

Analysis of the add-on effect of α -glucosidase inhibitor, acarbose in insulin therapy: A pilot study

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Abstract. The aim of the present study was to evaluate the add-on effect of acarbose therapy in oxidative stress, and the lipid and inflammatory profiles of patients with type 2 diabetes mellitus (T2DM) treated with insulin. This was an open and unblinded study. Patients (n=134) with T2DM (haemoglobin A_{1c} range, 9.0-12.0%) were recruited. After continuous subcutaneous insulin infusion for 7 days for initial rapid correction of hyperglycaemia, a premixed insulin titration period (duration, 4-6 days) subsequently followed. Patients were then randomized (1:1) into two groups as follows: An acarbose plus pre-mixed 30/70 insulin group or a pre-mixed 30/70 insulin only group; each group received treatment for 2 weeks. Plasma high-sensitivity C-reactive protein (Hs-CRP), 8-iso-prostaglandin F_{2 α} (8-iso PGF_{2 α}), tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , and IL-6 levels were measured before and after therapy. Patients that received acarbose plus insulin demonstrated greater reduction in 8-iso PGF_{2 α} , Hs-CRP, TNF- α , IL-1 β and IL-6 levels when compared with the insulin only patients. Thus, acarbose add-on insulin therapy was identified to be associated with greater improvements in oxidative stress and inflammation in patients with T2DM when compared with those that received insulin only therapy.

Introduction

Acarbose is an α -glucosidase inhibitor. It slows the breakdown of carbohydrates in the gut, and delays absorption of carbohydrates by inhibition of α -amylase and α -glucosidase activities, which reduces post-prandial hyperglycemia (1,2). The reduction of blood glucose concentration is accompanied by a decreased

insulin demand and increased insulin sensitivity in type 2 diabetes mellitus (T2DM) (3). Recently, by using a continuous glucose monitoring system (CGMS), further improvements in glucose fluctuation in T2DM patients treated with pre-mixed insulin therapy were observed (4). Microvascular and macrovascular complications are mainly (5,6) or partially (6,7) caused by hyperglycemia. Monnier *et al* (8) reported that acute glucose fluctuations during postprandial periods were crucial in oxidative stress. The rapid rise in postprandial blood glucose concentrations induces an overproduction of peroxynitrite and nitrotyrosine (8,9). As demonstrated by clinical trials, acarbose has been reported to improve postprandial endothelial dysfunction in newly diagnosed T2DM patients (10), as well as decreasing the lipid peroxidation and platelet activation in patients with T2DM (11). Furthermore, in patients with impaired glucose tolerance, acarbose was associated with a 49% relative risk reduction in the development of cardiovascular events (12).

A previous study demonstrated that exogenous hyperinsulinemia increases the activation of NAD(P)H in the rat aortic endothelium (13). However, at normal concentrations of insulin, the effect of insulin on activating oxidative stress is considered to be controversial (14). As insulin therapy is used for treating patients with T2DM worldwide, the administration of a combination of acarbose and insulin therapy is proposed.

Therefore an open and unblinded study was performed to investigate the add-on effect of acarbose on oxidative stress, and the lipid and inflammatory profiles in patients with T2DM treated with insulin.

Materials and methods

Patients. One hundred and thirty four patients (male: female, 67:67) with newly diagnosed T2DM were admitted to Nanjing First Hospital (Nanjing, China) between January, 2014 and May, 2015. Patients were aged 18-75 years with body mass index (BMI; calculated as weight in kilograms divided by the square of their height in meters), 18-40 kg/m² and hemoglobin A_{1c} (HbA_{1c}) range, 9.0-12.0%. Patients were excluded if they had acute or severe chronic diabetic complications, serious systemic disease or poor medication compliance. Patients with known cancers, known allergies to insulin or acarbose, or deemed not suitable to participate following an assessment

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by the researchers were excluded (15,16). The patients received therapy via continuous subcutaneous insulin infusion (CSII) for initial rapid correction of hyperglycaemia (defined as fasting plasma glucose between 7.0 and 8.0 mmol/l). Total daily doses for CSII were calculated as 0.4–0.6 IU/kg, and 50% of the total daily dose was administered as boluses with three meals at a fixed rate; the remaining insulin was administered over 24 h. Insulin doses were subsequently adapted according to blood glucose values that were obtained by self-monitoring. After rapid correction of hyperglycaemia, a premixed insulin titration period (duration, 4–6 days) subsequently followed. Patients were then randomized (1:1) into two groups: The acarbose plus insulin isophane protamine recombinant human insulin 30/70 (pre-mixed 30/70 insulin; twice-daily) group and a pre-mixed 30/70 insulin (twice-daily) only group. The initial pre-mixed 30/70 insulin doses were calculated according to the insulin dose used for the CSII, and subsequently adapted according to fasting capillary blood glucose and capillary blood glucose at 2 h after each of three meals (15). Investigators titrated the insulin doses on an individual patient basis with the titration algorithm (if the fasting blood glucose level was <4.4 mmol/l, the insulin dose was reduced by 2 units; if the fasting blood glucose level was within 4.4–6.1 mmol/l, the insulin dose was unchanged; if the fasting blood glucose level was within 6.2–7.8, 7.9–10.0, and >10.0 mmol/l, the insulin dose was subsequently increased by 2, 4, and 6 units, respectively). Pre-mixed insulin doses were unchanged and recorded if euglycemic control was achieved for two consecutive days. Patients were subsequently randomized to receive acarbose (100 mg, t.i.d; Glucobay; Bayer AG, Leverkusen, Germany) plus pre-mixed 30/70 (twice-daily) or pre-mixed insulin 30/70 (twice-daily) only. Treatment was maintained for 2 weeks. CGMS data were obtained using Medtronic MiniMed CGMS Gold (Medtronic Incorporated, Northridge, USA) for at least 3 days on completion of 2 weeks randomized treatment (17). All patients were subjected to 3 consecutive days of CGMS use in the hospital by a specialist nurse. Briefly, the CGMS sensor was subcutaneously embedded at day 0 around 16:00–17:00 PM. The patients continued with the sensor for 3 consecutive days if use of the CGMS was going well. Subjects were instructed to keep the sensor fixed and waterproof. The study nurse input a minimum of four calibration readings per day. At day 3, at around 16:00–17:00 PM, subjects had the sensor removed and the CGMS data were saved by the investigator. The 24-h mean amplitude of glycemic excursions (MAGE), the 24-h mean blood glucose (MBG), the percentage time duration (%) and the incremental area under the curve (AUC) of plasma glucose >10.0 and <3.9 mmol/l was calculated, and hypoglycemia episodes were also recorded. MAGE was calculated for each patient by measuring the arithmetic mean of the ascending and descending excursions between consecutive peaks and nadirs for the same 24 h period, only absolute excursion values >1 standard deviation (SD) were considered (18).

Ethical approval. The current study was approved by the ethics committee of Nanjing First Hospital (Nanjing, China), and was in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Written informed consent was obtained from the patients prior to participation in the present study.

Assays. The following plasma parameters were measured at baseline and upon completion of the study: Fasting plasma glucose, fasting plasma insulin, lipid profile, high-sensitivity C reactive protein (Hs-CRP), 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$), tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , IL-6, adiponectin (APN) and leptin. Plasma glucose concentrations were measured the day before and after therapy withdrawal using an Accu-Chek[®] Active glucometer (Roche Diagnostics GmbH, Mannheim, Germany). Fasting plasma insulin was determined using an insulin radioimmunoassay kit according to the manufacturer's instructions (Beijing Beifang Technology Co., Beijing, China). HbA_{1c} was measured using a Diastat HbA_{1c} analyzer (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at baseline. Hs-CRP was determined using radioimmunoassay kits provided by Beijing Onder High-Tech Co., Ltd. (Beijing, China) according to the manufacturer's instructions. The plasma 8-iso $PGF_{2\alpha}$ level was measured using an enzyme immunoassay method according to the manufacturer's instructions (Cayman Chemical Co., Ann Arbor, MI, USA) (19). TNF- α was measured using the human-specific Milliplex map kit (EMD Millipore, St. Charles, MO, USA) according to the manufacturer's instructions. IL-1 β was determined using enzyme-linked immunosorbent assay (ELISA) procedures (Orgenium Laboratories, Helsinki, Finland) and IL-6 was determined using commercially available ELISA kits (R&D Systems, Minneapolis, MN, USA) according to manufacturer's instructions. APN and leptin were measured using enzyme immunoassay kits (from R&D Systems, Inc., Minneapolis, MN, USA and Mercodia AB, Uppsala, Sweden, respectively) according to the manufacturer's instructions.

Statistical analysis. Statistical analysis was performed using SPSS software (version 17.0; SPSS, Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to assess the distribution of data. Normally distributed and continuous variables are presented as means \pm SD. Non-normally distributed variables are presented as medians (interquartile range) and were logarithmically transformed prior to analysis. The independent samples *t*-test was used to compare each group difference and Bonferroni correction was subsequently performed, with a significance level of 5%.

Results

Patient characteristics. The patients ($n=134$) were randomized into each of the two treatment groups. Their demographic characteristics and history of T2DM are presented in Table I. The two groups were well matched in terms of age, gender and BMI with no significant differences identified between the two groups.

Glucose control. Table II compares the 24-h mean glucose levels, and the percentage of time of significant hyperglycemia (glucose, >10 mmol/l) and significant hypoglycemia (glucose, <3.9 mmol/l) between the two groups. The differences between the acarbose plus insulin group and the monotherapy group were not statistically significant between the 24-h MBG, 24-h MBG SD, the hyperglycemic and hypoglycemic episodes, or the hyperglycemic and hypoglycemic duration (Table II). However, patients in the acarbose plus insulin

Table I. Baseline characteristics of the study population (n=134).

Characteristic	Acarbose +	Acarbose -	P-value
Patients (n)	68	66	-
Gender (male/female)	35/33	32/34	-
Age (years)	65.75±4.35	66.33±7.66	0.926
Body weight (kg)	60.15±10.33	62.15±10.86	0.845
Body mass index (kg/m ²)	23.63±3.93	24.23±2.21	0.729
HbA _{1c} (%)	9.66±1.90	9.79±1.19	0.721

Acarbose +, acarbose add-on insulin therapy group; Acarbose -, insulin monotherapy group. HbA_{1c}, haemoglobin A_{1c}. Data are presented as means ± standard deviation.

Table II. Glucose fluctuation parameters in the two groups upon completion of the follow-up period.

Parameter	Acarbose +	Acarbose -	P-value
Insulin dose/day (IU)	36.43±17.16	37.5±12.40	0.891
24-h MBG (mmol/l)	8.2±1.39	8.4±1.64	0.711
24-h MBG SD (mmol/l)	2.34±0.67	2.68±1.26	0.384
Hyperglycemic episodes, (n=16)	3.11±1.69	3.36±2.17	0.739
Hypoglycemic episodes, (n=8)	2.09±1.68	2.38±1.81	0.892
Hyperglycemic duration (%)	28.24±25.19	28.44±19.36	0.963
Hypoglycemic duration (%)	5.01±8.19	7.26±12.37	0.629

Hyperglycemic, >10 mmol/l glucose; hypoglycemic, <3.9 mmol/l glucose; Data are presented as means ± SD. Acarbose +, acarbose add-on insulin therapy group; Acarbose -, insulin monotherapy group. MBG, mean blood glucose; SD, standard deviation.

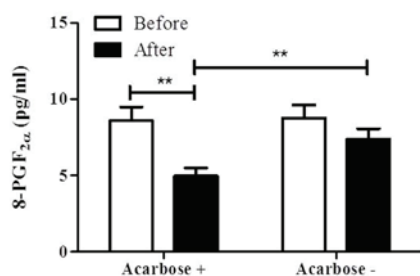


Figure 1. Plasma 8-PGF_{2α} levels in patients with type 2 diabetes mellitus before and after acarbose add-on insulin and insulin monotherapy treatment. 8-PGF_{2α}, 8-iso-prostaglandin F_{2α}.

group demonstrated significant decreases in the incremental AUC >10 mmol/l [0.85 (0.23, 1.4) mmol/l per day] and MAGE (7.50±3.28 mmol/l) compared with patients in the insulin only group.

BMI, blood glucose and insulin change. The BMI of patients from the two groups was calculated at the end of therapy. BMI was almost unchanged in the two groups, with no significant difference identified between groups ($P>0.05$). Fasting blood glucose significantly improved in each group after the 2-week treatment [acarbose plus insulin group: 8.21 ± 2.13 to 6.98 ± 1.53 mmol/l ($P<0.05$); insulin only group: 8.30 ± 2.32

to 7.16 ± 3.28 mmol/l ($P<0.05$)]. No significant difference in fasting blood glucose levels was identified between the two groups ($P>0.05$). Fasting insulin was assessed 2 days after completion of treatment. It was almost unchanged (8.14 ± 3.09 to 8.87 ± 4.11 μ U/ml; $P>0.05$) in the acarbose plus insulin group, whereas it slightly decreased from 7.78 ± 2.17 to 6.71 ± 3.34 μ U/ml ($P>0.05$) in the insulin only group. No statistical difference of fasting insulin concentration was identified between the two groups ($P>0.05$).

Oxidative stress. To determine the effect of co-administration of acarbose with insulin on oxidative stress in patients with T2DM, 8-PGF_{2α}, a well-recognized biomarker of oxidative stress, was measured. As shown in Fig. 1, 8-PGF_{2α} was significantly decreased in the acarbose plus insulin group from 8.55 ± 3.62 to 4.97 ± 2.16 pg/ml ($P<0.01$). Although it exhibited a decreasing tendency in the insulin group (8.79 ± 3.58 to 6.65 ± 2.45 pg/ml), no statistically significant difference was observed. Furthermore, the 8-PGF_{2α} level of the acarbose plus insulin group was significantly lower than that of the insulin group at 2 weeks ($P<0.01$; Fig. 1).

Inflammatory cytokines. Patients with T2DM have a significantly higher inflammatory score when compared with control subjects (20). Although significant decreases of inflammatory cytokines were found in the two groups, the addition of acarbose resulted in further reductions of

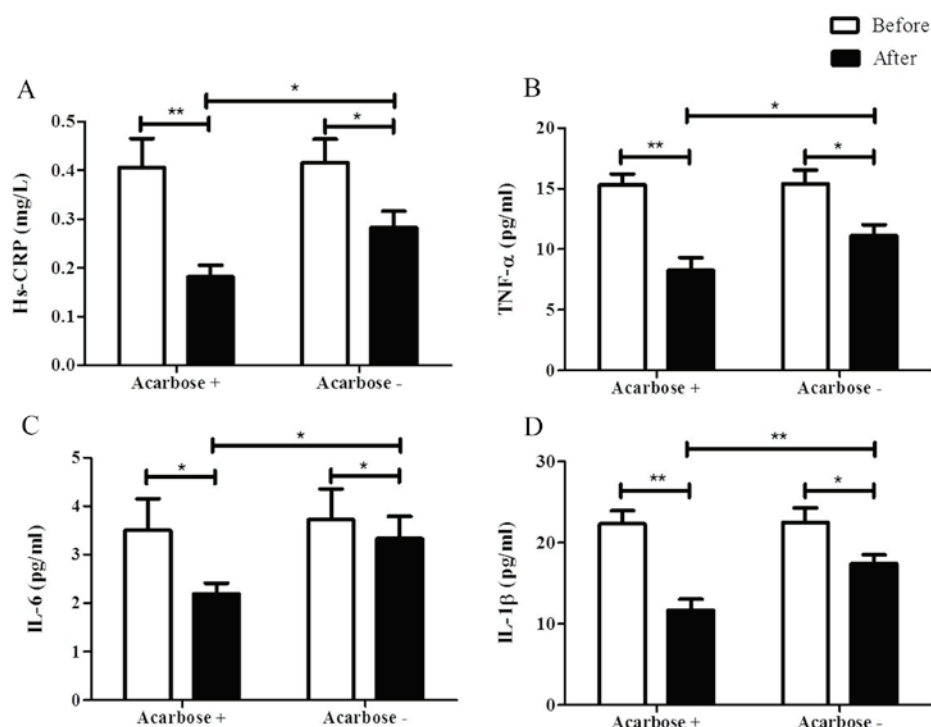


Figure 2. Plasma cytokine levels in patients with type 2 diabetes mellitus before and after therapy. (A) Hs-CRP, (B) TNF- α , (C) IL-6 and (D) IL-1 β . Data are presented as means \pm standard deviation. Hs-CRP, high-sensitivity C-reactive protein; TNF- α , tumor necrosis factor- α ; IL, interleukin.

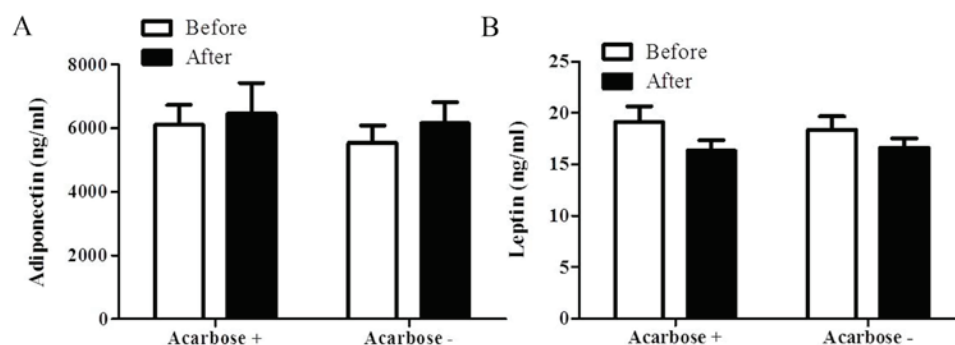


Figure 3. Plasma adipocytokine levels in patients with type 2 diabetes mellitus before and after therapy. (A) Adiponectin and (B) leptin.

inflammatory cytokine levels, when compared with the insulin only group at 2 weeks. The Hs-CRP level of the acarbose plus insulin group decreased from 0.41 ± 0.24 to 0.18 ± 0.10 mg/l ($P < 0.05$). This was also observed in the insulin only group (0.42 ± 0.20 to 0.28 ± 0.14 mg/l; $P < 0.05$). Notably, the Hs-CRP level of the acarbose plus insulin group was significantly lower than that of the insulin only group at 2 weeks ($P < 0.05$; Fig. 2A).

The TNF- α level was significantly reduced in the two groups at 2 weeks after treatment. It significantly reduced from 14.59 ± 4.56 to 8.13 ± 4.56 pg/ml ($P < 0.01$) in the acarbose plus insulin group, and it also significantly decreased from 13.84 ± 5.25 to 10.63 ± 4.31 pg/ml ($P < 0.05$) in the insulin only group (Fig. 2B). However, the addition of acarbose further reduced TNF- α levels as compared with the insulin group at 2 weeks ($P < 0.05$; Fig. 2B).

IL-6 significantly decreased in the two groups [3.50 ± 2.61 to 2.19 ± 0.91 pg/ml in the acarbose plus insulin group ($P < 0.05$) and from 3.72 ± 2.70 to 3.33 ± 1.94 pg/ml in the insulin only group ($P < 0.05$); Fig. 2C and D]. A lower level of IL-6 was observed in the acarbose plus insulin group when compared with the insulin only group ($P < 0.05$) at 2 weeks (Fig. 2C).

IL-1 β significantly decreased in the two groups [22.31 ± 6.48 to 11.66 ± 5.35 pg/ml in the acarbose plus insulin group ($P < 0.01$) and from 22.49 ± 7.61 to 17.43 ± 4.58 pg/ml in the insulin only group ($P < 0.05$)] in 2 weeks. However, IL-1 β in the acarbose plus insulin group was significantly lower than that of the insulin only group at 2 weeks ($P < 0.01$; Fig. 2D).

Adipocytokines. The APN level marginally increased from $5,539.06 \pm 2312.45$ to $6,156.94 \pm 2797.11$ ng/ml ($P > 0.05$) in the insulin only group following 2 weeks of treatment (Fig. 3A).

Similarly, the APN level increased from $6,110.31 \pm 2485.21$ to $6,458.88 \pm 3851.47$ ng/ml ($P > 0.05$) in the acarbose plus insulin group. However, no significant difference in the APN level was identified between the two groups at 2 weeks ($P > 0.05$; Fig. 3A). The leptin level in the acarbose plus insulin group marginally reduced from 19.11 ± 6.15 to 15.69 ± 4.11 ng/ml ($P > 0.05$). Similarly, the leptin level in the insulin only group decreased from 18.38 ± 5.43 to 16.61 ± 3.87 ng/ml following 2 weeks of treatment ($P > 0.05$). No significant difference in the level of leptin was observed between the two groups at 2 weeks ($P > 0.05$; Fig. 3B).

Safety. Adverse events, consisting of digestive disorders, were reported by nine of the patients in the acarbose plus insulin group and by three of the insulin only group patients.

Discussion

In the current study, the efficacy of acarbose on oxidative stress, and lipid and inflammatory profiles in patients with T2DM receiving insulin therapy was evaluated. The addition of acarbose with insulin therapy was observed to more effectively improve oxidative stress and the inflammatory profiles of patients with T2DM when compared with patients receiving insulin therapy alone.

Acute glucose fluctuations, other than chronic hyperglycemia, during postprandial periods more significantly affect oxidative stress in patients with T2DM (8). The rapid rise in postprandial blood glucose concentration induces an over production of peroxynitrite and nitrotyrosine (8,9,21). Thus, there are continued efforts aimed at suppressing postprandial hyperglycemia in patients with T2DM (22). Previous studies indicated that improved postprandial glycemic excursions smooth oxidative and nitrosative stress (23). Chiasson *et al* (24) reported that acarbose was able to normalize the PPG concentration in patients with impaired glucose tolerance (IGT). Furthermore, clinical trials have shown that acarbose treatment prevents cardiovascular complications in patients with T2DM and in those with IGT (12,25). Studies have also demonstrated that postprandial hyperglycemia concentration results in an increase of oxidative stress in patients with T2DM (26), which is associated with endothelial dysfunction (27). Kato *et al* (10) suggested that acarbose enhances postprandial endothelial function by improvement of postprandial hyperglycemia. Notably, postprandial hyperglycemia as a risk factor for cardiovascular disease was evaluated by the STOP-NIDDM (12) Trial and the data indicated that acarbose treatment was associated with a 49% relative risk reduction in the development of cardiovascular events. The current study presents evidence that the improved glucose fluctuation resulting from acarbose plus insulin therapy leads to a reduction of oxidative stress in patients with T2DM.

There is also evidence that hyperglycemia contributes to the inflammation experienced by patients with T2DM. It has been demonstrated that repeated fluctuations of glucose produce increased circulating levels of inflammatory cytokines when compared with stable, high glucose levels in normal subjects (28). Daniele *et al* (20) demonstrated that the inflammatory score, an integrated quantity of TNF- α , IL-6, monocyte chemoattractant protein-1, fractalkine, osteopontin

and APN, is increased in patients with T2DM and correlates with hyperglycemia (20). The link between hyperglycemia, increased oxidative stress and the higher level of inflammatory cytokines was first demonstrated by Esposito *et al* (28) who suggested that hyperglycemia increases the levels of inflammatory cytokines via an oxidative mechanism (28). Notably, in the present study, the levels of serum inflammatory markers (Hs-CRP, IL-1 β , IL-6 and TNF- α) were significantly decreased in patients with T2DM who were treated with acarbose plus insulin therapy when compared with those that received only insulin therapy, along with decreased oxidative stress levels. This may suggest that acarbose attenuates the oxidation that is caused by hyperglycemia or/and hyperinsulinemia. It was found that the level of APN, an anti-inflammatory cytokine (29), was not significantly increased in the current study.

In conclusion, the addition of acarbose to insulin therapy was associated with a reduction in oxidative stress and inflammation in patients with T2DM. However, future studies are required to clarify the underlying mechanisms.

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