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Acarbose Treatment Increases Serum Total Adiponectin Levels in Patients with Type 2 Diabetes

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Abstract. Adiponectin is an anti-diabetic and anti-atherogenic adipokine that serves as a major determinant of insulin sensitivity. Thiazolidine derivatives increase circulating adiponectin, particularly the high molecular weight isoform, which has been shown to well correlate with amelioration of insulin resistance by thiazolidines in diabetic patients. α-glucosidase inhibitors are another class of anti-diabetic agents that specifically reduce postprandial blood glucose elevations, but its effect on adiponectin is largely unknown. In the present study we investigated effect of an α-glucosidase inhibitor, acarbose, together with pioglitazone, the only thiazolidine derivative available in Japan, on serum concentrations of adiponectin. Seventeen patients with type 2 diabetes were treated with acarbose and sixteen with pioglitazone for three months. Treatment with acarbose and pioglitazone decreased HbA1c values by 0.49% and 0.63%, respectively. Pioglitazone, as expected, increased serum levels of total adiponectin by 2.1 fold and its high molecular weight isoform by 3.6 fold. We found that acarbose also caused a small but significant increase in serum concentrations of total adiponectin. However, in contrast to pioglitazone, no appreciable changes were observed in the levels of high molecular weight adiponectin. In conclusion, acarbose increases serum concentrations of total adiponectin without preference of the high molecular weight isoform in type 2 diabetic patients. Clinical relevance of the increased adiponectin to the acarbose effects remains to be elucidated.

Key words: Acarbose, Pioglitazone, Adiponectin, Isoform, Type 2 diabetes mellitus, α-Glucosidase inhibitor

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ADIPONECTIN (AdN) is an adipokine that has been identified independently by a Japanese group and others in the 1990s [1–3]. AdN-deficient mice generated by targeted gene deletion have been shown to develop insulin resistance, dyslipidemia, hypertension and atherosclerosis, demonstrating anti-diabetic and anti-atherogenic actions of AdN [4–6]. Moreover, decreased circulating levels of adiponectin have been observed in obese and type 2 diabetic patients [7–9]. Conversely, exogenous adiponectin administration to diabetic model mice ameliorates insulin resistance [10]. Based on these results, AdN has been proposed to play a critical role in the pathogenesis of obesity, type 2 diabetes and cardiovascular diseases involving atherosclerosis [11, 12].

It is known that several different drugs, including anti-diabetic agents, affect serum concentrations of AdN in humans. Among such drugs, thiazolidine derivatives, which improve insulin sensitivity through binding to a nuclear receptor PPARγ. have the highest capacity of increasing the circulating AdN levels indirectly by inducing "bad" large adipocytes into "good" small adipocytes [12, 13] and/or by directly inducing adiponectin gene transcription through PPARγ [14]. Pioglitazone, the only thiazolidine derivative clinically available in Japan, is no exception: it has been shown to increase blood levels of AdN, particularly its high molecular weight isoform (HMW-AdN) [15–18], raising a possibility that anti-diabetic and anti-atherogenic effects of pioglitazone may partly be mediated by increased AdN.

Acarbose is an α-glucosidase inhibitor, another class

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of anti-diabetic drugs that specifically suppress postprandial elevations of blood glucose levels. It inhibits a membrane-bound α-glucosidase and pancreatic α-amylase in a reversible manner, leading to delayed absorption of glucose in the small intestine. Administration of acarbose to a patient with type 2 diabetes causes a decrease in HbA1c by approximately 1% [19]. In the study to prevent non-insulin-dependent diabetes mellitus (STOP-NIDDM), acarbose was able to reduce the risk of diabetes by 25% in patients with impaired glucose tolerance (IGT) [20]. Acarbose treatment was further shown to result in a 49% and 34% relative risk reduction in the development of cardiovascular events and in the incidence of new cases of hypertension, respectively [21], and also to prevent the progression of atherosclerosis assessed by carotid intima-media thickness [22]. Although these results seem consistent with a role of postprandial hyperglycemia in the pathogenesis of cardiovascular diseases, precise mechanisms whereby acarbose counteracted cardiovascular diseases remain unclear.

Results from the STOP-NIDDM trial also demonstrated that combination of the G-allele of single nucleotide polymorphism (SNP) +45 and the T allele of SNP +276 of the adiponectin gene, both of which have been found to be associated with obesity and insulin resistance, is a predictor for the progression of IGT to type 2 diabetes [23]. Interestingly, this gene effect was not observed after acarbose treatment [23], which raises a possibility that adiponectin is involved in clinical effects of acarbose. There have been described virtually no studies that examined the effect of α-glucosidase inhibitors on the circulating levels of AdN except for negative reports that voglibose does not change serum AdN concentrations [15, 18]. Thus, in the present study, we investigated the effect of treatment with acarbose, and with pioglitazone as a positive control, on the serum levels of adiponectin in patients with type 2 diabetes.

Methods

Subjects

Thirty-three patients with type 2 diabetes, who had never been treated with insulin, pioglitazone or any α-glucosidase inhibitors during the past three months, were enrolled in this study. After obtaining informed consent, the subjects were divided into two groups: sixteen subjects in Group P were given 15 mg pioglitazone once daily, while the other seventeen in Group A were treated with 300 mg acarbose, immediately before every meal. During the study, all the drugs other than insulin, pioglitazone or α-glucosidase inhibitors were continued and kept unchanged. Blood samples were taken from every subject at the initiation of the study and after three months (11–14 weeks) of treatment. HbA1c was measured immediately after the blood was taken by an autoanalyzer. Serum was separated by centrifugation and stored at –70°C until adiponectin measurement. The study protocol was approved by the institutional review board at Teikyo University.

Measurement of serum adiponectin concentrations

Serum concentrations of total and HMW-AdN were measured by Human Adiponectin ELISA kit for Total and Multimers (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) according to the manufacturer's instruction. For HMW-AdN measurement, samples were first incubated with a protease solution provided by the company, which specifically degrades LMW (low molecular weight)- and MMW (mid molecular weight)-AdN. The residual HMW-AdN was then treated with SDS-containing acid buffer to convert it to a dimer form and measured by ELISA.

Statistical analysis

Statistical analysis was done with paired or unpaired t-test when appropriate using StatMate III software (ATMS Co., Ltd., Tokyo, Japan).

Results

Pretreatment levels of serum total AdN were 4.38 ± 3.58 μg/ml in Group P and 5.51 ± 2.76 μg/ml in Group A, which were both low and were not significantly different from each other. The initial HbA1c levels and the mean age of the subjects were slightly higher in Group P than Group A (Table 1). Three month's treatment with pioglitazone or acarbose significantly improved glycemic control and lowered HbA1c values by 0.63% and 0.42%, respectively.

We then measured and compared serum AdN concentrations before and after treatment. The results indicated that pioglitazone increased total AdN levels in

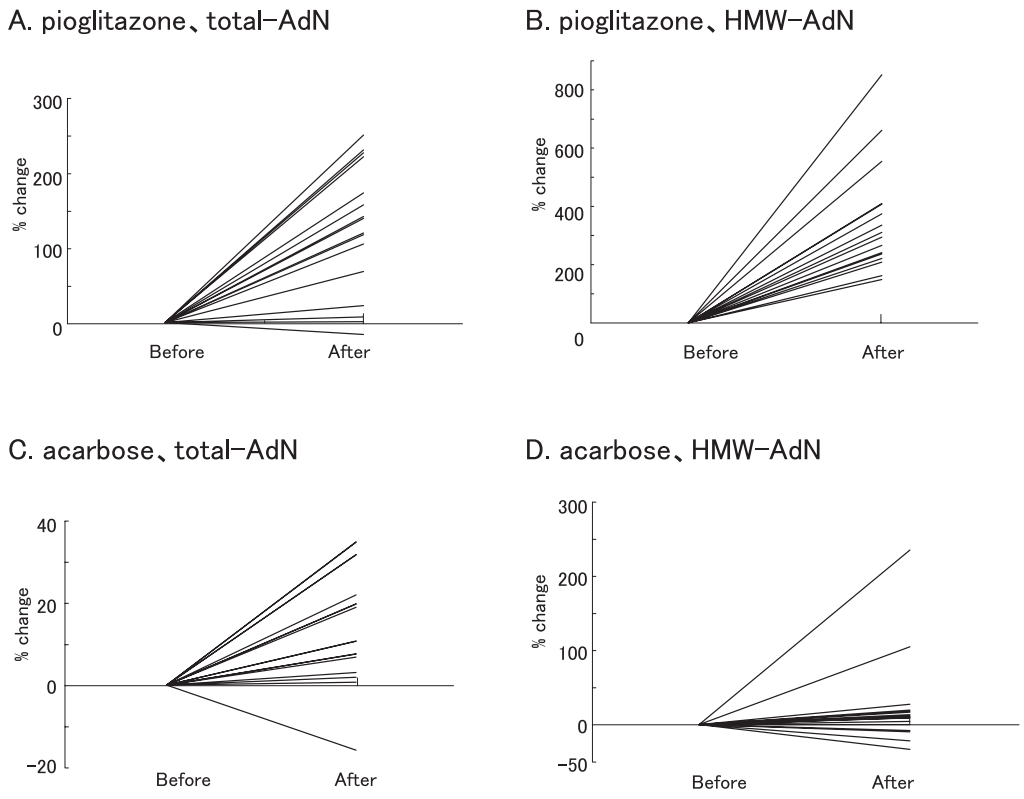

Fig. 1. Changes in serum concentrations of total and high molecular weight adiponectin
Per cent changes in serum concentrations of total-AdN (A, C) and HMW-AdN (B, D) in each individual are shown for Group P treated with pioglitazone (A, B) and Group A treated with acarbose (C, D).

Table 1. Basal characteristics of the subjects

	Group P	Group A
N	16	17
Age	63.19 ± 8.77	54.59 ± 17.74*
HbA1c (%)	8.09 ± 0.86	7.55 ± 0.73*
total-AdN (μg/ml)	4.38 ± 3.58	5.51 ± 2.76

Age, HbA1c and serum concentrations of total adiponectin in sixteen subjects given pioglitazone (Group P) and seventeen subjects given acarbose (Group A) at the initiation of the study are shown in mean ± S.D. *: Significantly different from Group P ($p<0.05$).

Table 2. Changes in serum concentrations of adiponectin after treatment

Group P (pioglitazone)	before	after
total-AdN (μg/ml)	4.38 ± 3.58	9.40 ± 7.23*
HMW-AdN (μg/ml)	1.51 ± 2.00	5.42 ± 5.44*
HMW/total (μg/ml)	0.29 ± 0.14	0.56 ± 0.28*

Group A (acarbose)	before	after
total-AdN (μg/ml)	5.51 ± 2.76	6.41 ± 3.69**
HMW-AdN (μg/ml)	1.83 ± 1.64	2.00 ± 1.78
HMW/total	0.32 ± 0.20	0.31 ± 0.18

Serum levels of total-AdN and HMW-AdN and the ratio of the latter to the former are shown in mean ± S.D. before and after treatment with pioglitazone (Group P) or acarbose (Group A).
* Significantly different from the value before treatment ($p<0.01$).
** Significantly different from the value before treatment ($p<0.05$).

every subject (Fig. 1) and, on the average, by 2.1 fold (Table 2), which was consistent with a known positive effect of pioglitazone on serum AdN concentrations. Interestingly, although to a lesser extent, acarbose also increased total AdN in all the subjects except one with a statistical significance.

Among multiple isoforms present in the circulating blood, HMW-AdN has been shown to best correlate with insulin sensitivity as well as clinical effects of thiazolidine derivatives. Consistently, we observed a strong positive effect of pioglitazone on HMW-AdN levels with a 3.6 fold increase, which was greater than that of total AdN, leading to a significant increase in the proportion of HWM-AdN to the total (Table 2). In

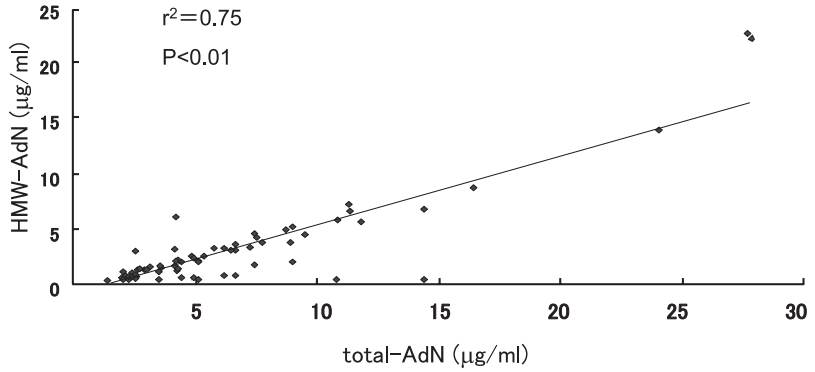

Fig. 2. Correlation between total and high molecular weight adiponectin
Relationship between total-AdN and HMW-AdN concentrations in all the serum samples tested in this study (N = 66) was analyzed in two-dimensional plots. There was a strong positive correlation between the two ($p<0.01$).

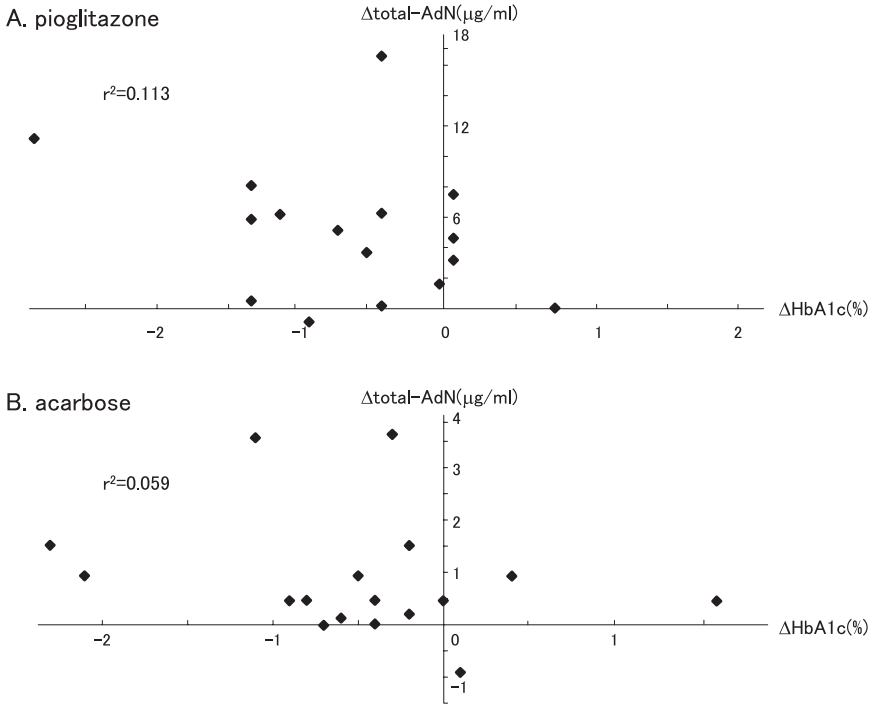

Fig. 3. Relationship between changes in HbA1c and those in total-AdN
Changes in HbA1c (%) and those in serum concentrations of total-AdN (μg/ml) were shown in two-dimensional plots. There was no significant correlation between them in pioglitazone-treated Group P (A) or in acarbose-treated Group A (B).

contrast, although there was a trend for up-regulation, acarbose treatment resulted in a decrease in the HMW-AdN levels in three cases (Fig. 1), and as a whole, difference between before and after treatment was not statistically significant (Table 2). Taken together, these results suggested that acarbose increases serum total AdN concentrations through a distinct mechanism from pioglitazone, involving a predominant effect on smaller isoforms including MMW- and LMW-AdN. When all the data were analyzed together, we found a good correlation between total and HMW-AdN (Fig. 2), demonstrating an overall consistency of the assays of AdN isoforms.

Finally, in order to clarify impact of AdN on the glucose metabolism, we examined correlation between changes in AdN and HbA1c caused by the two drugs.

As a result, we found that the changes in total AdN (Fig. 3), as well as HMW-AdN (data not shown), showed no significant correlation with the changes of HbA1c. Thus, from the current study, there was no evidence for a significant contribution of increased AdN to the metabolic improvement caused by pioglitazone or acarbose.

Discussion

In the present study, we demonstrated that acarbose treatment of patients with type 2 diabetes increases serum total AdN concentrations in three months. In general, improvement of glycemic controls by diet, exercise or medical interventions does not always lead to increase in serum AdN concentrations. In fact, there are some studies reporting negative effects of anti-diabetic agents on serum AdN levels, including glibenclamide [17], voglibose [18] and metformin [24, 25]. To our knowledge, this is the first clinical report of AdN-increasing effect of any α-glucosidase inhibitors. In the STOP-NIDDM trial, acarbose not only reduced the risk of development of diabetes from IGT, but also prevented cardiovascular events as well as development of hypertension [20]. Although its clinical significance remains to be established, the acarbose-induced increase in serum total AdN concentrations observed in the current study may contribute to the preventive effect of acarbose on cardiovascular diseases, which may not totally depend on its glucose-lowering effect.

In the circulation, adiponectin is present in various oligomeric complexes. Predominant isoforms include a low molecular weight trimer (LMW-AdN), a middle molecular weight hexamer (MMW-AdN), and a high molecular weight multimeric complex (HMW-AdN) that consists of 12–18 monomers [12, 26]. Among these isoforms, HMW-AdN has been suggested to play a particularly important physiological and pathological role in the glucose metabolism. Patients whose ability to generate HMW-AdN is genetically impaired develop diabetes [26]. Moreover, HMW-AdN has been shown to better correlate with and thus be a better clinical indicator of insulin sensitivity than total-AdN [27, 28]. There is however still some room for controversy on this matter: for example, in a recent study in which three groups with normal glucose metabolism, IGT and diabetes, each consisting of twenty subjects, were examined for relationship between various metabolic indices and serum AdN levels [29], they were able to confirm that AdN was indeed a good marker for insulin sensitivity, but were unable to obtain evidence for superiority of HMW-AdN to total-AdN as a predictor of insulin resistance. Thus, isoform-specific roles of AdN in glucose metabolism remain to be fully elucidated.

Several different classes of drugs have been shown to increase circulating levels of AdN, including pioglitazone and other thiazolidine derivatives [15–18, 27, 30], angiotensin II type I receptor blockers [31], sulfonylureas [25, 32], fenofibrate [33], and so on. Among them, thiazolidine derivatives are the most powerful stimulator of AdN, predominantly HMW-AdN over the other smaller oligomers, as reproduced in the present study. Such a predominant effect on HMW-AdN is however not always true for other drugs: most reports on drugs that increase AdN did not demonstrate a greater increase in HMW-AdN than smaller isoforms, except for a very recent paper analyzing fenofibrate effects [33]. Thiazolidine derivatives have been shown to promote AdN gene expression at the transcriptional level through PPARγ [14] and to up-regulate HMW-AdN production in adipocyte cultures *in vitro* [16]. Therefore, the effect of thiazolidine derivatives on AdN including pioglitazone is at least in part dependent on unique properties of this class of drugs including direct up-regulatory effects on cellular expression of AdN, and cannot be generalized to other anti-diabetic drugs.

The results of the present study suggest that in contrast to thiazolidine derivatives acarbose increases smaller isoforms of AdN rather than HMW-AdN. Similar effects of diet and exercise have been reported. Bluher *et al.* recently showed that exercise slightly increased serum concentrations of both total- and HMW-AdN but without any preference and that exercise-associated improvement of metabolic indices correlated better with total-AdN rather than HMW-AdN [29]. Polak *et al.* examined effects of twelve-week low calory diet in twenty obese women and demonstrated that the diet therapy preferentially increased LMW-AdN [34]. Molecular mechanisms as well as clinical significance of increased AdN upon various treatment modalities of diabetes require further investigation. Particularly, as for acarbose, it seems most important to determine whether or not its stimulatory effect on AdN is a class effect shared by other α-glucosidase inhibitors.

At present mechanisms whereby acarbose increased

AdN are totally unknown. Recently, it has been shown that posttranslational modifications including glycosylation of lysine residues are necessary for multimerization of AdN [35]. Such intracellular posttranslational reactions can be affected by hyperglycemia and has been implicated in functional impairment at the organ level in diabetic patients [36, 37], raising an interesting possibility that attenuation of postprandial hyperglycemia by acarbose may alter posttranslational modifications of AdN molecules and thereby their isoform composition in the circulation as well. Further studies are necessary to elucidate effects of extracellular glucose concentrations and acarbose itself on production of AdN isoforms at the cellular level.

The present study has limitations. First, the number of subjects analyzed in the study may be too small to draw definite conclusions. Even though statistically significant, on the average the increase in total-AdN by acarbose appeared minor. Our observation that acarbose increased total-AdN but not HMW-AdN, may be simply due to an insufficient statistical power that depends on the sample size. Second, because we did not analyze various metabolic indices that could affect serum AdN levels, we were unable to assess contribution of such factors to the acarbose-induced increase in AdN. It is particularly important to determine relationship between changes in total-AdN levels and insulin resistance, and ultimately cardiovascular events, which requires future large-scale studies. Despite such limitations, we would like to emphasize here that the acarbose-induced increase in total AdN levels were quite consistent at the individual level: positive trends of AdN were observed after three months' treatment in all the subjects but a single exception. And our study strongly suggests that acarbose, unlike thiazolidine derivatives, does not have a predominant effect on HMW-AdN. These observations will help elucidate new actions of acarbose and isoform-specific functions as well as usefulness as a metabolic marker of the circulating AdN.

In summary, three months' treatment of diabetic patients with acarbose led to a decrease in HbA1c and a significant increase in serum total-AdN concentrations without changing HMW-AdN, while, in contrast, treatment with pioglitazone caused a HMW-AdN-dominant increase. These observations warrant further studies to determine clinical significance of the positive effect of acarbose on serum concentrations of total-AdN.

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