureas stimulate insulin secretion from pancreatic b-cells by binding to the sulfonylurea receptor 1, which is part of the Kir6.2 adenosine triphosphate-sensitive potassium channel.

In literature there several concerns about the cardiovascular safety of this drugs. Kir6.2 adenosin triphosphate-sensitive potassium channel is also expressed in smooth muscle cells and cardiomyocytes and several authors have postulated that the increased CV mortality induced by sulphonylureas could be the result of an impaired vasodilatory response during acute myocardial ischemia. Although several hypotheses linking sulphonylureas to adverse cardiovascular effects exist, none provide conclusive evidence (45). Moreover in literature there are many conflicting data: some studies showed a negative effect on cardiovascular risk and others showed a neutral effect. It is to underline that the majority of these studies were not specifically designed to assess the effect of sulphonylureas on adverse cardiovascular event risk.

AIM OF THE STUDY

The aim of this study was to investigate the long-term effects of 5 years-treatment with pioglitazone plus metformin compared to treatment with glimepiride plus metformin on glycation processes and on lipid profile (quantitative and qualitative alterations) in type 2 diabetic patients.

MATERIALS AND METHODS

Subjects

The study was conducted within the framework of the TOSCA.IT trial (Thiazolidinediones Or Sulphonylureas and Cardiovascular Accidents. Intervention Trial; NCT00700856, ClinicalTrials.gov), a randomized clinical trial (RCT) designed to assess the cardiovascular effects of two different hypoglycemic drug regimens (sulfonylurea or pioglitazone) (46).

The protocol was approved by the Ethics Review Committee/Institutional Review Board of the Coordinating Center and each participating center. The study was conducted in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. Written informed consent was obtained from participants before beginning any protocol-specific procedure and participants were told that they had the right to withdraw from the study at any time.

The inclusion criteria were:

- Type 2 diabetes of at least 2 years duration
- Males and females, age 50-75 years
- BMI 20-45 Kg/m2
- Stable treatment for the last two months with metformin in monotherapy at 2 g/day
- HbA1c $\geq$ 7.0% and $\leq$ 9.0%

The exclusion criteria were:

- Type 1 diabetes
- Previous treatment with TDZs within the last six months
- Contraindication/intolerance to metformin or SUs or TZDs
- Documented coronary or cerebrovascular events within the previous 3 months
- Serum creatinine > 1.5 mg/dl
- History of congestive heart failure, NYHA class I or higher
- Chronic use of glucocorticoids

- Ischemic ulcer or gangrene of lower extremities
- Liver cirrhosis or severe hepatic dysfunction (ALT > 2.5 times the upper normal limit)
- Pregnancy or breast feeding
- Cancer, substance abuse, or any health problem that may interfere with the compliance to the study protocol or limit life expectancy

Eligible patients are randomized to one of two treatments: metformin plus sulfonylurea (glimepiride 2 mg) or metformin plus pioglitazone (15 mg). The treatment allocation schedule was computer-generated in blocks and stratified by clinical status and previous cardiovascular events. The participating centers were masked to the randomization sequences, which were generated at the Epidemiology Unit.

Data reported in this study were collected from the 96 patients recruited for the TOSCA.IT trial at the Diabetology and Dietetics Unit in Padua, Italy. A blood test was performed at the time of their randomization and after 5 years, in accordance with the RCT procedures, to obtain biochemical parameters of glyco-oxidation and lipid profiles.

Methods

At baseline and after 5 years of treatment in all patients we assessed: weight, height, body mass index (BMI), waist, systolic and diastolic blood pressure.

Basic blood chemistry parameters and markers of glyco-oxidation

HbA1c was measured by high-performance liquid chromatography (HPLC, Menarini Akray ADAM A1c HA-8180v), in line with IFCC standards (International Federation of Clinical Chemistry) (47).

Total cholesterol, LDL-C and HDL-C were measured using an enzymatic colorimetric method (COBAS 8000; Roche, Milan) (48), as were TG (GPO-PAP colorimetric enzyme tests; Roche Diagnostic System) (49).

An ELISA method was used to assay oxLDL, with 2 monoclonal antibodies specific for antigens on apolipoprotein B (Mercodia Oxidized LDL ELISA, Uppsala, Sweden), obtaining an intra-assay coefficient of variation <5% and an interassay coefficient of variation <10% (50).

Serum AGE were estimated by means of an ELISA assay, using a polyclonal antibody (51).

Statistical analysis

Continuous values are given as means ± standard deviations (SD). The comparison between continuous variables at baseline and after one year of treatment was calculated as the pre-post difference (HbA1c, TC, HDL, LDL, TG, oxLDL, AGE). Negative delta values (Δ) therefore indicate the actual decrease in the parameter depending on the treatment administered. The statistical significance of these differences (pre-post) in each treatment group was analyzed using Student's t-test for paired data, after checking the normality of the distribution of the parameters according to the skewness and kurtosis parameters, and after normalizing differences at the baseline.

The two treatment groups were compared with Student's t-test for unpaired data. All differences were considered statistically significant with a p-value <0.05.

For each parameter (SBP, DBP, BMI, HbA1c, TC, HDL, LDL, TG, oxLDL, AGE), an analysis of variance (ANOVA) between the two groups of patients (pioglitazone vs glimepiride) was performed at the baseline and after 5 years of treatment. For the nominal variables, the differences between the two groups (pioglitazone vs. glimepiride) were estimated with Fisher's exact test.

To prevent any interference of anthropometric and clinical characteristics in the associations between variables, a stepwise forward multiple regression analysis was performed, where appropriate.

RESULTS

The data for all 96 patients assessed at the baseline and after 5 years were considered in the statistical analysis. There were no dropouts from the study. 47 patients were treated with metformin plus pioglitazone and 49 patients were treated with metformin plus glimepiride.

The patients randomly assigned to the two treatments were similar as regards the following variables at the baseline (Table 1): male/female ratio, duration of diabetes, smoking habits, blood pressure, BMI and waist circumference (WC). The two groups were also comparable in terms of glycemic control, lipid profiles, markers of lipid oxidation and glyco-oxidation, and there were no differences in their treatment with statins.

Table I. Baseline anthropometric and clinical parameters of the two treatment groups. Continuous data are expressed as mean ± SD. Differences between groups were assessed with Student's t-test for continuous variables, and Fisher's exact test for nominal variables.

	Pioglitazone (n=47)	**Glimepiride (n= 49)**
Disease duration (y)	8.0±5.0	8.0±4.7
Gender (male/female)	28/19	26/23
Statin (yes/no)	25/22	27/20
SBP (mmHg)	131.1±10.7	135.1±13.7
DBP (mmHg)	80.5±7.0	80.8±7.1
BMI (kg/m^2)	29.3±4.0	29.6±4.2
Waist (cm)	101.7±11.2	99.7±10.2
HbA1c (%)	7.6±0.4	7.7±0.4
TC (mg/dl)	174.9±31.7	182.5±36.7
HDL (mg/dl)	47.1±11.7	48.6±14.1
LDL (mg/dl)	106.8±27.0	110.1±31.4
TG (mg/dl)	132.0±53.6	149.9±70.2
oxLDL (U/l)	40.1±7.9	40.9±8.9
AGE (ug/ml)	27.2±13.3	25.9±11.4

Table 2 shows the anthropometric and clinical parameters after 5 years of treatment. The two groups were similar for all the parameters evaluated except for oxLDL: values in the group treated

with pioglitazone were significant lower than the group treated with glimepiride (38.0±9.6 vs 44.1±12.5 U/l; p=0.04).

Table 2. Anthropometric and clinical parameters of the two treatment groups after 5 years of treatment. Continuous data are expressed as mean ± SD. Differences between groups were assessed with Student's t-test for continuous variables, and Fisher's exact test for nominal variables. *p<0.05

	Pioglitazone (n=47)	**Glimepiride (n= 49)**
Statin (yes/no)	31/16	37/12
SBP (mmHg)	135.1±12.6	138.9±10.4
DBP (mmHg)	80.9±6.6	80.7±7.1
BMI (kg/m^2)	30.4±4.6	30.1±4.3
Waist (cm)	102.3±11.9	101.2±9.6
HbA1c (%)	7.4±0.9	7.1±0.8
TC (mg/dl)	170.7±29.2	173.5±34.5
HDL (mg/dl)	51.3±15.7	45.8±12.7
LDL (mg/dl)	100.9±22.9	104.2±30.7
TG (mg/dl)	116.7±39.0	137.7±58.4
oxLDL (U/l)	38.0±9.6*	44.1±12.5
AGE (ug/ml)	8.2±7.1	9.2±5.9

Than we evaluated the comparison of the anthropometric and clinical parameters at baseline and after 5 years of treatment with pioglitazone plus metformin (Table 3). In particular there were: a significant increase of BMI (29.3 ± 4.0 vs 30.4 ± 4.6 kg/m^2; p<0.001); a significant increase of HDL (47.1 ± 11.7 vs 51.3 ± 15.7 mg/dl, p = 0.02); a significant reduction of AGEs (28.1±13.4 vs 7.5±5.6 ug ml; p <0.001); a reduction, even not significant, of oxLDL, TC, LDL, TG and HbA1c.

Table 3. Anthropometric and clinical parameters of the group treated with pioglitazone: comparison between baseline and after 5 years. Data are expressed as mean ± SD. Differences between groups were assessed with Student's t-test. *p<0.05; ** p<0.001

	Baseline	**After 5 years**
Statin (yes/no)	25/22	31/16
SBP (mmHg)	131.1/±10.7	135.1±12.6
DBP (mmHg)	80.5±7.0	80.9±6.6
BMI (kg/m^2)	29.3±4.0	30.4±4.6**
Waist (cm)	101.7±11.2	102.3±11.9**
HbA1c (%)	7.6±0.4	7.4±0.9
TC (mg/dl)	174.9±31.7	170.7±29.2
HDL (mg/dl)	47.1±11.7	51.3±15.7*
LDL (mg/dl)	106.8±27.0	100.9±22.9
TG (mg/dl)	132.0±53.6	116.7±39.0
oxLDL (U/l)	40.1±7.9	38.0±9.6
AGE (ug/ml)	27.2±13.3	9.2±5.9**

As regards the group treated with glimepiride plus metformin, after 5 years of treatment we observed, as shown in Table 4: a significant reduction of HbA1c (7.7±0.4 vs. 7.1±0.8%; p<0.001), a significant reduction of HDL (48.6±14.1 vs 45.8±12.7 mg/dl; p= 0.03), a significant reduction of AGEs (25.9±11.4 vs 9.2±5.9 ug/ml; p <0.001). In this group there was also a significant increase in statin use that was not observed in the group treated with pioglitazone.

Table 4. Anthropometric and clinical parameters of the group treated with glimepiride: comparison between baseline and after 5 years. Data are expressed as mean ± SD. Differences between groups were assessed with Student's t-test. *p<0.05; ** p<0.001

	Baseline	**After 5 years**
Statin (yes/no)	27/20	37/12*
SBP (mmHg)	135.1±13.7	138.9±10.4
DBP (mmHg)	80.8±7.1	80.7±7.1
BMI (kg/m^2)	29.6±4.2	30.1±4.3
Waist (cm)	99.7±10.2	101.2±9.6
HbA1c (%)	7.7±0.4	7.1±0.8**
TC (mg/dl)	182.5±36.7	173.5±34.5
HDL (mg/dl)	48.6±14.1	45.8±12.7*
LDL (mg/dl)	110.1±31.4	104.2±30.7
TG (mg/dl)	149.9±70.2	137.7±58.4
oxLDL (U/l)	40.9±8.9	44.1±12.5
AGE (ug/ml)	25.9±11.4	9.2±5.9**

To verify the possible difference in terms of effectiveness of the two different treatments (pioglitazone vs glimepiride) we compared the variations (after-minus-before treatment) of the parameters evaluated. Both treatments led to a reduction in HbA1c values with no significant difference between groups (Δgli=-0.6±0.8% vs Δpio=-0.3±0.9%; p=0.06).

Only patients treated with pioglitazone experienced an increase in HDL-C while patients treated with glimperide experience a reduction with a significant difference between the two groups (ΔHDLpio=+4.2± 10.5 mg/dl vs ΔHDLgli=-2.9±7.7 mg/dl; p=0.002) (Figure 3). No statistically significant differences were observed in terms of in CT, TG and LDL-C.

As regards oxLDL levels, a significant difference was observed between the two groups: treatment with pioglitazone shows a tendency to reduce oxidation of LDL, while treatment with glimepiride tends to increase them (ΔoxLDLpio = -2.1±9.8 U/ l vs ΔoxLDLgli = +3.6 11.4 U/ l, p between the two groups= 0.01). Finally, the two treatment leads a significant reduction of AGEs levels, with no significant difference between the two groups (Figure 3).

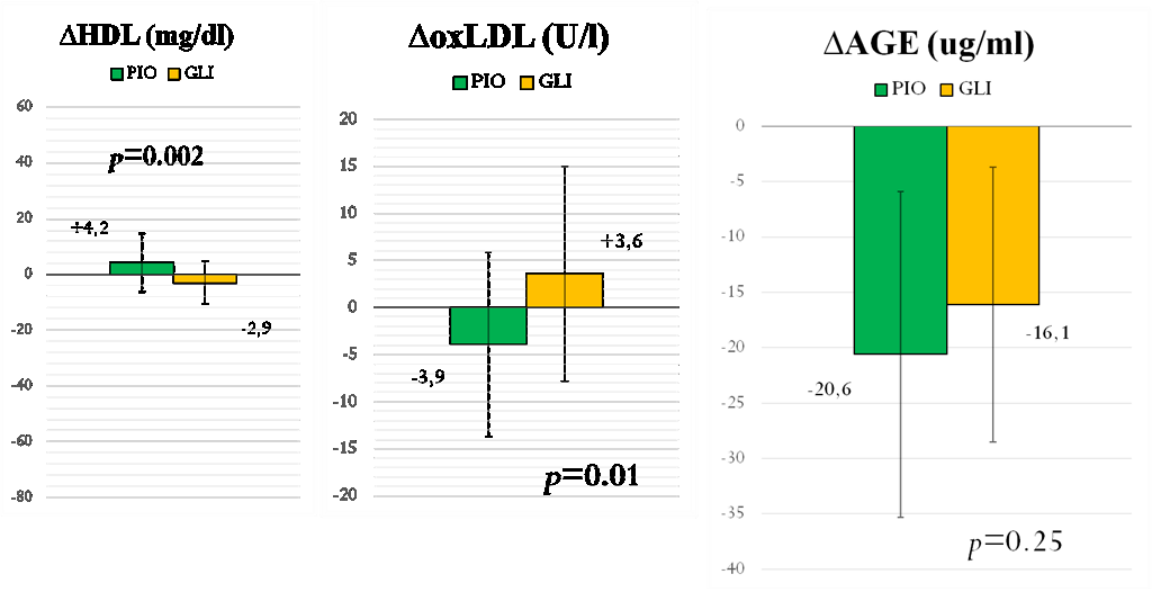

Figure 3. Comparison of the variations (after-minus-before treatment) between pioglitazone and glimepiride treatments of HDL, oxLDL and AGE.

To analyze better the reduction of AGEs obtained in both groups, a linear regression analysis was run between ΔHbA1c and ΔAGE and no significant association was found (r=1.22; *p*=0.59). Then a stepwise forward multivariate regression analysis was run with ΔAGE as the independent variable, while the other parameters (sex, duration of disease, use of statins, ΔHDL-C, ΔBMI, ΔHbA1c, ΔAGE) were included as covariates. This analysis confirmed no significant association between ΔHbA1c and ΔAGE (r = 1.70; p = 0.46) and showed a slight association, that failed to reach statistical significance, between ΔAGE and use of statin (r=8.69; *p*=0.07).

DISCUSSION

This study showed that long-term treatment with pioglitazone in addition to metformin is more effective than glimepiride in improving the quantitative and qualitative characteristics of lipoproetins in type 2 diabetic patients. Moreover it is effective in reducing the glyco-oxidation processes in terms of AGE formation.

Pioglitazone has been demonstrated positive CV outcomes in the long-term treatment of patients with DM2 (PROACTIVE) (42), as recently confirmed for cerebrovascular events even in patients without diabetes (52).

As already discusses, treatment with pioglitazone has demonstrated positive effects on the lipid profiles and oxidative stress of patients with DM2, irrespective of their glycemic control (53). Furthermore, treatment with pioglitazone results in a down-regulation of the AGE-RAGE system in patients with type 2 diabetes, probably due to the activation of antioxidant enzymes (especially glutathione peroxidase) as a consequence of PPAR-γ modulation (44). All these effects could explain the results of PROACTIVE and IRIS studies.

The results of our study are in line with data of the literature. We confirmed that a 5-years treatment with pioglitazone increases HDL levels and tends to decrease TG levels. Insulin resistance has a key role in the pathogenesis of diabetic dyslipidemia (high TG levels, low HDL levels), as previous described. Pioglitazone increase in insulin sensitivity of the liver, skeletal muscle, adipose tissue (39) so the quantitative effects on lipoprotieins demonstrated in our study are certainly partly due to the insulin-sensitizing effect of the drug.

It is to underline that patients of our study were free from cardiovascular events at baseline while in the PROACTIVE and IRIS studies patients had cardiovascular events at baseline. In addition in our study we had 5 years of treatment while all other studies had a shorter period of treatment. So we demonstrated that this 5-years treatment has positive quantitative effects of lipid profile in patients without a history of cardiovascular disease, a low-risk population.

On the other hand our results showed that 5-years treatment with glimepiride significantly decrease the HDL levels. This data could partly explain the negative effect on the cardiovascular risk of the patient with type 2 diabetes treated with sulphonylureas.

A recent published meta-analysis examined 59 randomized controlled trials to define the effect of sulfonylurea treatment on diabetic dyslipidemia and the results suggest that sulfonylureas increase significantly TG levels, while decrease the HDL levels (54).

In our study a 5-year treatment with pioglitazone had also qualitative effect on lipoproteins, in particular it reduces oxLDL levels while treatment with glimepiride increases their level with a significant difference between the two treatments. These data confirm the anti-oxidant effect of pioglitazone. PPARγ-agonists reduce, in a dose-dependent manner, the expression of lectin-type oxidized LDL receptor 1 (LOX1) (55). LOX1 is the main receptor of oxidized LDL and it is expressed on endothelial cells, macrophages and vascular smooth muscle cells. The oxLDL-LOX1 binding activates an intracellular signaling pathway that leads to the activation of NFKB and therefore to the production of proinflammatory cytokines and ROS, thus favoring the progression of the atherosclerotic process (56), as for the AGE-RAGE binding. So pioglitazone seems to reduce the oxidation of LDL and also to reduce the expression of the receptor for oxLDL: these effects could contain the atherosclerotic process and reduce the cardiovascular events.

The reduction of oxLDL obtained with pioglitazone in our study is slight but since LDL have a shorter half-life and a faster turnover, oxLDL may be a less reliable marker of long-term oxidative stress in patients with type 2 diabetes.

In our study we observed another important effect of piogliazone: the significant reduction of glycation processes, in terms of AGE levels that confirmed data available in literature. It is to underline that the data available in literature are obtained with studies *in vitro*. To our knowledge this is the first data obtained *in vivo* in type 2 diabetic patients.

We found that the reduction of AGE levels obtained with pioglitazone was independent from the reduction of HbA1c levels and so it appears unrelated to the degree of glycemic control.

This finding is in agreement with previous observations that have shown no correlation between glyco-oxidation products and the degree of glycemic control (57) and suggest a direct inhibitory effect of pioglitazone on AGE formation. In reducing the cardiovascular risk of pioglitazone, we can add the possible reduction of glico-oxidation processes.

Surprisingly in our study we obtained a significant reduction of AGE levels also in patients treated with glimepiride. Recently Nakamura et al have demonstrated that a 24 weeks treatment with glimepiride reduced the toxic AGE levels in type 2 diabetic patients. (58) However there are no evidence of a direct effect of sulphonylureas on AGE-RAGE system.

We hypothesized that the reduction of AGE levels obtained with glimepiride is partly due to the significant increase of statin use in patients from the baseline to the end of the study. In fact the results of the stepwise forward multivariate regression analysis with ΔAGE as the independent variable, showed a slight correlation between ΔAGE and use of statin, that failed to reach statistical significance probably because of the small sample size.

It has been demonstrated that after 12 months of treatment with cerivastatin the levels of AGEs are reduced by 21% compared to placebo in patients with type 2 diabetes (59).

Moreover a 4-months treatment with simvastatin reduces the AGE levels and the expression of RAGE in atherosclerotic plaques of diabetic patients with carotid stenosis (60).

This study has some limitations. First, the sample size is too small to draw definitive conclusions on the effect of the two treatments in patients with type 2 diabetes. Second, to better analyze the long-term effect of pioglitazone and glimpeiride on glyco-oxidation processes we have to study the whole AGE-RAGE system (RAGE, esRAGE, sRAGE).

On the other hand, this was the first study to examine the effect of pioglitazone, as compared with a sulfonylurea, on LDL oxidation and glyco-oxidation in patients with DM2

CONCLUSIONS

The present study confirms and extends the evidence of a positive effect of pioglitazone on the qualitative and qualitative features of lipid profile. A 5-years of treatment with pioglitazone in addition to metformin reduces oxidation of LDL (qualitative effect) and increase HDL levels (quantitative effect). In addition it has an effect on glyco-oxidation processes leading to a reduction of AGE levels independently from the degree of glycemic control.

The inhibition of glyco-oxidative processes is one of the mechanisms that may explain the drug ability to prevent cardiovascular events.

REFERENCES

1) Vaccaro O, Eberly LE, Neaton JD, Yang L, Riccardi G, Stamler J, for the Multiple Risk Factor Intervention Trial (MRFIT) Research Group. Impact of diabetes and previous myocardial infarction on long-term survival: 25-year mortality follow-up of primary screenees of the Multiple Risk Factor Intervention Trial. Arch Intern Med 2004; 164: 1438–43.
2) Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;364:937-952.
3) National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III), "Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report," Circulation, vol. 106, no. 25, pp. 3143–3144, 2002.
4) Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. Lancet 2011;378:169-81.
5) UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998; 352: 837–53.
6) Nathan DM, Cleary PA, Backlund JY, Genuth SM, Lachin JM, Orchard TJ, Raskin P, Zinman B; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643-53.

7) Goldin A, Beckman JA, Schmidt AM, Creager MA. Advanced glycation end products: sparking the development of diabetic vascular injury. Circulation 2006;114:597-605.

8) Koya D, King GL. Protein kinase C activation and the development of diabetic complications. Diabetes 1998;47:859-66.

9) Kolm-Litty V, Sauer U, Nerlich A, Lehmann R, Schleicher ED. High glucose-induced transforming growth factor beta1 production is mediated by the hexosamine pathway in porcine glomerular mesangial cells. J Clin Invest 2008;101:160-9.

10) Brownlee M. The pathobiology of diabetic complications. A unifying mechanism. Diabetes 2005;54:1615-25.

11) Kankova K. Diabetic threesome (hyperglycaemia, renal function and nutrition) and advanced glycation end products: evidence for the multiple-hit agent? Proc Nutr Soc 2008;67: 60-74.

12) Pugliese G. Do advanced glycation end products contribute to the development of long-term diabetic complications? Nutr Metab Cardiovasc Dis 2008;18:457-60.

13) Sell DR, Lapolla A, Odetti P, Fogarty J, Monnier VM. Pentosidine formation in skin correlates with severity of complications in individuals with long-standing IDDM. Diabetes 1992; 41:1286-92.

14) Piarulli F, Sartore G, Lapolla A. Glyco-oxidation and cardiovascular complications in type 2 diabetes: a clinical update. Acta Diabetol 2013;50:101-10.

15) Nakamura Y, Horii Y, Nishino T, Shiiki H, Sakaguchi Y, Kagoshima T, Dohi K, Makita Z, Vlassara H, Bucala R. Immunohistochemical localization of advanced glycosylation endproducts (AGEs) in coronary atheroma and cardiac tissue in diabetes mellitus. Am J Pathol 1993;143:1649–56.

16) Kiuchi K, Nejima J, Takano T, Ohta M, Hashimoto H. Increased serum concentrations of advanced glycation end products: a marker of coronary artery disease activity in type 2 diabetic patients. Heart 2001;85:87–91.

17) Lapolla A, Piarulli F, Sartore G, Ceriello A, Ragazzi E, Reitano R, Baccarin L, Laverda B, Fedele D. Advanced glycation end products and antioxidant status in type 2 diabetic patients with and without peripheral artery disease. Diabetes Care 2007;30:670-6.

18) Peng WH, Lu L, Hu J, Yan XX, Zhang Q, Zhang RY, Chen QJ, Shen WF. Decreased serum esRAGE level is associated with angiographically determined coronary plaque progression in diabetic patients. Clin Biochem 2009;42:1252-9.

19) Colhoun HM, Betteridge DJ, Durrington P, Hitman G, Neil A, Livingstone S, Charlton-Menys V, Bao W, Demicco DA, Preston GM, Deshmukh H, Tan K, Fuller JH. Total soluble and endogenous secretory receptor for advanced glycation end products as predictive biomarkers of coronary heart disease risk in patients with type 2 diabetes: an analysis from the CARDS trial. Diabetes 2011;60:2379-85.

20) Busch M, Franke S, Ru¨ster C, Wolf G. Advanced glycation endproducts and the kidney. Eur J Clin Invest 2010;40:742-55.

21) Jax TW. Metabolic memory: a vascular perspective. Cardiovasc Diabetol 2010;9:51.

22) Ceriello A, Inhat MA, Thorpe JE. The “metabolic memory”: is more than just tight glucose control necessary to prevent diabetic complications? J Clin Endocrinol Metab 2009;94:410-5.

23) Chilelli NC, Burlina S, Lapolla A. AGEs, rather than hyperglycemia, are responsible for microvascular complications in diabetes: a "glycoxidation-centric" point of view. Nutr Metab Cardiovasc Dis 2013;23:913-9.

24) Taskinen MR: Diabetic dyslipidaemia: from basic research to clinical practice. Diabetologia 2003; 46: 733-749; Rizzo M, Kotur-Stevuljenic J, Berneis K, Spinas G et al: Atherogenic dyslipidemia and oxidative stress: a new look. Translational Research 2009; 153: 217-223.

25) Assmann G, Schulte H. The Prospective Cardiovascular Munster (PROCAM) study: prevalence of hyperlipidemia in persons with hypertension and/or diabetes mellitus and the relationship to coronary heart disease. Am Heart J 1988;116:1713-24.

26) Cummings MH, Watts GF, Umpleby AM, Hennessy TR, Naoumova R, Slavin BM, et al. Increased hepatic secretion of very-low-density lipoprotein apolipoprotein B-100 in NIDDM. Diabetologia 1995;38:959-967.

27) Sniderman AD, Scantlebury T, Cianflone K. Hypertriglyceridemic hyperapob: the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. Ann Intern Med 2001;135:447-459.

28) Duvillard L, Pont F, Florentin E, Galland-Jos C, Gambert P, Verges B. Metabolic abnormalities of apolipoprotein B-containing lipoproteins in noninsulin- dependent diabetes: a stable isotope kinetic study. Eur J Clin Invest 2000;30:685-694.

29) Verges B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia 2015;58:886-899.

30) Vakkilainen J, Steiner G, Ansquer JC, Aubin F, Rattier S, Foucher C, et al. Relationships between low-density lipoprotein particle size, plasma lipoproteins, and progression of coronary artery disease: the Diabetes Atherosclerosis Intervention Study (DAIS). Circulation 2003;107:1733-1737.

31) Tani M, Kawakami A, Mizuno Y, Imase R, Ito Y, Kondo K, et al. Small dense LDL enhances THP-1 macrophage foam cell formation. J Atheroscler Thromb 2011;18:698-704.

32) Kita T, Kume N, Minami M, Hayashida K, Murayama T, Sano H, et al. Role of oxidized LDL in atherosclerosis. Ann N Y Acad Sci 2001;947:199-205.

33) Sartore G, Seraglia R, Burlina S, Bolis A, Marin R, Manzato E, et al. High-density lipoprotein oxidation in type 2 diabetic patients and young patients with premature myocardial infarction. Nutr Metab Cardiovasc Dis 2015;25:418-425.

34) Zheng L, Settle M, Brubaker G, Schmitt D, Hazen SL, Smith JD, et al. Localization of nitration and chlorination sites on apolipoprotein A-I catalyzed by myeloperoxidase in human atheroma and associated oxidative impairment in ABCA1-dependent cholesterol efflux from macrophages. J Biol Chem 2005;280:38-47.

35) UK Prospective Diabetes Study (UKPDS) Group. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 1998; 352: 837–53.

36) Action to Control Cardiovascular Risk in Diabetes Study Group, Gerstein HC, Miller ME, Byington RP, Goff DC,Jr, Bigger JT, et al. Effects of intensive glucose lowering in type 2 diabetes. N Engl J Med 2008;358:2545-2559.

37) ADVANCE Collaborative Group, Patel A, MacMahon S, Chalmers J, Neal B, Billot L, et al. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. N Engl J Med 2008;358:2560-2572.

38) Duckworth W, Abraira C, Moritz T, Reda D, Emanuele N, Reaven PD, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009;360:129-139.

39) Olefsky JM. Treatment of insulin resistance with peroxisome proliferator-activated receptor gamma agonists. J Clin Invest 2000;106:467-472.

40) Mukhtar, R., and Reckless, J.P.D. Dyslipidaemia in type 2 diabetes: effects of the thiazolidinediones pioglitazone and rosiglitazone. Diabetic Medicine 2005;22:1-20.

41) Lawrence JM, Reid J, Taylor GJ, Stirling C, Reckless JP. Favorable effects of pioglitazone and metformin compared with gliclazide on lipoprotein subfractions in overweight patients with early type 2 diabetes. Diabetes Care 2004;27:41–46.

42) Spanheimer R, Betteridge DJ, Tan MH, Ferrannini E, Charbonnel B, PROactive Investigators. Long-term lipid effects of pioglitazone by baseline anti-hyperglycemia medication therapy and statin use from the PROactive experience (PROactive 14). Am J Cardiol 2009;104:234-239.

43) Mirmiranpour H, Mousavizadeh M, Noshad S, Ghavami M, Ebadi M, Ghasemiesfe M, Nakhjavani M, Esteghamati A. Comparative effects of pioglitazone and metformin on

oxidative stress markers in newly diagnosed type 2 diabetes patients: a randomized clinical trial. J Diabetes Complications 2013;27:501-507.

44) Koyama H, Tanaka S, Monden M, Shoji T, Morioka T, Fukumoto S, Mori K, Emoto M, Shoji T, Fukui M, Fujii H, Nishizawa Y, Inaba M. Comparison of effects of pioglitazone and glimepiride on plasma soluble RAGE and RAGE expression in peripheral mononuclear cells in type 2 diabetes: randomized controlled trial (PioRAGE). Atherosclerosis 2014;234:329-334.

45) Abdelmoneim AS, Eurich DT, Light PE, et al. Cardiovascular safety of sulphonylureas: over 40 years of continuous controversy without an answer. Diabetes Obes Metab 2015; 17: 523–32.

46) Vaccaro O, Masulli M, Bonora E, Del Prato S, Giorda CB, Maggioni AP, Mocarelli P, Nicolucci A, Rivellese AA, Squatrito S, Riccardi G, TOSCA.IT study group (Thiazolidinediones Or Sulphonylureas and Cardiovascular Accidents. Intervention Trial). Addition of either pioglitazone or a sulfonylurea in type 2 diabetic patients inadequately controlled with metformin alone: Impact on cardiovascular events. A randomized controlled trial. Nutr Metab Cardiovasc Dis 2012;22: 997-1006.

47) Thevarajah TM, Nani N, Chew YY. Performance evaluation of the Arkray Adams HA-8160 HbA1c analyser. Malays J Pathol 2008;30: 81-6.

48) Lipid research clinics program: lipid and lipoprotein analysis. Washington DC. In: Hainline A, Karon J, Lippel K, editors. Manual of Laboratory Operations; 1982. p. 63-77.

49) Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982;28:2077-80.

50) Craig WY, Poulin SE, Nelson CP, Ritchie RF. ELISA of IgG antibody to oxidized low-density lipoprotein: Effects of blocking buffer and method of data expression. Clin Chem 1994;40(6):882-888.

51) Makita Z, Vlassara H, Cerami A, Bucala R. Immunochemical detection of advanced glycosylation end products in vivo. J Biol Chem 1992;267(8):5133-5138.

52) Kernan WN, Viscoli CM, Furie KL, Young LH, Inzucchi SE, Gorman M, Guarino PD, Lovejoy AM, Peduzzi PN, Conwit R, Brass LM, Schwartz GG, Adams HP Jr, Berger L, Carolei A, Clark W, Coull B, Ford GA, Kleindorfer D, O'Leary JR, Parsons MW, Ringleb P, Sen S, Spence JD, Tanne D, Wang D, Winderm TR; IRIS Trial Investigators. Pioglitazone after ischemic stroke or transient ischemic attack. NEJM 2016; 374: 1321-1331.

53) Mariz S, Urquhart R, Moules I, Tan MH, Edwards G. Effects of pioglitazone addition to metformin or sulfonylurea therapy on serum lipids in patients with type 2 diabetes mellitus: 2-year data. Diabetes 2004;53: 578P.

54) Chen YH, Du L, Geng XY, Peng YL, Shen JN, Zhang YG, et al. Effects of sulfonylureas on lipids in type 2 diabetes mellitus: a meta-analysis of randomized controlled trials. J Evid Based Med 2015;8:134-148.

55) Mehta JL, Hu B, Chen J, Li D. Pioglitazone inhibits LOX-1 expression in human coronary artery endothelial cells by reducing intracellular superoxide radical generation. Arterioscler Thromb Vasc Biol 2003;23:2203-2208.

56) Pirillo A, Norata GD, Catapano AL. LOX-1, OxLDL, and atherosclerosis. Mediators Inflamm 2013;2013:152786.

57) Lapolla A, Reitano R, Seraglia R, Sartore G, Ragazzi E, Traldi P. Evaluation of advanced glycation end products and carbonyl compounds in patients with different conditions of oxidative stress. Mol Nutr Food Res 2005;49:685-90.

58) Nakamura I, Oyama J, Komoda H, Shiraki A, Sakamoto Y, Taguchi I, et al. Possible effects of glimepiride beyond glycemic control in patients with type 2 diabetes: a preliminary report. Cardiovasc Diabetol 2014;13:15.

59) Scharnagl H, Stojakovic T, Winkler K, Rosinger S, Marz W, Boehm BO. The HMG-CoA reductase inhibitor cerivastatin lowers advanced glycation end products in patients with type 2 diabetes. Exp Clin Endocrinol Diabetes 2007;115:372-375.

60) Cuccurullo C, Iezzi A, Fazia ML, De Cesare D, Di Francesco A, Muraro R, et al. Suppression of RAGE as a basis of simvastatin-dependent plaque stabilization in type 2 diabetes. Arterioscler Thromb Vasc Biol 2006;26:2716-2723.