

# Measurement of the High-Molecular Weight Form of Adiponectin in Plasma Is Useful for the Prediction of Insulin Resistance and Metabolic Syndrome

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**OBJECTIVE** — The high-molecular weight (HMW) form of adiponectin, an adipocyte-derived insulin-sensitizing hormone, has been reported to be the most active form of this hormone. We investigated whether measurement of plasma HMW adiponectin levels, using our newly developed enzyme-linked immunosorbent assay system for selective measurement of human HMW adiponectin level, may be useful for the prediction of insulin resistance and metabolic syndrome.

**RESEARCH DESIGN AND METHODS** — A total of 298 patients admitted for diabetes treatment or coronary angiography served as study subjects. Receiver operator characteristic (ROC) curves for the HMW ratio (HMWR; ratio of plasma level of HMW adiponectin to that of total adiponectin) and plasma total adiponectin levels were plotted to predict the presence of insulin resistance and metabolic syndrome.

**RESULTS** — The area under the ROC curve (AUC) of the HMWR values to predict the presence of insulin resistance was significantly larger than that of plasma total adiponectin level in total subjects (0.713 [95% CI 0.620–0.805] vs. 0.615 [0.522–0.708],  $P = 0.0160$ ). The AUC for the HMWR values to predict the presence of metabolic syndrome was significantly larger than that for plasma total adiponectin levels in men (0.806 [0.747–0.865] vs. 0.730 [0.660–0.800],  $P = 0.0025$ ) and in women (0.743 [0.659–0.828] vs. 0.637 [0.532–0.742],  $P = 0.0458$ ).

**CONCLUSIONS** — The HMWR value has better predictive power for the prediction of insulin resistance and metabolic syndrome than plasma total adiponectin level.

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**A**diponectin (also known as ACRP30, GBP28, and AdipoQ) is a hormone secreted exclusively by adipocytes (1–4). Adiponectin replenishment has

been found to ameliorate the abnormalities of metabolic syndrome, including insulin resistance, hyperglycemia, and dyslipidemia, in a murine model of obe-

sity-linked metabolic syndrome associated with decreased adiponectin levels (5). Adiponectin-deficient mice (6,7) have been demonstrated to show features of metabolic syndrome, such as insulin resistance, glucose intolerance, dyslipidemia, and hypertension. In humans, decreased plasma adiponectin levels have been demonstrated in patients with obesity, diabetes, and coronary artery disease (8–10), all of which are linked to insulin resistance. Moreover, the degree of hypoadiponectinemia has been reported to be correlated with the degree of insulin resistance (11,12), and hypoadiponectinemia has been shown to be closely associated with the clinical phenotype of metabolic syndrome (13,14). The gene encoding adiponectin (*APM1*) has been mapped to chromosome 3q27, which has been reported to be linked to type 2 diabetes and metabolic syndrome by genome-wide scans in Japanese (15), American, (16), and French-Caucasian (17) populations. A single nucleotide polymorphism in the adiponectin gene was shown to be associated with hypoadiponectinemia, insulin resistance, and increased risk of type 2 diabetes (18,19), indicating that adiponectin may play a crucial role in the regulation of insulin sensitivity and glucose and lipid metabolism and that reduced plasma adiponectin levels caused by genetic and environmental factors may lead to the development of insulin resistance, type 2 diabetes, and metabolic syndrome (20). Indeed, a recent study demonstrated that individuals with high plasma adiponectin levels had a substantially lower relative risk of developing type 2 diabetes, even after adjustment for conventional risk factors, such as BMI (21,22).

We have reported that adiponectin forms multimers and is present in the serum as a trimer, hexamer, or as a high-molecular weight (HMW) form (23). The HMW isoform binds most avidly to its receptors and stimulates AMP-activated protein kinase, one of the key molecules mediating the metabolic actions of adiponectin (Y. Hada, T.Y., H. Waki, K.H.,

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**Abbreviations:** AUC, area under the curve; HMW, high molecular weight; HMWR, HMW ratio; HOMA-IR, homeostasis model assessment of insulin resistance; IDF, International Diabetes Federation; ROC, receiver operator characteristic.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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H.Y., O.M., H.E., T.K., unpublished observations). Mutations in the adiponectin gene that cause impaired multimerization and decreased plasma HMW adiponectin levels have been found to be associated with insulin resistance and type 2 diabetes (23), suggesting that alterations in plasma HMW adiponectin level may be more relevant in the prediction of insulin resistance than those in plasma total adiponectin levels. Indeed, a recent study has shown that the ratio of the plasma level of HMW adiponectin to that of total adiponectin level (HMWR) is significantly more useful for monitoring the improvement of insulin sensitivity in response to thiazolidinediones in cases of type 2 diabetes (24). The HMWR value has been also shown, by oral glucose tolerance tests, to be more significantly inversely correlated with 2-h glucose levels than total adiponectin level (25). However, in this study, the adiponectin multimers were separated by velocity sedimentation/gel filtration and quantified HMW adiponectin level by Western blotting. In the present study, we investigated the clinical usefulness of measurement of plasma HMW adiponectin level using a newly developed method, as compared with that of plasma total adiponectin level by analyzing the sensitivity or specificity of total adiponectin levels, HMW adiponectin levels, and HMWR values for the prediction of insulin resistance and metabolic syndrome. This study is the first to demonstrate the clinical usefulness of measuring HMW adiponectin levels in making precise prediction of insulin resistance and metabolic syndrome.

## RESEARCH DESIGN AND METHODS

The subjects of this study were 298 patients admitted to Tokyo University Hospital for the treatment of diabetes or coronary angiography. The present study was conducted according to the principles outlined in the Declaration of Helsinki. Informed consent was obtained from each of the study subjects. The protocol of the study was approved by the ethics review committee of Tokyo University School of Medicine.

Height, weight, hip, waist, fasting plasma glucose, serum insulin, serum total cholesterol, triglyceride, HDL cholesterol, and LDL cholesterol levels were measured the morning after an overnight fast. We modified the diagnostic criteria of the National Cholesterol Education Program Adult Treatment Panel III for

metabolic syndrome (26) by adopting a cutoff level for the waist circumference of 85 cm in men and 90 cm in women, in accordance with the recommendations for the Japanese population by the International Diabetes Federation (IDF) (27). The homeostasis model assessment of insulin resistance (HOMA-IR) (28) was determined in 171 subjects (110 men and 61 women); however, it could not be assessed in subjects being treated with insulin or in some of the subjects admitted for coronary angiography. We defined subjects with a HOMA-IR of  $>2.5$  as having insulin resistance. This cutoff has been adopted in the Japanese guideline for the treatment of diabetes. We analyzed the relationship between total adiponectin levels or HMWR values and severity and extent of coronary atherosclerosis using the score by Gensini (29). Subjects with a history of percutaneous coronary intervention were excluded from this analysis. Because thiazolidinediones have been reported to exhibit direct antirestenotic effects in the vasculature (30) and have protective effects against coronary artery disease (31), after the prescription of thiazolidinediones was incorporated into the model in addition to the conventional risk factors for atherosclerosis, stepwise regression analysis was done to analyze the relationship between HMWR values and Gensini score. The measurement method for plasma HMW adiponectin level is described elsewhere (H.E., O.M., H.Y., K.H., T.Y., T.K., unpublished observations) and coefficients of variations of the assay were 5.3% for total adiponectin and 3.3% for HMW adiponectin level.

## Statistical analyses

The values of the clinical parameters were expressed as means  $\pm$  SD. All statistical analyses were performed using JMP for Windows software (version 4.0; SAS Institute, Cary, NC). The significance of differences in plasma total adiponectin levels, HMW adiponectin levels, and HMWR values was analyzed by ANOVA. ROC curves were plotted and compared using the Stata software (College Station, TX). *P* values  $<0.05$  were considered to denote statistical significance.

**RESULTS**—As shown in online appendix Table 1 (available at <http://care.diabetesjournals.org>), there is no difference in the proportion of the subjects who took at least one of the drugs potentially affecting plasma adiponectin

levels, such as thiazolidinediones, biguanide, ACE inhibitors, and angiotensin receptor blockers, between subjects with and without metabolic syndrome (68 [42.5%] vs. 54 [39.1%], *P* = 0.686).

## Correlations between patient characteristics and plasma total adiponectin and HMW adiponectin levels and HMWR values

Women had higher plasma total adiponectin levels ( $5.59 \pm 0.31$  vs.  $4.55 \pm 0.22$   $\mu\text{g/ml}$ , *P* = 0.0069), HMW adiponectin levels ( $2.19 \pm 0.14$  vs.  $1.54 \pm 0.11$   $\mu\text{g/ml}$ , *P* = 0.0003), and HMWR values ( $35.9 \pm 1.1$  vs.  $29.9 \pm 0.8\%$ , *P* = 0.00001) than men. There was an inverse correlation between BMI and plasma total adiponectin levels ( $r = -0.29$ , *P* = 0.0001), HMW adiponectin levels ( $r = -0.27$ , *P* = 0.0001), and HMWR values ( $r = -0.13$ , *P* = 0.012). In multivariate analysis taking into account BMI, sex, and the interaction between sex and BMI, sex and BMI were independently correlated with plasma total adiponectin levels (*P* = 0.0001 and 0.0025, respectively), plasma HMW adiponectin levels (*P* = 0.0001 and 0.0001, respectively), and HMWR values (*P* = 0.0075 and 0.0001, respectively). However, since there was no interaction between sex and BMI for these three different measurements of plasma adiponectin (*P* = 0.29, 0.23, and 0.34, respectively), the inverse correlation between BMI and plasma total adiponectin level, plasma HMW adiponectin levels, or HMWR values were not affected by sex. Plasma total adiponectin level, plasma HMW adiponectin levels, and HMWR values did not vary with age in either sex (data not shown).

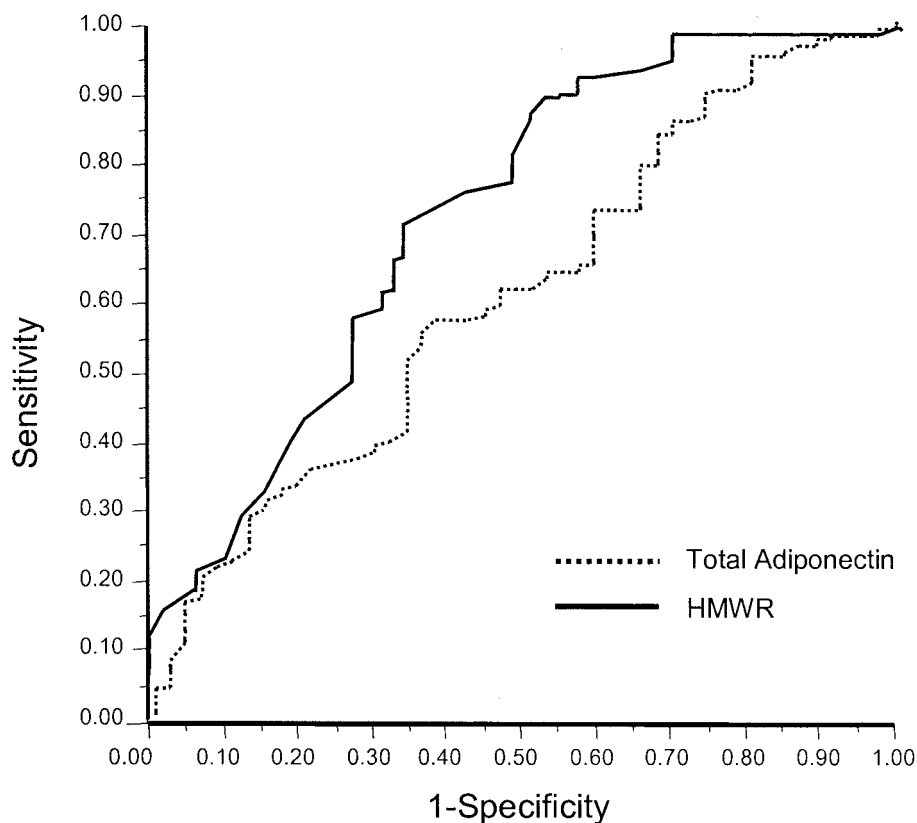
## HMWR value predicted insulin resistance more precisely than plasma total adiponectin level

Insulin resistance is closely linked with metabolic syndrome and is often observed even in the early stage of metabolic syndrome (32). We investigated the correlation between insulin resistance and each of the three different measurements of plasma adiponectin. The total adiponectin level was inversely correlated with the HOMA-IR (*P* = 0.0280) in 171 men and women, consistent with the results of previous studies (11,12); the HMW adiponectin levels (*P* = 0.0035) and HMWR values (*P* = 0.0008) were also inversely correlated with HOMA-IR. In 110 men, there was a significant inverse correlation between HOMA-IR and

plasma HMW adiponectin levels ( $P = 0.0083$ ) and HMWR values ( $P = 0.0010$ ), while there was only a tendency toward inverse correlation between HOMA-IR and plasma total adiponectin levels ( $P = 0.0613$ ). In 61 women, tendencies were found for decreased plasma total adiponectin levels and for HMW adiponectin levels or HMWR values to be associated with increased HOMA-IR values. HMWR values were significantly and inversely correlated with HOMA-IR, even after adjusting for age, sex, and BMI ( $P = 0.0304$ ) or adjusting for BMI alone ( $P = 0.0389$ ) in 171 men and women, suggesting that correlation between HMWR values and HOMA-IR was independent of the association between BMI and HOMA-IR. In contrast, the statistically significant association between total adiponectin and HOMA-IR disappeared after adjusting for age, sex, and BMI ( $P = 0.227$ ) or adjusting for BMI alone ( $P = 0.249$ ) in 171 men and women. These results are again consistent with the superiority of HMWR values over total adiponectin level to predict the presence of insulin resistance. We then plotted ROC curves to compare the power of plasma total adiponectin level and HMWR value to predict the presence of insulin resistance (Fig. 1). The AUC for HMWR values was significantly larger than that of plasma total adiponectin levels (0.713 [95% CI 0.620–0.805] vs. 0.615 [0.522–0.708],  $P = 0.0160$ ) (Fig. 1), suggesting that the HMWR value had better predictive power for the prediction of insulin resistance than plasma total adiponectin level. When a cutoff value of 35% was used, the HMWR value predicted the presence of insulin resistance with a sensitivity of 72% and specificity of 66%. On the other hand, at a cutoff level of 4.2  $\mu\text{g/ml}$ , plasma total adiponectin diagnosed insulin resistance with a sensitivity of 56% and specificity of 63%. When stratified according to sex, there was a significant difference between the AUCs for plasma total adiponectin levels and HMWR values in men (0.713 [0.605–0.821],  $P = 0.048$  vs. 0.624 [0.514–0.733],  $P = 0.048$ ), while we could detect no such difference in women (0.794 vs. 0.665,  $P = 0.09$ ).

#### Correlation between the number of risk factors and plasma total adiponectin levels, HMW adiponectin levels, or HMWR values

We then investigated the association between the number of risk factors defining the prediction of metabolic syndrome



**Figure 1**—ROC curves of plasma total adiponectin levels and HMWR values for the prediction of insulin resistance, defined as a HOMA-IR index  $>2.5$  ( $n = 171$ ). The AUC for the HMWR values was significantly larger than that for plasma total adiponectin levels (0.713 [95% CI 0.620–0.805] vs. 0.615 [0.522–0.708],  $P = 0.0160$ ).

(see RESEARCH DESIGN AND METHODS) in the subjects and plasma total adiponectin levels, HMW adiponectin levels, and HMWR values. The plasma total adiponectin levels ( $P = 0.0001$ ), HMW adiponectin levels ( $P = 0.0001$ ), and HMWR values ( $P = 0.0001$ ) decreased as the number of risk factors present increased; this tendency was observed irrespective of sex (data not shown).

#### ROC curves for models to predict the presence of metabolic syndrome

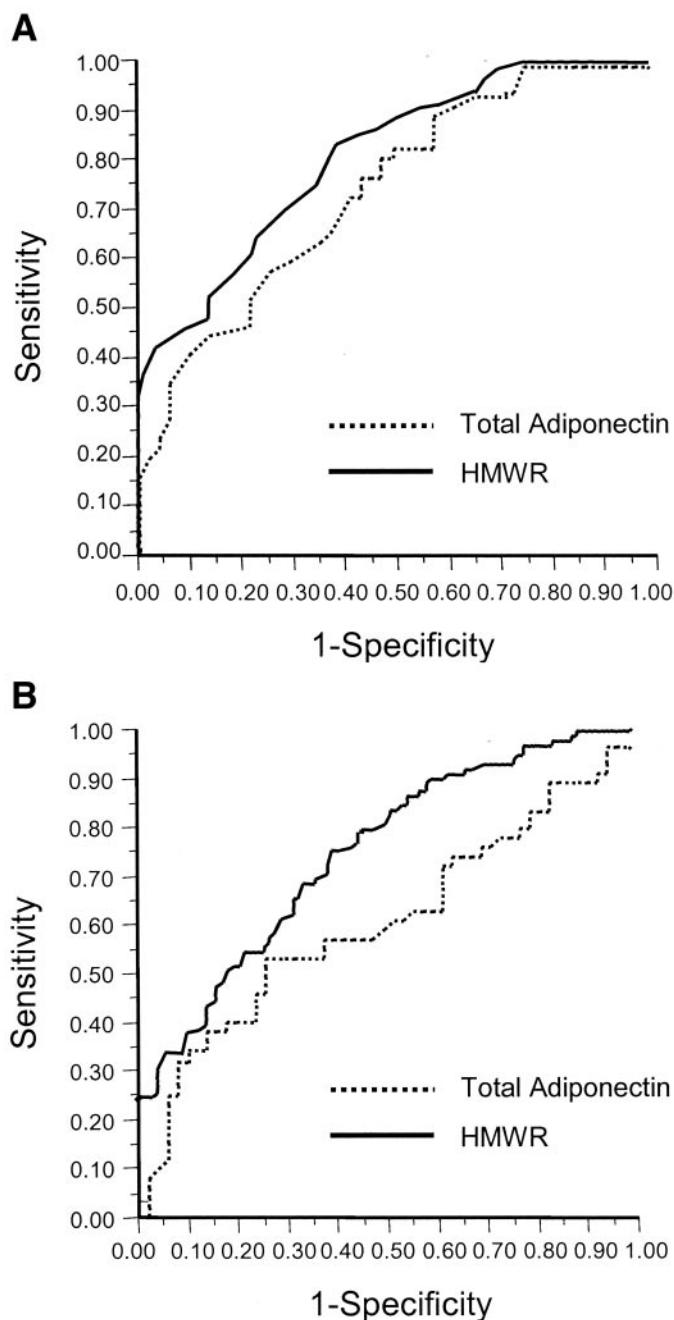
We performed an ROC analysis to quantify the power of the HMWR values to make a prediction of metabolic syndrome. ROC curves for plasma total adiponectin levels and HMWR values to discriminate between subjects with and without metabolic syndrome were plotted for total subjects (data not shown), men (Fig. 2A), and women (Fig. 2B). The areas under the curve for plasma total adiponectin level and HMWR values were then compared to determine whether the HMWR value had a better predictive power for the prediction of metabolic syndrome than plasma total adiponectin level. The AUC for the HMWR values was

significantly larger than that for plasma total adiponectin levels in all subjects (0.780 [95% CI 0.736–0.824] vs. 0.692 [0.632–0.751],  $P = 0.039$ ) (data not shown), in men (0.806 [0.747–0.865] vs. 0.730 [0.660–0.800],  $P = 0.0025$ ) (Fig. 2A), and in women (0.743 [0.659–0.828] vs. 0.637 [0.532–0.742],  $P = 0.0458$ ) (Fig. 2B). When a cutoff value of 32.0% for men and 40.2% for women was adopted to maximize the sensitivity (%) plus specificity (%), HMWR value predicted the presence of metabolic syndrome with a sensitivity of 83.2% and specificity of 62.0% in men and a sensitivity of 77.4% and specificity of 55.0% in women.

We have undertaken the subgroup analyses in both diabetic and nondiabetic subjects. In both groups, AUCs for the HMWR values to predict metabolic syndrome were larger than those for total adiponectin levels (type 2 diabetic group: 0.815 [95% CI 0.755–0.875] vs. 0.676 [0.616–0.736],  $P = 0.0013$ ; nondiabetic group: 0.843 [0.743–0.943] vs. 0.751 [0.651–0.851],  $P = 0.00475$ ).

We have also plotted ROC curves for





**Figure 2**—ROC curves of plasma total adiponectin levels and HMWR values for prediction of metabolic syndrome in men (A) ( $n = 193$ ) and women (B) ( $n = 105$ ). Prediction of metabolic syndrome was based on the criteria of the National Cholesterol Education Program Adult Treatment Panel III for prediction of metabolic syndrome. The AUC for the HMWR values was significantly larger than for total adiponectin in both men (0.806 [95% CI 0.747–0.865] vs. 0.730 [0.660–0.800],  $P = 0.0025$ ) and women (0.743 [0.659–0.828] vs. 0.637 [0.532–0.742],  $P = 0.0458$ ).

total adiponectin levels and HMWR values to predict metabolic syndrome defined by newly announced IDF criteria (27). Indeed, HMWR values were more predictive of metabolic syndrome defined by IDF than total adiponectin levels in total subjects (0.733 [95% CI 0.693–0.773] vs. 0.680 [0.640–0.720],  $P = 0.0037$ ).

#### HMWR value predicts the presence or absence of metabolic syndrome independently of plasma total adiponectin level

The HMWR values varied substantially even among subjects with similar total adiponectin levels in plasma. The present ROC analysis suggested the possibility that the HMWR value may be useful for

predicting the presence of metabolic syndrome among subjects with similar plasma levels of total adiponectin. We examined whether the HMWR values were different between subjects with and without metabolic syndrome after stratifying the subjects into quartiles of plasma total adiponectin levels. HMWR values were significantly lower in all the quartiles of total adiponectin in subjects with metabolic syndrome than in those without, suggesting that the HMWR value may be useful for the prediction of metabolic syndrome, irrespective of plasma total adiponectin level.

#### The relationship between HMWR value and the severity of coronary artery disease

We analyzed the relationship between total adiponectin levels or HMWR values and severity and extent of coronary atherosclerosis using the score by Gensini (29). Gensini scores were significantly associated with age ( $P = 0.0022$ ), prescription of thiazolidinediones ( $P = 0.0443$ ), and HMWR values ( $P = 0.0437$ ). Subjects with lower HMWR value had higher Gensini score, suggesting that HMWR value might be associated with the onset and development of coronary artery disease independently of age and prescription of thiazolidinediones. Total adiponectin level was not correlated with Gensini score before and after adjustment for the conventional risk factors for atherosclerosis and prescription of thiazolidinediones.

**CONCLUSIONS**— Prediction of metabolic syndrome, defined by the presence of a cluster of metabolic abnormalities, including impaired glucose metabolism, high BMI and abdominal fat distribution, dyslipidemia, and hypertension, is very important because of its association with the subsequent development of type 2 diabetes and cardiovascular disease (28). Because of the epidemic of obesity and a sedentary lifestyle worldwide, metabolic syndrome is becoming increasingly commonly recognized. According to the National Cholesterol Education Program (NCEP) definition, roughly one-fourth of middle-aged men and women in the U.S. have metabolic syndrome (33). Development of a method for convenient prediction of metabolic syndrome in daily clinical practice presents a major challenge for physicians and public health policy makers facing

the epidemic of obesity and a sedentary lifestyle.

There is a mounting body of evidence to suggest that adiponectin is an insulin-sensitizing hormone and that the plasma level of this hormone is the best predictor of the subsequent development of type 2 diabetes among the various plasma biomarkers (34). Recently, however, it has been reported that adiponectin forms a wide range of multimers in plasma and that mutations in the adiponectin gene that inhibit the formation of HMW adiponectin are closely associated with the subsequent development of type 2 diabetes. Therefore, it was considered that HMW adiponectin value might be an attractive biomarker for the prediction of insulin resistance and metabolic syndrome. Indeed, the present study demonstrated that the AUC of HMWR values was significantly larger than that of plasma total adiponectin levels and that the sensitivity of the HMWR value for predicting the presence of metabolic syndrome reached 80%. Thus, this study is the first to demonstrate that HMWR value is more closely associated with insulin resistance and the presence of metabolic syndrome than plasma total adiponectin level.

The present study provided evidence of the usefulness of a newly developed method of measurement of plasma HMW adiponectin level as a convenient and sensitive biomarker for the prediction of insulin resistance and metabolic syndrome.

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