Insulin resistance and Alzheimer's disease: the role of defective insulin signaling and inflammation

Suzan N. Abou-Raya[a], Ashraf M. Abdou[b], Maher Abdel-Nabi Kamel[c], Ali M.A. Ramadan[d]

[a]Geriatric Unit, Department of Internal Medicine, [b]Department of Neuropsychiatry, Faculty of Medicine, [c]Department of Medical Biochemistry, Medical Research Institute, [d]Department of Internal Medicine, Faculty of Mediciney, University of Alexandria, Alexandria, Egypt

Correspondence to Ali Mahmoud Ali Ramadan, MBBCh, Alex. Master Degree of Internal Medicine Assistant Lecturer Alexandria University Hospitals, Department of Internal Medicine Faculty of Medicine, University of Alexandria, Egypt. Tel: 01002235145; e-mail: Ramadan_2707@outlook.com

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Introduction
Alzheimer's disease (AD) is the most common form of dementia in the elderly, accounting for 60–80% of cases. The present study was to explore the role of insulin resistance and inflammatory processes in AD patients and to assess the effect of an insulin sensitizer (pioglitazone) on cognition and plasma levels of the amyloid beta derivative. Also, the study aimed to verify experimentally the effect of pioglitazone on the components of brain insulin signaling pathway and inflammatory pathway.
Materials and methods
We studied the impact of pioglitazone treatment on diabetic AD patients for 6 months with concomitant study of pioglitazone effect on insulin signaling pathway on diabetic AD rats.
Results
We report that pioglitazone 6 months treated patients has a positive effect on cognitive deficit, improve neurometabolic and decreasing neuroinflammation in diabetic AD patients, and it also was associated with a positive effect on insulin-signaling pathway plus its antioxidant effect on the brain of rats.
Conclusion
There is a strong association between AD and type 2 diabetes mellitus indicating that they share similar underlying pathophysiological mechanisms. Pioglitazone-treated diabetic AD patients were associated with improvement in cognition.

Keywords:
Alzheimer's disease, pioglitazone, treated, type 2 diabetes

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Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly, accounting for 60–80% of cases. As the population ages, the overall burden of dementia is increasing worldwide. As the number of older Egyptians grow rapidly, so too will the numbers of new and existing cases of Alzheimer's. United States develops Alzheimer's every 65 s [1]. Regular physical activity and management of cardiovascular risk factors especially diabetes, obesity, smoking, and hypertension, reduce the risk of cognitive decline and may reduce the risk of dementia [2]. Insulin resistance, the compensatory hyperinsulinemia, and other components are associated with an increased risk of cardiovascular disease; endothelial dysfunction is a prominent feature of insulin resistance syndrome [3].

Insulin in the brain

The presence of insulin in the brain was first detected by Havrankova and colleagues, who used radioimmunoassay to determine high levels of insulin in brain extracts. Likewise, they reported that insulin content in the brain was independent of the peripheral insulin, since circulating insulin levels had no effect on the brain's insulin concentration [4].

Brain insulin receptors

The molecular events through which insulin functions in the brain are the same as those operating in the periphery. However, certain insulin actions are different in the central nervous system, such as the hormone-induced glucose uptake due to a low insulin-sensitive glucose transporter (GLUT-4) activity, and because of the predominant presence of GLUT-1 and GLUT-3. In addition, insulin in the brain contributes to the control of nutrient homeostasis, reproduction, cognition, and memory, as well as to neurotrophic, neuromodulatory, and neuroprotective effects. Although the presence of insulin receptors (IRs) in many tissues in the periphery and their main function of mediating glucose transport into cells are well known, the existence of IRs within the brain was poorly understood [5].

Insulin effects on cognition and memory

It has been widely reported that the peripheral or central administration of insulin by Intracerebroventricular (ICV) or intrahippocampal routes to experimental animals has positive effects on memory and learning processes. The improvement in these activities is related with an increase in both IR expression and its signal transduction pathways in the hippocampus, and the loss of memory due to ischemic lesions in this structure can be avoided by insulin administration [6,7].

Inflammation, insulin resistance, and the brain

There is experimental evidence to indicate that inflammatory responses are closely associated with the development of insulin resistance in peripheral and central tissues. Chronic inflammation may contribute to the appearance of insulin resistance and type 2 diabetes, as well as to the association of AD and type 2 diabetes mellitus (T2DM) [8,9]. Alterations of some of the insulin-signaling pathways such as PI3K/Akt and GSK-3 are recorded in central inflammation and insulin resistance [10]. It is well accepted that neuroinflammation occurs in AD. Inflammation is needed for the expression of insulin resistance, as demonstrated by the inhibition of inflammatory pathways, which avoid diet-induced insulin resistance in experimental animals [11].

Relationships between alterations of insulin signaling and Alzheimer's disease pathogenesis

Insulin resistance is a risk factor for AD, it being a common feature of AD patients with or without T2DM. Cognitive deficits are associated with insulin signaling abnormalities. The close relationship between these two pathological disturbances, because of the common presence of insulin resistance, has led to the use of the term type 3 diabetes, which means it is considered a neuropathogenic expression of AD [12].

Materials and methods

The study was conducted on two phases: clinical and experimental.

Clinical study

The study was carried out on 60 aged male patients (65 years and above) divided into:

(1) Group I (control), which included 20 normal participants.
(2) Group II (diabetic), which included 20 insulin resistant type 2 diabetic patients.
(3) Group III (diabetic/AD), which included 10 insulin resistant participants with AD/diabetic (Mini-Mental State Exam score between 12 and 26).
(4) Group IV (pioglitazone treated), which included 10 insulin-resistant participants with AD/diabetic treated with pioglitazone for 6 months.

Alzheimer's patients were recruited from the Geriatric Unit and Neurology Department, Faculty of Medicine, University of Alexandria. The two most commonly used criteria for the diagnosis of AD are those of the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition and those created by the National Institute on Aging and the Alzheimer's Association [13,14].

Cognitive assessment was done using:

(1) General Practitioner Assessment of Cognition [15].
(2) Folstein Mini-Mental Status Examination [16].
(3) Alzheimer's Disease Assessment Scale-Cognitive [17].
(4) Mini-Cognitive Assessment Instrument [18].

The following laboratory investigations were done for control and cases:

(1) Fasting blood glucose.
(2) Postprandial blood glucose.
(3) Glycated hemoglobin.
(4) Fasting insulin concentration.
(5) Lipid profile [serum triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol].
(6) High-sensitivity C-reactive protein (hs-CRP).
(7) Plasma levels of the amyloid protein-beta (Aβ) derivative ($A\beta_{1-17}$) as a useful marker for the diagnosis of AD [19].
(8) Insulin resistance index: calculated as the homeostasis model of assessment (HOMA-IR).
(9) Computed tomography of brain.

Experimental phase

The rats were divided into four main groups:

Group I (control), 10 normal healthy male rats.
Group II (diabetic), 10 diabetic rats [high fat diet (HFD) induced and streptozotocin (STZ) induced].
Group III (diabetic/AD), 10 diabetic rats (HFD induced and STZ induced) in which AD will be induced by $CuSO_4$.
Group IV (pioglitazone treated): 10 diabetic rats (HFD induced and STZ induced) in which AD will

Table 1 Age and diabetic profile of the studied groups

			Diabetic patients with AD		
				Pioglitazone-treated patients (*n*=20)	
Parameters	Control (*n*=20)	Diabetic patients (*n*=20)	Pioglitazone untreated patients (*n*=10)	Before	After
Age (year)	66.2±1.27	68.4±7.50	70.2±5.34	70.8±5.90	–
FBS (mg/dl)	98.2±8.6	211.5±10.9[a]	224.8±42.1[a]	205.9±17.8[a]	160.6±14.8[a,b,c,d]
PPS (mg/dl)	149.2±9.6	282.4±12.2[a]	296.1±36.8[a,b]	292.4±19.5[a]	215.1±17.5[a,b,c,d]
Insulin (mIU/ml)	6.3±1.2	13.5±2.4[a]	17.8±3.5[a,b]	18.2±3.2[a,b]	16.3±2.2[a,b]
HOMA-insulin resistance index	1.52±0.31	7.01±1.14[a]	9.95±2.86[a,b]	9.21±1.63[a,b]	6.34±0.87[a,c,d]
HBA1C (%)	5.59±0.44	7.57±0.37[a]	8.12±0.33[a,b]	8.03±0.42[a,b]	7.39±0.37[a,c,d]
Medications	No medications	Metformin Short acting sulfonyl urea DPP4 inhibitors insulin	DPP4 inhibitors Insulin metformin Short acting sulfonyl urea	Insulin Metformin DPP4 inhibitors Short acting sulfonyl urea	Pioglitazone Insulin DPP4 inhibitors

Data presented as mean±SD. AD, Alzheimer disease; ANOVA, analysis of variance; FBS, fasting blood sugar; HBA1C, glycated hemoglobin; HOMA, homeostasis model of assessment; PPS, postprandial blood sugar. [a]Significantly different from control group by ANOVA. [b]Significantly different from diabetic patients by ANOVA. [c]Significantly different from untreated diabetic patients with AD by ANOVA. [d]Significantly different from pioglitazone-treated patients before treatment by paired *t* test.

be induced by $CuSO_4$ and will be treated with pioglitazone for 1 month.

Behavioral assessment of the rats

(1) Object recognition test [20].
(2) Morris water maze [21].

Biochemical assays

At the end of the experiment the rats were killed and the blood and brain tissues were obtained for the analysis of:

(1) Blood parameters:
    (a) Fasting blood glucose.
    (b) Fasting insulin concentration.
    (c) Lipid profile (serum triglyceride, total cholesterol, HDL-cholesterol, and LDL-cholesterol).
    (d) hs-CRP.
    (e) Insulin resistance index: calculated as HOMA-IR.
(2) Brain insulin signaling parameters:
    (a) Phospho-insulin receptor (P-IR) [22].
    (b) Phospho-inositol 3 kinase [23].
    (c) GLUT [24].

Results

Human results

Age of the studied groups

There were no statistical significant differences between the studied groups as regards age (Table 1 and Fig. 1).

Diabetic profile of the studied groups

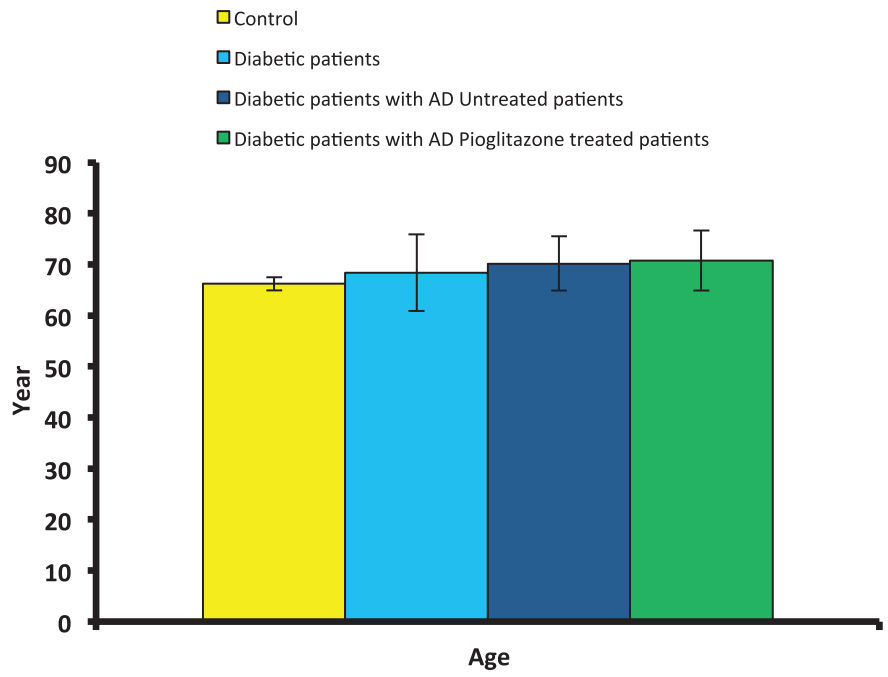
Fasting blood sugar (FBS) was significantly lower in the pioglitazone-treated group in relation to the other three groups (Table 1 and Fig. 2). Postprandial sugar was significantly lower in the pioglitazone-treated group in relation to diabetics without AD and diabetics with AD untreated groups (Table 1 and Fig. 2). The mean insulin was significantly higher in the pioglitazone-treated group in comparison to control and diabetic patients without AD (Table 1 and Fig. 3). HOMA was significantly lower in the pioglitazone-treated group in comparison to diabetic patients with AD and before treatment with pioglitazone (Table 1 and Fig. 3).

Glycated hemoglobin was significantly lower in the pioglitazone-treated group in comparison to diabetic patients with AD and before treatment with pioglitazone (Table 1 and Fig. 4).

Serum levels of amyloid β protein peptide (1–42) high-sensitive C-reactive protein in the studied groups

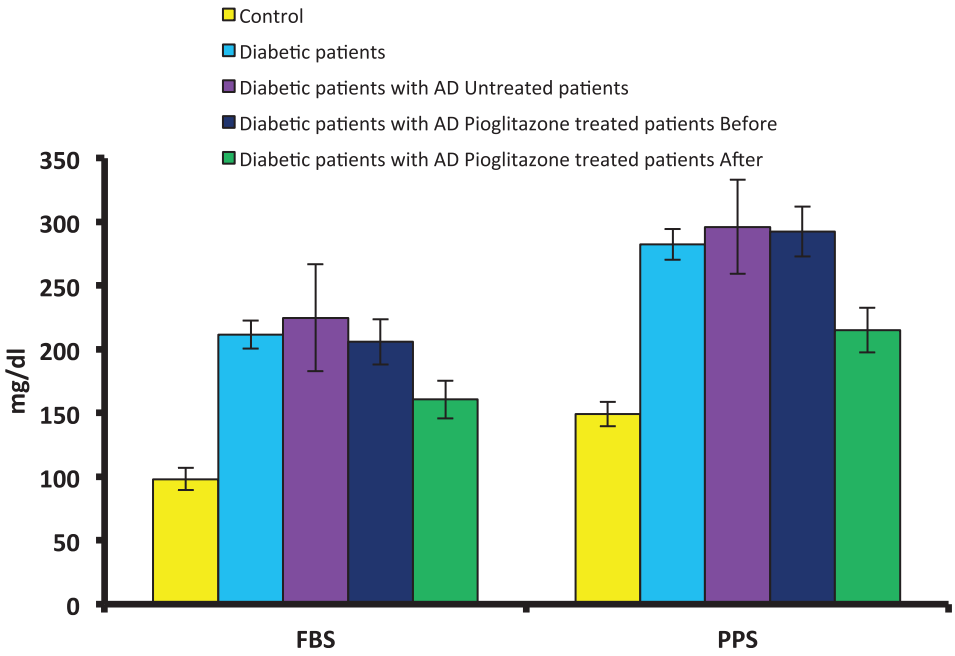
Comparing the mean of $A\beta_{1-42}$ in different groups it was significantly lower in the pioglitazone-treated group in relation to diabetics with AD untreated. It was significantly higher in the pioglitazone-treated group in relation to control and diabetics without AD (Table 2 and Fig. 5). Comparing the mean of hs-CRP in different groups it was significantly higher in pioglitazone-treated in relation to control and diabetics without AD (Table 2 and Fig. 6).

Figure 1



Age of the studied population.

Figure 2

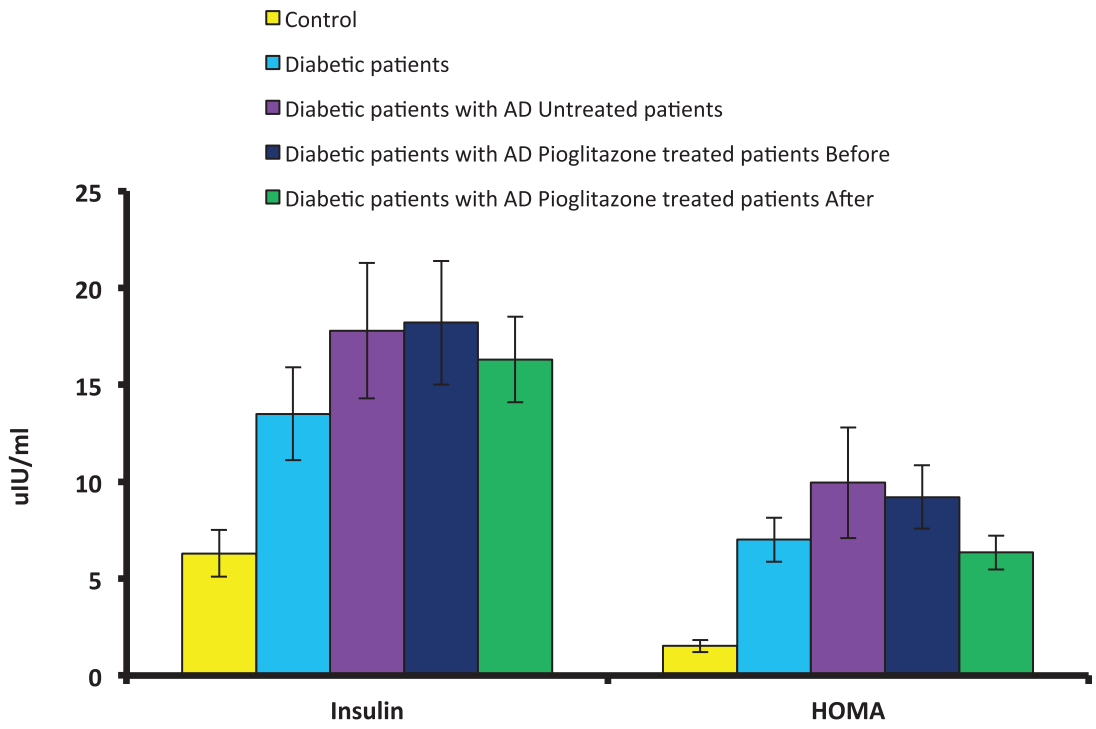


Fasting blood sugar (FBS) and postprandial blood sugar (PPS) of the studied groups.

Behavioral tests of the studied groups

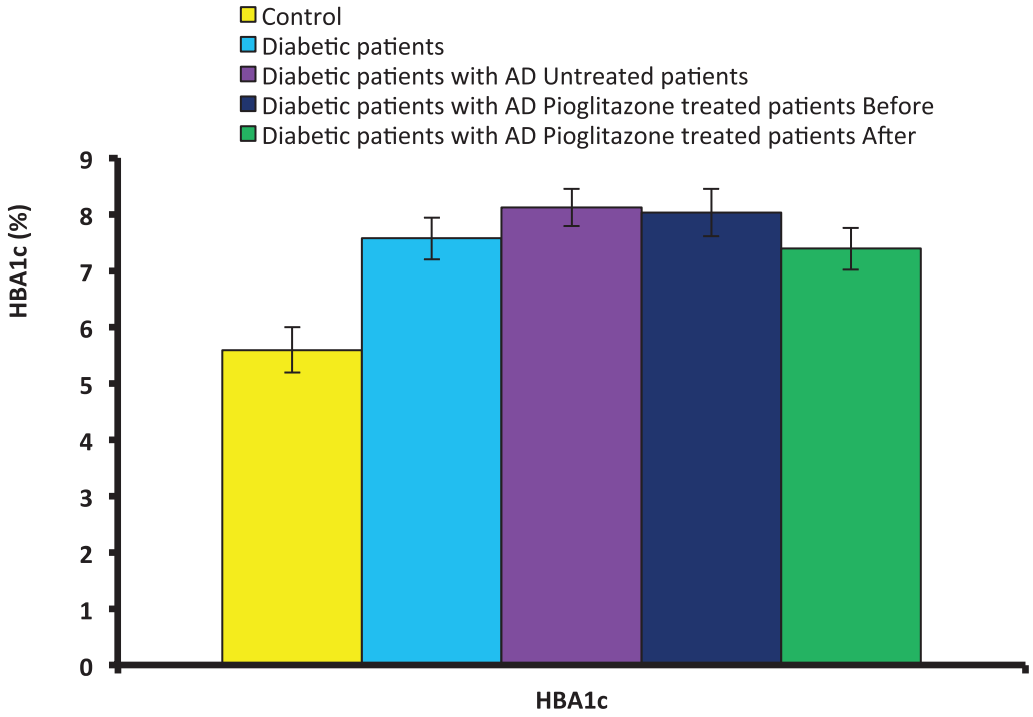
Alzheimer's Disease Assessment Scale-Cognitive was significantly lower in the pioglitazone-treated in relation to untreated diabetics with AD group, but was significantly higher in relation to control group and diabetics without AD group (Table 3 and Fig. 7). Folstein Mini-Mental Status Examination was significantly higher in the pioglitazone-treated group in relation to the same group before treatment and untreated diabetic with the AD group, but was significantly lower in relation to control group and diabetic without AD group (Table 3 and Fig. 7). Comparing General Practitioner Assessment of Cognition, Mini-Cognitive Assessment Instrument were significantly higher in the pioglitazone-treated group in relation to the same group before treatment and untreated diabetic with AD group but was significantly lower

Figure 3



Insulin and HOMA-insulin resistance index of the studied groups. HOMA, homeostasis model of assessment.

Figure 4



Glycated hemoglobin (HBA1C) of the studied groups.

in relation to control group and diabetics without AD (Table 3 and Fig. 8).

Serum lipid profile of the studied groups

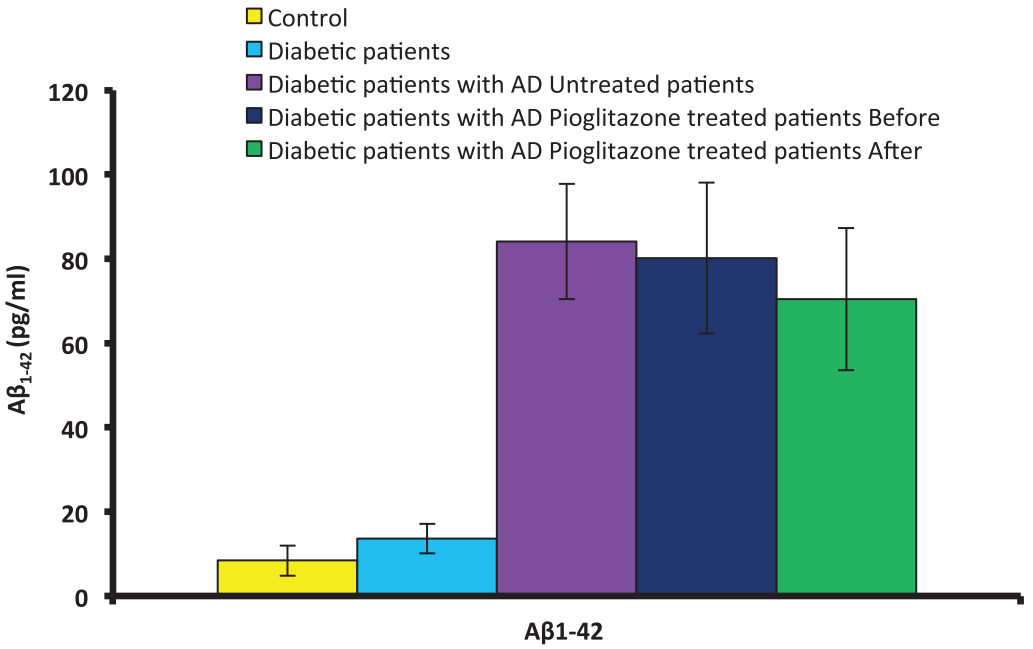
Triglycerides were significantly lower in the pioglitazone-treated group in relation to diabetic patients with AD without treatment and before treatment in the same group. It was significantly higher in pioglitazone-treated group in relation to control group (Table 4 and Fig. 9). Cholesterol was significantly lower in pioglitazone-treated group in relation to diabetic patients without AD, diabetic

Table 2 Serum levels of amyloid β protein peptide (1–42) high-sensitivity C-reactive protein in the studied groups

			Diabetic patients with AD		
				Pioglitazone-treated patients (*n*=20)	
Parameters	Control (*n*=20)	Diabetic patients (*n*=20)	Pioglitazone untreated patients (*n*=10)	Before	After
$A\beta_{1-42}$ (pg/ml)	8.4±3.6	13.6±3.5	84.1±13.7[a,b]	80.2±17.9[a,b]	70.4±16.9[a,b,c]
hs-CRP (mg/dl)	1.8±0.47	3.0±0.46[a]	3.9±0.72[a]	4.0±1.1[a,b]	3.6±0.81[a,b]

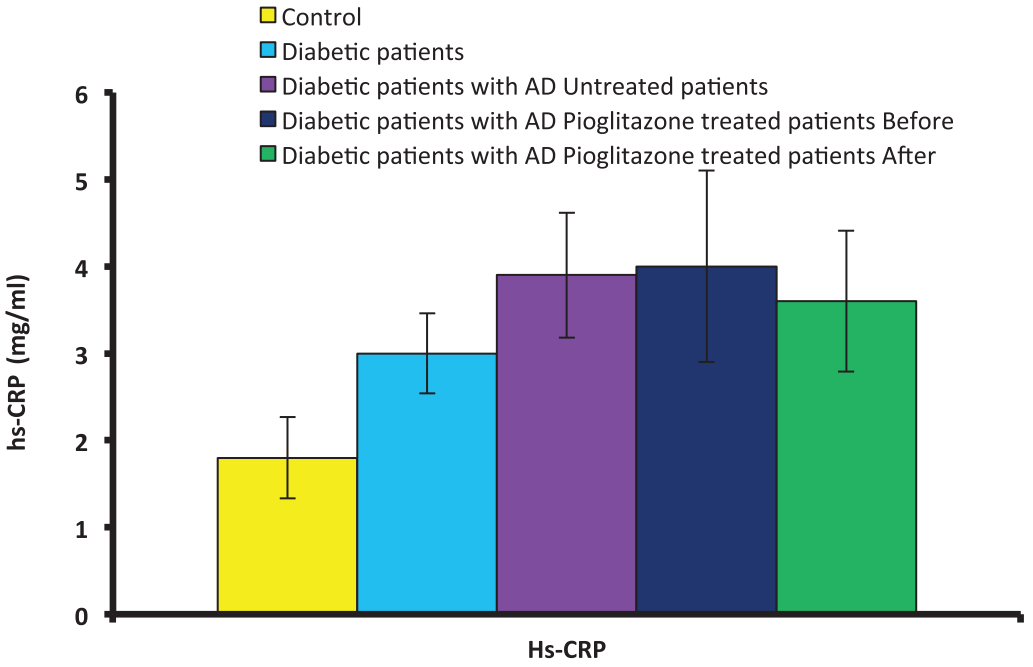
Data are presented as mean±SD. AD, Alzheimer disease; $A\beta_{1-42}$, amyloid β protein peptide (1–42); ANOVA, analysis of variance; hs-CRP, high-sensitivity C-reactive protein. [a]Significantly different from control group by ANOVA. [b]Significantly different from diabetic patients by ANOVA. [c]Significantly different from untreated diabetic patients with AD by ANOVA. [d]Significantly different from pioglitazone treated patients before treatment by paired *t* test.

Figure 5



Serum level of amyloid β peptide 1–42 ($A\beta_{1-42}$) of the studied groups.

Figure 6



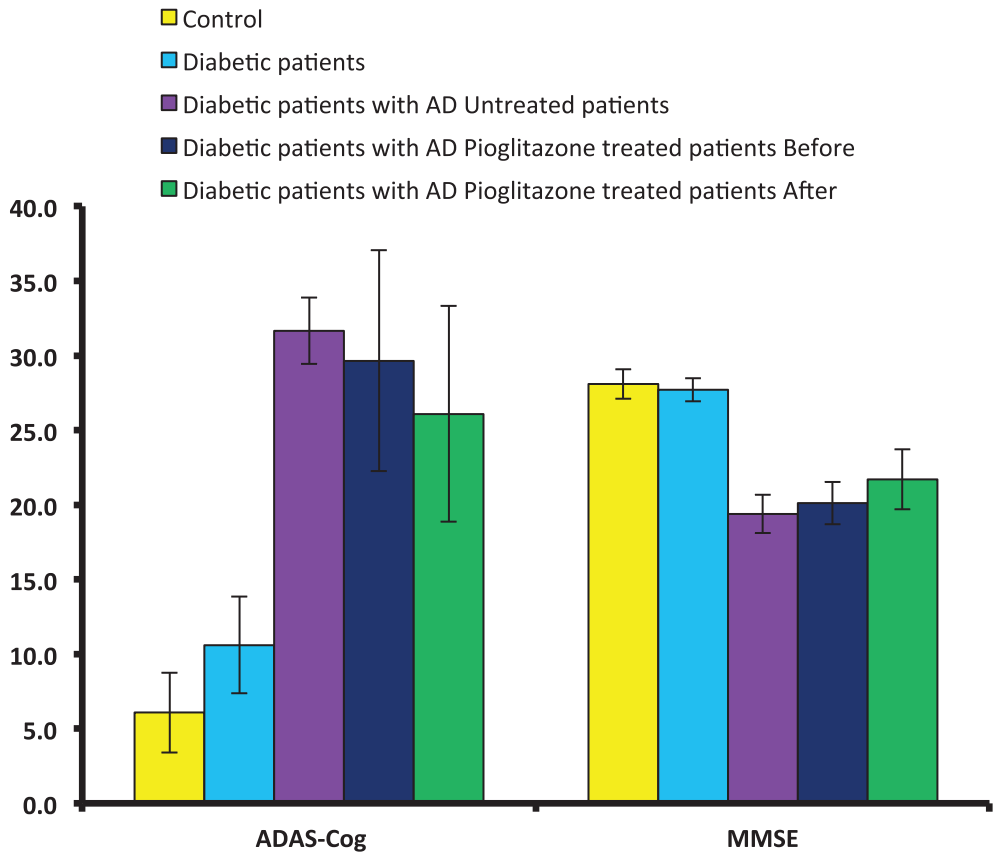
Serum level of high-sensitivity C-reactive protein (hs-CRP) of the studied groups.

Table 3 Behavioral tests of the studied groups

			Diabetic patients with AD		
				Pioglitazone-treated patients (*n*=20)	
Parameters	Control (*n*=20)	Diabetic patients (*n*=20)	Pioglitazone untreated patients (*n*=10)	Before	After
GPCOG	8.3±0.47	8.2±0.59	2.5±0.61[a,b]	2.65±0.75[a,b]	3.7±0.80[a,b,c,d]
Mini-Cog	4.4±0.40	4.6±0.42	1.5±0.43[a,b]	1.8±0.91[a,b]	2.6±1.02[a,b,c,d]
ADAS-Cog	6.1±2.7	10.6±3.2	31.7±2.2[a,b]	29.7±7.4[a,b]	26.1±7.2[a,b,c]
MMSE	28.1±1.0	27.7±0.77	19.4±1.29[a,b]	20.1±1.42[a,b]	21.7±2.05[a,b,c,d]

Data are presented as mean±SD. AD, Alzheimer disease; ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive; ANOVA, analysis of variance; GPCOG, General Practitioner Assessment of Cognition; Mini-Cog, Mini-Cognitive Assessment Instrument; MMSE, Folstein Mini-Mental Status Examination. [a]Significantly different from the control group by ANOVA. [b]Significantly different from diabetic patients by ANOVA. [c]Significantly different from untreated diabetic patients with AD by ANOVA. [d]Significantly different from pioglitazone-treated patients before treatment by paired *t* test.

Figure 7



ADAS-Cog and MMSE psychological test of the studied groups. ADAS-Cog, Alzheimer's Disease Assessment Scale-Cognitive; MMSE, Folstein Mini-Mental Status Examination.

patients with AD, and before treatment. Cholesterol was significantly higher in the pioglitazone-treated group in relation to the control group (Table 4 and Fig. 9).

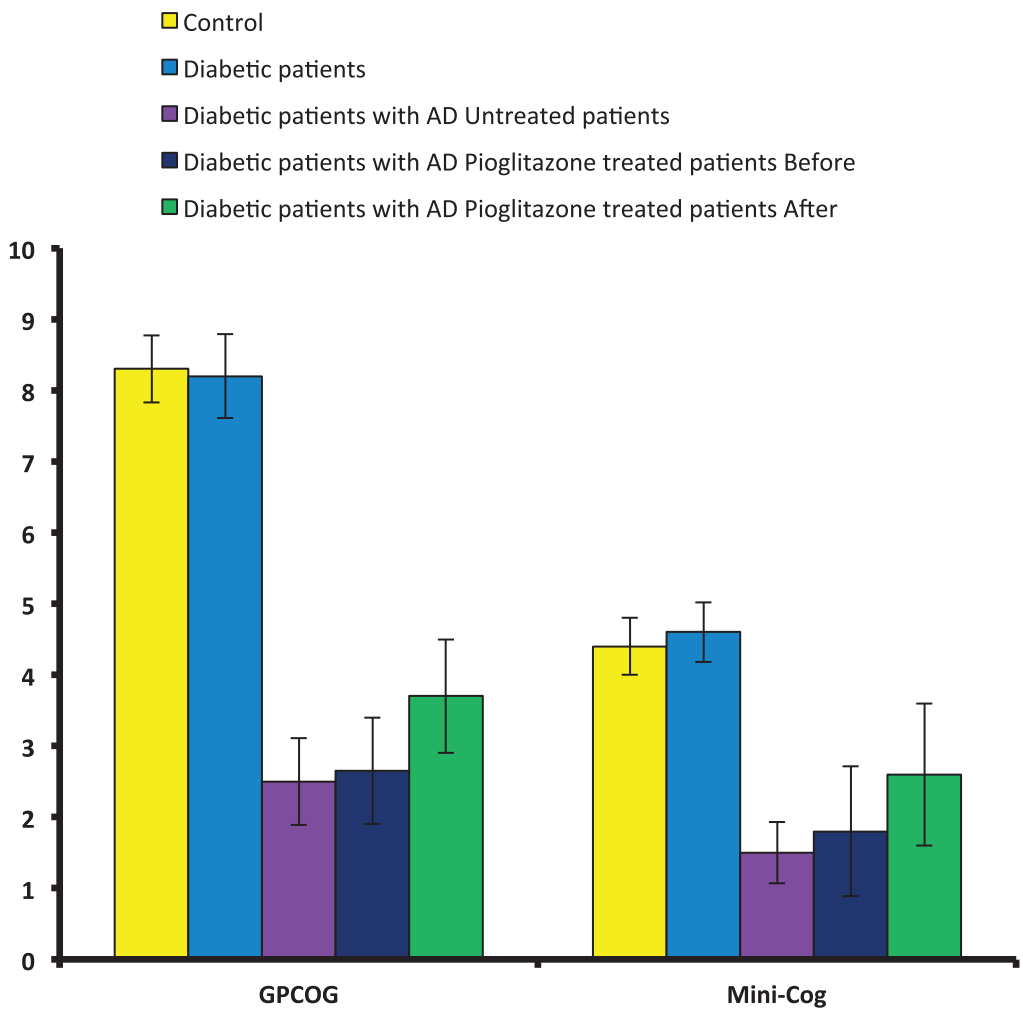
HDL-cholesterol was significantly higher in the pioglitazone-treated group in relation to diabetic patients without AD and untreated diabetic patients with AD, but it was significantly lower in the pioglitazone-treated group in relation to the control group (Table 4 and Fig. 10). LDL-cholesterol was significantly lower in the pioglitazone-treated group in relation to diabetic patients without AD, untreated diabetic patients with AD, and before treatment but it was significantly higher in the pioglitazone-treated group in relation to the control group (Table 4 and Fig. 10).

Experimental results

Body weight

The results of body weight are summarized in Table 5 and Fig. 10. T2DM rats maintained on HFD showed significantly higher body weight than control rats while AD rats showed lower body weight than diabetic rats.

Figure 8



GPCOG and Mini-Cog psychological test of the studied groups. GPCOG, General Practitioner Assessment of Cognition; Mini-Cog, Mini-Cognitive Assessment Instrument.

Table 4 Serum lipid profile of the studied groups

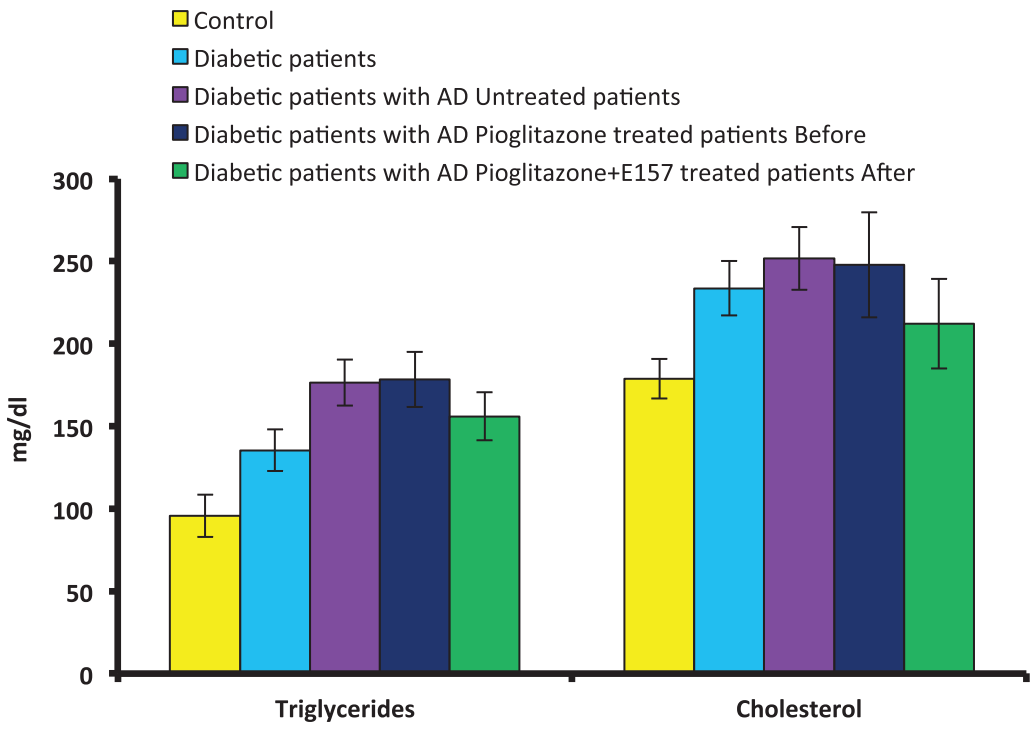
			Diabetic patients with AD		
				Pioglitazone-treated patients (*n*=20)	
Parameters	Control (*n*=20)	Diabetic patients (*n*=20)	Pioglitazone untreated patients (*n*=10)	Before	After
Triglycerides (mg/dl)	95.6±12.9	135.3±12.6[a]	176.4±13.9[a,b]	178.2±16.7[a,b]	155.9±14.6[a,c,d]
Cholesterol (mg/dl)	178.8±12.0	233.6±16.5[a]	251.7±9.1[a]	247.7±31.8[a]	212.2±27.2[a,b,c,d]
HDL-cholesterol (mg/dl)	58.1±5.5	42.2±3.4[a]	41.2±6.4[a]	43.4±3.8[a]	47.4±2.7[a,b,c]
LDL-cholesterol (mg/dl)	101.6±14.1	164.3±17.9[a]	175.1±11.5[a]	168.6±31.8[a]	133.6±25.5[a,b,c,d]

Data are presented as mean±SD. AD, Alzheimer disease; ANOVA, analysis of variance; HDL, high-density lipoprotein; LDL, low-density lipoprotein. [a]Significantly different from the control group by ANOVA. [b]Significantly different from diabetic patients by ANOVA. [c]Significantly different from untreated diabetic patients with AD by ANOVA. [d]Significantly different from pioglitazone treated patients before treatment by paired *t* test.

The treatment of AD rats with pioglitazone results in significant increase in body weight compared with untreated rats (Table 5 and Fig. 11).

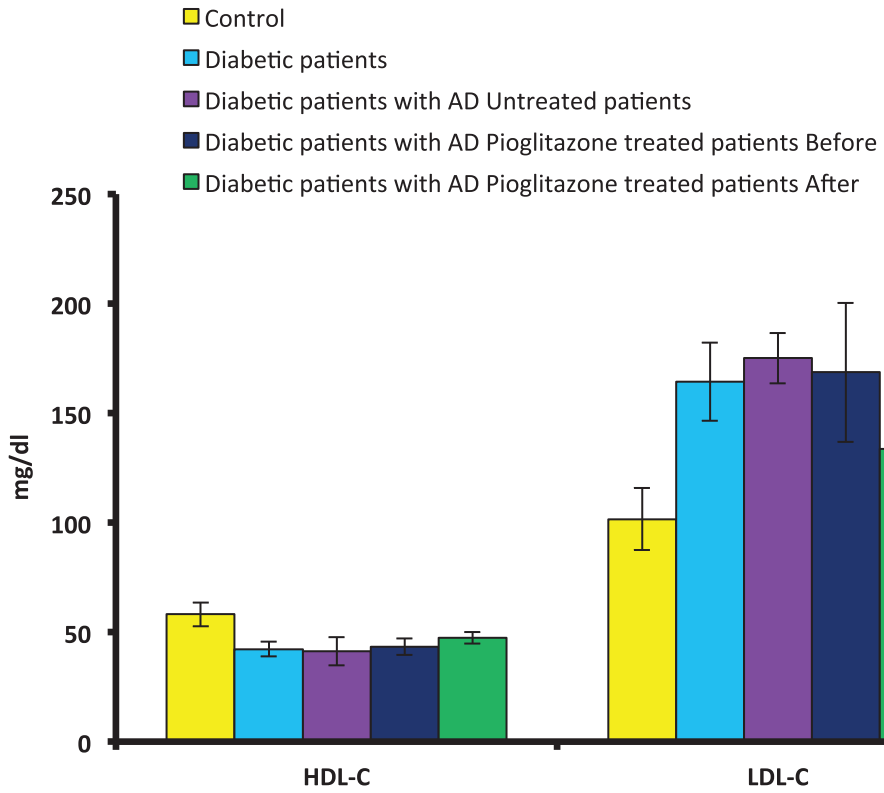
FBS was significantly lower in pioglitazone-treated rats than FBS in diabetic rats without AD and untreated diabetic rats with AD but FBS was significantly higher

Figure 9



Serum level of triglycerides and total cholesterol of the studied groups.

Figure 10



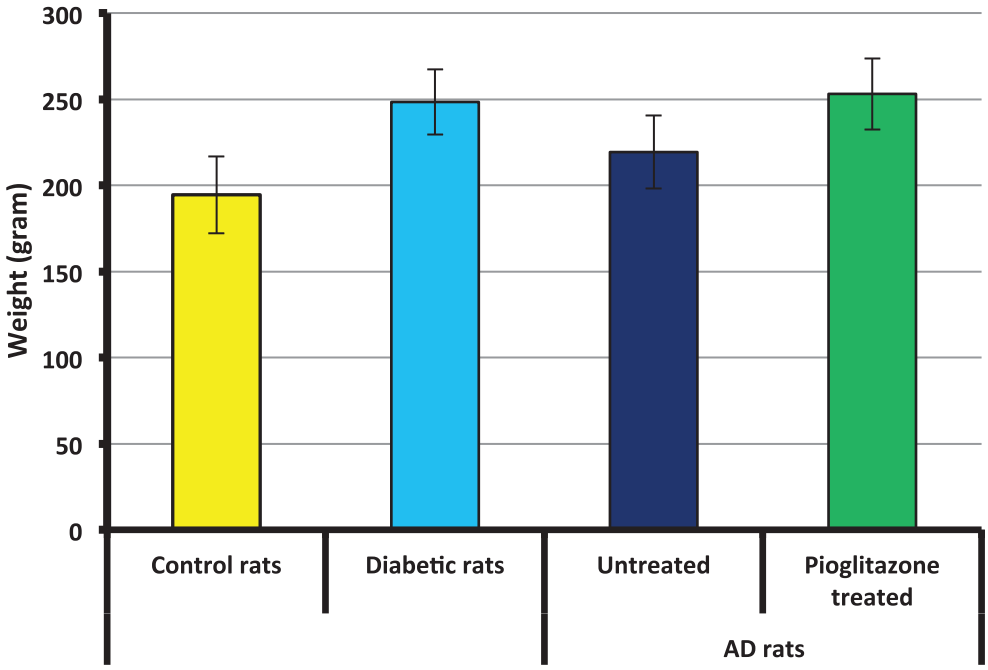
Serum level of HDL cholesterol and LDL cholesterol of the studied groups. HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 5 Body weight (g) and glucose homeostasis parameters of studied groups of rats

Parameters	Control rats	Diabetic rats	AD rats	
			Untreated	Pioglitazone treated
Body weight (g)	194.6±22.4	248.5±18.9[a]	219.4±21.2[a]	253.2±20.7[a]
FBS (mg/dl)	85.0±11.2	237.1±28.1[a]	306.1±37.9[a,b]	149.4±13.3[a,b,c]
Insulin (mIU/ml)	3.97±0.63	7.31±1.17[a]	7.26±0.74[a]	4.49±0.67[b,c]
HOMA-insulin resistance index	0.83±0.17	4.27±0.79[a]	5.50±0.97[a]	1.65±0.24[a,b,c]

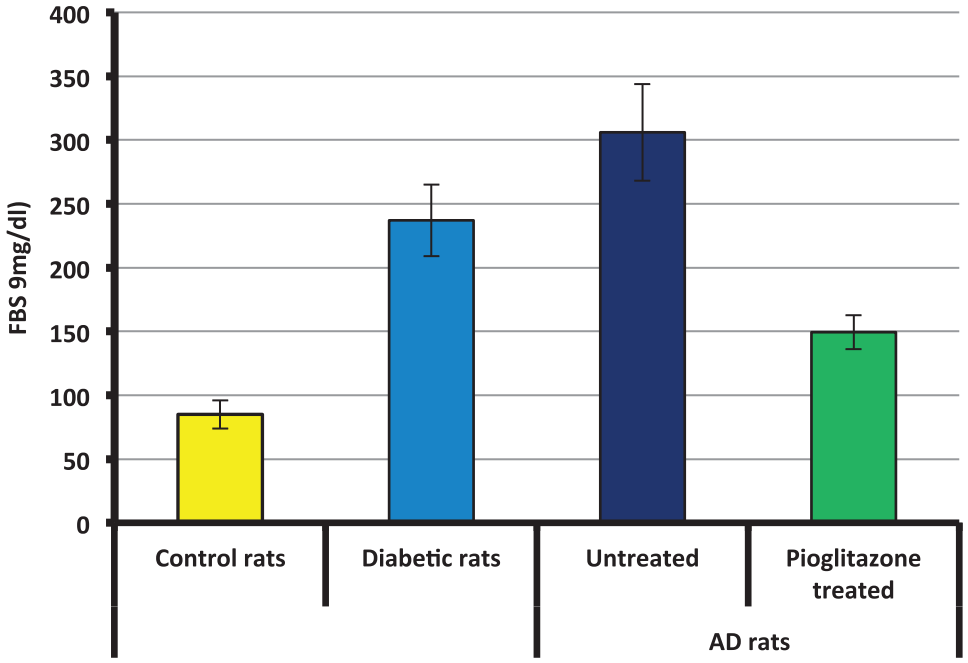
Data are presented as mean±SD. AD, Alzheimer disease; ANOVA, analysis of variance; FBS, fasting blood sugar; HOMA, homeostasis model of assessment. [a]Significantly different from control rats by ANOVA. [b]Significantly different from diabetic rats by ANOVA. [c]Significantly different from AD rats by ANOVA.

Figure 11



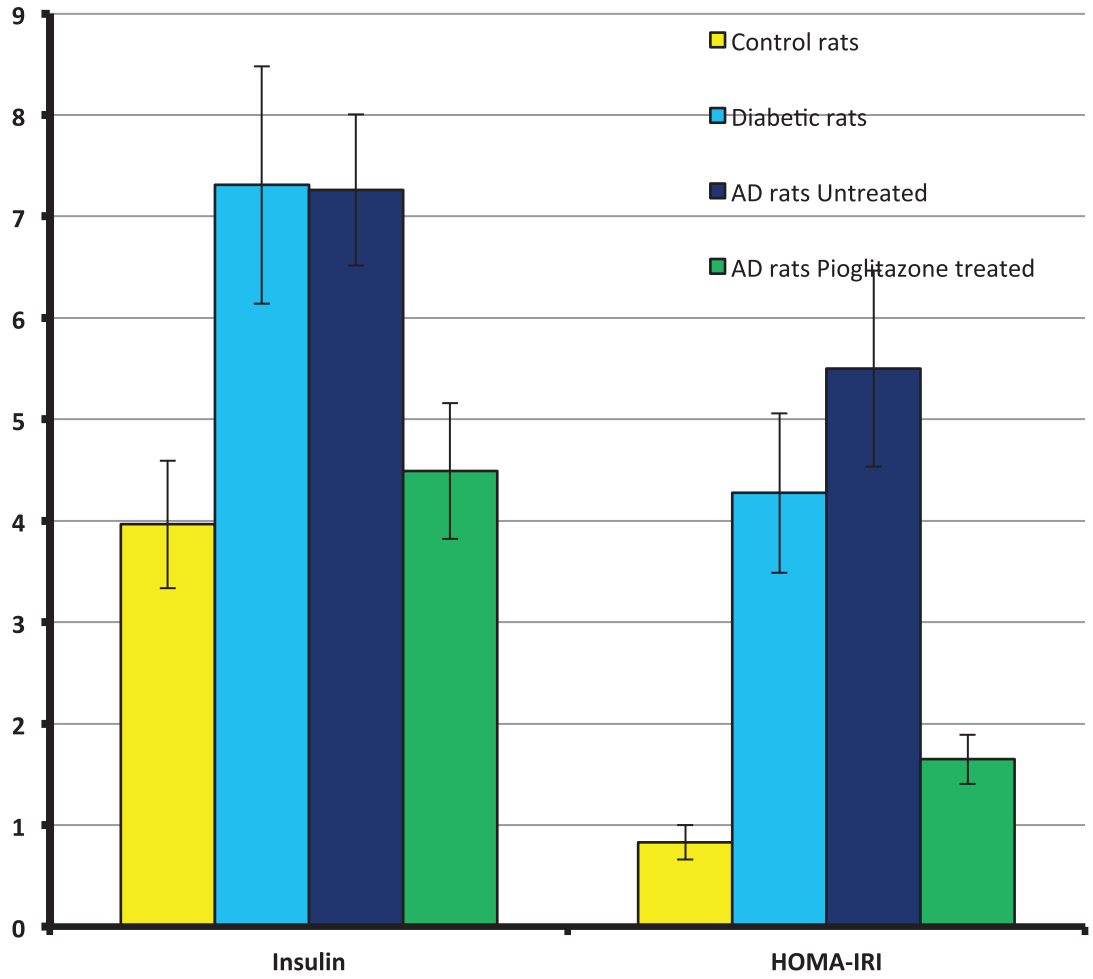
Body weight of control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Figure 12



Fasting blood sugar of control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Figure 13



Serum insulin level (μIU/ml) and HOMA-insulin resistance index of control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer disease; HOMA, homeostasis model of assessment.

Table 6 Serum levels of high-sensitivity C-reactive protein and brain content of amyloid β protein peptide (1–42) and Nrf2 in the studied groups of rats

Parameters	Control rats	Diabetic rats	AD rats	
			Pioglitazone untreated	Pioglitazone treated
hs-CRP (mg/ml)	2.94±0.61	10.14±1.36[a]	19.90±2.49[a,b]	14.82±14.82[a,b,c]
$A\beta_{1-42}$ (ng/mg protein)	19.8±3.8	28.5±2.8[a]	52.3±8.4[a,b]	37.7±4.7[a,b,c]
Nrf2 (μg/mg protein)	4.1±0.63	2.2±0.24[a]	2.1±0.29[a]	2.6±0.31[a,c]

Data are presented as mean±SD. AD, Alzheimer disease; $A\beta_{1-42}$, amyloid β-protein peptide (1–42); ANOVA, analysis of variance; hs-CRP, high-sensitivity C-reactive protein. [a]Significantly different from control rats by ANOVA. [b]Significantly different from diabetic rats by ANOVA. [c]Significantly different from AD rats by ANOVA.

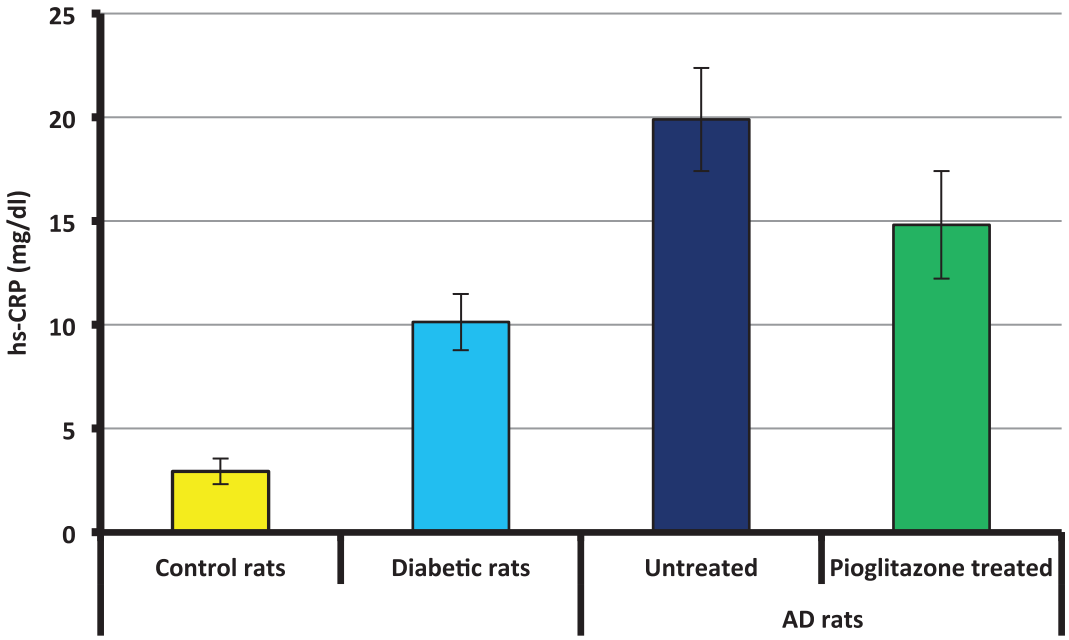
in pioglitazone-treated rats than in control rats (Table 5 and Fig. 12).

Insulin was significantly lower in pioglitazone-treated rats than in diabetic rats without AD and untreated diabetic rats with AD, but there is no significant difference between pioglitazone-treated rats and control. HOMA-insulin resistance index was significantly lower in pioglitazone treated rats than in diabetic rats without AD and untreated diabetic rats with AD, but it was significantly higher in pioglitazone-treated rats than in control rats (Table 5 and Fig. 13).

Serum levels of high-sensitivity C-reactive protein and brain content of amyloid β-protein peptide (1–42) and Nrf2 in the studied groups of rats

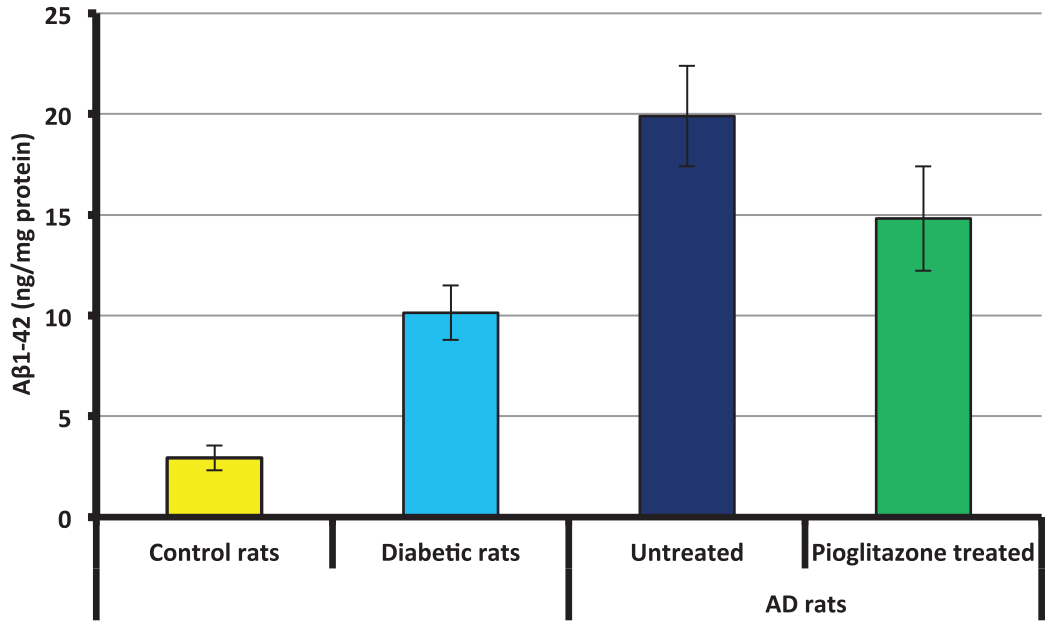
hs-CRP was significantly lower in pioglitazone-treated rats in comparison to untreated diabetic rats with AD, but significantly higher in relation to control and diabetic rats without AD (Table 6 and Fig. 14). $A\beta_{1-42}$ was significantly lower in pioglitazone-

Figure 14



Serum level of hs-CRP of control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease; hs-CRP, high-sensitivity C-reactive protein.

Figure 15



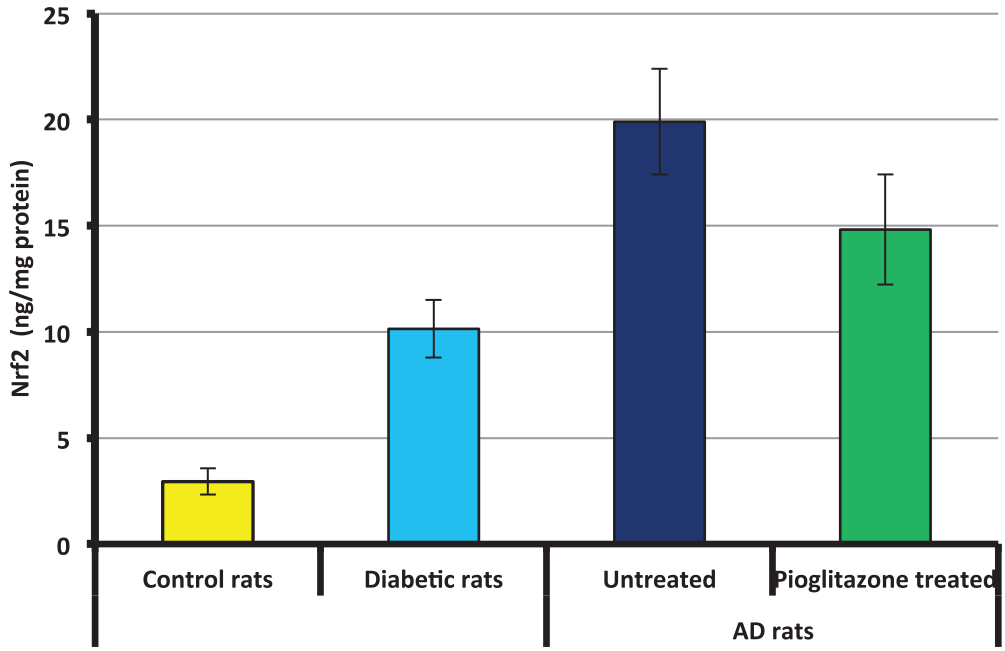
Brain level of $A\beta_{1-42}$ of control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease; $A\beta_{1-42}$, amyloid β protein peptide (1–42).

treated rats in relation to untreated diabetic rats with AD, but significantly higher in relation to control and diabetic rats without AD (Table 6 and Fig. 15). Nrf2 was significantly lower in pioglitazone-treated rats in relation to control rats, but it was significantly higher in relation to diabetic untreated AD rats (Table 6 and Fig. 16).

Lipid profile of the studied groups of rats

Triglycerides were significantly lower in pioglitazone-treated diabetic rats with AD in relation to diabetic rats without AD and untreated AD diabetic rats, but in comparing pioglitazone-treated diabetic rats with AD and control it was significantly higher (Table 7 and Fig. 17). Total cholesterol was significantly lower

Figure 16



Brain level of Nrf2 control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Table 7 Lipid profile of the studied groups of rats

Parameters	Control rats	Diabetic rats	AD rats	
			Pioglitazone untreated	Pioglitazone treated
Triglycerides (mg/dl)	49.4±7.1	135.7±9.1[a]	147.2±6.2[a,b]	108.6±10.6[a,b,c]
Total cholesterol (mg/dl)	122.2±12.7	168.0±9.3[a]	175.4±12.1[a]	155.3±6.6[a,c]
HDL-cholesterol (mg/dl)	36.8±2.5	29.2±3.5[a]	28.3±3.0[a]	31.9±3.6[a]
LDL-cholesterol (mg/dl)	75.5±15.6	111.7±9.5[a]	117.7±13.8[a]	101.6±7.2[a,c]

Data are presented as mean±SD. AD, Alzheimer disease; ANOVA, analysis of variance; HDL, high-density lipoprotein; LDL, low-density lipoprotein. [a]Significantly different from control rats by ANOVA. [b]Significantly different from diabetic rats by ANOVA. [c]Significantly different from AD rats by ANOVA.

in pioglitazone-treated diabetic rats with AD in relation to untreated AD diabetic rats but it was significantly higher in pioglitazone-treated diabetic rats with AD in relation to control rats (Table 7 and Fig. 17). HDL-cholesterol was significantly lower in pioglitazone-treated diabetic rats with AD in relation to control rats (Table 7 and Fig. 18). LDL-cholesterol was significantly lower in pioglitazone-treated diabetic rats with AD in relation to untreated AD diabetic rats, but it was significantly higher in pioglitazone-treated diabetic rats with AD in relation to control rats (Table 7 and Fig. 18).

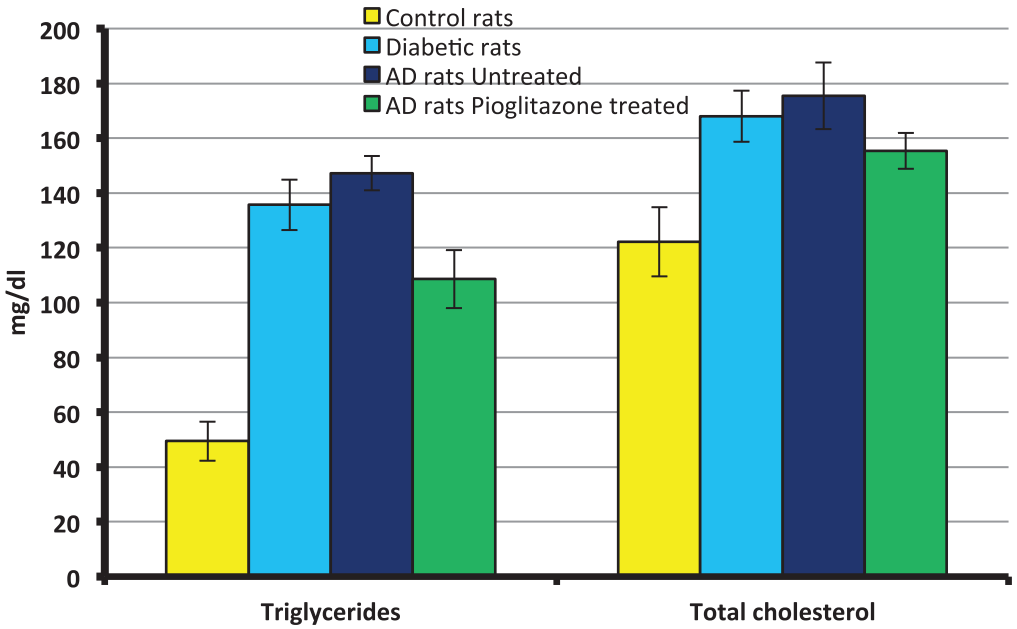
Behavioral tests of the studied groups of rats

Object recognition test (discrimination index) was significantly lower in pioglitazone-treated diabetic rats with AD in comparison to control rats and diabetic rats without AD, but it was significantly higher in pioglitazone-treated diabetic rats with AD in comparison to untreated diabetic rats with AD (Table 8 and Fig. 19). Morris water maze (MWM) (trailing trail, s) in day 3 was significantly higher in pioglitazone-treated diabetic rats with AD in comparison to control rats and diabetic rats without AD, but it was significantly lower in pioglitazone-treated diabetic rats with AD in comparison to untreated diabetic rats with AD (Table 8 and Fig. 20). MWM (probe trail, %) was significantly lower in pioglitazone-treated diabetic rats with AD in comparison to control rats and diabetic rats without AD, but it was significantly higher in pioglitazone-treated diabetic rats with AD in comparison to untreated diabetic rats with AD (Table 8 and Fig. 21).

Insulin signaling parameters

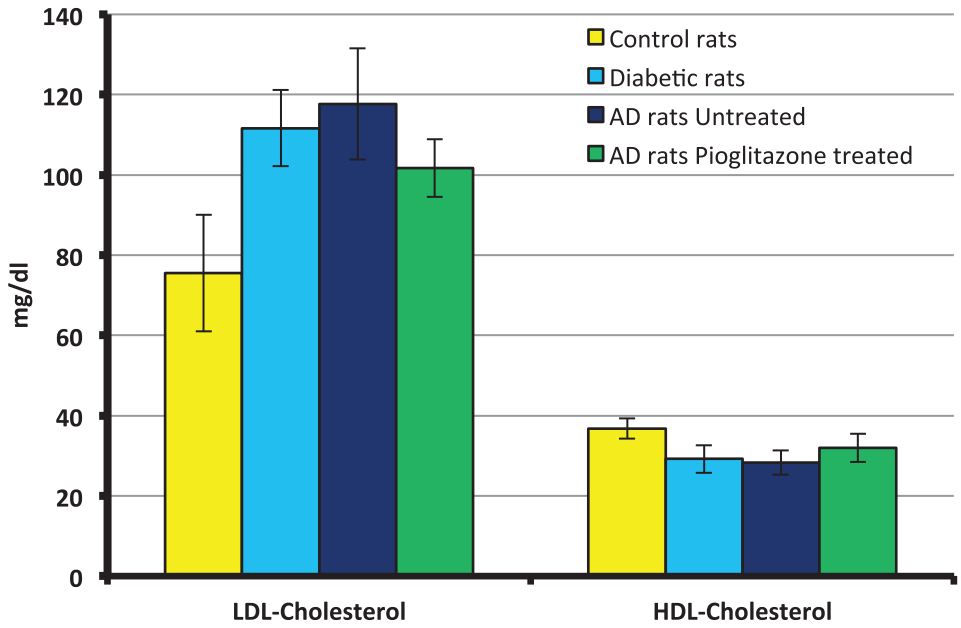
IR was significantly higher in pioglitazone-treated diabetic rats with AD in relation to diabetic rats without AD and untreated diabetic rats with AD, but it was significantly lower in pioglitazone-treated diabetic rats with AD in comparison to control (Table 9 and Fig. 22). P-IR was significantly higher

Figure 17



Serum triglycerides and total cholesterol levels in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Figure 18



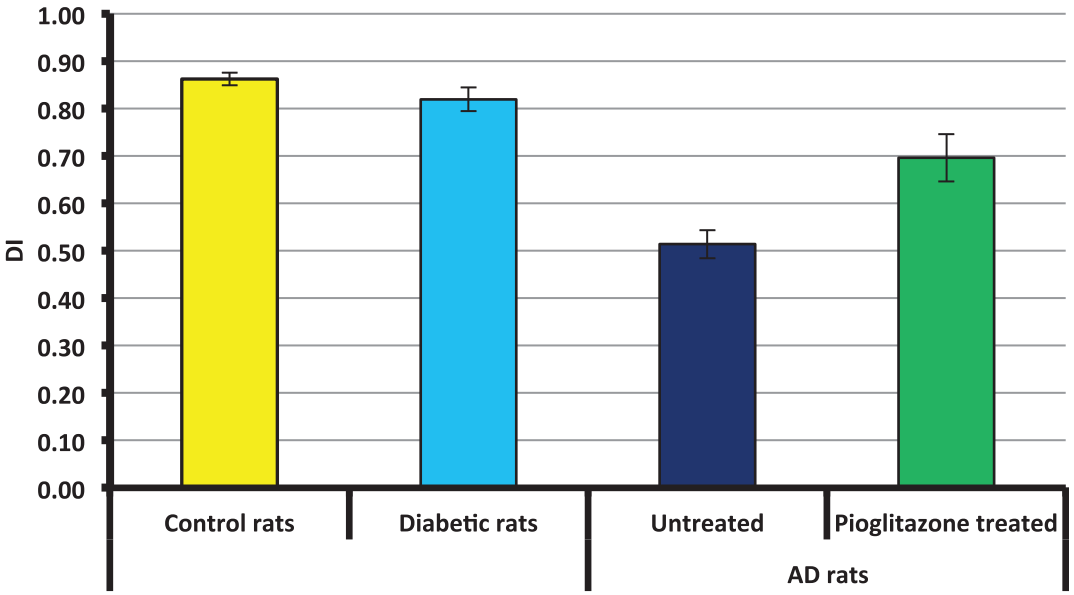
Serum LDL-cholesterol and HDL-cholesterol levels in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer disease; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 8 Behavioral tests of the studied groups of rats

Parameters	Control rats	Diabetic rats	AD rats	
			Pioglitazone untreated	Pioglitazone treated
Object recognition test (discrimination index)	0.86±0.01	0.82±0.03[a]	0.51±0.03[a,b]	0.70±0.05[a,b,c]
MWM (trailing trail, s)				
Day 1	43.4±7.2	45.3±9.1	52.6±8.7	50.4±8.9
Day2	32.0±6.9	35.2±8.5	52.0±6.9[a,b]	44.9±8.1[a,b]
Day3	22.3±3.7	27.2±6.8	52.1±7.4[a,b]	39.6±7.6[a,b,c]
MWM (probe trail, %)	44.4±4.27	40.20±2.57	26.00±2.67[a,b]	35.30±5.19[a,b,c]

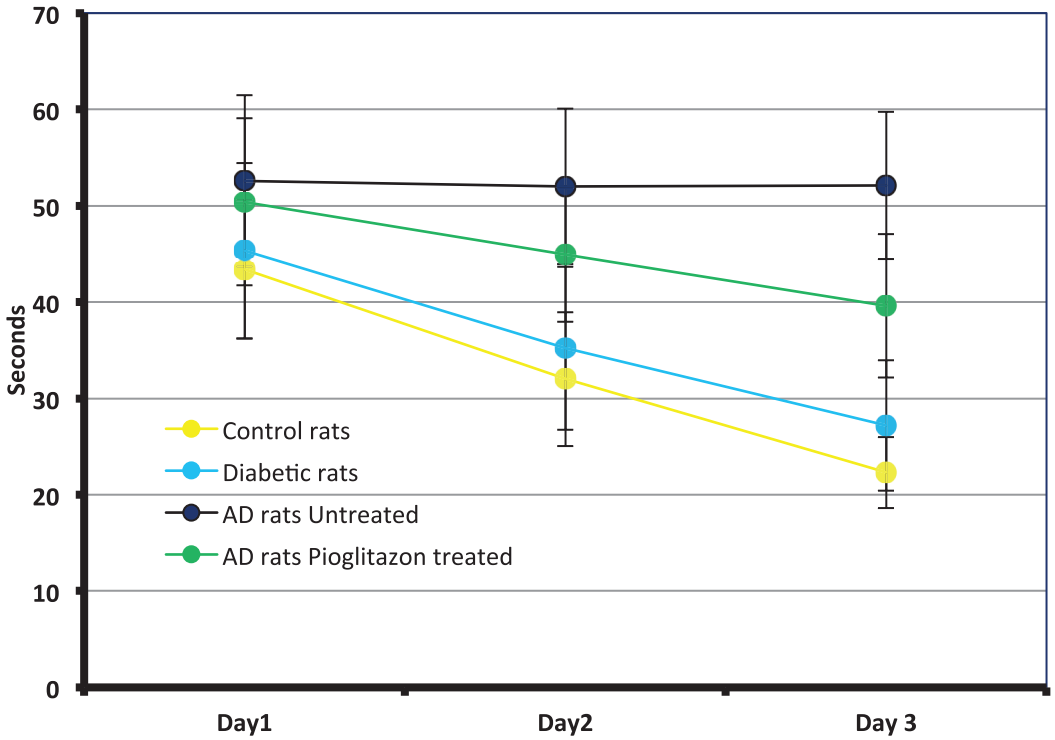
Data are presented as mean±SD. AD, Alzheimer disease; ANOVA, analysis of variance. [a]Significantly different from control rats by ANOVA. [b]Significantly different from diabetic rats by ANOVA. [c]Significantly different from AD rats by ANOVA.

Figure 19



Object recognition test of control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

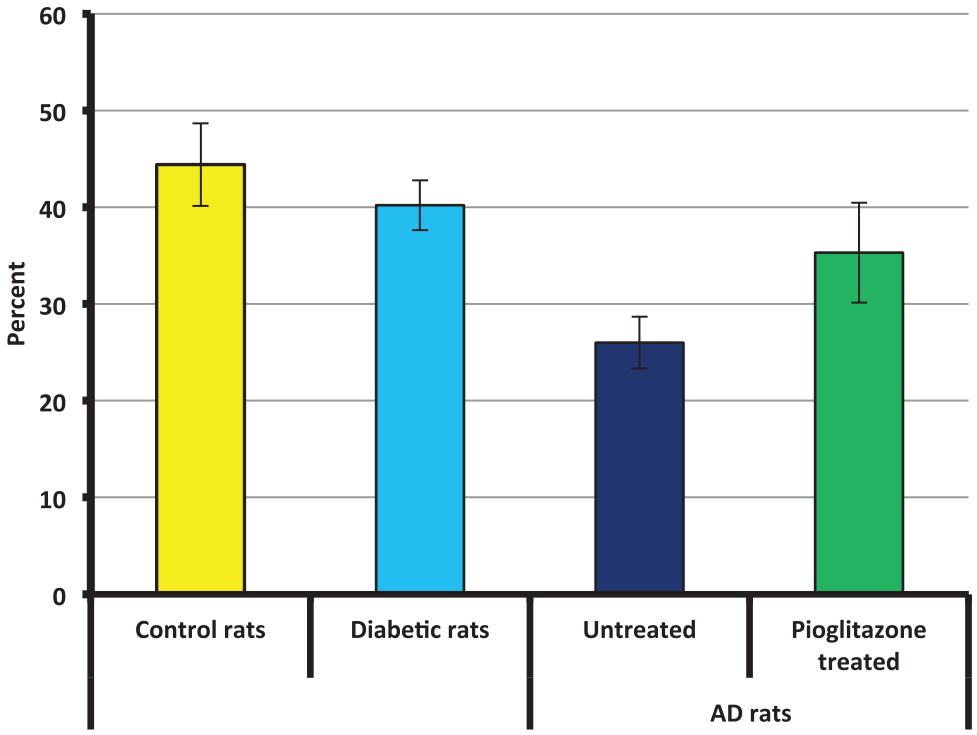
Figure 20



MWM (trailing) test in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

in pioglitazone-treated diabetic rats with AD in relation to untreated diabetic rats with AD, but it was significantly lower in pioglitazone-treated diabetic rats with AD in relation to control (Table 9 and Fig. 23). GLUT-3 was significantly higher in pioglitazone-treated diabetic rats with AD in comparison to diabetic rats without AD and untreated diabetic rats with AD, but it was significantly lower in pioglitazone-treated diabetic rats with AD in comparison to control (Table 9 and Fig. 24). P-Akt (Thr308) was significantly higher in pioglitazone-treated diabetic rats with AD in relation to untreated diabetic rats with AD, but it was significantly lower in pioglitazone-treated diabetic rats with AD in relation to control (Table 9 and Fig. 25).

Figure 21



MWM (probe trail) test in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Table 9 Insulin signaling parameters

Parameters	Control rats	Diabetic rats	AD rats	
			Pioglitazone untreated	Pioglitazone treated
Insulin receptor (μg/mg protein)	0.46±0.06	0.32±0.03[a]	0.27±0.03[a,b]	0.39±0.03[a,b,c]
Phospho-insulin receptor (U/mg protein)	52.52±10.75	35.32±4.58[a]	25.77±3.87[a,b]	36.09±3.70[a,c]
GLUT-3 (μg/mg protein)	0.41±0.06	0.21±0.04[a]	0.14±0.03[a,b]	0.31±0.04[a,b,c]
P-Akt (Thr308) (U/mg protein)	53.1±5.10	35.1±4.59[a]	27.9±3.35[a,b]	40.2±3.94[a,c]

Data are presented as mean±SD. AD, Alzheimer disease; ANOVA, analysis of variance. [a]Significantly different from control rats by ANOVA. [b]Significantly different from diabetic rats by ANOVA. [c]Significantly different from AD rats by ANOVA.

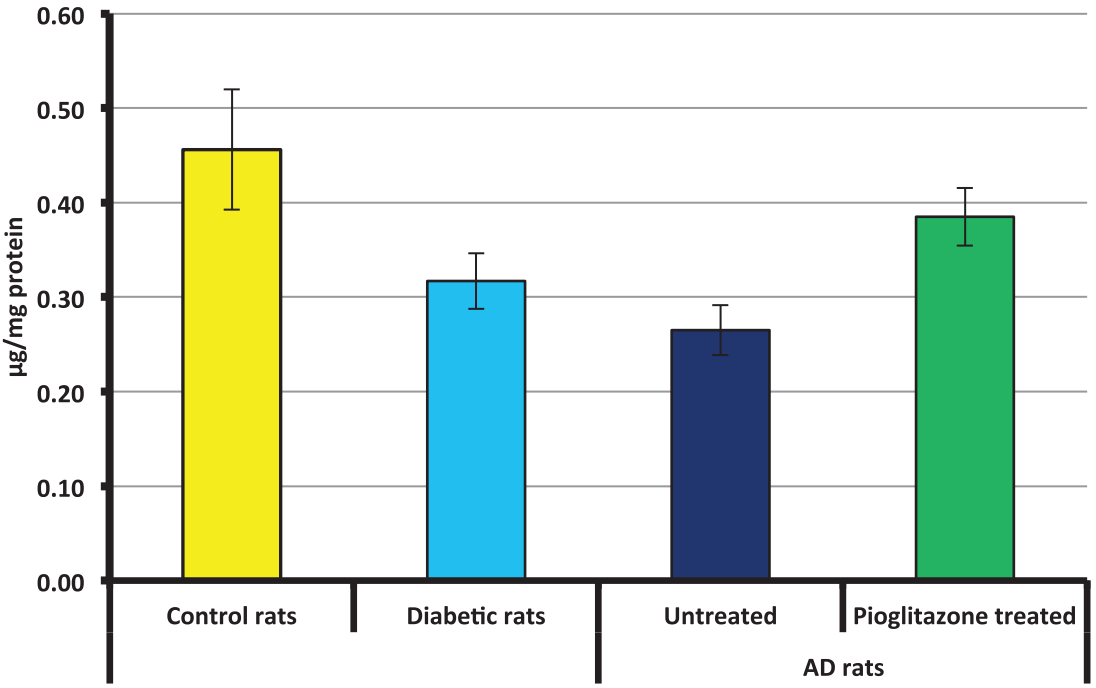
Discussion

In the human section of the present study, it was found that pioglitazone improves cognition of Alzheimer's due to its multiple positive benefits including increasing insulin sensitivity, improving lipid profiles, and acting as an anti-inflammatory agent. The finding demonstrates the strong relationship between insulin resistance in diabetes type 2 and AD. Pioglitazone in the human section was found to have a positive neurometabolic effect that can be attributed to the effect of pioglitazone on several glucose parameters including FBS, postprandial blood sugar, the insulin level, and HOMA-insulin resistance index. In addition, pioglitazone has another positive neurometabolic effect explained by increased HDL-C and the decreased triglycerides, cholesterol, and LDL-C levels in the pioglitazone-treated group. Similarly, a pilot study with pioglitazone in AD patients with DM indicated that pioglitazone for 6 months improved cognition and cerebral blood flow in the parietal lobe compared with controls. Also, pioglitazone treatment resulted in a decrease in the fasting plasma insulin levels, indicating enhanced insulin sensitivity [25].

The study demonstrates the positive effects of pioglitazone on neuroinflammation in humans that appear in the form of decreasing hs-CRP level and $A\beta_{1-42}$ in the pioglitazone-treated diabetic AD patients in comparison to untreated diabetic AD patients.

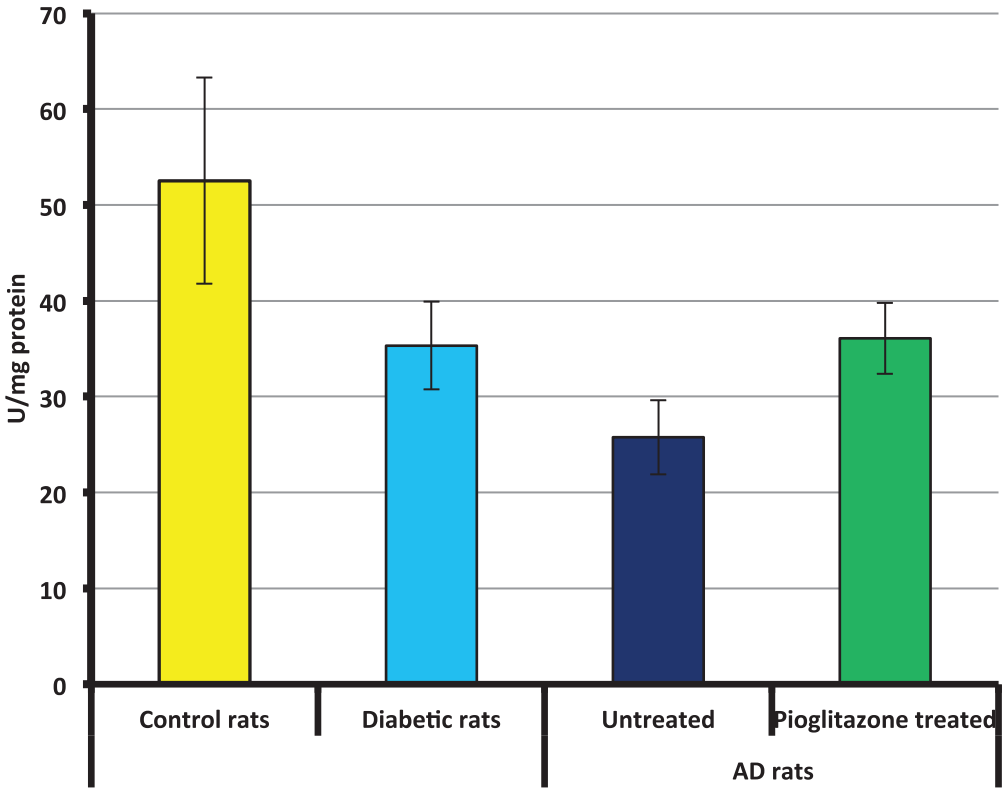
In the present study, pioglitazone was found to have a positive effect on cognitive deficit noted in improved different behavioral tests in the pioglitazone-treated diabetic AD. These findings are in agreement with the results of Cheng *et al.* [26] study in which a recent

Figure 22



Brain insulin receptor level in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Figure 23

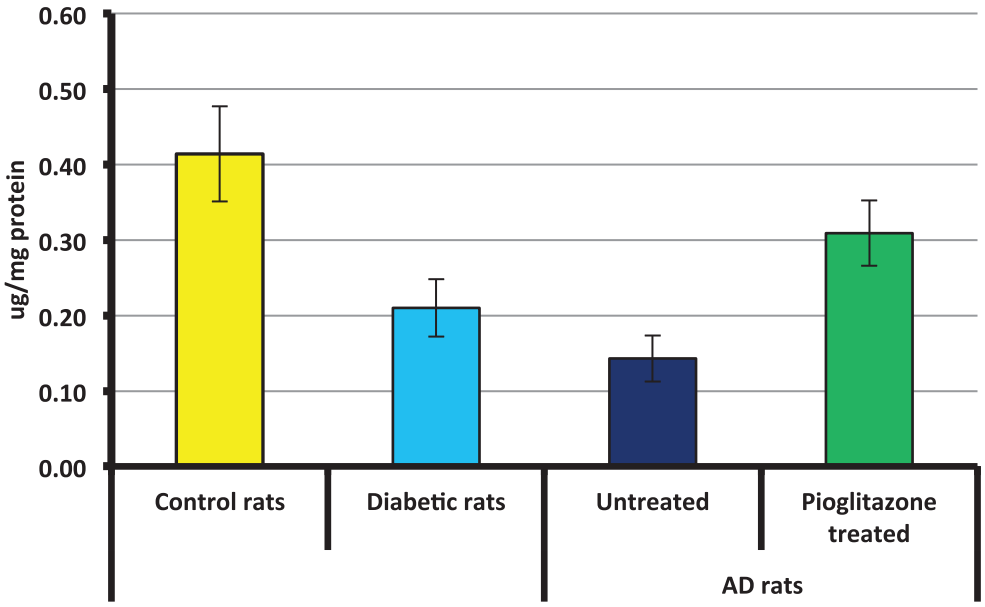


Brain content of P-IR (Tyr 1162/1163) in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease; P-IR, Phospho-insulin receptor.

meta-analysis on PPAR-γ agonists in AD suggests that only pioglitazone may offer an improvement in the early stages of AD and in mild-to-moderate AD.

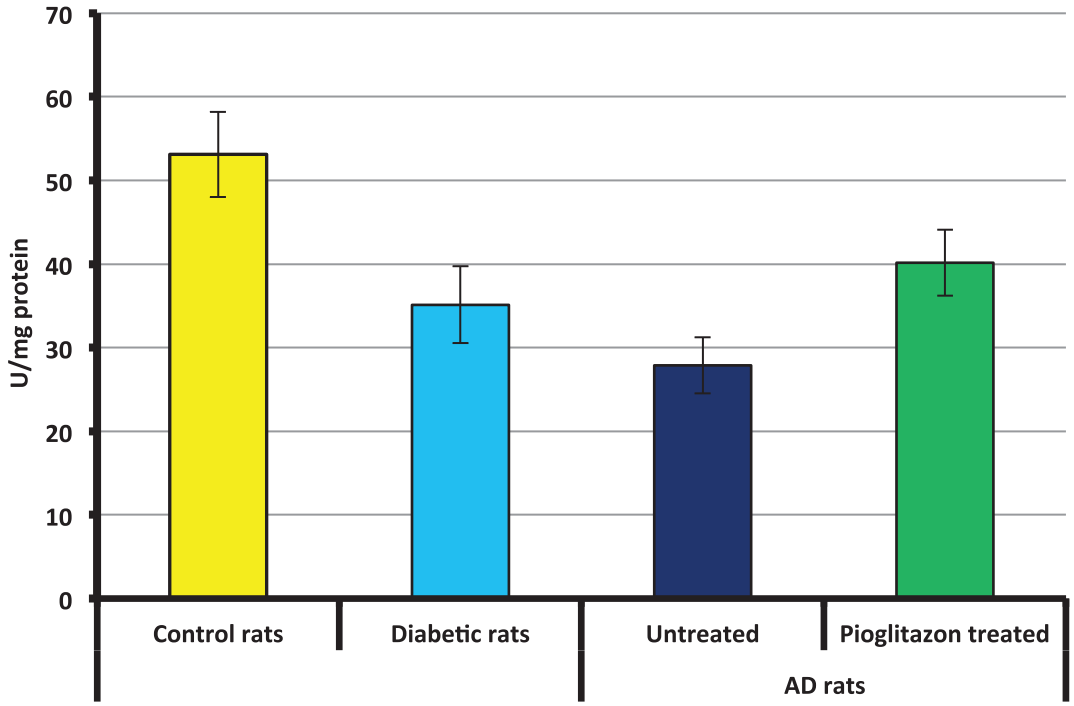
Since, we cannot assess the different receptor changes in a live human brain, we did an experimental section where we were able to access and assess the receptor

Figure 24



Brain content of GLUT-3 in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

Figure 25



Brain content of p-Akt in control rats, diabetic rats, and AD rats treated or untreated with pioglitazone. AD, Alzheimer's disease.

changes. The positive relationship was also confirmed again at the experimental level by using rats where induction of T2DM and AD was done.

In the experimental section, we found the same beneficial effect of pioglitazone as regards its positive neurometabolic and neuroinflammatory effects in treated diabetic AD rats in comparison to untreated diabetic AD rats. These effects were reflected in the decreased levels of hs-CRP, $A\beta_{1-42}$, and improved different glucose homeostasis parameters of treated diabetic AD rats.

Also, improved cognitive deficits were evident in pioglitazone-treated diabetic AD rats. The positive effect of pioglitazone appeared at different behavioral tests in comparison to untreated diabetic rats with AD.

These significant findings in the experimental section can be explained by the same changes occurred in the human section plus the improvement of insulin signaling pathways in the brain in the form of increased number of IR, GLUT-3, P-IR, and Phospho-Akt in pioglitazone-treated diabetic rats with AD when compared with untreated diabetic rat AD brains. The experimental findings were supported by a recent preclinical study in which Fernandez-Martos *et al*. [27] reported that pioglitazone showed beneficial effects on the preclinical APPswe/PS1dE9 mice model of familial AD improving cognition and decreasing Aβ levels.

A recent study in which Galimberti and Scarpini found a very relevant effect of the drug reversing the damage that neuroinflammation causes in the structural plasticity of the dendrites. Thus, it has been observed that treatment with pioglitazone can reverse the loss of synaptic density induced by Aβ peptide generation [28]. Another support for the current study finding was the Sato *et al*. [25] study which showed that pioglitazone treated 3×Tg-AD mice for 4 months was associated with improvement in brain spatial learning impairment, TAU hyperphosphorylation, and neuroinflammation.

Zhang and colleagues found that pioglitazone inhibits advanced glycation end product-induced matrix metalloproteinases and apoptosis by suppressing the activation of MAPK and NF-κB. In relationship to AD, the treatment with pioglitazone has been shown to reduce glial pro-inflammatory activity and the Aβ peptide levels due to the phagocytic activity of microglia [29].

In the Jahrling and colleagues study, pioglitazone has been shown to improve amyloid deposits. Moreover, these molecules can modify gene expression and restore both memory and cognition impairment in AD mouse models. Additionally, PPAR-γ stimulation also improves synapse density in cell cultures and reduces Aβ levels in AD transgenic mice [30].

Conclusion

Insulin resistance and inflammation associated with diabetes are risk factors for the development of AD and the usage of the insulin sensitizer, pioglitazone for 6 months at a dose of 15–30 mg/day was associated with positive neurometabolic as well as positive effects on neuroinflammation and a positive effect on cognition in humans. Pioglitazone was associated with positive neurometabolic, positive effects on neuroinflammation, positive effect on cognitive deficit, positive antioxidant effect, and in improving insulin signaling pathways in the brain of rats.

Financial support and sponsorship

Nil.

Conflicts of interest

Although Pioglitazone caused improvement in cognition and decreased Aβ levels a large studies for longer time and higher doses still needed to confirm our results.

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