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Predictive impact of adiponectin for detecting dysglycemia in non-fasting Japanese employees with metabolic risk factors

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Abstract. The screening of dysglycemia in the non-fasting state is a challenging issue in healthcare practice. We investigated whether the additional measurement of circulating adiponectin levels could improve screening performance. We used a database of 1190 Japanese employees with metabolic risk factors, who underwent a 75-g oral glucose tolerance test (OGTT), following non-fasting health check-ups. Dysglycemia was defined as fasting glucose levels ≥6.1 mmol/L or 2-hr glucose levels ≥7.8 mmol/L during the OGTT. Logistic regression analysis revealed that decreased adiponectin levels were associated with dysglycemia, independently of postprandial glucose (PG) and hemoglobin A1c (HbA1c) levels, as well as other health check-up data ($p<0.01$). The trivariate model with PG, HbA1c, and adiponectin levels (PG+A1c+ADN model) had a larger area under the receiver operating characteristic curves (AUC) than the bivariate model with only PG and HbA1c levels (0.746 *vs.* 0.729; $p=0.01$). However, the AUC of the multivariate model with PG, HbA1c, and other health check-up data, but not adiponectin levels (PG+A1c+Other model) was 0.815, much larger than that of the PG+A1c+ADN model ($p<0.01$). The addition of adiponectin levels to the PG+A1c+Other model had a significantly larger AUC than the A1c+PG+Other model only in the subgroup without abdominal obesity ($p=0.01$), but not in the overall population ($p=0.06$) or in the subgroup with abdominal obesity ($p=0.62$). In conclusion, circulating adiponectin levels were independently associated with dysglycemia in non-fasting Japanese employees with metabolic risk factors, but they improved the screening capacity only in those without abdominal obesity.

Key words: Dysglycemia, Screening performance, Non-fasting state, Adiponectin

ALTHOUGH workplaces are one of the best places to screen people for their risk of lifestyle-related diseases, including glucose intolerance and diabetes mellitus [1], some employees have difficulty in undergoing health check-ups in the fasting state and prefer non-fasting examinations. In these subjects, the risk of dysglycemia is often assessed by postprandial plasma glucose levels as well as hemoglobin A1c levels. However, the screening performance of postprandial glucose levels is inferior compared to fasting glucose levels [2], and screening of dysglycemia in the non-fasting state is still one of the challenging issues in healthcare practice.

Recent studies have revealed that decreased circulating adiponectin levels are associated with glucose intolerance and diabetes mellitus [3-8]. However, it remains unclear whether its measurement would be useful for the screening of dysglycemia. The aim of the current study was to investigate whether the additional measurement of circulating adiponectin levels could improve the screening performance for dysglycemia in Japanese employees attending health check-ups in the non-fasting state.

Materials and Methods

Study population and definitions

We used a cross-sectional database of the Amagasaki Visceral Fat Study, registered as UMIN000002391. The study was approved by the human ethics committee of Osaka University and written informed consent was obtained from every participant. The study subjects were a total of 1190 Japanese employees of the city office of Amagasaki, Hyogo, who had never been diagnosed or treated with diabetes mellitus. All the subjects

underwent a 75-g oral glucose tolerance test (OGTT), following a non-fasting health check-up. The subjects had one or more of the following metabolic risk factors: (1) postprandial glucose levels ≥ 7.8 mmol/L (140 mg/dL), (2) hemoglobin A1c levels ≥ 5.6%, (3) elevated blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, or taking medications of hypertension), (4) abnormal lipid metabolism (high-density-lipoprotein (HDL) cholesterol levels < 1.0 mmol/L (40 mg/dL), postprandial triglycerides levels ≥ 2.3 mmol/L (200 mg/dL), or taking medications of dyslipidemia), (5) body mass index (BMI) ≥ 25 kg/m^2, (6) abdominal obesity (waist circumference ≥ 85 cm in males and ≥ 90 cm in females), and/or (7) serum alanine aminotransferase levels ≥ 31 U/L [9-15]. We included these subjects, simply because they were considered to have a high risk for dysglycemia, and to be a high priority of the screening. Indeed, OGTT is recommended in those with postprandial glucose levels ≥ 7.8 mmol/L or hemoglobin A1c levels ≥ 5.6% [9]. Furthermore, it is known that metabolic syndrome and obesity are associated with insulin resistance and diabetes mellitus [14-17]. It is also known that individual components of metabolic syndrome are associated with insulin resistance [17-19]. We therefore adopted these components as inclusion criteria in the current study. We used 2.3 mmol/L (200 mg/dL) as a cutoff point of postprandial triglycerides levels [10] , providing a high specificity for hypertriglyceridemia defined by fasting triglycerides levels ≥ 1.7 mmol/L (150 mg/dL) [20]. We also adopted elevated alanine aminotransferase levels as an inclusion criterion, because alanine aminotransferase is known to be a marker of non-alcoholic fatty liver disease, which is associated with insulin resistance and diabetes mellitus [11-13]. Note that these data were obtained in the preceding non-fasting health check-up (see below). Subjects with hepatic disease, renal disease, and/or malignant neoplasms were excluded for the current study.

In the preceding health check-ups, anthropometric measurements, sphygmomanometry, and laboratory examinations, as well as checking of medical history, were performed. Laboratory examinations included plasma glucose, hemoglobin A1c, serum total cholesterol, HDL cholesterol, triglycerides, transaminase, and adiponectin levels. Serum concentrations of adiponectin were measured by a latex particle-enhanced turbidimetric assay (Otsuka Pharmaceutical and Mitsubishi Chemical Medience, Tokyo, Japan) [21]. Hemoglobin

A1c levels were measured by high-performance liquid chromatography (HPLC) and were converted to a National Glycohemoglobin Standardization Program equivalent value with the conversion equation reported by the Japan Diabetes Society [22].

The outcome measure was dysglycemia, determined by the subsequent 75-g OGTT as either fasting plasma glucose levels ≥ 6.1 mmol/L (110 mg/dL) or 2-hour plasma glucose levels ≥ 7.8 mmol/L (140 mg/dL) (*i.e.*, not "normal type") [9]. We investigated whether circulating adiponectin levels would have a significant association with, and have a significant predictive performance for the outcome.

Statistical analysis

Logistic regression models were used to develop predictive tools for detecting undiagnosed dysglycemia. The dependent variable was the presence of OGTT-detected dysglycemia and the explanatory variables were the laboratory and non-laboratory data obtained from the preceding health check-ups. We first developed a bivariate model in which explanatory variables were plasma glucose and hemoglobin A1c levels (PG+A1c model). We subsequently developed a trivariate model which also included adiponectin levels (PG+A1c+ADN model). We also developed a multivariate stepwise model in which other health check-up data instead of adiponectin levels were added to plasma glucose and hemoglobin A1c levels (PG+A1c+Other model). The candidates for the explanatory variables other than plasma glucose and hemoglobin A1c levels were: sex, age, BMI, systolic blood pressure, serum total cholesterol, HDL cholesterol, triglycerides, alanine aminotransferase, and presence of hypertension and dyslipidemia under treatment. We finally developed a full model in which both adiponectin levels were added to the PG+A1c+Other model (Full model). The predictive capability of these developed models for dysglycemia was evaluated using receiver operating characteristic (ROC) curves, and the area under the curves (AUC) was calculated. We also investigated the predictive capability of the subgroups with and without abdominal obesity.

Data are given as means and standard deviations (SD) for continuous variables or as percentages for dichotomous variables, if not mentioned otherwise. Serum adiponectin levels, as well as serum triglycerides and alanine aminotransferase levels, had a right-skewed distribution and therefore was log-trans-

Table 1 Characteristics of the study population

	Overall population (n = 1190)	Subjects without dysglycemia (n = 894)	Subjects with dysglycemia (n = 296)	p value
Males	1015 (85%)	759 (85%)	256 (86%)	0.57
Age (years)	50 ± 9	48 ± 9	54 ± 7	< 0.01
Body mass index (kg/m^2)	25.1 ± 3.2	24.9 ± 2.9	25.7 ± 3.7	< 0.01
Systolic blood pressure (mmHg)	130 ± 18	128 ± 17	136 ± 19	< 0.01
Plasma glucose (mmol/L)	5.8 ± 1.1	5.6 ± 1.0	6.3 ± 1.3	< 0.01
Hemoglobin A1c (%)	5.6 ± 0.4	5.5 ± 0.4	5.8 ± 0.4	< 0.01
Total cholesterol (mmol/L)	5.4 ± 0.9	5.4 ± 0.9	5.5 ± 1.0	0.13
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.27
Triglycerides (mmol/L)	1.8 (1.2 – 2.7)	1.7 (1.1 – 2.6)	2.1 (1.4 – 2.8)	
Log (Triglycerides (mmol/L))	0.25 ± 0.26	0.23 ± 0.26	0.29 ± 0.24	< 0.01
Alanine aminotransferase (IU/L)	21 (16 – 34)	21 (15 – 32)	26 (19 – 41)	
Log (Alanine aminotransferase (IU/L))	1.37 ± 0.23	1.35 ± 0.23	1.44 ± 0.25	< 0.01
Adiponectin (µg/mL)	6.3 (4.7 – 8.3)	6.4 (4.8 – 8.6)	6.0 (4.4 – 7.6)	
Log (Adiponectin (µg/mL))	0.80 ± 0.20	0.81 ± 0.20	0.76 ± 0.19	< 0.01
Hypertension under treatment	169 (14%)	92 (10%)	77 (26%)	< 0.01
Dyslipidemia under treatment	52 (4%)	31 (3%)	21 (7%)	0.01

Data are means ± SD or n (%), except for original values of triglycerides, alanine aminotransferase, and adiponectin levels, which are presented as medians (quartiles). p values are between those with and without dysglycemia.

formed. Differences of continuous variables between two groups were evaluated by the unpaired t test, whereas dichotomous variables between two groups were compared by the Fisher's exact test. A p value for trend among more than two groups was assessed by the linear trend test in one-way ANOVA for continuous variables, and by the linear-by-linear association chi-squared test for dichotomous variables. In logistic regression analysis, continuous variables were not transformed into categorical variables but were entered as they were [23, 24]. Odds ratios of the continuous variables were shown as those per one-SD increment, rather than those per one-unit increment [25], to make an easier inter-variable comparison in the association with dysglycemia. A p value of less than 0.05 was considered to be significant and 95% confidence intervals (CI) were given when required. The statistical difference in the AUC was assessed through 10000-time bootstrap resampling, with the use of R version 2.12.1 (R Development Core Team), whereas the other statistical analyses were performed using IBM SPSS Statistics Version 19 (SPSS Inc.).
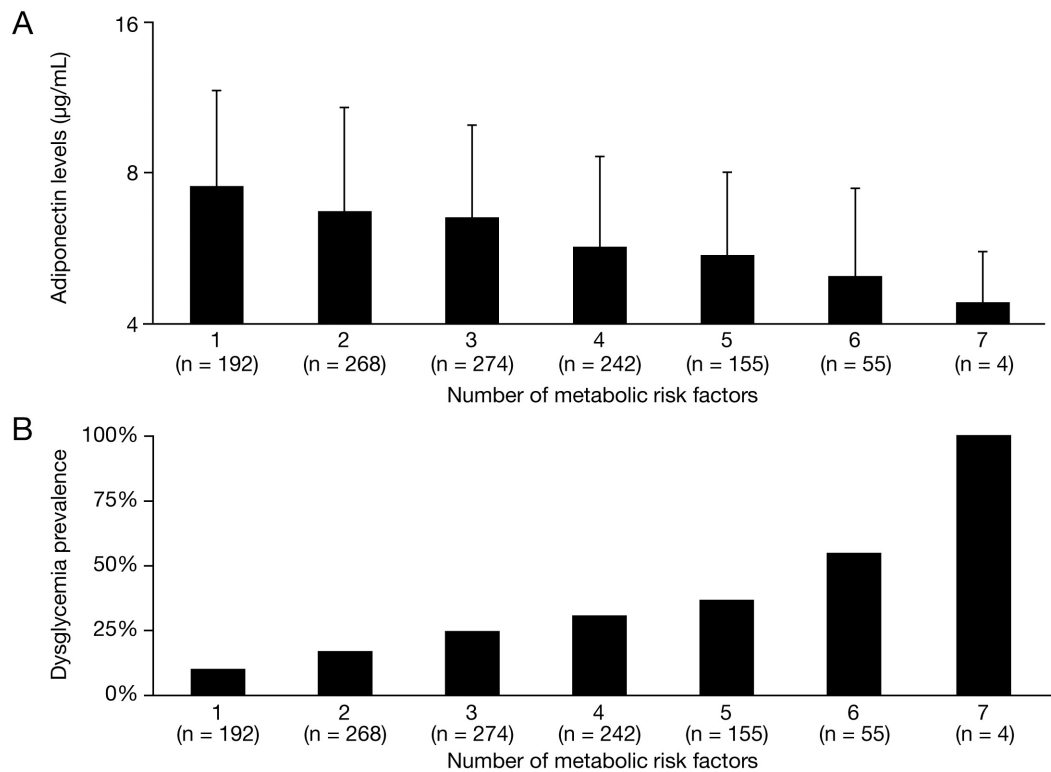
Results

The characteristics of the study population are shown in Table 1. They were 50 ± 9 years old and 1015 (85%) were male. The prevalence of dysglycemia detected by the 75-g OGTT was 25% (n = 296). Circulating adiponectin levels were significantly lower in the subjects with dysglycemia compared to those without it (p < 0.01). The accumulation of metabolic risk factors was significantly associated with low circulating adiponectin levels and a high prevalence of dysglycemia (both p for trend < 0.01) (Fig. 1).

Table 2 shows the association of characteristics with dysglycemia in the logistic regression models. Increased adiponectin levels had an inverse association with the risk of dysglycemia, independently of plasma glucose and hemoglobin A1c levels, as well as other covariates. The adjusted odds ratio and its 95% CI of log-transformed adiponectin levels per one-SD increment were 0.7 [0.6, 0.8] in the PG+A1c+ADN model, and 0.8 [0.6, 0.9] in the Full model (both p < 0.01).

The ROC curves of these developed models for detecting dysglycemia are shown in Fig. 2A. The PG+A1c+ADN model had a significantly larger AUC than the PG+A1c model (0.746 vs. 0.729). The difference of the AUC and its 95% CI were 0.017 [0.004, 0.031] (p = 0.01). However, the AUC of the PG+A1c+Other model was 0.815 and was much larger than that of the PG+A1c+ADN model; the difference of AUC and its 95% CI were 0.069 [0.039, 0.099] (p < 0.01). On the other hand, the AUC of the Full model
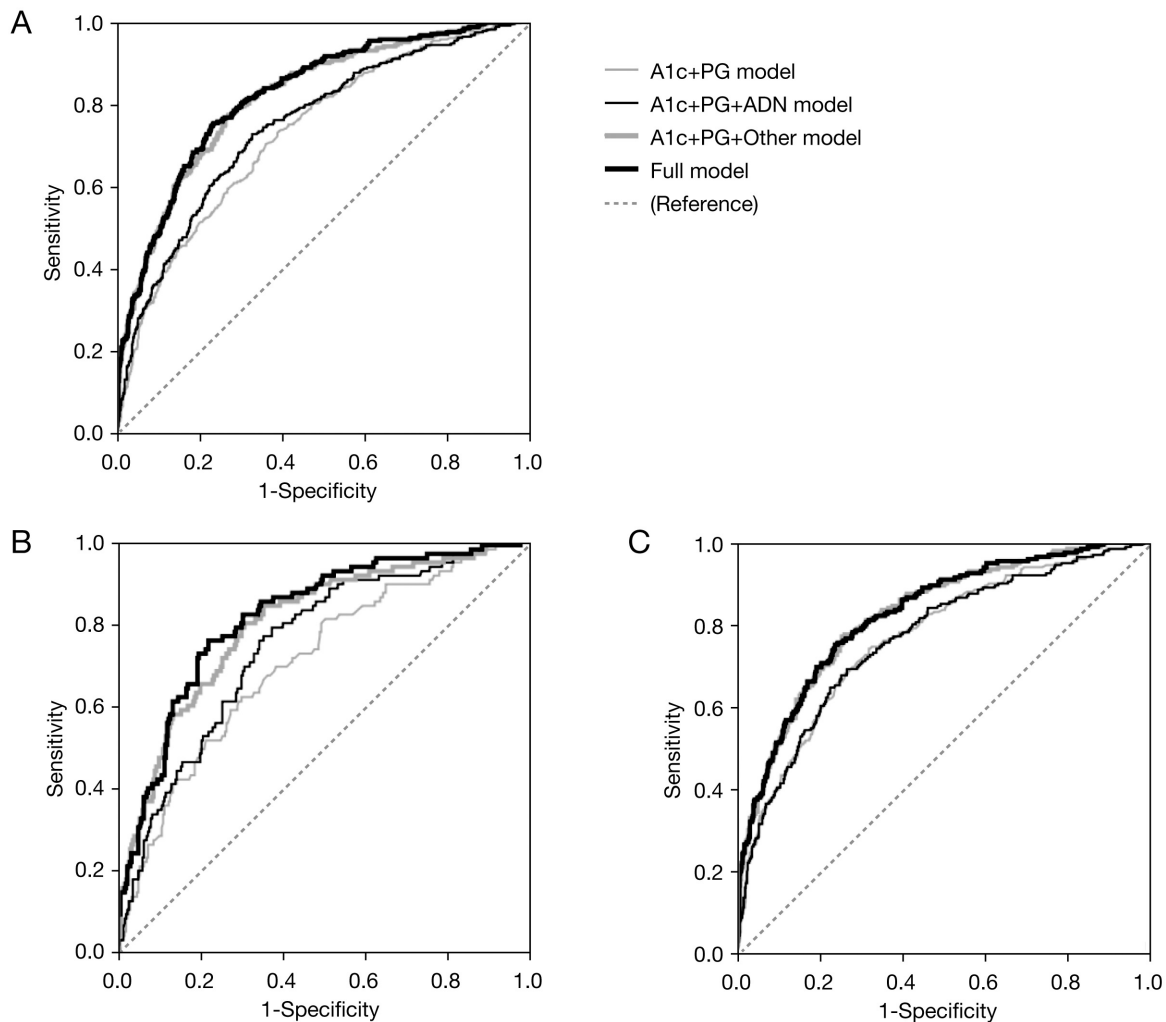
Fig. 1 Association of accumulating metabolic risk factors with circulating adiponectin levels (A) and the prevalence of dysglycemia (B)
The study population was classified according to the number of accumulating metabolic risk factors. The metabolic risk factors were as follows (see Methods): (1) postprandial glucose levels ≥ 7.8 mmol/L, (2) hemoglobin A1c levels ≥ 5.6%, (3) elevated blood pressure, (4) abnormal lipid metabolism, (5) BMI ≥ 25 kg/m^2, (6) abdominal obesity, and/or (7) elevated serum alanine aminotransferase levels. Note that all individuals in the study population had one or more metabolic risk factors.

Table 2 Logistic regression models for dysglycemia

	Univariate model	PG+A1c model	PG+A1c+ADN model	PG+A1c+Other model	Full model
Males	1.1 [0.8, 1.7]	---	---	N/I	N/I
Age	2.0 [1.7, 2.3]*	---	---	1.8 [1.5, 2.2]*	1.9 [1.6, 2.4]*
Body mass index	1.3 [1.1, 1.5]*	---	---	1.3 [1.1, 1.5]*	1.3 [1.1, 1.5]*
Systolic blood pressure	1.5 [1.3, 1.7]*	---	---	1.3 [1.1, 1.5]*	1.3 [1.1, 1.5]*
Plasma glucose	1.7 [1.5, 2.0]*	1.6 [1.4, 1.8]*	1.6 [1.4, 1.8]*	1.6 [1.4, 1.9]*	1.6 [1.4, 1.9]*
Hemoglobin A1c	2.1 [1.8, 2.5]*	2.0 [1.7, 2.3]*	2.1 [1.8, 2.5]*	2.1 [1.7, 2.5]*	2.1 [1.8, 2.5]*
Total cholesterol	1.1 [1.0, 1.3]	---	---	N/I	N/I
HDL cholesterol	0.9 [0.8, 1.1]	---	---	N/I	N/I
Log (Triglycerides)	1.3 [1.1, 1.4]*	---	---	N/I	N/I
Log (Alanine aminotransferase)	1.5 [1.3, 1.7]*	---	---	1.8 [1.5, 2.2]*	1.7 [1.4, 2.0]*
Log (Adiponectin)	0.8 [0.7, 0.9]*	---	0.7 [0.6, 0.8]*	---	0.8 [0.6, 0.9]*
Hypertension under treatment	3.1 [2.2, 4.3]*	---	---	1.8 [1.2, 2.6]*	1.7 [1.2, 2.6]*
Dyslipidemia under treatment	2.1 [1.2, 3.8]*	---	---	N/I	N/I

Data are unadjusted odds ratios (OR) [95% CI] in the Univariate model, and adjusted OR [95% CI] in the other models. The OR [95% CI] of continuous variables are presented per one-SD increment. Asterisks show that the OR are statistically significant ($p < 0.05$). N/I, a candidate but not included in the final stepwise model.

Fig. 2 Screening performance for detecting undiagnosed dysglycemia

ROC curves of the developed models are shown in the overall population (**A**), the subgroup without abdominal obesity (**B**), and those with abdominal obesity (**C**). Thin gray line, the A1c+PG model; thin black line, the A1c+PG+ADN model; thick gray line, the A1c+PG+Other model; thick black line, the Full model. The AUC of the A1c+PG+ADN model (thin black line) was significantly larger than that of the A1c+PG model (thin gray line) in the overall population (**A**) and in the subgroup without abdominal obesity (**B**) (both *p* = 0.01), but not in the subgroup with abdominal obesity (**C**) (*p* = 0.80). The Full model (thick black line) had a significantly larger AUC than the A1c+PG+Other model (thick gray line) in the subgroup without abdominal obesity (**B**) (*p* = 0.01), but not in the overall population (**A**) (*p* = 0.06) or in the subgroup with abdominal obesity (**C**) (*p* = 0.62).

was 0.821, which was not significantly larger than that of the PG+A1c+Other model; the difference and its 95% CI were 0.006 [0.000, 0.012] (*p* = 0.06).

We subsequently assessed the predictive capability in the subgroups with and without abdominal obesity. Fig. 2B and C show the ROC curves of the developed models for dysglycemia in the subgroups without and with abdominal obesity, respectively. In the subjects without abdominal obesity (*n* = 396), as shown in Fig. 2B, the PG+A1c+ADN model had a significantly larger AUC than the PG+A1c model (0.747 *vs*. 0.705;

p = 0.01). Furthermore, the AUC of the Full model was significantly larger than that of the PG+A1c+Other model (0.817 *vs*. 0.800; *p* = 0.01).

On the other hand, in the subjects with abdominal obesity (*n* = 794), the addition of adiponectin levels did not improve the predictive performance (Fig. 2C). The AUC of the A1c+PG+ADN model was 0.764 and was not significantly different from that of the A1c+PG model (*p* = 0.80, *vs*. 0.766). Neither was there any significant difference in the AUC between the Full model and the A1c+PG+Other model (0.824 *vs*. 0.822; *p* = 0.62).

Discussion

The current study revealed that circulating adiponectin levels were independently associated with dysglycemia in non-fasting Japanese employees with metabolic risk factors, but that they improved the screening capacity only in those without abdominal obesity.

Early detection of dysglycemia is now regarded as an important strategy in healthcare fields [2], and health check-ups of employees has been widely performed at the workplace in Japan. However, there remains one challenging issue concerning health check-ups at the workplace; some employees have difficulty in undergoing health check-ups in the fasting state. In these subjects, the risk of dysglycemia is often assessed by postprandial plasma glucose levels, as well as hemoglobin A1c levels. As is often pointed out, the screening performance of postprandial examinations is inferior compared to fasting glucose levels [2]. A strategy to improve the screening performance in the non-fasting state is now highly desired in healthcare practice.

Circulating adiponectin level is one of the most promising biomarkers very close to practical use. Adiponectin is an adipocytokine constitutively produced by adipose tissue, whose circulating levels are inversely correlated with adiposity [26-28]. Recent studies demonstrated that its decreased levels are associated with various diseases, and dysglycemia is no exception [3-8]. However, most previous studies only investigated the relative risk of decreased adiponectin levels for dysglycemia, and did not assess its predictive performance, i.e, the area under the ROC curves (AUC). As recent studies demonstrated, a significant and independent association of a biomarker with an outcome, assessed with its relative risk, will not always guarantee its significant improvement of the predictive performance [29, 30]. To assess its usefulness as a predictor, an evaluation of its predictive performance is needed [31]. Furthermore, it remains unknown whether the measurement of adiponectin levels is useful for the screening of dysglycemia in the non-fasting state. To the best of our knowledge, this is the first report concerning the improvement of screening performance by adiponectin levels in the non-fasting state.

In the current study, the PG+A1c+ADN model had a significantly larger AUC than the PG+A1c model in the overall population (Fig. 2A), indicating that the additional measurement of circulating adiponectin levels would significantly improve the screening performance compared to the combination of only postprandial glucose and hemoglobin A1c levels. However, the screening performance of the PG+A1c+ADN model was significantly inferior compared to the PG+A1c+Other model (Fig. 1A). That is to say, the additional consideration of other routine health check-up data would be more informative than the additional measurement of adiponectin levels in the screening of dysglycemia. Furthermore, the AUC of the Full model (*i.e.*, adding adiponectin levels to the PG+A1c+Other model) was not significantly different from that of the PG+A1c+Other model (Fig. 1A), although adiponectin levels were independently associated with dysglycemia (Table 2). These findings indicate that adiponectin levels would provide little additive information to other routine health check-up data in the screening of dysglycemia in the overall population. It is possible that adiponectin explains some effects of conventional risk factors on the development of dysglycemia, which would obscure the clinical usefulness of adiponectin measurements for predicting dysglycemia.

The current study also investigated whether the healthcare usefulness of adiponectin measurements would be consistent in subjects with and without abdominal obesity. Numerous previous studies have shown a strong inverse association between adiponectin and abdominal obesity [28]. In general, a ROC curve of a test value for an outcome could depend on the distribution of the test value [32]. Given that subjects with abdominal obesity are likely to have lower circulating adiponectin levels than those without it, adiponectin measurements might have different impacts on the predictive performance for dysglycemia between the two subgroups. Indeed, our study revealed that adding adiponectin measurements significantly improved the predictive performance in the subjects without abdominal obesity, but not in those with abdominal obesity (Fig. 2B and C). It is possible that in the subjects with abdominal obesity, coexisting metabolic abnormalities would already be so informative in predicting dysglycemia that adiponectin measurements would have little room for adding predictive information. Another possible explanation is that adiponectin levels might be low in most of the subjects with abdominal obesity [33]. Their relatively narrow distribution within a low range might cause their limited discriminative capability for dysglycemia prediction.

The current study had some limitations. First, the subjects in the current study had one or more metabolic

risk factors. It still remains unknown whether the measurement of adiponectin levels would be useful in the subjects with no metabolic risk factors. Second, the current study did not assess high-molecular-weight adiponectin levels. They might show a different screening performance from that of total adiponectin levels. Third, the prevalence of male sex was high and relatively few females were included in the current study. Future studies with a sufficient number of females will be needed to validate the current findings.

In conclusion, circulating adiponectin levels were independently associated with dysglycemia in non-fasting Japanese employees with metabolic risk factors, but their measurements improved the screening capacity only in those without abdominal obesity.

Acknowledgements

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