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The ratio of leptin to adiponectin can be used as an index of insulin resistance

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Abstract

The level of leptin increases with obesity, whereas that of adiponectin decreases with obesity. It is reported that the ratio of leptin to adiponectin (L/A) is associated with insulin resistance. It is difficult to evaluate insulin resistance in diabetic patients who have a dysfunction of insulin secretion. The aim of this study was to examine whether the L/A ratio is a useful marker for insulin resistance in diabetic patients. We examined L/A in the serum of a total of 139 Japanese patients with type 2 diabetes mellitus (66 women and 73 men) and 7 healthy individuals recruited in our hospital. Changes in the levels of leptin and adiponectin were observed using the oral glucose tolerance test and a hyper- and euglycemic clamp test. Twenty-one patients with type 2 diabetes mellitus were observed for more than 6 months after treatment with pioglitazone, and 31 patients with type 2 diabetes mellitus were observed for more than 6 months after the treatment with metformin. The mean value of L/A in 139 Japanese patients with type 2 diabetes mellitus was 1.22 ± 1.41 (1.68 ± 1.76 in women, 0.81 ± 0.80 in men; $P = .0002$). In the clamp tests, L/A correlated with glucose infusion rate (GIR) ($r^2 = 0.26$, $P = .0034$). The correlation of L/A and GIR indicated a stronger correlation than either leptin ($r^2 = 0.144$, $P = .03$) or adiponectin alone ($r^2 = 0.023$, $P = .41$), or the homeostasis model assessment of insulin resistance ($r^2 = 0.103$, $P = .08$). The average hemoglobin A_{1c} (HbA_{1c}) improved from 10.2% ± 1.2% to 9.2% ± 1.6% ($P = .0037$) in 6 months after treatment with pioglitazone. Our results indicate pioglitazone to be effective for HbA_{1c} improvement in subjects with high L/A and low L/A. The average HbA_{1c} improved from 9.2% ± 0.9% to 8.0% ± 1.2% ($P = .0002$) in 6 months after treatment with metformin. Our results indicate metformin to be effective for HbA_{1c} improvement in subjects with a low L/A. In conclusion, we demonstrate that L/A is different between male and female subjects. The correlation of L/A and GIR by the euglycemic hyperinsulinemic clamp test suggests that L/A is a useful indicator for the choice of drug to treat diabetes mellitus.

1. Introduction

Obesity is defined by an accumulation of adipocytes throughout the body and is also associated with a variety of metabolic diseases. Insulin resistance or metabolic syndrome is now thought to be triggered by deposition of fat in the major target organs for insulin such as liver or muscle [1]. To improve the adverse metabolic state, it is necessary to create a negative balance in energy intake and energy consumption (ie, by exercising or by enhancing the oxidation of fatty acid in the tissues using medication), thereby leading to weight loss. Leptin and adiponectin are important hormones derived from fat cells and secreted into the serum. Both hormones improve insulin resistance [2,3], although the blood concentrations are contradictory depending on adipocyte deposition. Specifically, the level of leptin increases with obesity, whereas that of adiponectin decreases [4]. Moreover, adiponectin acts against arterial sclerosis as a "good hormone" [5]. It was recently reported that the ratio of leptin to adiponectin (L/A) could act as a useful marker for metabolic disease [6,7]. Indeed, L/A was reported to display a better correlation to insulin resistance than the level of leptin or adiponectin alone [8,9]. The ratio of leptin to adiponectin is an excellent indicator of obesity and could be a useful marker for the progression of arterial sclerosis

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Table 1
Changes in the concentration of leptin and adiponectin during the OGTT

	0 min	60 min	120 min
Plasma glucose (mg/dL)	106.1 ± 10.9	191.0 ± 37.4	131.7 ± 50.3
Insulin (mol/L)	32.5 ± 14.6	364.4 ± 255.0	209.1 ± 99.0
C-peptide (mol/L)	301.2 ± 86.0	1641.7 ± 711.6	1472.9 ± 516.3
Leptin (ng/mL)	6.34 ± 2.89	5.88 ± 2.69	5.41 ± 2.31
Adiponectin (μg/mL)	7.87 ± 3.12	8.04 ± 3.43	8.07 ± 2.87

The number of subjects was 7. All values are the mean ± SD. The serum level of both leptin and adiponectin showed no significant change. Comparison was made by dependent *t* test.

because the levels of the 2 hormones fluctuate in the opposite direction depending on the amount of visceral fat. Here, we evaluate L/A in patients with type 2 diabetes mellitus to assess the clinical significance.

We measured the levels of leptin and adiponectin during an oral glucose tolerance test (OGTT) and hyper- and euglycemic clamp test. In addition, we examined the relationship between insulin resistance in the muscles and L/A during the euglycemic hyperinsulinemic clamp test. We then measured the levels of leptin and adiponectin in diabetic patients and examined the possible selectivity of diabetic drugs using this index.

2. Subjects and methods

A total of 139 Japanese patients with type 2 diabetes mellitus (66 women and 73 men) agreed to take part in this study. The mean age of the female subjects was 62.8 ± 11.6 years, with a mean body mass index (BMI) of 24.6 ± 5.8 kg/m^2. The mean age of the male subjects was 59.2 ± 12.8 years, with a mean BMI of 23.8 ± 3.7 kg/m^2. The mean age of diabetes onset for the female and male subjects was 51.7 ± 11.7 and 50.5 ± 11.6 years, respectively. The mean hemoglobin A_{1c} (HbA_{1c}) of the female and male subjects was 8.0 ± 1.6 and 7.8 ± 2.0, respectively. In addition, 7 healthy individuals (6 women and 1 man) who received an OGTT in our hospital also participated in this study. At the time of recruitment, informed consent was obtained from each subject. All the patients had their serum leptin and adiponectin levels measured. The OGTT was performed with 75 g glucose; and the levels of leptin and adiponectin were measured at 0, 60, and 120 minutes. Fifteen patients (7 women and 8 men) with type 2 diabetes mellitus (mean age of female and male subjects: 64.4 ± 10.2 and 44.5 ± 9.7 years, respectively; mean BMI of female and male subjects: 23.6 ± 5.1 and 25.7 ± 3.2 kg/m^2, respectively) received a hyperglycemic clamp test. A total of 31 patients (14 women and 17 men) with type 2 diabetes mellitus (mean age of female and male subjects: 64.8 ± 9.8 and 48.7 ± 11.7 years, respectively; mean BMI of female and male subjects: 25.1 ± 4.9 and 25.4 ± 3.8 kg/m^2, respectively) received a euglycemic hyperinsulinemic clamp test. The hyperglycemic-euglycemic insulin clamp study was performed according to the standard protocol [10]. We maintained the hyperglycemic clamp at the glucose level of 225 mg/dL for 60 minutes. The levels of

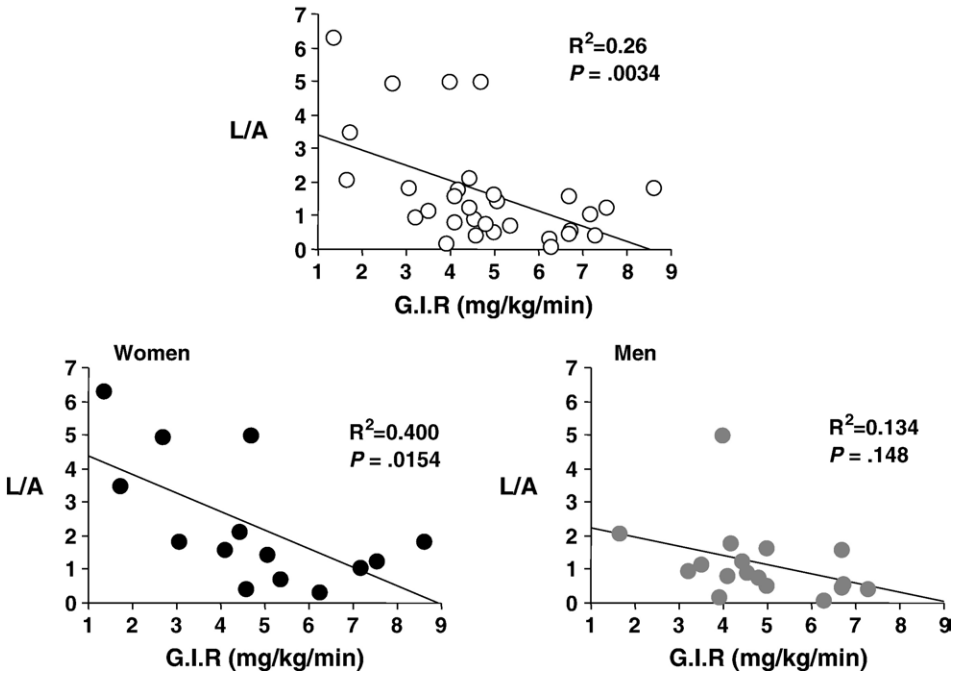

Fig. 1. Leptin to adiponectin ratio and GIR by hyperinsulinemic glucose clamp test. Comparison was made by simple linear regression analyses. Results were considered statistically significant at $P < .05$. Results examined separately for female and male subjects gave $r^2 = 0.400$ ($P = .0154$) for women and $r^2 = 0.134$ ($P = .148$) for men. Thus, a stronger correlation was found in female subjects.

Table 2
The changes of leptin and adiponectin concentrations during the hyperglycemic glucose clamp

	0 min	60 min
Leptin (ng/mL)	6.22 ± 4.62	5.51 ± 4.39 *
Adiponectin (μg/mL)	5.39 ± 3.54	5.21 ± 3.40

The number of subject was 15. All values are the mean ± SD. Comparison was made by dependent *t* test.

* $P < .05$, compared with 0 min.

leptin and adiponectin were measured at 0 and 60 minutes. We set the euglycemic hyperinsulinemic clamp test (average insulin level of 98 mU/mL) at a glucose level of 100 mg/dL, which was maintained for 90 minutes; and the levels of leptin and adiponectin were measured at 0 and 90 minutes. Glucose infusion rate (GIR) at the end of the euglycemic hyperinsulinemic clamp test was also measured. Twenty-one patients (7 women and 14 men) with type 2 diabetes mellitus were observed for more than 6 months after treatment with pioglitazone (15 mg/d). Thirty-one patients (14 women and 17 men) with type 2 diabetes mellitus were observed for more than 6 months after treatment with metformin (500 mg/d). Almost all subjects in both groups of patients were taking a sulfonylurea. Serum leptin levels were measured by using the human leptin radioimmunoassay kit (LINCO Research, St Charles, MO). Serum adiponectin levels were measured by using the adiponectin enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical, Tokyo, Japan).

3. Statistical analyses

The clinical variables were compared by dependent and independent *t* test or by simple linear regression analysis. All statistical analyses were performed by the StatView 5.0 software (SAS Institute, Cary, NC). All values are the mean ± SD, and a value of $P < .05$ was considered statistically significant. Statistical methods are included in the tables and figures.

4. Results

4.1. Changes of serum leptin and adiponectin during OGTT and hyper- and euglycemic hyperinsulinemic clamp tests

Changes in the mean values of leptin and adiponectin during OGTT are shown in Table 1. During the test, the mean plasma glucose values at 0, 60, and 120 minutes were 106, 191, and 131 mg/dL, respectively. The serum level of both leptin and adiponectin showed no significant change.

Changes in the mean values of leptin and adiponectin during the hyper- and euglycemic hyperinsulinemic clamp tests are shown in Table 2. The level of leptin decreased significantly 60 minutes ($P = .0017$) after the hyperglycemic clamp test, but no significant change in the level of adiponectin was observed. Indeed, our results concur with a previous study that concluded that the serum level of adiponectin is unaffected by hyperinsulinemia and hyperglycemia [11].

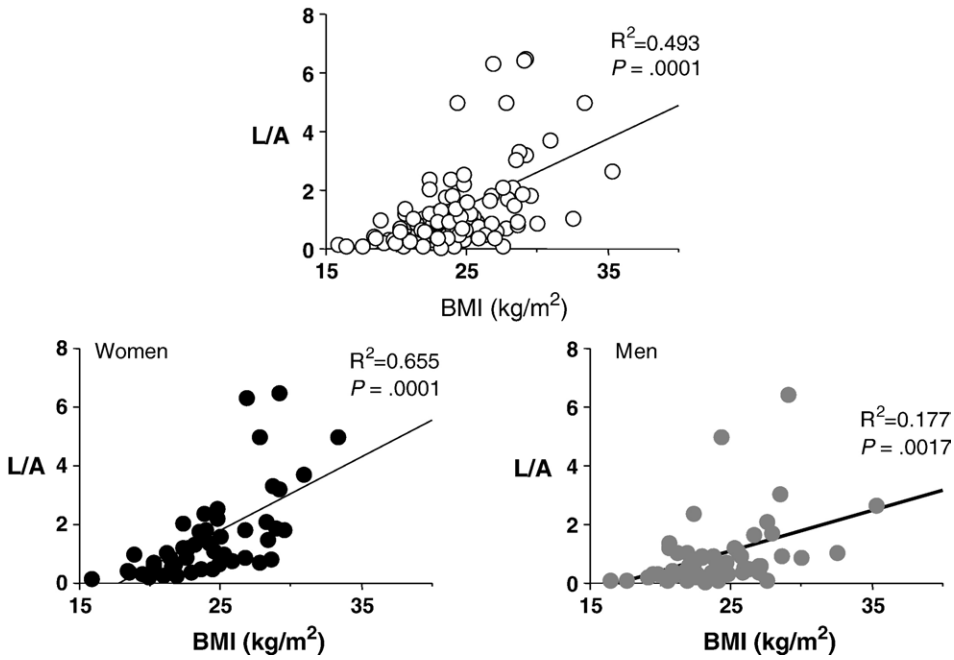

Fig. 2. Leptin to adiponectin ratio and BMI. Comparison was made by simple linear regression analyses. Results were considered statistically significant at $P < .05$. Results examined separately for female and male subjects gave $r^2 = 0.655$ ($P = .0001$) for women and $r^2 = 0.177$ ($P = .0017$) for men. Thus, a stronger correlation was found in female subjects. The correlation of L/A and BMI indicated a stronger correlation than leptin ($r^2 = 0289$, $P = .001$) or adiponectin alone ($r^2 = 0.052$, $P = .020$).

Table 3
Clinical characteristics of the study population before treatment

	Treatment by pioglitazone	Treatment by metformin
Sex (F/M)	7/14	14/17
Age (y; F/M)	60.0 ± 13.1/59.2 ± 14.3	58.9 ± 9.2/58.4 ± 8.8
BMI (kg/m^2; F/M)	28.0 ± 3.1/26.9 ± 4.4	24.3 ± 3.5/24.1 ± 4.6
Onset of diabetes (y; F/M)	51.2 ± 9.2/49.2 ± 8.3	49.1 ± 8.3/48.5 ± 8.8
HbA_{1c} (%; F/M)	10.1 ± 0.7/10.7 ± 1.1	9.2 ± 1.0/9.3 ± 0.9
Leptin (ng/mL; F/M)	11.4 ± 2.6/3.9 ± 1.8	11.9 ± 5.5/4.0 ± 2.5
Adiponectin (μg/mL; F/M)	6.2 ± 2.4/5.0 ± 1.6	8.1 ± 3.0/7.4 ± 2.9
L/A ratio (F/M)	2.06 ± 0.79/0.92 ± 0.77	1.87 ± 1.54/0.79 ± 0.84

All values are the mean ± SD. F/M indicates female/male.

4.2. L/A correlated with GIR by euglycemic hyperinsulinemic clamp test

The mean values of L/A, BMI, and GIR in this clamp test were 1.62 ± 1.57 (2.18 ± 1.88 in women, 1.14 ± 1.12 in men), 25.4 ± 4.44 kg/m^2 (25.4 ± 4.69 in women, 25.4 ± 4.38 in men) and 4.76 ± 1.75 mg/(kg min) (4.82 ± 2.08 in women, 4.72 ± 1.48 in men), respectively. In the euglycemic hyperinsulinemic clamp tests, L/A correlated with GIR (r^2 = 0.26, P = .0034) (Fig. 1). The correlation of leptin and GIR was weak (r^2 = 0.144, P = .035), and adiponectin (r^2 = 0.023, P = .41) or the homeostasis model assessment of insulin resistance (HOMA-IR) (r^2 = 0.103, P = .08) was not correlated with GIR. The L/A correlation in women (r^2 = 0.400, P = .0154) was stronger than that in men (r^2 = 0.134, P = .148) (Fig. 1). The L/A was also correlated with BMI (r^2 = 0.240, P = .003). The relationship is particularly good for female patients. It was also reported that L/A in women with polycystic ovary syndrome is related with insulin resistance [12]. However, these sex-based differences might be related to age variation within the subject group.

4.3. L/A in different BMI populations with type 2 diabetes mellitus

The L/A correlated with BMI (r^2 = 0.279, P < .001), as shown in Fig. 2. The mean L/A was 1.22 ± 1.41. The mean L/A was significantly higher in women (1.68 ± 1.76) than in men (0.81 ± 0.80). However, this sex difference was not apparent when BMI ranged from 20 to 24. We then divided the subjects into 3 groups: BMI less than 20 (9 women and 8 men), BMI of 20 to 24 (24 women and 31 men), and BMI more than 24 (31 women and 31 men). The L/As of women and men were 0.48 ± 0.37/0.27 ± 0.11 for BMI less than 20 (P = .078), 0.91 ± 0.60/0.75 ± 0.92 for BMI 20 to 24 (P = .480), and 2.64 ± 2.13/0.95 ± 0.69 for BMI more than 24 (P = .0001), respectively. No differences were observed in terms of the BMI between men and women in the 3 groups. Comparison was made by independent t test.

4.4. L/A ratio and the effects of pioglitazone and metformin

The clinical parameters of the patients before treatment are shown in Table 3. The average HbA_{1c} improved from 10.25% ± 1.2% to 9.2% ± 1.6% (P = .0037) 6 months after treatment with pioglitazone. The average weight significantly changed from 66.4 ± 11.7 to 69.7 ± 12.9 kg (P = .0025). Treatment with pioglitazone was considered effective because an HbA_{1c} decline was observed in subjects with high L/A and low L/A (Fig. 3A). The average HbA_{1c} improved from 9.2% ± 0.9% to 8.0% ± 1.2% (P = .0002) 6 months after treatment with metformin. No change in average weight was observed (from 60.2 ± 11.9 to 60.5 ± 12.4 kg, P = .66). Treatment with metformin was considered effective because an HbA_{1c} decline was observed in subjects with a low L/A (Fig. 3B). Comparison was made by dependent t test.

5. Discussion

Methods to alleviate or prevent insulin resistance and obesity have been intensively studied. The target molecules of these therapies are leptin, adiponectin, tumor necrosis factor α, and plasminogen activator inhibitor 1 derived from

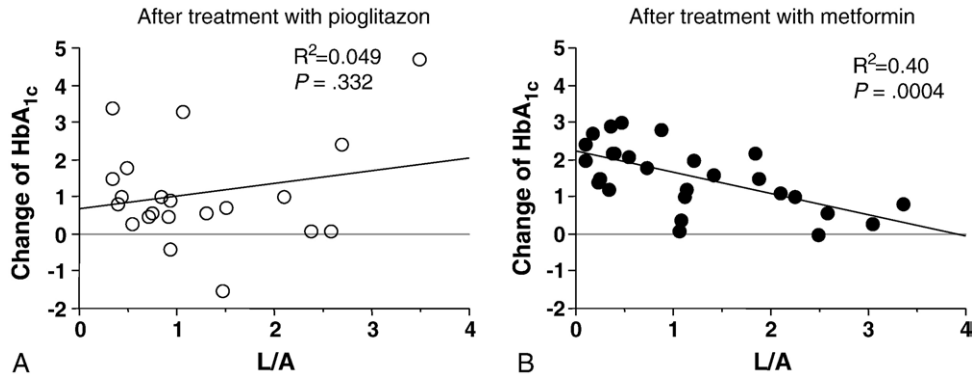

Fig. 3. Correlation of L/A and change of HbA_{1c} after treatments with pioglitazone and metformin. In Fig. 2, the L/A ranges in 50% middle persons are from 0.580 to 2.100 for women and 0.335 to 1.041 for men. In this study, *low L/A subjects* are defined as those with less than 0.580 for women and 0.335 for men; *high L/A subjects* are those with more than 2.100 in women and 1.041 in men. Number of subjects undergoing treatment with pioglitazone having low L/A or high L/A was 3 and 7, respectively. Number of subjects undergoing treatment with metformin having low L/A or high L/A was 8 and 11, respectively. Comparison was made by simple linear regression analyses. Results were considered statistically significant at P < .05.

fat cells [13-16]. In the present study, we measured leptin and adiponectin, which regulate insulin sensitivity, during the OGTT and hyper- and euglycemic clamp test. Our results show that the level of leptin decreases significantly during the hyperglycemic clamp test, but the change is not significant during the OGTT. These observations indicate that the duration of hyperglycemia is the determining factor of leptin concentration in the serum. Thus, long-term hyperglycemic conditions may obstruct appetite suppression as the level of leptin decreases. However, the precise mechanism remains unclear.

Leptin has been reported to be a good hormone because it improves insulin resistance [2]. Adiponectin is also a good hormone because it improves insulin resistance and has action on anti–arterial sclerosis [3]. However, the level of leptin increases with obesity, whereas that of adiponectin decreases [4]. Thus, we reasoned that the L/A ratio may be an excellent predictor for insulin resistance in diabetic patients. When diabetic patients are evaluated for insulin resistance using HOMA-R, it is essential that the fasting plasma glucose is greater than 140 mg/dL to avoid erroneous results [17]. This study clearly demonstrates that L/A correlates with GIR more closely than leptin and adiponectin alone or HOMA-R. We therefore conclude that L/A could be a useful index for insulin resistance in clinical practice.

It has been reported that both leptin and adiponectin in the peripheral tissues indicate oxidation enhancement of fatty acid through adenosine monophosphate–activated protein kinases [18,19], resulting in an improvement of insulin resistance and obesity. Metformin has been reported to decrease gluconeogenesis in the liver and increase uptake of glucose in the peripheral tissues through adenosine monophosphate–activated protein kinases [20]. Moreover, it has also been confirmed that pioglitazone, an insulin-sensitizing drug, is a powerful tool to increase plasma adiponectin. We reasoned that the balance of leptin and adiponectin in the body could influence the effect of antidiabetic drugs. Therefore, we examined the effect of these drugs using L/A as an index. Our results show that pioglitazone was particularly potent in subjects with a high L/A and in those with a low L/A compared with the midrange L/A population. This may be because many subjects with high L/A are low in adiponectin, making them particularly receptive to increases in the level of adiponectin. However, because the sample number is too small, we cannot explain why low L/A subjects are more amenable to HbA_{1c} improvement. Further studies involving greater subject numbers will be required to investigate this mechanism in more detail.

On the other hand, metformin was potent in subjects with low L/A. Low L/A subjects in this study had a high serum adiponectin and low BMI. This may be because the condition of high adiponectin is working as a good balance for the communication of the signal. These results appear to contradict previous studies using metformin, which concluded that the drug is particularly effective for obese individuals [21]. However, more recent studies reported that there was no difference in the change of HbA_{1c} between nonobese and obese subjects with type 2 diabetes mellitus [22]. Finally, we anticipate reinforcement of the effect of using metformin after an increase in adiponectin after treatment with pioglitazone. Further studies are required to confirm this hypothesis. In conclusion, we found that L/A is a good predictor for insulin resistance in diabetic patients. In addition, L/A may be a good indicator for assessing the effectiveness of antidiabetic drugs.

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