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

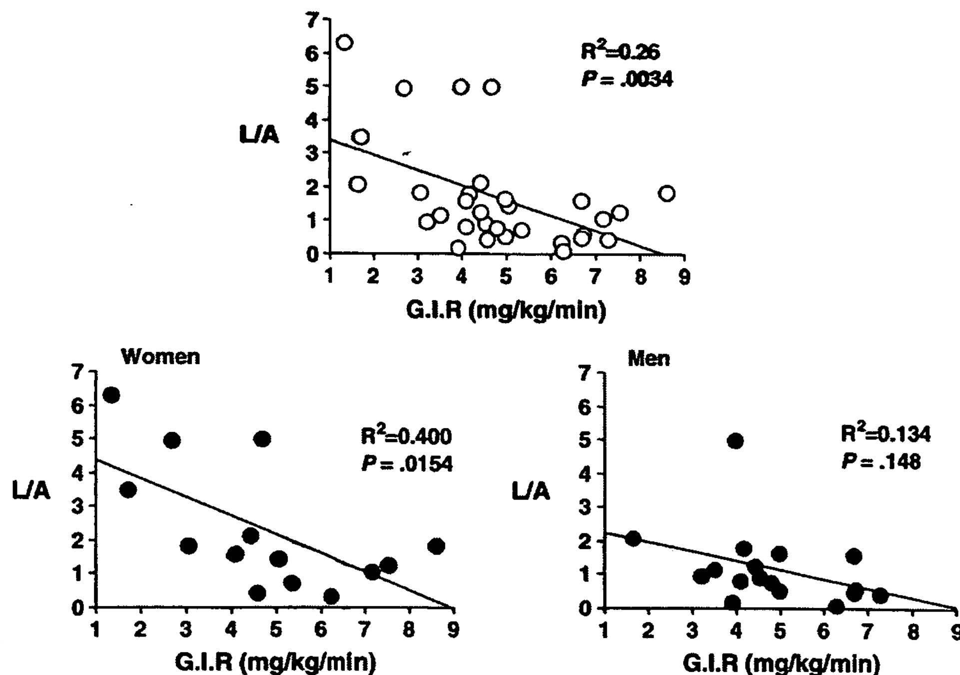

Fig. 1. Leptin to adiponectin ratio and GIR by hyperinsulinemic glucose clamp test. Comparison was made by simple linear regression analyses. Results were considered statistically significant at $P < .05$. Results examined separately for female and male subjects gave $r^2 = 0.400$ ($P = .0154$) for women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

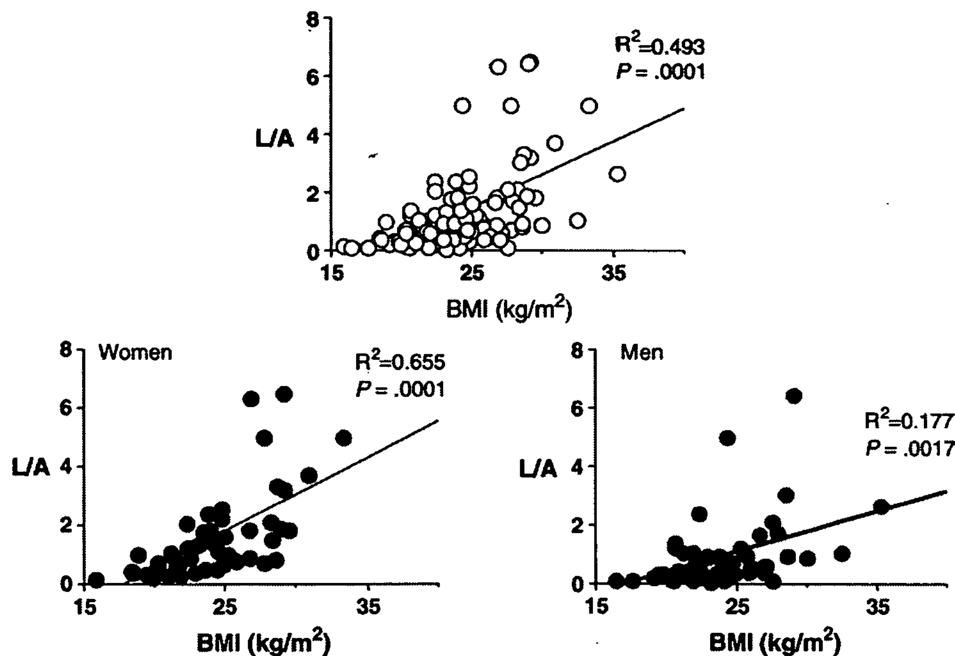

Fig. 2. Leptin to adiponectin ratio and BMI. Comparison was made by simple linear regression analyses. Results were considered statistically significant at $P < .05$. Results examined separately for female and male subjects gave $r^2 = 0.655$ ($P = .0001$) for women and $r^2 = 0.177$ ($P = .0017$) for 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

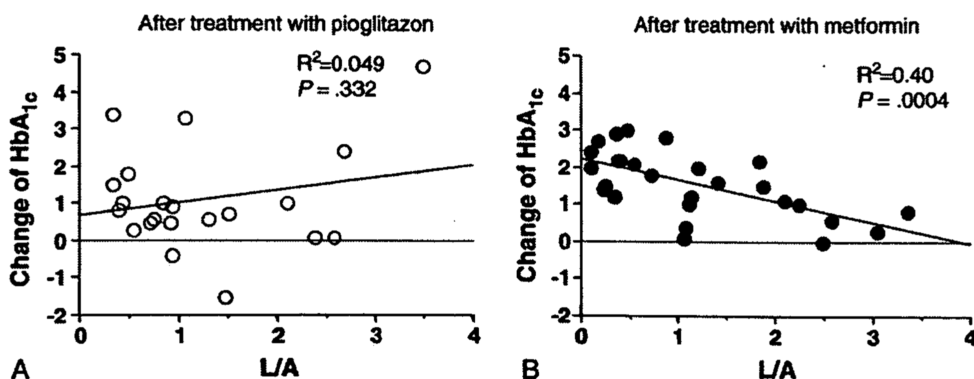

Fig. 3. Correlation of L/A and change of HbA_{1c} after treatments with pioglitazone and metformin. In Fig. 2, the L/A ranges in 50% middle persons are from 0.580 to 2.100 for women and 0.335 to 1.041 for men. In this study, *low L/A subjects* are defined as those with less than 0.580 for women and 0.335 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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ChREBP: A glucose-activated transcription factor involved in the development of metabolic syndrome

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Keywords: ChoRE, ChREBP, L-PK, Metabolic Syndrome,

Abbreviations: Carbohydrate Response Element Binding Protein (ChREBP), Carbohydrate Response Element (ChoRE), Liver type pyruvate kinase (LPK), Fatty Acid synthase (FAS), Acetyl CoA Carboxylase (ACC), Malic Enzyme (ME), Hexose Monophosphate Shunt (HMP Shunt), Malate–Pyruvate Shunt (MP Shunt), Glucokinase

(GCK), glucose 6 phosphatase (G6Pase), Glucose 6 phosphate (G6P), AMP activated protein kinase (AMPK), Xylulose-5-phosphate (Xu-5-P), Protein phosphatase 2A delta (PP2Adelta), cAMP-activated protein kinase (PKA), Phosphoenol Pyruvate (PEP),

Abstract

Excess carbohydrate intake leads to fat accumulation and insulin resistance. Glucose and insulin coordinately regulate *de novo* lipogenesis from glucose in the liver, and insulin activates several transcription factors including SREBP1c and LXR, while those activated by glucose remain unknown. Recently, a carbohydrate response element binding protein (ChREBP), which binds to the carbohydrate response element (ChoRE) in the promoter of rat liver type pyruvate kinase (LPK), has been identified. The target genes of ChREBP are involved in glycolysis, lipogenesis, and gluconeogenesis. Although the regulation of ChREBP remains unknown in detail, the transactivity of ChREBP is partly regulated by a phosphorylation/dephosphorylation mechanism. During fasting, protein kinase A and AMP-activated protein kinase phosphorylate ChREBP and inactivate its transactivity. During feeding, xylulose-5-phosphate in the hexose monophosphate pathway activates protein phosphatase 2A, which dephosphorylates ChREBP and activates its transactivity. ChREBP controls 50% of hepatic lipogenesis by regulating glycolytic and lipogenic gene expression. In $ChREBP^{-/-}$ mice, liver triglyceride content is decreased and liver glycogen content is increased compared to wild-type mice. These results indicate that ChREBP can regulate metabolic gene expression to convert excess carbohydrate into triglyceride rather than glycogen. Furthermore, complete inhibition of ChREBP in ob/ob mice reduces the effects of the metabolic syndrome such as obesity, fatty liver, and glucose intolerance. Thus, further clarification of the physiological role of ChREBP may be useful in developing treatments for the metabolic syndrome.

Carbohydrate Response Element Binding Protein (ChREBP)

Increased consumption of high-carbohydrate and high-fat diets (so-called cafeteria diet) is one of the most important risk factors in the development of the metabolic syndrome. Excess carbohydrate is mainly converted to triglyceride in the liver, and excess fat accumulation in the body leads to insulin resistance and metabolic syndrome [1]. When a high-carbohydrate diet is ingested, carbohydrate is converted into triglyceride in the liver by key glycolytic enzymes such as glucokinase and liver-type pyruvate kinase (L-PK) and enzymes of *de novo* lipogenesis such as acetyl CoA carboxylase (ACC) and fatty acid synthase (FAS) [2]. Both insulin and glucose are potent factors in inducing the transcription of these key enzyme genes (Fig. 1). Insulin signaling regulates transcription of these glycolytic and lipogenic enzymes by activation of SREBP1c and liver X receptor (LXR), respectively [3, 4]. In contrast, the mechanism by which glucose signaling activates the expression of these enzymes is poorly understood. For example, transcription of Spot14 (S14) in liver is induced by a carbohydrate diet. Towle et al. reported that the carbohydrate response element (ChoRE) of the S14 gene consists of two E-box-like consensus sequences (CAYGNGN5CNCRTG) [5]. Like S14, many glucose response genes (such as LPK, FAS, and ACC) contain a similar ChoRE in their promoter regions [2]. This suggests that a common transcription factor binds to the conserved ChoRE, and many researchers have addressed this problem over a long period [6]. Uyeda et al. successfully purified a transcription factor that binds to the rat L-PK ChoRE [7]. This transcription factor is in the same region as the Williams–Beuren syndrome critical region 14 (WBSCR14)

protein, which is now renamed the carbohydrate response element binding protein (ChREBP) [7, 8]. Williams–Beuren syndrome is a neurodevelopmental disorder affecting several systems, and is caused by a heterozygous deletion in chromosomal region 7q11.23 in human. WBSCR14/ChREBP is expressed as a 4.2 kb transcript, and the WBSCR14/ChREBP locus encompasses 33 kb of genomic DNA with 17 exons [9]. The distribution of ChREBP mRNA is ubiquitous, but it is most abundant in lipogenic organs such as liver, brown and white adipose tissues, small intestine, kidney, and muscle [7, 10]. ChREBP is a member of the basic helix–loop–helix/leucine zipper (bHLH/ZIP) family of transcription factors with Mr = 94,600, and forms heterodimers with the bHLHZip protein Mlx to bind the ChRoE [8, 11]. Target genes of ChREBP are involved in glycolysis (L-PK), the NADPH supply system (glucose-6-phosphate dehydrogenase, transketolase, malic enzyme, etc.), gluconeogenesis (G6Pase), and lipogenesis (ACC, FAS) [10, 12].

Glucose and insulin signals coordinately regulate lipogenesis

In the fed state, glucose and insulin coordinate hepatic lipogenesis by regulating glycolytic and lipogenic gene expression at the transcriptional level. ChREBP and SREBP1c share lipogenic genes and genes related to the hexose monophosphate (HMP) shunt (Fig. 2) [12, 13]. Some groups have reported that hepatic glucokinase is required for the synergistic effects of ChREBP and SREBP1c on glycolytic and lipogenic gene expression (Fig. 2) [14, 15]. Uyeda et al. showed that glucose-activated ChREBP directly binds the ChoRE of the L-PK promoter and

activates L-PK gene expression [7]. However, whether SREBP1c physiologically mediates the action of insulin on glucokinase remains controversial. We reconfirmed that the overexpression of dominant active SREBP1c induces glucokinase gene expression in hepatocytes, which we previously confirmed as unpublished data. However, Liang et al. reported that the response of glucokinase to high-carbohydrate diet refeeding is still conserved in SREBP1c knockout mice [13]. In addition, Iynedjian et al. reported that SREBP1c cannot bind to liver-type glucokinase promoter [16], and Pichard et al. reported that SREBP1c knockdown by small interfering RNAs results in impaired induction of the FAS gene in response to glucose and insulin but does not prevent induction of the glucokinase gene [17]. Glucokinase is a key molecule regulating glycolytic flux, and it is important to identify the various transcription factors that mediate the activation of glucokinase gene expression by insulin.

Regulation of ChREBP transcriptional activity

Many glycolytic and lipogenic enzymes are induced by high-carbohydrate feeding and suppressed by fasting and starvation [6]. During starvation, hepatic glycolysis and *de novo* lipogenesis are suppressed. In contrast, gluconeogenesis, the beta-oxidation of fatty acyl CoA, and ketogenesis are upregulated. This change from anabolism to catabolism is regulated by stress hormone and AMP accumulation. During starvation, concentrations of plasma glucagon and epinephrine are increased. Glucagon and epinephrine increase the intracellular cAMP concentration and activate cAMP-activated protein kinase (PKA). PKA phosphorylates ChREBP, inactivating it

[18]. Phosphorylation of ChREBP at Serine residue 196 (Ser196) inactivates nuclear import, and phosphorylation at Threonine residue 666 (Thr666) prevents DNA binding by ChREBP (Fig. 3A). Similarly, intracellular AMP accumulation inhibits ChREBP transactivity by activating AMP-activated protein kinase (AMPK) and phosphorylating ChREBP (Fig. 3A) [19]. In contrast, glucose activates ChREBP transactivity. Glucose is converted to xylulose-5-phosphate (Xu-5-P) in the hexose monophosphate (HMP) shunt, and Xu-5-P activates protein phosphatase 2A delta (PP2Adelta) and dephosphorylates ChREBP protein (Fig. 3B) [20]. Xu-5-P-mediated PP2A activation also is seen in the activation of 6-phosphofructo-2, 6-kinase/bisphosphatase [21, 22, 23]. Xu-5-P is a key molecule in regulating not only transcription but also enzyme activity in glycolysis. Thus, ChREBP, by a phosphorylation/dephosphorylation mechanism, would seem to regulate the expression of glycolytic and lipogenic enzyme genes (L-PK, FAS, ACC, S14, etc.). However, some groups have reported evidence against such a phosphorylation/dephosphorylation mechanism. Despite lacking phosphorylation sites by PKA, a S196A/T666A mutant of ChREBP retains glucose responsiveness and cAMP-dependent inhibition of ACC promoter transactivity [24]. Nevertheless, the ChREBP protein contains a glucose-sensing module that mediates its glucose responsiveness (Fig. 3A and 3B) [25], so it is clear that PKA and AMPK inhibit ChREBP transactivity and that PP2A activates it. However, the mechanism by which glucose activates the transactivity of ChREBP remains unknown (Fig. 4).

At the transcriptional level, ChREBP also is regulated loosely in several conditions. The level of ChREBP mRNA in liver in the fed state is the same as or twice

as high as the level during fasting [26]. Repa et al. reported that LXR directly regulates ChREBP gene expression at the transcriptional level [27]. The mouse ChREBP gene promoter contains an LXR response element at about 2.4 kbp, and LXR agonists increase hepatic ChREBP mRNA in wild-type mice but not in LXR-α, βdouble knockout mice. Moreover, Saez et al. reported that LXR is activated by glucose and that high-glucose treatment increased ChREBP mRNA two-fold in HepG2 cells [28]. Insulin also regulates the expression and transactivity of the LXR gene [29]. However, despite the hyperinsulinemia and hyperglycemia seen in ob/ob mice, the level of ChREBP mRNA in liver of ob/ob mice is only twice as high as in liver of wild-type mice [30]. These results suggest that ChREBP transactivity is regulated mainly at the post-transcriptional level rather than at the transcriptional level.

ChREBP knockout mice

To identify the physiological role of ChREBP in hepatic glucose and lipid metabolism, we established ChREBP knockout mice (ChREBP$^{-/-}$) [10]. ChREBP$^{-/-}$ mice are viable and appear to have a normal lifespan. These mice show a phenotype with hepatic lipogenesis from glucose 65% lower than in wild-type mice, and adipose tissue weight correspondingly lower. In addition, the mRNAs of many glycolytic and lipogenic enzymes in liver of ChREBP$^{-/-}$ mice are suppressed [10]. Consistent with the *in vivo* data, our CHIP and EMSA assays show that ChREBP binds directly to ChoREs in the promoters of LPK, ACC, and FAS [31]. These data also indicate that ChREBP directly regulates the expression of glycolytic and lipogenic enzyme genes.

In contrast to decreased lipid content, liver glycogen content is increased and hepatomegaly appears in $ChREBP^{-/-}$ mice [10]. The mechanism by which glycogen content in ChREBP knockout mice is increased is partly understood (Fig. 5). In these mice, most metabolites in the glycolytic pathway, except for pyruvate, are increased. Pyruvate is converted from phosphoenol pyruvate (PEP) by L-PK. In addition, L-PK activity in liver of $ChREBP^{-/-}$ mice was markedly lower and the PEP/pyruvate ratio was higher. G6Pase activity also was decreased in these mice, and the G6P content was increased. Because G6P activates glycogen synthase and stimulates glycogen synthesis in liver, glycogen accumulates in the liver of $ChREBP^{-/-}$ mice. These results indicate that ChREBP is a transcription factor that preferentially regulates triglyceride storage (Fig. 5).

Both glucose and insulin are required for full induction of most lipogenic enzyme genes. In addition, the actions of insulin in regard to lipid metabolism are mediated through the transcription factor SREBP1c, and in $ChREBP^{-/-}$ mice, SREBP1 mRNA and protein are unchanged compared with wild-type mice. Furthermore, as with glycolytic genes, SREBP and ChREBP regulate glucokinase and LPK, respectively. These data indicate that ChREBP and SREBP independently regulate *de novo* lipogenesis.

Inhibition of ChREBP as a treatment for metabolic syndrome

Excess fat accumulation in the liver (fatty liver) leads to insulin resistance in the body, and reducing the fat content of the liver readily improves insulin sensitivity [1]. We

intercrossed ChREBP$^{-/-}$ mice with ob/ob mice and established ob/ob ChREBP double-cross (ob/ob ChREBP$^{-/-}$) mice [30]. In ob/ob ChREBP$^{-/-}$ mice, complete inhibition of the ChREBP gene throughout the body ameliorated symptoms of the metabolic syndrome such as obesity, insulin resistance, fatty liver, and glucose intolerance (Fig. 6). Consistently, glycolytic and lipogenic gene expression was normalized in ob/ob ChREBP$^{-/-}$ mice. Postic et al. reported that delivery of adenovirus-bearing ChREBP short hairpin RNA (Ad-shChREBP) into the liver effectively improved the metabolic syndrome in ob/ob mice [32]. Their data suggest that liver-specific inhibition of ChREBP ameliorates both fatty liver and also glucose intolerance. In contrast, liver-specific inhibition of SREBP1c and PPARγ was found to ameliorate fatty liver but not glucose intolerance[33, 34]. Although the source of the phenotypic differences between these liver-specific SREBP1c or PPARγ knockout mice and Ad-shChREBP mice is not known, ChREBP might regulate G6Pase gene expression at the transcriptional level. G6Pase is a key enzyme in the regulation of gluconeogenesis and glucose output in liver, and decreased G6Pase activity in Ad-shChREBP mice can lower the plasma glucose concentration [32]. Comparison of liver-specific ChREBP knockdown mice with liver-specific SREBP1c or PPARγ knockout mice should provide a clearer understanding of the roles of these substances in the metabolic syndrome. In addition, food intake in ob/ob ChREBP$^{-/-}$ mice was lower than in ob/ob mice, which was not the case in ob/ob mice infected with Ad-shChREBP. In additon, since ChREBP is also expressed in the brain, ChREBP may regulate appetite control, likely in the hypothalamus (Fig. 6).

Marked hepatomegaly and massive glycogen accumulation are thought to be effects of ChREBP inhibition. Although hepatoma was not visible, excess glycogen accumulation could well finally induce liver fibrosis and carcinogenesis. With the aim of applying these results on ChREBP inhibition to the treatment of metabolic syndrome, we are now identifying ChREBP target genes to suggest new drug therapies based on inhibition of ChREBP transactivity.

Activation of PKA and AMPK has been used in the treatment of obesity-related disorders [35, 36]. Exedine-4 (GLP-1 analogue) increases hepatic cAMP content and ameliorates fatty liver by suppressing *de novo* lipogenesis in ob/ob mice [35]. Metformin also inhibits *de novo* lipogenesis and ameliorates fatty liver by AMPK in genetically obese mice [37]. In addition, acetate can be taken daily in the form of vinegar; when acetate is converted to acetyl CoA, the AMP/ATP ratio is increased and AMPK is activated [38, 39]. Intake of acetate reduces lipogenesis and improves fatty liver in obese mice and rats. In addition, polyunsaturated fatty acids (PUFA) also can be taken daily in the form of fish oil to improve obesity-related disorders [40, 41]. Since these drugs and foods modulate transactivity not only of SREBP but also of ChREBP, they are promising means of mitigating the metabolic syndrome, but the mechanisms by which they act remain unclear.

Role of ChREBP in other tissues

ChREBP is expressed ubiquitously, but mainly in lipogenic organs such as liver, intestine, and white adipose tissues. Interestingly, ChREBP also is expressed in pancreatic islets [42, 43]. In islets, glucose stimulates insulin secretion and is an important signal for cellular events. Using DNA microarrays, many researchers have identified glucose responsive genes in islets that are common to those in liver [44]. In insulin-producing INS-1 cells, overexpression of ChREBP was found to upregulate LPK, FAS, and ACC1 mRNAs, but the insulin response to glucose in these cells was the same as in control cells [42]. In islets of ChREBP$^{-/-}$ mice, glucose-stimulated insulin secretion was the same as in wild-type mice (unpublished data). ChREBP also regulated lipogenic genes in islets, but only overexpression of ChREBP prevented the accumulation of lipid droplets, unlike overexpression of SREBP1c [45]. These findings suggest the action of an insulin signal in addition to ChREBP activation that is important in the induction of lipogenesis.

ChREBP also is abundantly expressed in adipose tissues. During 3T3-L1 preadipocyte adipogenesis, ChREBP is dramatically induced and the expression of its gene in 3T3L1 cells is modulated by various factors including glucose, free fatty acids, insulin, and the antidiabetic agent troglitazone [46]. However, the expression of ChREBP mRNA in adipose tissue *in vivo* is barely responsive to changes in nutrient status. Moreover, ChREBP mRNA is induced in the late stage of adipogenesis and ChREBP has little part in this process. Thus, the physiological role of ChREBP in adipose tissue remains unclear.

Conclusion

The liver is an important organ in the maintenance of glucose homeostasis and energy storage. Excess triglyceride in the liver induces fatty liver and eventually insulin resistance. To prevent the metabolic syndrome, it is important to gain understanding of the mechanism by which certain glucose/insulin-regulated transcription factors coordinate hepatic energy metabolism. Among these transcription factors, glucose-activated transcription factor ChREBP regulates the balance between glycogen and triglyceride storage by coordinately regulating glycolytic and lipogenic gene expression. In genetically obese mice, complete deficiency of ChREBP ameliorates glucose intolerance, fatty liver, and obesity, although hepatomegaly and liver glycogen accumulation develop. Thus, the identification of the roles of ChREBP and its target genes in glucose and lipid metabolism should be useful in developing treatments for the metabolic syndrome.

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