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Effects of sitagliptin or metformin added to pioglitazone monotherapy in poorly controlled type 2 diabetes mellitus patients

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Abstract

The aim of the study was to compare the effects of the addition of sitagliptin or metformin to pioglitazone monotherapy in poorly controlled type 2 diabetes mellitus patients on body weight, glycemic control, β-cell function, insulin resistance, and inflammatory state parameters. One hundred fifty-one patients with uncontrolled type 2 diabetes mellitus (glycated hemoglobin [HbA_{1c}] >7.5%) in therapy with pioglitazone 30 mg/d were enrolled in this study. We randomized patients to take pioglitazone 30 mg plus sitagliptin 100 mg once a day, or pioglitazone 15 mg plus metformin 850 mg twice a day. We evaluated at baseline and after 3, 6, 9, and 12 months these parameters: body weight, body mass index, HbA_{1c}, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting plasma insulin (FPI), homeostasis model assessment insulin resistance index (HOMA-IR), homeostasis model assessment β-cell function index, fasting plasma proinsulin (Pr), Pr/FPI ratio, adiponectin, resistin (R), tumor necrosis factor-α (TNF-α), and high-sensitivity C-reactive protein. A decrease of body weight and body mass index was observed with metformin, but not with sitagliptin, at the end of the study. We observed a comparable significant decrease of HbA_{1c}, FPG, and PPG and a significant increase of homeostasis model assessment β-cell function index compared with baseline in both groups without any significant differences between the 2 groups. Fasting plasma insulin, fasting plasma Pr, Pr/FPI ratio, and HOMA-IR values were decreased in both groups even if the values obtained with metformin were significantly lower than the values obtained with sitagliptin. There were no significant variations of ADN, R, or TNF-α with sitagliptin, whereas a significant increase of ADN and a significant decrease of R and TNF-α values were recorded with metformin. A significant decrease of high-sensitivity C-reactive protein value was obtained in both groups without any significant differences between the 2 groups. There was a significant correlation between HOMA-IR decrease and ADN increase, and between HOMA-IR decrease and R and TNF-α decrease in pioglitazone plus metformin group after the treatment. The addition of both sitagliptin or metformin to pioglitazone gave an improvement of HbA_{1c}, FPG, and PPG; but metformin led also to a decrease of body weight and to a faster and better improvement of insulin resistance and inflammatory state parameters, even if sitagliptin produced a better protection of β-cell function.

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1. Introduction

Targeting glycated hemoglobin (HbA_{1c}) levels less than 7.0% is considered a primary goal of diabetes care, given its importance to obtain a sustained reduction in microvascular and possibly macrovascular complications. However, maintaining an adequate metabolic control is still a challenge in many patients with type 2 diabetes mellitus (T2DM) [1]. The importance of an early, intensified approach to metabolic control has been also clearly demonstrated by the long-term results of the United Kingdom Prospective Diabetes Study, showing that the benefits of tight blood glucose control extended well beyond the end of the study and persisted after more than 10 years [2]. Initial antihyperglycemic monotherapy is often unsuccessful at getting patients with T2DM to glycemic goals; and as the glycemic targets recommended by standard guidelines are lowered, even fewer patients will achieve the goal with single-agent treatment [3,4]. The combination therapy has emerged as an alternative approach, getting more patients to goal initially and avoiding or delaying the need for subsequent treatment regimen changes to maintain glycemic targets [5]. In this regard, recent breakthroughs in the understanding of incretin-based therapies have provided additional options for the treatment of T2DM. Incretins are gastrointestinal hormones released during nutrient absorption to increase insulin secretion. The 2 gut peptides accounting for most of the incretins effect are glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). In response to a meal, GLP-1 and GIP are released and, in turn, stimulate insulin (both in a glucose-dependent manner), delay gastric emptying, and increase satiety [6,7]; furthermore, GLP-1 acts on α cells and inhibits the secretion of glucagons [8]. Within some minutes of release from their intestinal sites, GIP and GLP-1 undergo rapid metabolism (proteolytic cleavage) to inactive metabolites by the enzyme dipeptidyl peptidase–4 (DPP-4). In T2DM, GLP-1 concentrations are reduced in response to a meal, whereas GIP concentrations are normal or increased. This observation suggests resistance to the action of GIP, making GLP-1 the favored potential therapeutic target [9]. Because GLP-1 is rapidly degraded by DPP-4 [10], a new class of compounds has been developed: DPP-4 inhibitors that delay endogenous degradation of GLP-1 and GIP. Sitagliptin is the first in this class of drugs. It is orally administered, and it is indicated for the treatment of T2DM at the recommended dose of 100 mg once daily either as monotherapy or in combination with metformin and/or sulfonylureas or thiazolidinediones in patients poorly controlled on the maximum doses of these drugs [11]. After an oral glucose tolerance test, sitagliptin produced 2-fold increases in intact (active) GLP-1 and GIP concentrations and, in a glucose-dependent manner, enhanced insulin release and reduced glucagon secretion compared with placebo in patients with T2DM [12]. These changes contributed to the significant reduction in postprandial glucose concentration in these patients.

Metformin is the most commonly used oral antihyperglycemic agent, both as monotherapy and in combination with other agents such as sulfonylureas or thiazolidinediones [13-16]. Metformin reduces elevated blood glucose levels by reducing hepatic glucose output and also by enhancing peripheral glucose uptake, improving insulin resistance [17]. In addition, metformin has been reported to increase active GLP-1 concentrations by 1.5- to 2-fold after an oral glucose load in obese, nondiabetic subjects [18] even if this effect on GLP-1 was not the result of inhibiting DPP-4 activity [19,20].

Pioglitazone targets insulin resistance by binding to the transcription factor peroxisome proliferator-activated receptor–γ that is involved in the regulation of carbohydrate and lipid metabolism [21,22], promoting synthesis of glucose transporters, and activating adipocyte differentiation [23-25].

The aim of this study was to compare the effects of the addition of sitagliptin or metformin to pioglitazone monotherapy in poorly controlled diabetic patients on body weight, glycemic control, insulin resistance, and β-cell function, but also on some insulin resistance and inflammatory state parameters like adiponectin (ADN), resistin (R), tumor necrosis factor-α (TNF-α), and high-sensitivity C-reactive protein (hs-CRP).

2. Material and methods

2.1. Study design

This multicenter, randomized, double-blind clinical trial was conducted at the Department of Internal Medicine and Therapeutics, University of Pavia (Pavia, Italy); the "G Descovich" Atherosclerosis Study Center, Department of Internal Medicine, Aging and Kidney Diseases, University of Bologna (Bologna, Italy); the Diabetes Care Unit, S Carlo Hospital (Milano, Italy); the Pesenti Fenaroli Hospital (Alzano Lombardo [Bergamo], Italy); the Metabolic Unit, Regional Hospital (Varese, Italy); the Division of Medicine, Civic Hospital (Cittiglio [Varese], Italy); the RSA Don Leone Porta (Milano, Italy); and the Fondazione Ospedale della Carità (Casalbuttano [Cremona], Italy).

The study protocol was approved at each site by institutional review boards and was conducted in accordance with the Declaration of Helsinki and its amendments.

2.2. Patients

We enrolled 151 white T2DM patients aged at least 18 years of either sex (Table 1) according to the European Society of Cardiology and the European Association for the Study of Diabetes guidelines criteria [26] with uncontrolled T2DM (HbA_{1c} >7.5%) in therapy with pioglitazone. All the patients were not well controlled with diet, physical activity, and pioglitazone at the dosage of 30 mg/d. Suitable patients, identified from review of case notes and/or computerized

Table 1
Subjects characteristics at baseline in the study

	Pioglitazone + sitagliptin	Pioglitazone + metformin
n	75	76
Sex (male-female)	37/38	39/37
Age (y)	57 ± 5	58 ± 6
Smoking status (male-female)	12/15	16/14
Diabetes duration (y)	5 ± 2	6 ± 3
Height (m)	1.68 ± 0.05	1.67 ± 0.04
Weight (kg)	78.7 ± 6.2	77.3 ± 5.4
BMI (kg/m^2)	27.9 ± 1.5	27.7 ± 1.3
HbA_{1c} (%)	8.5 ± 0.9	8.4 ± 0.8
FPG (mg/dL)	143 ± 19	142 ± 18
PPG (mg/dL)	189 ± 26	186 ± 24
FPI (μU/mL)	18.4 ± 3.6	18.2 ± 3.4
HOMA-IR	6.7 ± 2.5	6.4 ± 2.3
HOMA-β	54.6 ± 49.9	52.1 ± 47.8
FPPr (pmol/L)	42.2 ± 29.1	41.4 ± 26.9
Pr/FPI ratio	0.38 ± 1.48	0.38 ± 1.49
ADN (μg/mL)	5.4 ± 0.9	5.3 ± 0.8
R (ng/mL)	7.7 ± 0.8	7.8 ± 0.9
TNF-α (pg/mL)	3.8 ± 1.1	4.0 ± 1.4
hs-CRP (mg/L)	2.1 ± 1.0	2.0 ± 0.9

Data are means ± SD.

clinic registers, were contacted by the investigators in person or by telephone.

Patients were excluded if they had a history of ketoacidosis or had unstable or rapidly progressive diabetic retinopathy, nephropathy, or neuropathy; *impaired hepatic function* (defined as plasma aminotransferase and/or γ-glutamyltransferase level higher than the upper limit of normal for age and sex); *impaired renal function* (defined as serum creatinine level higher than the upper limit of normal for age and sex); or severe anemia. Patients with serious cardiovascular disease (eg, New York Heart Association class I-IV congestive heart failure or a history of myocardial infarction or stroke) or cerebrovascular conditions within 6 months before study enrolment also were excluded. Women who were pregnant or breastfeeding or of child-bearing potential and not taking adequate contraceptive precautions were also excluded. All patients provided written informed consent to participate.

2.3. Treatments

Patients were randomly assigned to receive pioglitazone 30 mg plus sitagliptin 100 mg once a day, or pioglitazone 15 mg plus metformin 850 mg twice a day, for 12 months. Both sitagliptin and metformin were supplied as identical, opaque, white capsules in coded bottles to ensure the blind status of the study. Randomization was done using a drawing of envelopes containing randomization codes prepared by a statistician. A copy of the code was provided only to the responsible person performing the statistical analysis. The code was only broken after database lock, but could have been broken for individual subjects in cases of an emergency. Medication compliance was assessed by counting the number of pills returned at the time of specified clinic visits. At baseline, we weighed participants and gave them a bottle containing a supply of study medication for at least 100 days. Throughout the study, we instructed patients to take their first dose of new medication on the day after they were given the study medication. At the same time, all unused medication was retrieved for inventory. All medications were provided free of charge.

2.4. Diet and exercise

Subjects began a controlled-energy diet (near 600 kcal daily deficit) based on American Heart Association recommendations [27] that included 50% of calories from carbohydrates, 30% from fat (6% saturated), and 20% from proteins, with a maximum cholesterol content of 300 mg/d and 35 g/d of fiber. Patients were not treated with vitamins or mineral preparations during the study.

Standard diet advice was given by a dietician and/or specialist physician. Dietician and/or specialist physician periodically provided instructions on dietary intake recording as part of a behavior modification program and then later used the subject's food diaries for counseling. Individuals were also encouraged to increase their physical activity by walking briskly for 20 to 30 minutes, 3 to 5 times per week, or by cycling. The recommended changes in physical activity throughout the study were not assessed.

2.5. Assessments

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs, a 12-lead electrocardiogram, measurements of body mass index (BMI), HbA_{1c}, fasting plasma glucose (FPG), postprandial plasma glucose (PPG), fasting plasma insulin (FPI). Insulin resistance and β-cell function were evaluated by the homeostasis model assessment (HOMA) method, in particular we considered homeostasis model assessment insulin resistance index (HOMA-IR) and homeostasis model assessment β-cell function index (HOMA-β). We evaluated body weight and BMI, HbA_{1c}, FPG, PPG, FPI, HOMA-IR, HOMA-β, fasting plasma proinsulin (FPPr), proinsulin (Pr) to FPI ratio, ADN, R, TNF-α, and hs-CRP at baseline and after 3, 6, 9, and 12 months. To evaluate the tolerability assessments, all adverse events were recorded. All plasmatic parameters were determined after a 12-hour overnight fast, with the exception of PPG, which was determined 2 hours after a standardized meal. Venous blood samples were taken for all patients between 8:00 and 9:00 AM. We used plasma obtained by addition of Na_2-EDTA, 1 mg/mL, and centrifugation at 3000*g* for 15 minutes at 4°C. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C for no more than 3 months. All measurements were performed in a central laboratory.

Body mass index was calculated as weight in kilograms divided by the square of height in meters. Glycated hemoglobin level was measured by a high-performance liquid chromatography method (DIAMAT; Bio-Rad, Richmond, CA; normal values, 4.2%-6.2%) with intra- and interassay coefficients of variation (CVs) of less than 2% [28]. Plasma glucose was assayed by glucose-oxidase method (GOD/PAP; Roche Diagnostics, Mannheim, Germany) with intra- and interassay CVs of less than 2% [29]. Plasma insulin was assayed with Phadiaseph Insulin RIA (Pharmacia, Uppsala, Sweden) by using a second antibody to separate the free and antibody-bound 125 I-insulin (intra- and interassay CVs, 4.6% and 7.3%, respectively) [30].

The estimate of insulin resistance was calculated using the HOMA-IR, with the following formula: insulin resistance = FPI (microunits per milliliter) × FPG (millimoles per liter)/22.5, as described by Matthews et al [31] (normal if <2.5, marker of insulin resistance if ≥2.5). The HOMA-β index was calculated as the product of 20 and basal insulin levels (microunits per milliliter) divided by the value of basal glucose concentrations (millimoles per liter) minus 3.5; this formula has been proposed to be a good measure of β-cell function [32].

Proinsulin was determined using an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). The intra- and interassay CVs were 2.4% and 8.9%, respectively, [33].

Adiponectin level was determined using enzyme-linked immunoassay (ELISA) kits (B-bridge International, Sunnyvale, CA). Intraassay CVs were 3.6% for low- and 3.3% for high-control samples, whereas interassay CVs were 3.2% for low- and 7.3% for high-control samples, respectively [34].

Resistin value was measured by a commercially available ELISA kit (BioVendor Laboratory Medicine, Brno, Czech Republic). Intraassay CV was 3.4% and interassay CV was 6.9% [35].

The TNF-α level was assessed using commercially available ELISA kits according to manufacturer's instructions (Titer-Zyme EIA kit; Assay Designs, Ann Arbor, MI). Intraassay CVs were 4.5% for low- and 3.6% for high-concentration samples, whereas the interassay CVs were 6.0% for low and 11.8% for high-concentration samples, respectively [36].

High-sensitivity C-reactive protein was measured with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, DE). The intra- and interassay CVs were 5.7% and 1.3%, respectively [37].

2.6. Statistical analysis

An intention-to-treat analysis was conducted in patients who had received at least 1 dose of study medication and had a subsequent efficacy observation. Patients were included in the tolerability analysis if they had received at least 1 dose of trial medication and had undergone a subsequent tolerability observation. Continuous variables were compared by analysis of variance. Intervention effects were adjusted for additional potential confounders using analysis of covariance. Analysis of variance was also used to assess significance within and between groups. The statistical significance of the independent effects of treatments on the other variables was determined using analysis of covariance. A 1-sample t test was used to compare values obtained before and after treatment administration; 2-sample t tests were used for between-group comparisons. The Bonferroni correction for multiple comparisons was also carried out [38]. Statistical analysis of data was performed using the Statistical Package for Social Sciences software version 11.0 (SPSS, Chicago, IL). Data are presented as mean ± standard deviation (SD). For all statistical analyses, $P < .05$ was considered statistically significant.

3. Results

3.1. Study sample

One hundred fifty-one patients were enrolled in the study. Of these, 137 completed the study; and 69 (50.4%) were allocated in the sitagliptin group and 68 (49.6%) in the metformin group. There were 14 patients (7 men and 7 women) who did not complete the study; and the reasons for premature withdrawal included adverse effects such as diarrhea (1 woman in sitagliptin group and 1 woman in metformin group, after 3 months), nausea (1 man and 1 woman in metformin group, after 6 months), vomiting (1 woman in sitagliptin group, after 3 months; and 1 man in metformin group, after 3 months), and gastrointestinal discomfort (1 woman in metformin group, after 9 months; 1 man in metformin group, after 9 months; and 1 man in metformin group, after 12 months) and being lost to follow-up (2 women in sitagliptin group, after 6 months; and 1 woman in metformin group, after 6 months). Furthermore, 2 men had hypoglycemia (FPG <60 mg/dL) in sitagliptin group, after 3 and 9 months, respectively. The characteristics of the patient population at study entry are shown in Table 1.

3.2. Body weight and BMI

Body mass index and body weight did not show any significant change after 3, 6, 9, and 12 months compared with baseline in the sitagliptin group, whereas there was a significant decrease after 12 months in the metformin group ($P < .05$); and the values were significantly lower than with sitagliptin after 12 months ($P < .05$) (Tables 2 and 3).

3.3. Glycemic parameters

We observed a statistically significant improvement of HbA_{1c} after 9 and 12 months ($P < .05$ and $P < .01$, respectively, for both groups) compared with baseline in both groups, without any significant differences between the 2 groups.

Table 2
Patients' data during the study in pioglitazone + sitagliptin group

	Pioglitazone + sitagliptin group			
	3 mo	6 mo	9 mo	12 mo
n	72	70	69	69
Sex (male-female)	36/36	36/34	35/34	35/34
Smoking status (male-female)	12/15	11/15	10/15	10/14
Weight (kg)	78.2 ± 6.0	77.6 ± 5.7	77.3 ± 5.4	77.1 ± 5.2
BMI (kg/m^2)	27.7 ± 1.3	27.5 ± 1.2	27.4 ± 1.1	27.3 ± 1.0
HbA_{1c} (%)	8.2 ± 0.7	7.7 ± 0.5	7.4 ± 0.4*	7.1 ± 0.3†
FPG (mg/dL)	139 ± 17	133 ± 15	128 ± 13*	123 ± 11†
PPG (mg/dL)	178 ± 23	169 ± 22	161 ± 20*	156 ± 18†
FPI (μU/mL)	17.9 ± 3.3	16.9 ± 3.2	15.6 ± 2.7	14.3 ± 2.4*
HOMA-IR	6.2 ± 2.1	5.6 ±1.7	5.0 ± 1.2	4.3 ± 0.8*
HOMA-β	56.1 ± 50.6	59.9 ± 53.7	64.6 ± 56.4*	69.8 ± 59.7†
FPPr (pmol/L)	40.3 ± 27.4	38.6 ± 25.5	34.7 ± 23.2	31.5 ± 20.6*
Pr/FPI ratio	0.37 ± 1.48	0.38 ± 1.46	0.37 ± 1.41	0.36 ± 1.39*
ADN (μg/mL)	5.4 ± 0.9	5.5 ± 1.0	5.6 ± 1.1	5.7 ± 1.2
R (ng/mL)	7.6 ± 0.7	7.6 ± 0.7	7.5 ± 0.6	7.4 ± 0.5
TNF-α (pg/mL)	3.7 ± 1.0	3.7 ± 1.0	3.6 ± 0.9	3.6 ± 0.9
hs-CRP (mg/L)	2.0 ± 0.9	1.7 ± 0.8	1.5 ± 0.6	1.4 ± 0.5*

Data are means ± SD.
* $P < .05$ vs baseline.
† $P < .01$ vs baseline.

There was a statistically significant decrease of FPG after 9 and 12 months ($P < .05$ and $P < .01$, respectively, for both groups) compared with baseline in both groups, and we did not observe any significant differences between the 2 groups.

Table 3
Patients' data during the study in pioglitazone + metformin group

	Pioglitazone + metformin group			
	3 mo	6 mo	9 mo	12 mo
n	74	71	69	68
Sex (male-female)	38/36	36/35	35/34	34/34
Smoking status (male-female)	15/14	15/14	14/13	14/13
Weight (kg)	76.7 ± 5.0	75.9 ±4.7	75.0 ± 4.3	74.5 ± 4.1*,§
BMI (kg/m^2)	27.5 ± 1.2	27.2 ± 0.9	26.9 ± 0.8	26.7 ± 0.7*,§
HbA_{1c} (%)	8.0 ± 0.6	7.8 ± 0.5	7.3 ± 0.4*	7.0 ± 0.2‡
FPG (mg/dL)	137 ± 16	131 ± 14	125 ± 12*	120 ± 10‡
PPG (mg/dL)	177 ± 23	167 ± 21	159 ± 19*	150 ± 17‡
FPI (μU/mL)	16.4 ± 3.1	16.1 ± 3.0	14.6 ± 2.5*	12.7 ± 1.6‡,§
HOMA-IR	6.0 ± 2.0	5.2 ± 1.4	4.5 ± 0.9*	3.8 ± 0.6‡,§
HOMA-β	54.8 ± 49.2	57.1 ± 51.1	60.8 ± 53.9	66.9 ± 57.6*
FPPr (pmol/L)	36.2 ± 24.4	35.1 ± 22.7	29.9 ± 18.6*	25.1 ± 17.2‡,§
Pr/FPI ratio	0.37 ± 1.47	0.36 ± 1.38*	0.35 ± 1.29†	0.33 ± 1.20‡,§
ADN (μg/mL)	5.5 ± 1.0	5.9 ± 1.3	6.2 ± 1.5	6.6 ± 1.7*,§
R (ng/mL)	7.7 ± 0.8	7.1 ± 0.4	6.3 ± 0.3	5.4 ± 0.2*,§
TNF-α (pg/mL)	3.8 ± 1.1	3.5 ± 0.8	3.2 ± 0.6	3.0 ± 0.5*,§
hs-CRP (mg/L)	2.0 ± 0.9	1.7 ± 0.8	1.6 ± 0.7	1.3 ± 0.4*

Data are means ± SD.
* $P < .05$ vs baseline.
† $P < .02$ vs baseline.
‡ $P < .01$ vs baseline.
§ $P < .05$ vs pioglitazone + sitagliptin.

A significant decrease of PPG was obtained after 9 and 12 months ($P < .05$ and $P < .01$, respectively, for both groups) compared with baseline in both groups, and no significant differences between the 2 groups were recorded.

Fasting plasma insulin was significantly decreased, compared with baseline, after 12 months ($P < .05$) in the group treated with sitagliptin and after 9 and 12 in the group treated with metformin ($P < .05$ and $P < .01$, respectively); furthermore, FPI obtained with metformin was significantly lower than the value obtained with sitagliptin after 12 months ($P < .05$).

3.4. β-Cell function

A HOMA-β increase was present after 9 and 12 months ($P < .05$ and $P < .01$, respectively) compared with baseline in the group treated with sitagliptin and after 12 months ($P < .05$) in the group treated with metformin. No significant differences between the 2 groups were recorded.

There was a statistically significant decrease of FPPr compared with baseline after 12 months ($P < .05$) in the group treated with sitagliptin and after 9 and 12 months in the group treated with metformin ($P < .05$ and $P < .01$, respectively), and the decrease recorded in the group treated with metformin was significantly higher than the decrease observed in the group treated with sitagliptin.

We observed a statistically significant decrease of Pr/FPI ratio compared with baseline after 12 months ($P < .05$) in the group treated with sitagliptin and after 6, 9, and 12 months in the group treated with metformin ($P < .05$, $P < .02$, and $P < .01$, respectively). The Pr/FPI ratio in the group treated with metformin was significantly lower than the value obtained in the group treated with sitagliptin after 12 months ($P < .05$) (Tables 2 and 3).

3.5. Insulin resistance parameters

A statistically significant decrease of HOMA-IR was recorded after 12 months ($P < .05$) compared with baseline in the group treated with sitagliptin and after 9 and 12 months in the group treated with metformin ($P < .05$ and $P < .01$, respectively). The HOMA-IR in the metformin group was significantly lower than that in the sitagliptin group after 12 months ($P < .05$) (Tables 2 and 3).

There were no significant variations of ADN in the group treated with sitagliptin compared with baseline, whereas a statistically significant increase of ADN was recorded after 12 months ($P < .05$) in the group treated with metformin; and it was significantly higher than in the group treated with sitagliptin after 12 months ($P < .05$).

No R variation was observed in the group treated with sitagliptin compared with baseline, whereas R decreased in the group treated with metformin after 12 months ($P < .05$); and it was significantly lower compared with the value recorded in the group treated with sitagliptin ($P < .05$) (Tables 2 and 3).

3.6. Inflammatory state

We did not obtain any TNF-α variations compared with baseline in the group treated with sitagliptin, whereas we registered a significant decrease of TNF-α after 12 months with metformin ($P < .05$); and the decrease was significantly higher than the value obtained with sitagliptin after 12 months ($P < .05$).

A significant decrease of hs-CRP value was obtained after 12 months compared with baseline in both groups ($P < .05$ for both), and there were no significant differences between the 2 groups (Tables 2 and 3).

3.7. Correlations

There was a significant correlation between HOMA-IR decrease and ADN increase ($r = -0.58$, $P < .01$) (Fig. 1), R decrease ($r = 0.56$, $P < .01$) (Fig. 2), and TNF-α decrease ($r = 0.54$, $P < .01$) (Fig. 3) in pioglitazone plus metformin group after the 1-year treatment.

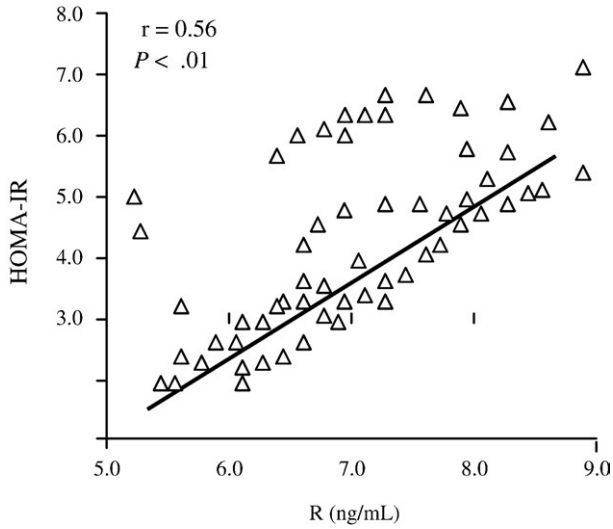

Fig. 2. Correlation between HOMA-IR and R in pioglitazone plus metformin group after the 1-year treatment.

4. Discussion

In larger clinical trials, sitagliptin provided clinically meaningful reductions in HbA_{1c}, FPG, and PPG concentrations and was well tolerated either as monotherapy or as an add-on therapy to metformin or pioglitazone [39-43]. Raz et al [39] reported a significant decrease of HbA_{1c}, FPG, and PPG with sitagliptin treatment; the reduction in FPG can be a proof that the enhancement of active incretin concentrations in the fasting state also leads to glucose lowering. Because hepatic glucose production is an important determinant of FPG and because higher active GLP-1 levels lower glucagon concentrations, the likely mechanism of the lowered FPG with sitagliptin is that higher insulin secretion coupled with reduced glucagon levels leads to reduced overnight hepatic glucose production. They also observed that there was no significant effect of sitagliptin treatment compared with placebo on HOMA-IR, implying that sitagliptin may not affect peripheral insulin sensitivity, whereas there was a rise in HOMA-β suggesting improved FPI, which probably contributed to the observed reduction in FPG. These results were confirmed also by Aschner et al [40]: they recorded that, as with other antihyperglycemic agents, sitagliptin lowered HbA_{1c} even more in patients with

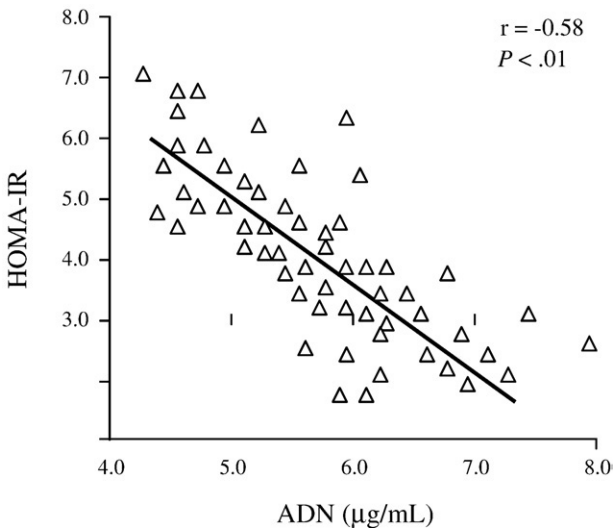

Fig. 1. Correlation between HOMA-IR and ADN in pioglitazone plus metformin group after the 1-year treatment.

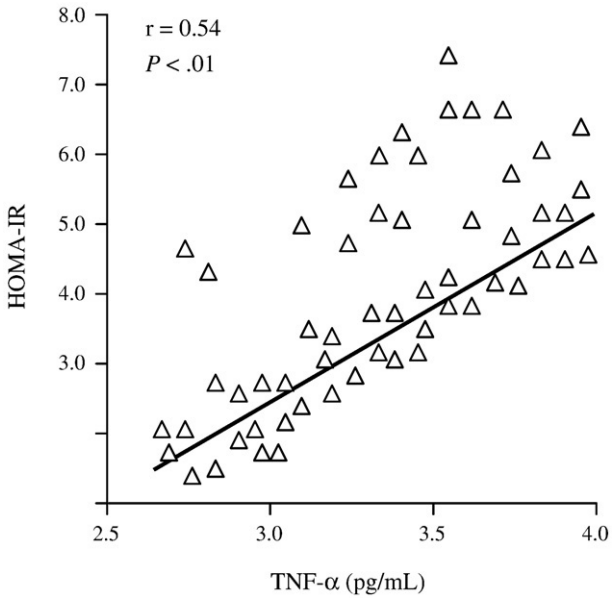

Fig. 3. Correlation between HOMA-IR and TNF-α in pioglitazone plus metformin group after the 1-year treatment.

higher baseline HbA_{1c}, with a 1.5% reduction in patients with a baseline HbA_{1c} of at least 9%. The increases in HOMA-β and in the amount of insulin secreted relative to glucose levels during a meal tolerance test and the reduction in the Pr/FPI ratio support the conclusion that sitagliptin improved β-cell function. Poorly functioning β-cells in patients with T2DM secrete greater amounts of Pr relative to insulin, and a decline in this ratio has been suggested to be a marker of improved β-cell function [44]. They also reported that treatment with sitagliptin had a neutral effect on body weight relative to baseline, consistent with results from earlier studies [45,46].

Our study confirmed these data: both sitagliptin and metformin gave a similar improvement of glycemic control, improving $HbA_{1c,}$ FPG, and PPG, even if metformin has more positive effects compared with sitagliptin on body weight, insulin resistance, and inflammation state parameters. We observed that after 12 months of treatment there was a body weight decrease of 2.8 kg with metformin treatment, whereas there were no variations with sitagliptin.

Regarding insulin resistance and β-cell function parameters, it has been already reported in literature that in T2DM patients the HOMA-β was reduced compared with the subjects with normal glucose tolerance, whereas HOMA-IR was increased [47]. Data from our study showed that both metformin and sitagliptin improved HOMA-IR, even if metformin improved it in a faster and better way. This is in contrast to what has already been observed by Raz et al [39] about the neutral effect of sitagliptin on HOMA-IR; the improvement on HOMA-IR we recorded is probably due to the association of sitagliptin with pioglitazone that has been reported to reduce both peripheral and hepatic insulin resistance [48]. Regarding HOMA-β, sitagliptin gave a faster and better increase of this index compared with metformin, confirming what has already been recorded in literature [39,40].

It has been also observed that both FPPr concentration and the ratio of Pr/FPI insulin of the T2DM patients are significantly higher compared with subjects with normal glucose tolerance [47] because of the defective conversion of Pr to insulin. Proinsulin has also been demonstrated to be an independent cardiovascular risk factor by stimulating plasminogen activator inhibitor–1 secretion and blocking fibrinolysis [49]; both sitagliptin and metformin improved these parameters, even if metformin reached the goal in a faster and better way.

Compared with the other studies reported above, our study also analyzed the effects of sitagliptin and metformin on some insulin resistance and inflammatory state parameters like ADN, R, TNF-α, and hs-CRP. Adiponectin is a protein exclusively synthesized by adipocytes; it is decreased in obesity and inversely related to glucose and insulin [50]. Ablation of the ADN gene in mice resulted in insulin resistance, glucose intolerance, dyslipidemia, and increased susceptibility to vascular injury and atherosclerosis [51-53]. Adiponectin reverses these abnormalities by stimulating oxidation of fatty acids; suppressing gluconeogenesis; and inhibiting monocyte adhesion, macrophage transformation, and proliferation and migration of smooth muscle cells in blood vessels [34,51,54]. On the other side, R is produced by mononuclear cells and activated macrophages: it has been demonstrated that overexpression of R decreases the ability of insulin to suppress hepatic glucose output or increase glucose uptake by muscle [55-57]. Available data support also a role of R in determining an increase of inflammation and atherosclerosis [58]. We observed a neutral effect of sitagliptin on ADN and R values, whereas there was an increase of ADN and a decrease of R with metformin, confirming what we have already reported in 2 previous studies conducted on T2DM patients treated with the association of a thiazolidinedione plus metformin [59,60].

On the other side, TNF-α was the first adipose-secreted product proposed to represent a molecular link between obesity and insulin resistance [61,62]; TNF-α is also a macrophage-derived inflammatory factor. It alters insulin signal in cultured cells and in vivo [63], and it has been reported that chronic exposure of cells or whole animals to TNF-α induces insulin resistance [61]. High-sensitivity C-reactive protein, instead, has been shown to independently predict myocardial infarction, stroke, and peripheral artery disease [64]. In our study, sitagliptin had a neutral effect on TNF-α, whereas metformin decreased TNF-α levels after 12 months of treatment. Regarding hs-CRP, both drugs instead decreased hs-CRP on a similar level.

Regarding adverse reactions to sitagliptin, we did not register any particular adverse effects, confirming what already reported in literature [39,40], whereas we observed more gastrointestinal disorders with metformin, even if none of these adverse experiences was reported as serious.

Of course our study has some limitations: for example, we did not assess the recommended changes in physical activity throughout the study. Moreover, we did not evaluate if the beneficial effects on β-cell function, glycemic control, body weight, and inflammatory parameters were sustained after the cessation of therapy. Another limitation is that we dosed a limited number of inflammation biomarkers, concentrating our attention on a few of these.

However, to the best of our knowledge, this is the first study investigating the effect of sitagliptin on inflammation and insulin resistance parameters.

5. Conclusion

The addition of both sitagliptin or metformin to pioglitazone gave a similar improvement of HbA_{1c}, FPG, and PPG; but metformin led also to a decrease of body weight and to a faster and better improvement of insulin resistance and inflammatory state parameters, even if sitagliptin produced a better protection of β-cell function compared with metformin.

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References

[1] Nicolucci A, Rossi MC. Incretin-based therapies: a new potential treatment approach to overcome clinical inertia in type 2 diabetes. Acta Biomed 2008;79:184-91.
[2] Holman RR, Paul SK, Bethel MA, Matthews DR, Neil HA. 10-Year follow-up of intensive glucose control in type 2 diabetes. N Engl J Med 2008;359:1577-89.
[3] Liebl A, Mata M, Eschwege E. Evaluation of risk factors for development of complications in type II diabetes in Europe. Diabetologia 2002;45:S23-8.
[4] Saydah SH, Fradkin J, Cowie CC. Poor control of risk factors for vascular disease among adults with previously diagnosed diabetes. JAMA 2004;291:335-42.
[5] Canadian Diabetes Association, Clinical Practice Guidelines Expert Committee: Canadian Diabetes Association. Clinical practice guidelines for the prevention and management of diabetes in Canada. Can J Diabetes 2003;27(Suppl 2):S1-S152.
[6] Drucker DJ. Biological actions and therapeutic potential of the glucagon-like peptides. Gastroenterology 2002;122:531-44.
[7] Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. Am J Physiol Endocrinol Metab 2004;287:E199-206.
[8] Drucker DJ, Nauck MA. The incretin system: glucagonlike peptide–1 receptor agonists and dipeptidyl peptidase–4 inhibitors in type 2 diabetes. Lancet 2006;368:1696-705.
[9] Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide–1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocrinol Metab 1995;80:952-7.
[10] Triplitt CL. New technologies and therapies in the management of diabetes. Am J Manag Care 2007;13(Suppl 2):547-54.
[11] Triplitt C, McGill JB, Porte Jr D, Conner CS. The changing landscape of type 2 diabetes: the role of incretin-based therapies in managed care outcomes. J Manag Care Pharm 2007;13:S2-16.
[12] Herman GA, Bergman A, Stevens C, Kotey P, Yi B, Zhao PL, et al. Effect of single oral doses of sitagliptin, a dipeptidyl peptidase–4 inhibitor, on incretin and plasma glucose levels following an oral glucose tolerance test in patients with type 2 diabetes. J Clin Endocrinol Metab 2006;91:4612-9.
[13] Setter SM, Iltz JL, Thams J, Campbell RK. Metformin hydrochloride in the treatment of type 2 diabetes mellitus: a clinical review with a focus on dual therapy. Clin Ther 2003;25:2991-3026.
[14] Rosenstock J, Rood J, Cobitz A, Biswas N, Chou H, Garber A. Initial treatment with rosiglitazone/metformin fixed-dose combination therapy compared with monotherapy with either rosiglitazone or metformin in patients with uncontrolled type 2 diabetes. Diabetes Obes Metab 2006;8:650-60.
[15] Garber AJ, Donovan Jr DS, Dandona P, Bruce S, Park JS. Efficacy of glyburide/metformin tablets compared with initial monotherapy in type 2 diabetes. J Clin Endocrinol Metab 2003;88:3598-604.
[16] Deeks ED, Scott LJ. Pioglitazone/metformin. Drugs 2006;66:1863-77.
[17] Hundal RS, Inzucchi SE. Metformin: new understandings, new uses. Drugs 2003;63:1879-94.
[18] Mannucci E, Ognibene A, Cremasco F, Bardini G, Mencucci A, Pierazzuoli E, et al. Effect of metformin on glucagon-like peptide 1 (GLP-1) and leptin levels in obese nondiabetic subjects. Diabetes Care 2001;24:489-94.
[19] Hinke SA, Kuhn-Wache K, Hoffmann T, Pederson RA, McIntosh CH, Demuth HU. Metformin effects on dipeptidylpeptidase IV degradation of glucagon-like peptide–1. Biochem Biophys Res Commun 2002; 291:1302-8.
[20] Lenhard JM, Croom DK, Minnick DT. Reduced serum dipeptidyl peptidase–IV after metformin and pioglitazone treatments. Biochem Biophys Res Commun 2004;324:92-7.
[21] Elbrecht A, Chen Y, Cullinan CA, Hayes N, Leibowitz M, Moller DE, et al. Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors gamma 1 and gamma 2. Biochem Biophys Res Commun 1996;224:431-7.
[22] Spiegelman BM. PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. Diabetes 1998;47:507-14.
[23] Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM, Kliewer SA. An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma. J Biol Chem 1995;270:12953-6.
[24] Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type 2 diabetes. Diabetes 1996;45:1661-9.
[25] Young PW, Cawthorne MA, Coyle PJ, Holder JC, Holman GD, Kozka IJ. BRL 49653C normalizes glycemic control in Zucker fatty fa/fa rats by improving hepatic and peripheral tissue sensitivity to insulin. Diabetologia 1993;36:A184.
[26] Rydén L, Standl E, Bartnik M, Van den Berghe G, Betteridge J, de Boer MJ, et al. Guidelines on diabetes, pre-diabetes, and cardiovascular diseases: executive summary. The Task Force on Diabetes and Cardiovascular Diseases of the European Society of Cardiology (ESC) and of the European Association for the Study of Diabetes (EASD). Eur Heart J 2007;28:88-136.
[27] Summary of American Heart Association diet and lifestyle recommendations revision 2006. Arterioscler Thromb Vasc Biol 2006;26: 2186-91.
[28] Bunn HF, Gabbay KH, Gallop PM. The glycosylation of haemoglobin. Relevance to diabetes mellitus. Science 1978;200:21-7.
[29] European Diabetes Policy Group. A desktop guide to type 2 diabetes mellitus. Diabet Med 1999;16:716-30.
[30] Heding LG. Determination of total serum insulin (IRI) in insulin-treated diabetic patients. Diabetologia 1972;8:260-6.
[31] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412-9.
[32] Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care 2004;27:1487-95.
[33] Houssa P, Dinensen B, Deberg M, Frank BH, Van Schravendijk C, Sodoyez-Goffaux F, et al. First direct assay for intact human proinsulin. Clin Chem 1998;44:1514-9.
[34] Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-6.
[35] Yannakoulia M, Yiannakouris N, Bluher S, Matalas AL, Klimis-Zacas D, Mantzoros CS. Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metabol 2003;88:1730-6.
[36] Zhang M, Tracey K. The cytokine handbook. 3rd ed. San Diego: Academic Press; 1988.
[37] Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. Clin Chem 1999;45:2136-41.
[38] Winer BJ. Statistical principles in experimental design. 2nd ed. New York: McGraw-Hill; 1971.
[39] Raz I, Hanefeld M, Xu L, Caria C, Williams-Herman D, Khatami H. Efficacy and safety of the dipeptidyl peptidase–4 inhibitor sitagliptin as monotherapy in patients with type 2 diabetes mellitus. Diabetologia 2006;49:2564-71.
[40] Aschner P, Kipnes MS, Lunceford JK, Sanchez M, Mickel C, Williams-Herman DE. Effect of the dipeptidyl peptidase–4 inhibitor sitagliptin as monotherapy on glycemic control in patients with type 2 diabetes. Diabetes Care 2006;29:2632-7.

[41] Charbonnel B, Karasik A, Liu J, Wu M, Meininger G. Efficacy and safety of the dipeptidyl peptidase–4 inhibitor sitagliptin added to ongoing metformin therapy in patients with type 2 diabetes inadequately controlled on metformin alone. Diabetes Care 2006;29:2638-43.
[42] Nauck MA, Meininger G, Sheng D, Fanurik D, Stein PP. Efficacy and safety of the dipeptidyl peptidase–4 inhibitor, sitagliptin, compared with the sulfonylurea, glipizide, in patients with type 2 diabetes inadequately controlled on metformin alone: a randomized, double-blind, noninferiority trial. Diabetes Obes Metab 2007;9:194-205.
[43] Rosenstock J, Brazg R, Andryuk PJ, Lu K, Stein P. Efficacy and safety of the dipeptidyl peptidase–4 inhibitor sitagliptin added to ongoing pioglitazone therapy in patients with type 2 diabetes: a 24-week, multicenter, randomized, double-blind, placebo-controlled parallel group study. Clin Ther 2006;28:1556-68.
[44] Hansen PA, Corbett JA. Incretin hormones and insulin sensitivity. Trends Endocrinol Metab 2005;16:135-6.
[45] Scott R, Herman GA, Zhao PL, Chen X, Wu M, Stein PP. Twelve-week efficacy and tolerability of MK-0431, a dipeptidyl peptidase IV (DPP-IV) inhibitor, in the treatment of type 2 diabetes (abstract). Diabetes 2005;54(Suppl 1):A10.
[46] Herman GA, Hanefeld M, Wu M, Chen X, Zhao PL, Stein PP. Effect of MK-0431, dipeptidyl peptidase IV (DPP-IV) inhibitor, on glycemic control after 12 weeks in patients with type 2 diabetes (abstract). Diabetes 2005;54(Suppl 1):A134.
[47] Li YB, Zhu DL, Tian HM, Shi LX, Luo ZJ, Yan L, et al. Characteristics of dysfunction of islet beta-cell in newly diagnosed type 2 diabetic patients. Zhonghua Yi Xue Za Zhi 2006;86:2537-41.
[48] Miyazaki Y, Mahankali A, Matsuda M, Glass L, Mahanlaki S, Ferrannini E. Improved glycemic control and enhanced insulin sensitivity in type 2 diabetic subjects treated with pioglitazone. Diabetes Care 2001;24:710-9.
[49] Pfützner A, Pfützner AH, Larbig M, Forst T. Role of intact proinsulin in diagnosis and treatment of type 2 diabetes mellitus. Diabetes Technol Ther 2004;6:405-12.
[50] Jackson MB, Ahima RS. Neuroendocrine and metabolic effects of adipocyte-derived hormones. Clin Sci 2006;110:143-52.
[51] Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002;13:84-9.
[52] Bouskila M, Pajvani UB, Scherer PE. Adiponectin: a relevant player in PPARγ–agonist mediated improvements in hepatic insulin sensitivity? Int J Obes Relat Metab Disord 2005;29(Suppl 1):S17-23.
[53] Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8:731-7.
[54] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947-53.
[55] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature (London) 2001;409:307-12.
[56] Satoh H, Nguyen MT, Miles PD, Imamura T, Usui I, Olefsky JM. Adenovirus-mediated chronic 'hyper-resistinemia' leads to in vivo insulin resistance in normal rats. J Clin Invest 2004;114:224-31.
[57] Rangwala SM, Rich AS, Rhoades B, Shapiro JS, Obici S, Rossetti L, et al. Abnormal glucose homeostasis due to chronic hyperresistinemia. Diabetes 2004;53:1937-41.
[58] Reilly MP, Lehrke M, Wolfe M, Rohatgi A, Lazar MA, Rader DJ. Resistin is an inflammatory marker of atherosclerosis in humans. Circulation 2005;111:932-9.
[59] Derosa G, Fogari E, Cicero AF, D'Angelo A, Ciccarelli L, Piccinni MN, et al. Blood pressure control and inflammatory markers in type 2 diabetic patients treated with pioglitazone or rosiglitazone and metformin. Hypertens Res 2007;30:387-94.
[60] Derosa G, Salvadeo SAT, D'Angelo A, Fogari E, Ragonesi PD, Ciccarelli L, et al. Rosiglitazone therapy improves insulin resistance parameters in overweight and obese diabetic patients intolerant to metformin. Arch Med Res 2008;39:412-9.
[61] Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. Nature 1997;389:610-4.
[62] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
[63] Hotamisligil GS. The role of TNF-alpha and TNF receptors in obesity and insulin resistance. J Intern Med 1999;245:621-5.
[64] Zwacka TP, Hornbach V, Torzewski J. C-reactive protein-mediated lipoprotein uptake by macrophages. Circulation 2001;103:1194-7.