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ORIGINAL PAPER

Insulin-dependent actions of pioglitazone in newly diagnosed, drug naïve patients with type 2 diabetes

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Abstract The aim of this study was to study the effects of pioglitazone on several diabetic parameters with subjects possessing distinct levels of insulin. Treatment naive patients with type 2 diabetes received 15–30 mg/day pioglitazone monotherapy. At 3 months, levels of insulin, C-peptide, HbA1c, HOMA-R, HOMA-B and BMI were compared with those at baseline between the low (below 5.9 μU/ml, $n = 48$), medium (11.9–6 μU/ml $n = 39$) and high (above 12 μU/ml, $n = 33$) insulin groups. At baseline, differences existed in the levels of HbA1c, insulin, C-peptide, HOMA-R, HOMA-B, and BMI between these groups. In the high-insulin group significant reductions of insulin/C-peptide levels were observed, while in the low-insulin group significant increases of insulin/C-peptide were observed. In the medium-insulin group, no significant changes were observed. In contrast, the HbA1c levels significantly and similarly decreased in all the groups. Significant correlations between the changes of insulin/C-peptide levels with pioglitazone and the baseline insulin/C-peptide levels were observed. HOMA-R showed greater reductions in the high-insulin group, while HOMA-B showed greater increases in the low-insulin group in comparison to other groups. Multiple regression analysis revealed that the baseline insulin level is the predominant determinant of the changes of insulin levels with pioglitazone. These results suggest that pioglitazone appears to have two effects: to reduce insulin resistance (and lower insulin) and to improve beta-cell function (and increase insulin). The predominance of these effects appears to be determined by the insulin levels. Based on these data, a novel physiological model showing that pioglitazone may shift the natural history of diabetes toward an earlier stage (rejuvenation of beta-cell function) will be presented.

Keywords Insulin resistance · Pioglitazone · PPARγ · History of type 2 diabetes

Abbreviations

FBG	Fasting blood glucose
GOT	Glutamic oxalacetic transaminases
GPT	Glutamic pyruvic transaminases
CRE	Creatinine
BNP	Brain natrium peptide
HOMA-R and -B	Homeostasis model assessment-R and -beta
BMI	Body mass index

Introduction

Type 2 diabetes develops from progressive failure of pancreatic beta-cell function in the presence of insulin resistance. From the early to mid phase of diabetic history, insulin secretion is increased in response to insulin resistance, demonstrating beta-cell compensation for the resistance. This represents an adaptive response of beta-cells that are trying to maintain normal glucose levels. However, these compensatory demands of insulin secretion by chronic insulin resistance will be a burden for beta-cell in the long run. When insulin secretion is not able to match insulin resistance, hyperglycemia/diabetes will develop. If insulin resistance causes or accelerates beta-cell failure in

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an active manner, then relieving insulin resistance could preserve beta-cell function and reverse/prevent the development of diabetes. Indeed, insulin resistance has been shown to have adverse effects on beta-cells, including hypertrophy, apoptosis and those caused by lipotoxicity and glucotoxicity [1, 2].

Since the number of newly diagnosed patients with type 2 diabetes is increasing worldwide, it is important to establish the therapeutic strategies for those patients. Currently metformin is regarded as the first drug of choice [3], although other drugs (e.g., thiazolidinediones, alpha glucosidase inhibitors, meglinitide analogs, combination of metformin and thiazolidinediones) could also be candidates. Thiazolidinediones (TZDs), a class of insulin-sensitizing agents, are recently introduced for the treatment of type 2 diabetes. Currently pioglitazone and rosiglitazone are available in the market. A large amount of basic and clinical studies have shown that TZDs ameliorate insulin resistance and improve hyperglycemia, hyperinsulinemia, and dyslipidemia in patients with type 2 diabetes [4]. Recent studies suggest that TZDs may have direct beneficial effects on pancreatic beta-cells [2, 5]. Troglitazone, for example, demonstrated improvements in insulin secretion in isolated pancreatic islets from Wistar rats and hamster beta-cell line [6, 7]. A report using db/db mice suggests that long-term treatment with pioglitazone is effective in decreasing hyperglycemia, protecting against beta-cell damage and improving glucose-induced insulin secretion [8, 9]. It was also reported that in human islets rosiglitazone inhibits h-IAPP-induced islet cell apoptosis, and may have the potential role to decrease beta-cell apoptosis in type 2 diabetes and reduce loss of beta-cell mass [10]. Given these backgrounds, TZDs may have direct beneficial effects for preventing or delaying the decline in pancreatic beta-cell function and may slow the progression to type 2 diabetes.

As indicated above, TZDs are well known to ameliorate hyperinsulinemia as a result of decreased insulin resistance [4]. However, many patients with type 2 diabetes possess substantially low-levels of insulin. Nevertheless, pioglitazone was shown to be effective in these patients [11]. It is of significance to study the effects of pioglitazone on the levels of insulin as well as other parameters with these patients. This work was initiated in order to answer this question.

Results

Characteristics of the subjects

Pioglitazone monotherapy was performed with newly diagnosed, drug naïve patients and the metabolic parameters were measured and the analysis was performed as described in "Subjects and methods". The subjects were divided into three groups according to the baseline insulin values; low (L: below 5.9 μU/ml, $n = 48$) medium (M: between 6 and 11.9 μU/ml, $n = 39$), and high (L: above 12 μU/ml, $n = 33$) insulin groups. Baseline characteristics of the subjects in each group are summarized in Table 1.

Baseline comparisons

At baseline, differences existed in the levels of HbA1c, insulin, C-peptide, HOMA-R, HOMA-B, and BMI between these three groups (for each value and its statistical significance, see Fig. 1a–e). The HbA1c levels were in negative proportion to the insulin (or C-peptide) levels (see Fig. 1a, b). The magnitude of insulin resistance (HOMA-R) was in proportion to the insulin (or C-peptide) levels (Fig. 1d, b, c). Similarly, the magnitude of beta-cell function (HOMA-B) was in proportion to the insulin (or C-peptide) levels (Fig. 1e, b and c). Taken together these data so far, compensatorily activated beta-cell function followed by elevated insulin levels in response to insulin resistance may have resulted in the decreased glucose levels. BMI was in proportion to the insulin or C-peptide levels.

Differential regulation of insulin and C-peptide levels with pioglitazone

Pioglitazone treatment had caused the changes of HbA1c, insulin, and C-peptide as presented in Fig. 2a–c. Briefly, in the high-insulin group, significant reduction of insulin (−38.7%, $P < 0.00001$) and C-peptide (−27.6%, $P < 0.00001$) levels were observed (Fig. 2b), while in the low-insulin group, significant increase in insulin (+113.5%, $P < 0.00001$) and C-peptide (+32.1%, $P = 0.034$) were observed (Fig. 2c). In the medium-insulin group, no significant changes were observed (+18.1%, $P = 0.22$ for insulin and +2.0%, $P = 0.76$ for C-peptide). In contrast to this, the HbA1c levels

Table 1 Baseline characteristics of the subjects are described in low (L), medium (M) and high (H) insulin groups

	L	M	H
Age	55.1 ± 10.7	50.3 ± 12.8	52.4 ± 13.1
Numbers (male/female)	38/10	25/14	22/11
Insulin (μU/ml)	3.53 ± 1.31	8.38 ± 2.08	23.06 ± 10.89
C-peptide (ng/ml)	1.99 ± 0.81	2.92 ± 099	4.92 ± 1.88
HbA1c (%)	10.28 ± 2.43	10.13 ± 1.98	9.35 ± 2.07
HOMA-R	2.20 ± 1.61	5.27 ± 2.42	14.08 ± 8.53
HOMA-B	10.30 ± 7.89	20.81 ± 13.30	55.86 ± 36.68
BMI	22.80 ± 3.81	26.41 ± 4.85	28.79 ± 5.74

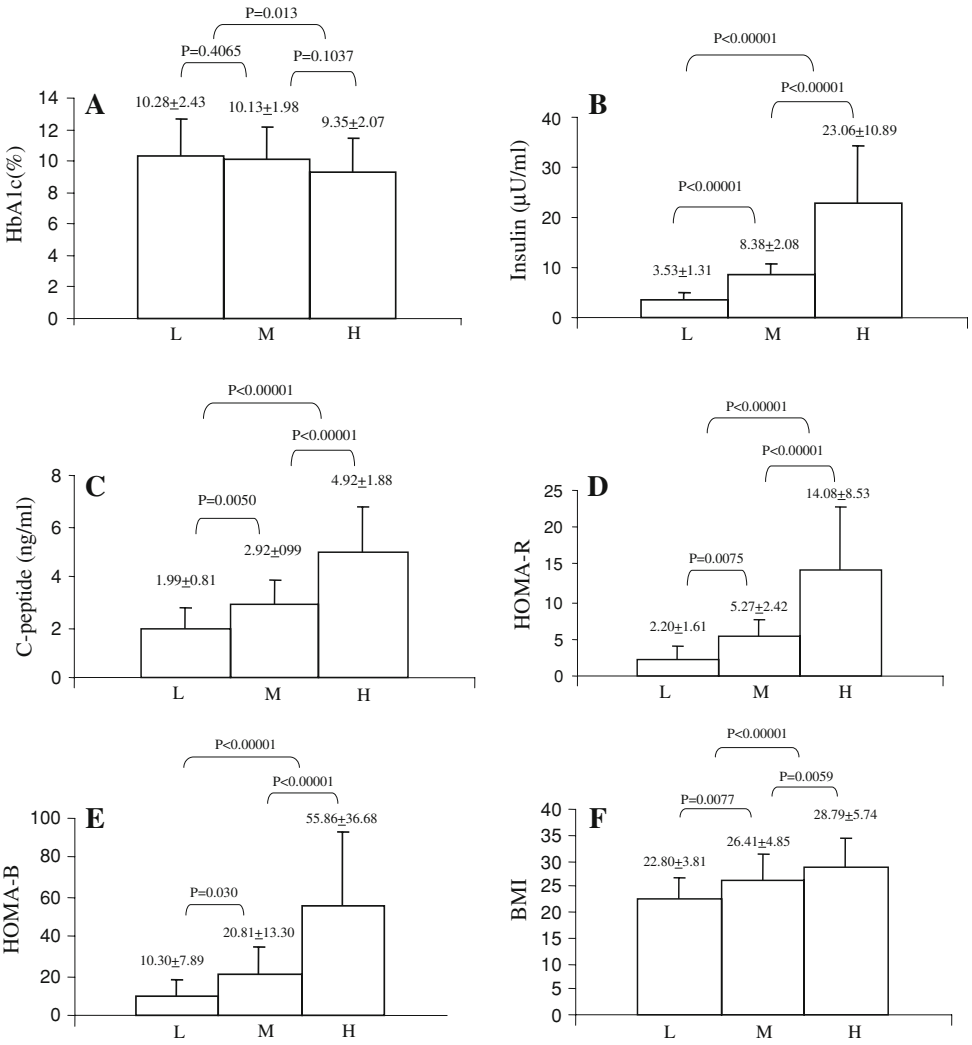

Fig. 1 Baseline characteristics. Baseline values of each parameter are shown in low (L), medium (M), and high (H) insulin groups. Panel **A**: HbA1c. Panel **B**: insulin. Panel **C**: C-peptide. Panel **D**: HOMA-R. Panel **E**: HOMA-B. Panel **F**: BMI

significantly and effectively decreased in all the groups (high: −14.9%, medium: −19.0%, low: −16.6%, $P < 0.00001$ for all the groups, Fig. 2a).

The above mentioned results showing that pioglitazone could down-regulate hyperinsulinemia (low C-peptide), while it could up-regulate hypoinsulinemia (high C-peptide), were obtained by grouping the subjects with different insulin levels. A linear model analysis may strengthen this data. For this purpose, simple linear regression analysis was performed as described in "Subjects and methods". As shown in Fig. 3a, the regression equation between the changes of insulin levels with pioglitazone (y-axis) and the baseline insulin levels (x-axis) was: $y = -0.5979x + 5.9086$ ($R = 0.6438$, $P < 0.00001$, $n = 120$). Identical analysis was performed with C-peptide (Fig. 3b). The regression equation between the changes of C-peptide levels with pioglitazone (y-axis) and the baseline C-peptide levels (x-axis) was: $y = -0.5147x + 1.4982$ ($R = 0.5501$, $P < 0.00001$, $n = 112$). These data suggest that significant correlations between the changes of insulin/C-peptide levels and the baseline insulin/C-peptide levels exist. Consequently, they have strengthened the previous data (Fig. 2b, c) that pioglitazone is indeed able to down-regulate hyperinsulinemia (or high C-peptide levels) but up-regulate hypoinsulinemia (or low C-peptide levels).

Differential effect on insulin resistance and beta-cell function with pioglitazone

Pioglitazone has been shown to reduce insulin resistance and/or improve beta-cell function (see "Introduction"). A question arises whether the magnitudes of these activities are distinct according to the baseline insulin levels. In order to answer this question, HOMA-R and HOMA-B were compared before and after the treatment in the high low, and medium insulin groups (Fig. 2d, e). In the high-insulin group, HOMA-R significantly decreased (−32.3%, $P = 0.00042$) and HOMA-B slightly increased (+11.2%, $P = 0.34$). In the low-insulin group, HOMA-R significantly decreased (−18.6%, $P = 0.00060$) and HOMA-B

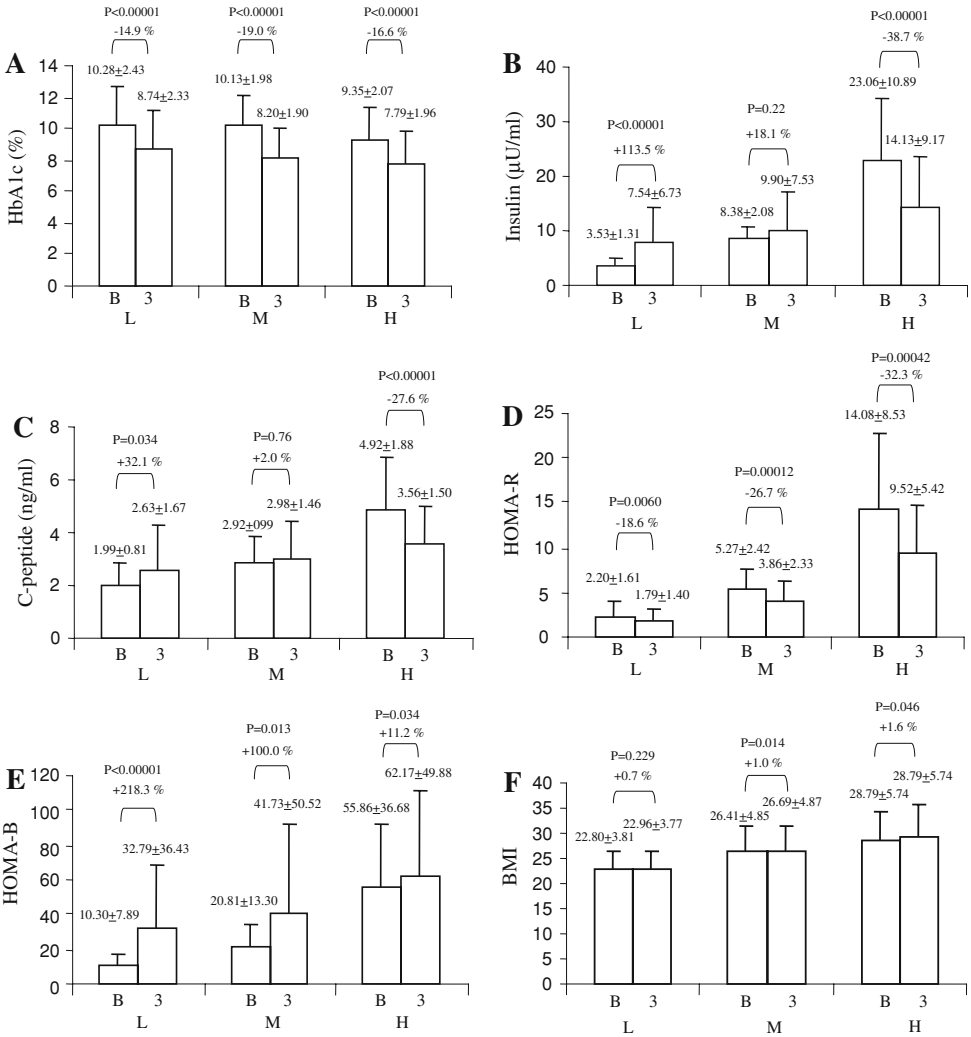

Fig. 2 Changes with pioglitazone treatment. Changes of each parameter with pioglitazone are shown in low (I), medium (M) and high (H) insulin groups at baseline (B) and 3 months [3]. Panel **A**: HbA1c. Panel **B**: insulin. Panel **C**: CPR. Panel **D**: HOMA-R. Panel **E**: HOMA-B. Panel **F**: BMI

significantly increased (+218.3%, $P < 0.00001$). In the medium-insulin group, HOMA-R significantly decreased (−26.7%, $P = 0.00012$) and HOMA-B significantly increased (+100%, $P = 0.013$). These results indicate that pioglitazone differentially exerts its effects on reducing insulin resistance and improving beta-cell function according to baseline insulin levels; HOMA-R showed greater reductions in the high-insulin group while HOMA-B showed greater increases in the low-insulin group in comparison to other groups.

Differential effect on BMI with pioglitazone

Pioglitazone is known to cause weight gain. We addressed a question whether the increase in BMI is differentially regulated according to the baseline insulin levels. As shown in Fig. 2f, the degree of BMI increase was in proportion to the baseline insulin levels (+0.7%, $P = 0.229$ for low-insulin group, +1.0%, $P = 0.014$ for medium-insulin group, and +1.6%, $P = 0.046$ for high-insulin group).

Possible contributors to the change of insulin levels with piogliatzone

The above results have shown that pioglitazone has differential effect on insulin/C-peptide levels according to the baseline insulin levels (down-regulate hyperinsulinemia but up-regulate hypoinsulinemia). Since there were significant differences in the levels of HbA1c, C-peptide, HOMA-R, HOMA-B, and BMI among these three groups at baseline, it is important to include these confounding factors when testing the relationship between the change of insulin levels with pioglitazone and the baseline insulin levels. For this purpose, multiple regression analysis was performed to detect any significant independent variables that had an influence on the changes of insulin levels. Changes of insulin levels were set as dependent variable and HbA1c, HOMA-R, HOMA-B, and BMI as independent variables. As shown in Table 2, this analysis revealed that the baseline insulin level is the independent contributor to the changes of insulin levels with pioglitazone.

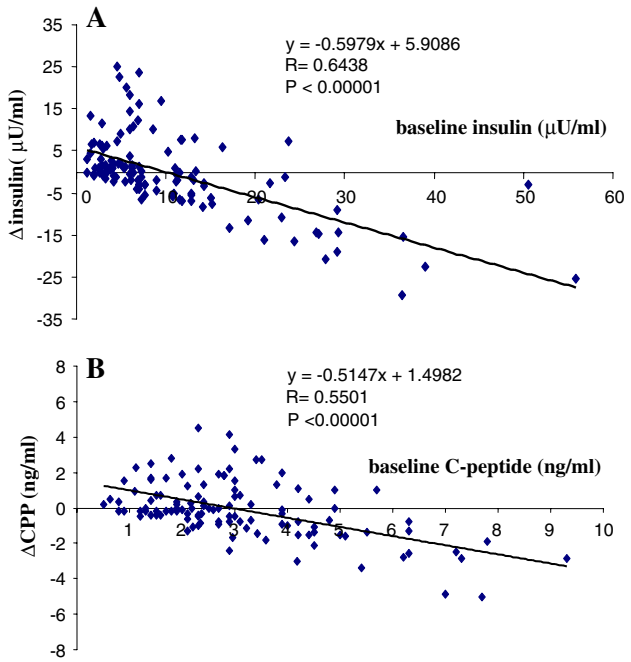

Fig. 3 Simple linear regression analysis. Panel **A**: changes of insulin levels with pioglitazone (Δ—insulin; *y*-axis) and baseline insulin levels (*x*-axis) were plotted. Panel **B**: changes of C-peptide levels with pioglitazone (Δ—peptide; *y*-axis) and baseline C-peptide levels (*x*-axis) were plotted

Discussion

One novel and intriguing finding in this study is that pioglitazone is able to differentially regulate insulin levels depending upon the baseline values (down-regulation of hyperinsulinemia and up-regulation of hypoinsulinemia, Fig. 2b). This conclusion is further strengthened by the results showing that (1) C-peptide exhibited similar change (Fig. 2c) and (2) significant correlations between the changes of insulin (or C-peptide) levels with pioglitazone and the baseline insulin (or C-peptide) levels (see Fig. 3a, b) were observed. (3) Multiple regression analysis revealed that the baseline insulin level was selected as the significant contributory factor to the change of insulin levels with pioglitazone, although other untested independent variables may be involved.

Effect of pioglitazone on insulin resistance (HOMA-R) and beta-cell function (HOMA-B) were also investigated. In the low-insulin group, the decrease of HOMA-R is less than other groups (see Fig. 2d) because it is already low enough to begin with. In contrast to this, in the high-insulin group, the increase of HOMA-B is less than other groups because it is already high enough to begin with (see Fig. 2e). Interestingly, HOMA-R and HOMA-B showed distinct changes in these groups (see Fig. 2d, e). These results suggest that pioglitazone appears to have two effects: to improve insulin resistance (and lower insulin) and to improve beta-cell function (and increase insulin). Further, the effect of insulin resistance appears to predominate if the subjects have high insulin levels and the effect of on beta-cell function seems to predominate if subjects have low-insulin levels.

The fact that pioglitazone can increase insulin levels is surprising. In any case, pioglitazone is effective in decreasing the HbA1c levels regardless of the baseline insulin levels (Fig. 2a). Initially pioglitazone was believed to exert its glucose lowering effect more efficiently with obese and hyperinsulinemic subjects. Many Japanese patients with type 2 diabetes are often lean and hypoinsulinemic. The result presented in this work showing that pioglitazone is effective in decreasing glucose levels regardless of the baseline insulin levels or BMI is consistent with other work carried out with Japanese populations (PRACTICAL, 11), and may be a favorable profile of this drug.

From a physiological point of view, it may be illustrated that pioglitazone is be able to shift the history of diabetes towards an earlier stage (rejuvenation of beta-cell), thereby changing the levels of insulin resistance, glucose and beta-cell function (insulin) towards the direction as depicted in Fig. 4 (see the arrows). This is somewhat in contrast with the current commonly accepted idea that once diabetes, hyperglycemia, and/or beta-cell impairment develop, they

Table 2 Multiple regression analysis: analysis of factors associated with the changes of insulin levels with pioglitazone. Dependent variable: change of insulin levels independent variables: insulin, HbA1c, HOMA-R, HOMA-B, and BMI

	Regession coefficients	SE	*P*-values	Lower 95%	Upper 95%
Insulin	−0.985	0.432	0.024	−1.842	−0.127
HbA1c	0.219	0.352	0.533	−0.478	0.917
HOMA-R	0.263	0.427	0.538	−0.582	1.11
HOMA-B	0.084	0.08	0.293	−0.074	0.243
BMI	0.166	0.141	0.241	−0.114	0.447
Total R2 = 0.47					

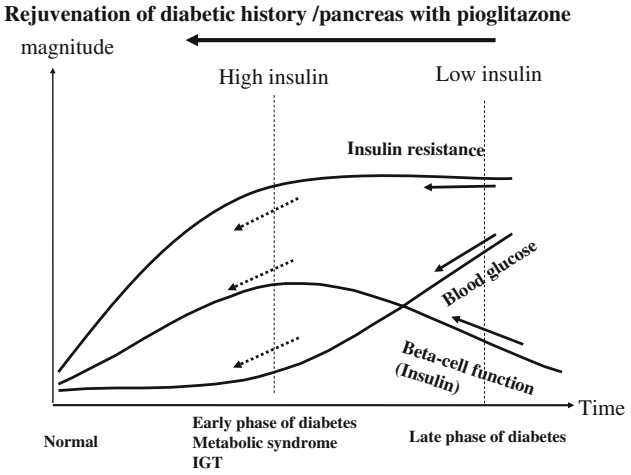

Fig. 4 Rejuvenation of diabetic history with pioglitazone. Arrows show the changes of each metabolic parameter (insulin resistance, blood glucose and beta-cell function) with pioglitazone treatment

are not curable. As a matter of fact, most patients who are newly diagnosed as type 2 diabetes do not actually have "new-onset" diabetes. Diabetes and/or hyperglycemia have been for many years at the time of clinical diagnosis. At the early stage of diabetes, the patients are asymptomatic and difficult to detect, leading to delay in diagnosis. With time, as the beta-cell defect becomes more severe, impaired insulin secretion leads to hyperglycemia and hyperglycemic symptoms at the clinical diagnosis. Thus, the idea that pioglitazone may be able to change the natural history of diabetes is attractive.

Although the precise mechanism of this differential regulation of insulin remains elusive, this can be explained as follows from a molecular and biochemical point of view (see Fig. 5).

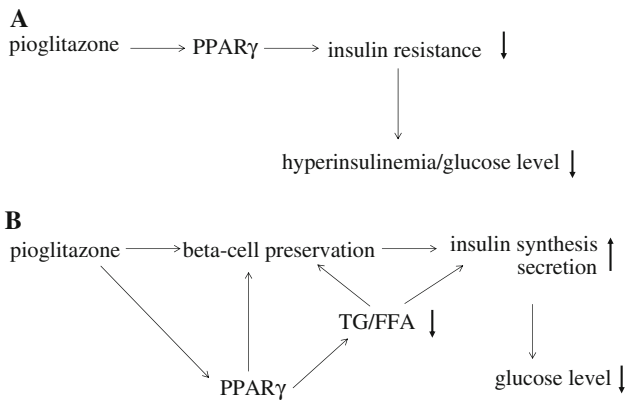

Fig. 5 Mechanism of differential regulation. Potential molecular and physiological mechanisms depending on the high and low insulin levels are depicted. Panel **A**: high insulin subjects. Panel **B**: low insulin subjects

High-insulin group (Fig. 5a)

In the high insulin group, pioglitazone decreased insulin levels (Fig. 2b). This is well in agreement with the action of TZDs, which ameliorate hyperinsulinemia. The increased insulin sensitivity (or reduced insulin resistance) with pioglitazone via the activation of PPARγ leads to unburden beta-cell demand because of the effect of insulin sensitivity to modulate beta-cell responsiveness. Thus, pioglitazone-associated decrease in beta-cell demand could potentially lead to reduced levels of insulin and glucose (see the arrow of Fig. 4 in the high insulin/early phase of diabetes/IGT/metabolic syndrome group).

Low-insulin group (Fig. 5b)

In contrast, in the low-insulin group, pioglitazone enhanced insulin levels possibly by acting directly or indirectly on beta-cells with a mechanism that is dependent or independent of PPARγ activation, thereby lowering glucose levels. The enhanced insulin levels by pioglitazone may be linked to a reduction in chronic insulin resistance that can damage or decline beta-cell function. Pioglitazone treatment as a consequence, improves beta-cell function, restores insulin secretory capacity, and decreases glucose levels (Fig. 4 see arrows in the low insulin/late phase of diabetes). However, it is still possible that these effects (enhanced insulin levels or secretory capacity) are independent from increased insulin sensitivity by pioglitazone.

It is well established that pioglitazone is able to ameliorate lipid profiles including TG and FFAs [4]. Increased insulin secretion by pioglitazone may result from the decreased TG or FFA levels with pioglitazone, since TG and FFA have been shown to reduce both insulin synthesis and glucose-stimulated insulin secretion [12, 13]. In these reports, the authors speculate that by impairing the ability of the beta-cell to compensate for insulin resistance, FFA contributes directly to the deterioration of beta-cell function that accompanies the development of diabetes. Alternatively, eliminated glucotoxicity with pioglitazone may have resulted in increased insulin secretion. At the molecular level, insulin secretion is directly controlled by pancreatic sulfonylurea receptor (SUR1, [14]). Thus, it can be speculated that SUR1 could be involved for this up-regulation, implicating a possible regulation of SUR1 by pioglitazone through PPARγ. Further basic research, for example studying the regulation of the SUR1 promoter or mRNA by pioglitazone will answer this question.

So far, most studies regarding the beta-cell protection by TZDs were done in vitro or in animal models [5–8]. Thus, this study showing that pioglitazone can improve beta-cell function and increase insulin levels in patients with type 2 diabetes may be an important observation. Since this work

only measures the steady-state insulin levels, further robust studies and observations are required to conclude that pioglitazone is capable of restoring the first-phase insulin response, improving secretory responses to plasma glucose levels, and consequently preventing or delaying the development of type 2 diabetes.

Although this study is merely an uncontrolled, observational study, one can assume that the observed changes were caused exclusively by pioglitazone based on the design of the study (monotherapy with drug naïve patients). Further randomized, double-blind, controlled study with a larger number of subjects should be performed to strengthen the finding in this study.

Subjects and methods

Subjects

Currently a project of monitoring the effect of pioglitazone monotherapy with newly diagnosed, drug naïve subject with type 2 diabetes is ongoing. The subjects are recently diagnosed as type 2 diabetes by the definition of American Diabetes Association [15] and Japan Diabetes Society [16] and have not received any regularly prescribed drugs in the last 6 months. Briefly, these subjects received 15–30 mg/day pioglitazone monotherapy and a number of sub-analysis are being undertaken. The work described in this study is one among them and is aimed to study the change of insulin levels depending on its baseline values as well as the effect on glucose (HbA1c) and insulin resistance (HOMA-R) and beta-cell function (HOMA-B). The subjects were divided into three groups; those with low- (below 5.9 μU/ml, $n = 48$) medium- (between 6 and 11.9 μU/ml, $n = 39$), and high- (above 12 μU/ml, $n = 33$) insulin levels. In the case of unacceptable or undesirable therapeutic outcome, they were free to leave this therapy whenever they wished. Exclusion criteria were clinically significant renal (CRE > 1.5 mg/dl), hepatic (GOT/GPT > 70/70 IU/l), heart (BNP > 70 pg/ml), or hypertensive (blood pressure above 150/100) disorders. Subjects who require insulin therapy (e.g., pregnant women, extremely poorly controlled subjects) were also excluded. Informed consents were obtained from the patients and all the procedures were followed by Helsinki Declaration [17]. This study was approved by the Ethical Committee.

Laboratory measurements

Measurements of FBG and HbA1c were performed once in a month. Insulin and C-peptide were measured at the start and at 3 months of the study. C-peptide was measured in most, not all of the subjects (112 out of 120 subjects; 47 in the low, 33 in the medium and 32 subjects in the high-insulin group). Anti-GAD antibody was also measured in some suspected subjects in order to exclude patients with type 1 diabetes. The blood was collected at the fasting state and the analysis was performed at Mitsubishi BCL (Tokyo, Japan) or BML (Saitama, Japan) laboratories using standard techniques. FBG and HbA1c were measured using Adams system (ARKRAY, Shiga, Japan). Insulin and C-peptide were measured using insulin and C-peptide kits (Roche Diagnostics for insulin and Siemens Medical Solutions for C-peptide with ARCHITECT analyzer). The minimum detectable concentrations were 0.5 μU/ml and <0.1 ng/ml for insulin and for C-peptide, respectively. Normal range of insulin and C-peptide levels according to this system was 2.5–15 μU/ml for insulin and 0.7–3.5 ng/ml for C-peptide. The cut-off value for the high (12 μU/ml) and low (6 μU/ml) insulin level was determined 25% from the upper limit (15 μU/ml) and 25% from the lower limit (2.5 μU/ml). HOMA-R and HOMA-B were calculated as described (ref: [18, 19] HOMA-R = IRI (μU/ml) × FBG (mg/dl)/405, HOMA-B = IRI (μU/ml) × 360/FBG(mg/dl)−63.

Hepatic (GOT, GPT, ALP, γ-GTP) and renal (BUN, CRE) functions were also monitored monthly. In case of any significant increase of these parameters, administration of pioglitazone was planned to discontinue. The drop-out subjects were excluded from the data analysis.

Data analyses

One-way analysis of variance (ANOVA) was used to compare the differences at baseline between these groups, followed by multiple range test (Ryan's method). Change was calculated as the values at 3 months (post-therapy) minus those at baseline (pre-therapy). Paired Student's *t*-test was used to analyze the changes in each group. The results were expressed as mean ± SD. Simple regression analysis was used to show whether the changes of insulin/C-peptide levels with pioglitazone are correlated with the baseline insulin/C-peptide levels. Multiple regression analysis was performed to determine the contributing factors to the changes of insulin. We used the following independent variables: HbA1c, HOMA-R, HOMA-B and BMI. Values of $P < 0.05$ were considered significant.

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