Original Article

Ezetimibe Ameliorates Early Diabetic Nephropathy in db/db Mice

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Aim: Lipid-lowering medications have been suggested to have a potential benefit in the treatment of chronic kidney disease (CKD) such as diabetic nephropathy. Although ezetimibe has been widely used to lower serum cholesterol levels, the effect of this drug on diabetic nephropathy remains unclear. In the present study, therefore, we examined the protective effect of ezetimibe on diabetic nephropathy in db/db mice.
Method: Db/db mice were fed a standard diet with 0.01% (w/w) of ezetimibe for 8 weeks from 8 weeks of age.
Results: Treatment with ezetimibe did not affect food intake, body weight gain, adiposity, or blood pressure in db/db mice. Ezetimibe also had no effect on glucose metabolism such as fasting plasma glucose and insulin; however, it markedly reduced plasma lipid levels and hepatic lipid contents and reduced the urinary excretion of albumin by 50% in db/db mice, suggesting the effect of ezetimibe on diabetic nephropathy. Furthermore, ezetimibe improved glomerular hypertrophy. Although ezetimibe had no effect on oxidative stress measured by urinary 8-OHdG in db/db mice, the plasma adiponectin level was normalized, and the expression of adiponectin receptor 1 in the kidney was increased by ezetimibe treatment.
Conclusion: Our data suggest that ezetimibe can improve early diabetic nephropathy through its hypolipidemic effect, and the amelioration of adiponectin resistance may also be responsible for the renoprotective effect of ezetimibe as its underlying mechanism.

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Key words; Ezetimibe, Diabetic nephropathy, Albuminuria, Adiponectin

Introduction

Diabetic nephropathy is one of the most common forms of chronic kidney disease (CKD) and the most frequent cause of mortality in patients with diabetes[1, 2]. The number of people affected by diabetic nephropathy or who need renal replacement is steadily increasing[3]. Furthermore, CKD such as diabetic nephropathy is strongly associated with the development of cardiovascular disease[4, 5]; therefore, the establishment of therapeutic strategies for diabetic nephropathy is awaited. Diabetic nephropathy results from complex interactions among genetic, metabolic, and hemodynamic factors, and can be characterized by mesangial expansion followed by glomerulosclerosis and a decline in renal function. The development of glomerulosclerosis in diabetes mellitus is preceded by persistent albuminuria and glomerular hypertrophy[2]; therefore, these two manifestations are promising therapeutic targets for the treatment of diabetic nephropathy.

Hypercholesterolemia has been suggested to be associated with the development of diabetic nephropathy[6]. In fact, lipid-lowering therapy using 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reduc-

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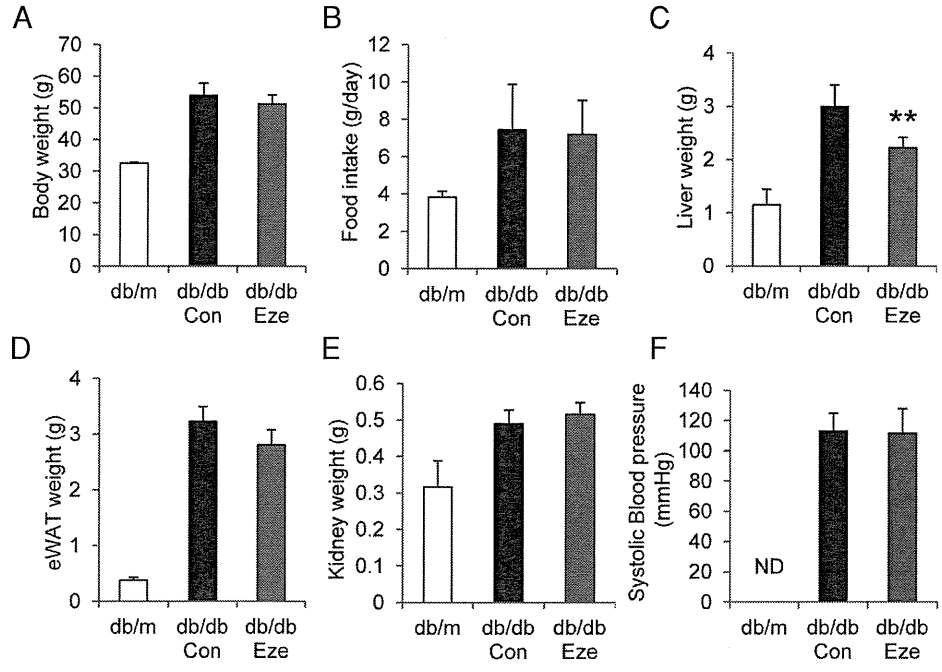

Fig. 1. Effect of ezetimibe on body weight, adiposity, and blood pressure in db/db mice at 16 weeks of age. The graphs show body weight (A), food intake (B), liver weight (C), epididymal white adipose tissue (eWAT) weight (D), kidney weight (E), systolic blood pressure (F) in db/m mice and non-treated (Con) or ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D. $^{**}p<0.01$ vs. non-treated db/db mice ($n=6$ in each group). ND: not determined.

tase inhibitors (statins) has been successful for the amelioration of diabetic nephropathy[7, 8]. Ezetimibe, another lipid-lowering drug that selectively inhibits cholesterol absorption by inhibiting Niemann-Pick C1-Like 1 (NPC1L1) protein, is also used for the treatment of dyslipidemia[9, 10]. In addition to its effect on hyperlipidemia, ezetimibe has been reported to ameliorate renal dysfunction such as non-diabetic nephropathy[11] and nephropathy after transplantation[12]; however, the effects of ezetimibe on diabetic nephropathy remain undetermined. In the present study, therefore, we examined the renoprotective effects of ezetimibe on diabetic nephropathy in db/db mice.

Methods

Animal Procedure and Experimental Design

Male db/db mice ($n=12$) and their lean control db/+m ($n=6$) mice were obtained from Charles River Laboratories Japan, Inc. (Yokohama, Japan) at 6 weeks of age. Db/db mice were fed with normal chow without additional supplementation (non-treated group, $n=6$) or with chow supplementation with 0.01% (w/w) ezetimibe ($n=6$) for 8 weeks from 8 weeks of age. Animals were provided with the diet and water ad libitum and were maintained on a 12-hour light/dark cycle. All animal experiments were conducted according to the Guidelines for Animal Experiments at Kyoto University.

Analysis of Metabolic Parameters

Blood samples were collected after fasting the mice for 16 h. Fasted plasma glucose concentration was measured with Glutest Ace (Sanwa Kagaku Kenkyusho Co, Ltd, Nagoya, Japan). Fasted plasma insulin concentration was measured with an insulin assay kit (Morinaga Institute of Biological Science, Yokohama, Japan). Serum total cholesterol (T-Cho), triglyceride (TG) and cholesterol contents of each lipoprotein fraction were analyzed by Skylight Biotech, Inc. (Tokyo, Japan). Serum adiponectin was measured with a Mouse/Rat Adiponectin ELISA kit (Otsuka Pharmaceuticals, Tokushima, Japan). Serum total protein, creatinine, and BUN were analyzed by SRL, Inc. (Tokyo, Japan).

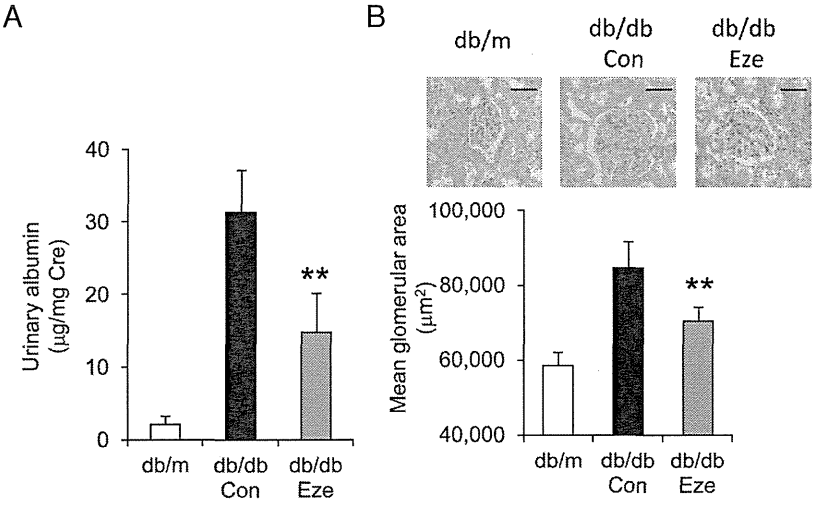

Fig. 2. Effect of ezetimibe on renal function in db/db mice at 16 weeks of age. Urinary excretion of albumin (A), HE staining of glomeruli (magnification ×400, bar; 50 μm) and mean glomerular surface area (B) of db/m mice, non-treated (Con), ezetimibe-treated (Eze) db/db mice. Fifty glomeruli per mouse were analyzed. Results are expressed as the mean ± S.D. $^{**}p<0.01$ vs. non-treated db/db mice ($n=6$ in each group).

Table 1. Effect of ezetimibe treatment on blood chemistry in db/db mice

	db/m	db/db Con	db/db Eze
Total protein (g/dL)	5.0±0.5	5.9±0.5	5.1±0.7
BUN (mg/dL)	19.1±3.5	20.1±2.4	27.2±7.6
Creatinine (mg/dL)	0.11±0.03	0.08±0.02	0.09±0.03

Results are expressed as the means ± S.D. ($n=6$ in each group)

Measurement of Hepatic Lipid Content

Hepatic triglyceride and cholesterol contents were measured with Triglyceride E and Cholesterol E test (Wako Pure Chemical Industries Ltd., Osaka, Japan) as previously described[13]. Tissue triglyceride and cholesterol contents were expressed as mg/mg protein.

Measurement of Urinary Albumin and Creatinine

Urinary albumin and creatinine were measured at 16 weeks age using 24-h collection samples from mice housed in individual metabolic cages. During the urine collection, the mice were allowed free access to food and water. Albumin concentration in the urine was measured by Albuwell (Exocell Inc., Philadelphia, PA). Urinary creatinine was measured with a Hitachi Mode 736 analyzer (Hitachi, Tokyo, Japan). Urinary albumin concentration was adjusted by the urinary creatinine concentration.

Measurement of Urinary Oxidative Stress

Urinary 8-OHdG concentrations were measured at 16 weeks of age using a competitive enzyme-linked immunosorbent assay kit (8OHdG Check; Japan Institute for the Control of Aging, Fukuroi, Japan). Urinary 8-OHdG excretion was expressed as the total amount excreted in 24 h.

Measurement of Glomerular Size

Mice were euthanized at 16 weeks of age. The kidneys were rapidly fixed in 10% formaldehyde and embedded in paraffin. Paraffin sections were cut at 3 μm. To measure glomerular size, paraffin sections were stained with hematoxylin and eosin. The glomerular area was measured using Image Pro plus software version 3.0.1 (Media Cybernetics Inc., Bethesda, MD).

Quantitative Real-Time PCR

Total RNA was extracted from frozen adipose tissue (100 mg) and kidney tissue (30 mg) using an

RNeasy mini kit (Qiagen, Valencia, CA). The cDNA was synthesized from total RNA using Super Script III (Invitrogen). Real-time polymerase chain reaction was performed on an ABI PRISM 9700 using the SYBR GREEN polymerase chain reaction Master Mix (Applied Biosystems, Warrington, UK). Primer sets were as follows: TNF alpha forward: CCCAGACCCTCACACTCAGATC, reverse: GCCACTCCAGCTGCTCCTC, Nox2 forward: TTGGGTCAGCACTGGCTCTG, reverse: TGGCGGTGTGCAGTGCTATC, Nox4 forward: ATTTGGATAGGCTCCAGGCAAAC, reverse: CACATGGGTATAAGCTTTGTGAGCA, $p22^{phox}$ forward: GTCCACCATGGAGCGATGTG, reverse: CAATGGCCAAGCAGACGGTC adiponectin receptor 1 (AdipoR1) forward: ACGTTGGAGAGTCATCCCGTA, reverse: CTCTGTGTGGATGCGGAAGAT, adiponectin receptor 2 (AdipoR2) forward: TGCCAGGAAGATGAAGGGTTTAT, reverse: TTCCATTCGTTCGATAGCATGA, β-actin forward: TACCACAGGCATTGTGATGG, reverse: TTTGATGTCACGCACGATTT. The mRNA levels were normalized relative to the amount of β-actin mRNA and expressed in arbitrary units.

Statistical Analysis

Data are expressed as the mean ± S.D. Multiple comparisons among the groups were conducted by one-way analysis of variance with Fisher's PLSD test for post-hoc analysis. Pearson's correlation was used to find a correlation between two continuous variables. $P<0.05$ was considered significant.

Results

Effect of Ezetimibe Treatment on Body Weight, Adiposity, and Systolic Blood Pressure

In db/db mice fed with a standard diet for 8 weeks until 16 weeks of age, body weight, epididymal white adipose tissue (eWAT) weight, liver weight, and kidney weight were increased compared with db/m mice (**Fig. 1A-E**). Although ezetimibe treatment reduced liver weight in db/db mice, body weight, food intake, eWAT weight, and kidney weight were not changed (**Fig. 1A-E**). In addition, there was no difference in systolic blood pressure between ezetimibe-treated and non-treated db/db mice (**Fig. 1F**).

Effect of Ezetimibe Treatment on Renal Dysfunction in db/db Mice

Because albuminuria reflects renal dysfunction at early diabetic nephropathy[14], we measured urinary excretion of albumin in normal chow-fed db/db mice at 16 weeks of age. Urinary excretion of albumin was markedly increased in db/db mice compared with db/+m mice (**Fig. 2**); however, ezetimibe treatment reduced urinary excretion of albumin by 50% in db/db mice (**Fig. 2**). There was no difference in serum total protein, creatinine, or BUN levels between db/m and non-treated db/db mice. Ezetimibe treatment also had no effect on these variables in db/db mice (**Table 1**). These data suggest that ezetimibe ameliorates early diabetic nephropathy in db/db mice.

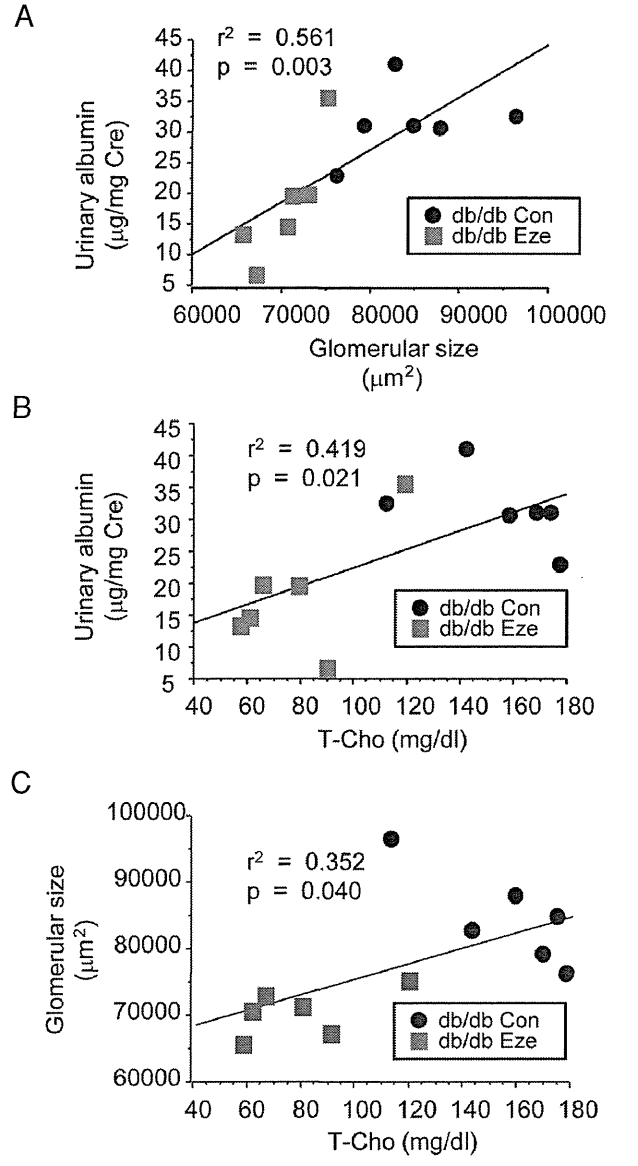

Fig. 3. The correlation between the effect of ezetimibe on urinary albumin and glomerular size (A), urinary albumin and serum T-Cho level (B), glomerular size and serum T-Cho level (C) in db/db mice.

Table 2. Effect of ezetimibe treatment on serum lipid in db/db mice

	db/m	db/db Con	db/db Eze
Triglyceride (mg/dL)	50.9±10.2	59.0±23.3	64.8±27.4
Total Cholesterol (mg/dL)	77.8±2.2	150.3±26.8	78.7±23.1**
Chylomicron (mg/dL)	0.8±0.5	1.9±0.6	0.9±0.5*
VLDL cholesterol (mg/dL)	4.2±1.5	7.0±1.6	4.3±3.0
LDL cholesterol (mg/dL)	6.7±1.1	30.4±11.1	7.1±4.8**
HDL cholesterol (mg/dL)	66.1±1.5	111.0±17.6	66.4±16.2**
Small dense LDL cholesterol (mg/dL)	2.4±0.4	5.0±2.1	1.2±0.9**

Results are expressed as the means±S.D. (n=6 in each group)
*p<0.05, **p<0.01 vs db/db Con group

Effect of Ezetimibe Treatment on Glomerular Hypertrophy in db/db Mice

Glomerular hypertrophy is a marker of diabetic nephropathy along with albuminuria; therefore, we checked glomerular hypertrophy in db/db mice and the effect of ezetimibe by measuring the glomerular surface area. Mean glomerular surface area size in db/db mice was increased compared with db/m mice; however, ezetimibe treatment suppressed glomerular hypertrophy in db/db mice (**Fig. 2B**). Furthermore, there was a significant correlation in the effect of ezetimibe treatment on glomerular hypertrophy and albuminuria in db/db mice (**Fig. 3A**).

Effect of Ezetimibe Treatment on Lipid Metabolism in db/db Mice

To clarify the mechanisms by which ezetimibe improves renal dysfunction, we next examined the effect of ezetimibe treatment on lipid metabolism in db/db mice. Serum TG levels were not affected by ezetimibe treatment in db/db mice (**Table 2** and **Fig. 4**). Serum T-Cho levels were increased in non-treated db/db mice compared with in db/m mice; however, ezetimibe treatment normalized T-Cho levels in db/db mice (**Table 2** and **Fig. 4**). Furthermore, ezetimibe treatment reduced chylomicron, LDL, small dense LDL, and HDL cholesterol levels in db/db mice (**Table 2** and **Fig. 4**). In addition, hepatic TG and T-Cho contents in db/db mice were reduced by ezetimibe treatment (**Fig. 5**), suggesting that ezetimibe treatment improves hepatic steatosis.

Effect of Ezetimibe Treatment on Insulin Resistance in db/db Mice

It has been reported that ezetimibe treatment improves insulin resistance, which is associated with the development of diabetic nephropathy[15, 16]; therefore, we next examined the effect of ezetimibe treatment on glucose metabolism in db/db mice. Fasted

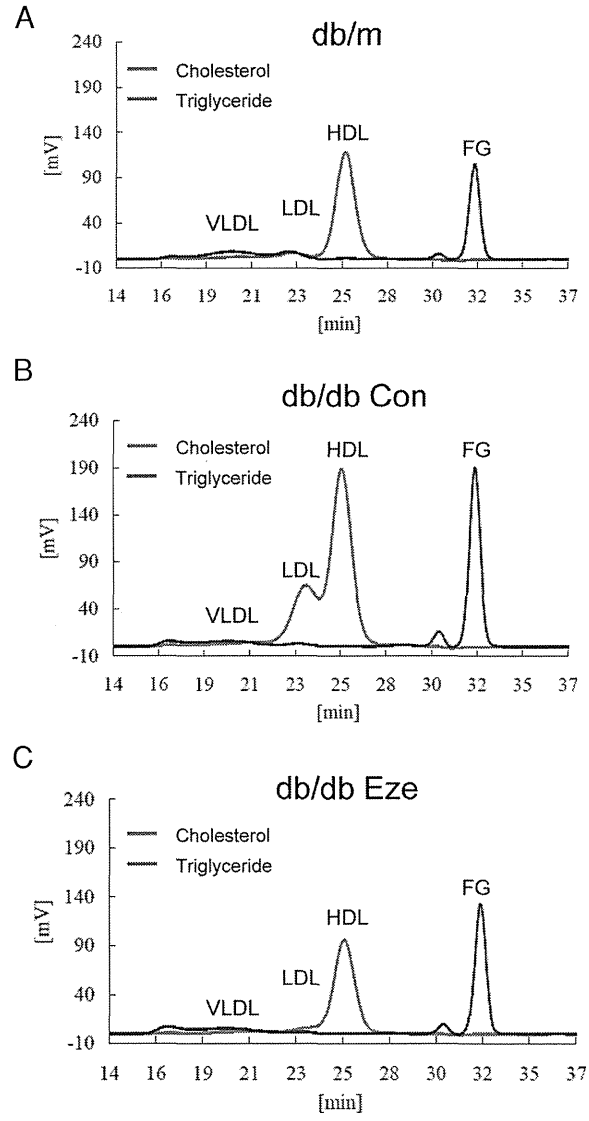

Fig. 4. Graph of cholesterol (pink line) and triglyceride (blue line) contents in each fraction of lipoprotein in db/m (A), non-treated db/db mice (B) and ezetimibe-treated db/db mice (C). FG: free glycerol.

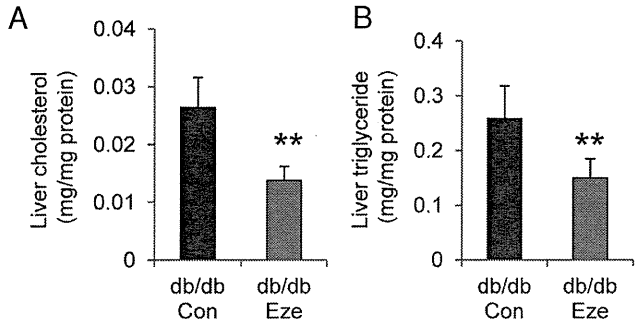

Fig. 5. Effect of ezetimibe on hepatic steatosis in db/db mice at 16 weeks of age. Hepatic triglyceride content (A), hepatic cholesterol content (B) in db/m mice, non-treated (Con), ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D. $^{**}p<0.01$ vs. non-treated db/db mice ($n=6$ in each group).

plasma glucose, the plasma insulin level and HOMA-IR were markedly increased in db/db mice compared with db/m mice, indicating an increase in insulin resistance (**Fig. 6**); however, ezetimibe treatment had no effect on glucose metabolism in db/db mice.

Effect of Ezetimibe Treatment on Oxidative Stress in Kidney of db/db Mice

To examine the effect of ezetimibe treatment on oxidative stress, we measured urinary 8-OHdG levels in db/db mice. Urinary 8-OHdG levels in non-treated db/db mice were significantly higher than those in db/m mice; however, ezetimibe had no effect on urinary 8-OHdG levels in db/db mice (**Fig. 7A**). Furthermore, mRNA expressions of Nox2 and Nox4, the substrate of NADPH oxidase, were not altered by ezetimibe treatment in the whole kidney of db/db mice (**Fig. 8**). These data suggest that ezetimibe has no effect on oxidative stress in the kidney of db/db mice.

Effect of Ezetimibe Treatment on Hypoadiponectinemia

Because hypoadiponectinemia is associated with the development of kidney disease[17], we examined the effect of ezetimibe treatment on serum adiponectin levels in db/db mice. In non-treated db/db mice, serum adiponectin levels were decreased compared with db/m mice; however, ezetimibe treatment normalized serum adiponectin levels in db/db mice (**Fig. 7B**).

Effect of Ezetimibe on Inflammation and Oxidative Stress in Adipose Tissue

Because inflammation and oxidative stress in adipose tissue are a major cause of hypoadiponectinemia[18], we examined the effect of ezetimibe treatment on the expression of inflammatory cytokines and an oxidative stress marker in db/db mice. TNF-α mRNA expression was markedly increased in non-treated db/db mice compared with in db/m mice (**Fig. 9A**); however, ezetimibe treatment reduced TNF-α mRNA expression in db/db mice, suggesting that it suppresses adipose tissue inflammation in db/db mice. Furthermore, oxidative stress markers such as Nox2 and $p22^{phox}$ mRNA expression were decreased by ezetimibe treatment in db/db mice (**Fig. 9B, C**), suggesting that ezetimibe treatment suppresses oxidative stress in adipose tissue of db/db mice.

Effect of Ezetimibe Treatment on the Expression of Adiponectin Receptor (AdipoR) in the Kidney and Adipose Tissue in db/db Mice

We next examined the effect of ezetimibe on the expression of AdipoR1 and AdipoR2 in the kidney

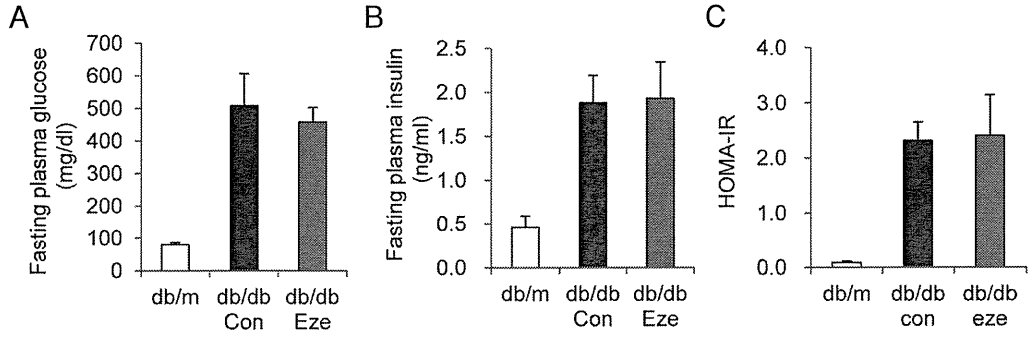

Fig. 6. Effect of ezetimibe on glucose metabolism in db/db mice at 16 weeks of age. Fasted plasma glucose (A), Fasted plasma insulin level (B) and HOMA-IR (C) in db/m mice, non-treated (Con), ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D. $^{*}p<0.05$ vs. non-treated db/db mice ($n=6$ in each group).

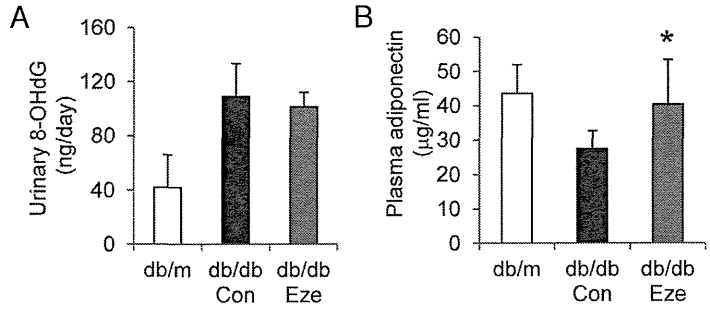

Fig. 7. Effect of ezetimibe on renal oxidative stress and hypoadiponectinemia in db/db mice at 16 weeks of age. Urinary 8-OHdG level (A) and serum adiponectin level (B) in db/m mice, non-treated (Con), ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D. $^{*}p<0.05$ vs. non-treated db/db mice ($n=6$ in each group).

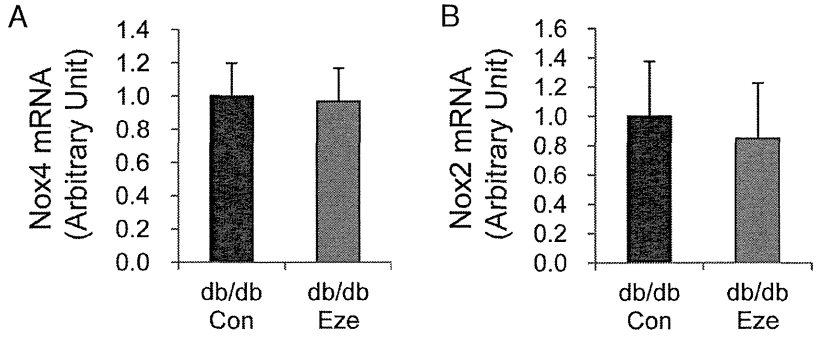

Fig. 8. Effect of ezetimibe on oxidative stress in whole kidney of db/db mice. Expression of Nox4 mRNA (A) and Nox2 mRNA (B) in non-treated (Con) or ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D.

and adipose tissue of db/db mice. AdipoR1, but not AdipoR2, was abundantly expressed in the kidney as well as in adipose tissue (**Fig. 10**). There were no differences in mRNA expression of AdipoR1 and AdipoR2 in adipose tissue and the expression of AdipoR2 in the kidney among db/m mice, non-treated and ezetimibe-treated db/db mice; however, mRNA expression of AdipoR1 was decreased in the kidney of db/db mice compared with db/+m mice. Intriguingly, ezetimibe treatment significantly increased mRNA expression of AdipoR1 in the kidney of db/db mice (**Fig. 10**).

Discussion

In the present study, we showed that ezetimibe treatment improved hyperlipidemia, albuminuria, and glomerular hypertrophy in db/db mice, implying a beneficial role of ezetimibe in early diabetic nephropathy[19].

Ezetimibe is an anti-hyperlipidemic medication that is used to lower cholesterol levels in addition to statins[9]. Specifically, it appears to bind to a critical mediator of cholesterol absorption, NPC1-L1, on gastrointestinal tract epithelial cells[10]. In the present study, we observed a correlation between the effect of ezetimibe on albuminuria and on the T-Cho level in db/db mice (**Fig. 3B**). These data suggest that ezetimibe improves diabetic nephropathy through its hypolipidemic action, and provide further evidence for the importance of hyperlipidemia in the development of diabetic nephropathy. Ezetimibe treatment also markedly reduced LDL cholesterol and small dense LDL cholesterol in db/db mice, consistent with the effect in humans[8, 20]. In the present study, we found a correlation between the effect of ezetimibe treatment on albuminuria and on the LDL cholesterol level, but not on the small dense LDL cholesterol level

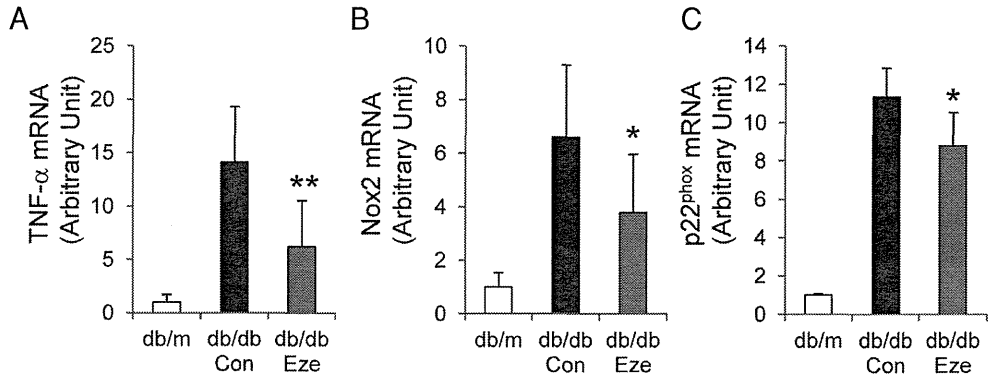

Fig. 9. Effect of ezetimibe on inflammation and oxidative stress in adipose tissue of db/db mice. The mRNA expression of TNF-α (A), Nox2 (B) and $p22^{phox}$ (C) in db/m mice, non-treated (Con), ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D. $^{*}p < 0.05$ vs. non-treated db/db mice ($n = 6$ in each group).

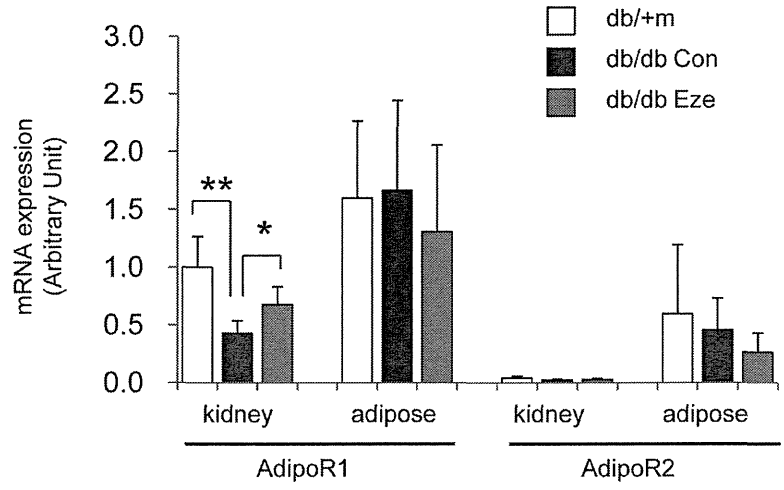

Fig. 10. Effect of ezetimibe on mRNA expression of adiponectin receptor 1 (AdipoR1) and 2 (AdipoR2) in the kidney and adipose tissue of db/m mice and non-treated (Con) and ezetimibe-treated (Eze) db/db mice. Results are expressed as the mean ± S.D. $^{*}p < 0.05$, $^{**}p < 0.01$ ($n = 6$ in each group).

in db/db mice (**Supplementary Fig. 1**); therefore, the effect of ezetimibe on LDL cholesterol might be associated with the amelioration of diabetic nephropathy in this model.

In this study, we found no effect of ezetimibe on the urinary 8-OHdG level and the expression of oxidative stress markers in the kidney of db/db mice, whereas ezetimibe significantly reduced oxidative stress in the adipose tissue of db/db mice, suggesting that the renoprotective effect of ezetimibe is not directly due to reduced oxidative stress in the kidney. Nevertheless, ezetimibe has been reported to have an anti-oxidative effect in non-diabetic individuals[11].

Several human and animal studies have reported that hypoadiponectinemia is associated with renal dysfunction[21-23]. In the present study, we observed the improvement of hypoadiponectinemia by ezetimibe treatment in db/db mice; however, we did not find a correlation between the effect of ezetimibe on albuminuria and on adiponectin in db/db mice (data not shown), suggesting that improvement of hypoadiponectinemia may not be responsible for the renoprotective effect of ezetimibe in db/db mice. In contrast, we found a negative correlation between the effect of ezetimibe on albuminuria and AdipoR1 expression in the kidney of db/db mice (**Supplementary Fig. 2A**).

Furthermore, we also observed a negative correlation between the effect of ezetimibe on AdipoR1 expression in the kidney and the LDL cholesterol level in db/db mice (**Supplementary Fig. 2B**). Guo *et al.* reported decreased adipoR1 expression in the kidney of diabetic rats, suggesting the existence of adiponectin resistance in diabetic nephropathy[24]. Taken together, whether renoprotection by ezetimibe occurs through alteration of the adiponectin effect remains to be determined.

We also observed that ezetimibe treatment suppressed the expression of pro-inflammatory cytokines such as TNF-α in adipose tissue of db/db mice. TNF-α can dose-dependently reduce the expression of adiponectin in adipocytes by suppressing its promoter activity[25]; however, we did not find any correlations among the effect of ezetimibe on the serum adiponectin level, TNF-α expression in adipose tissue, and serum lipid profiles in db/db mice. We thus speculate that ezetimibe has pleiotropic effects, which might not be mutually interrelated.

Insulin resistance is also associated with the development of renal dysfunction in type 2 diabetes. It has been shown that insulin resistance correlates with the onset of microalbuminuria in patients with type 2 diabetes as well as in non-diabetic subjects[16]. In the present study, we observed marked elevation of plasma glucose and insulin in db/db mice, indicating the development of insulin resistance. Because it has been reported that ezetimibe improved hepatic insulin resistance[15], we examined the effect of ezetimibe on the glucose metabolism; however, ezetimibe had no effect on systemic insulin resistance in db/db mice despite the increase in serum adiponectin. These data indicate that the renoprotective effect of ezetimibe in db/db mice was independent of systemic insulin-sensitizing action.

Ezetimibe can be used for hypercholesterolemic patients who exhibit statin resistance or suffer adverse effects of statin treatment[26]. Recently, combination therapy with a statin and ezetimibe showed efficacy and safety compared with high-dose statin therapy in patients with hypercholesterolemia[27]. Therefore, combination therapy of statins and ezetimibe as well as ezetimibe monotherapy might be useful for the treatment of diabetic nephropathy associated with hyperlipidemia[28].

In conclusion, our data suggest that ezetimibe can improve diabetic nephropathy through its hypolipidemic action, and the amelioration of adiponectin resistance may be responsible for the renoprotective effect of ezetimibe as its underlying mechanism.

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Disclosure

None.

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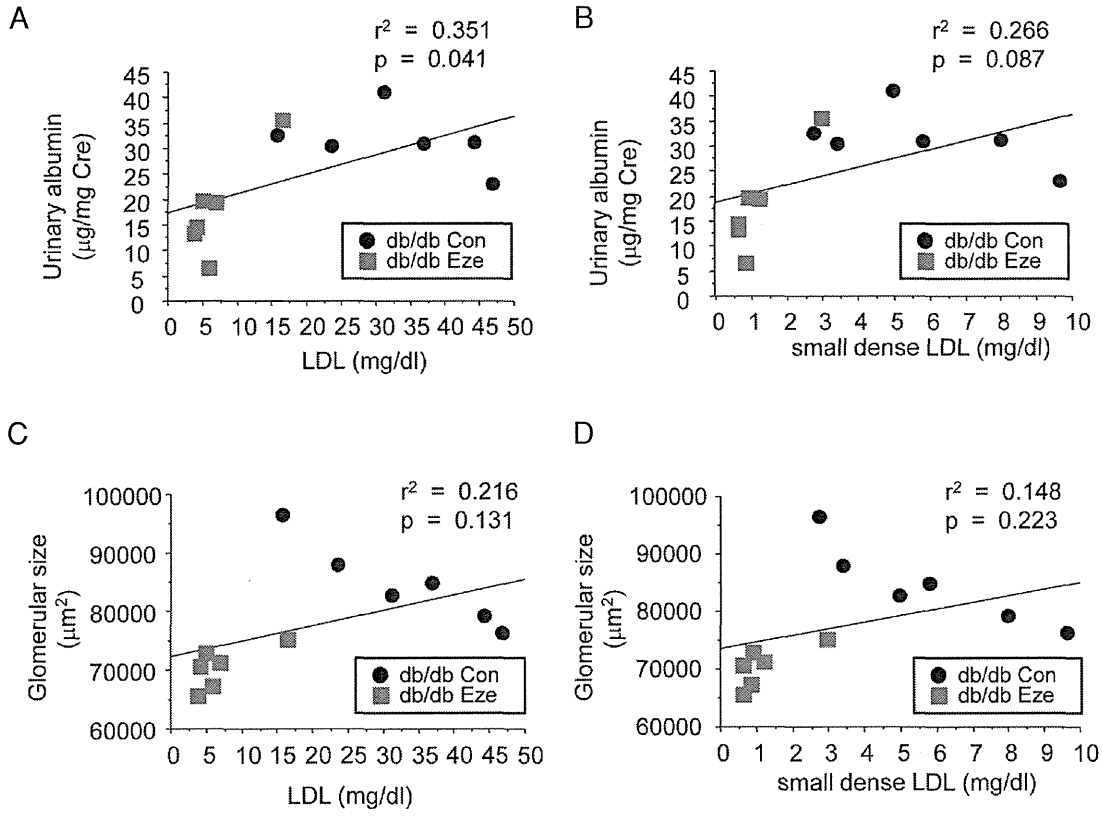
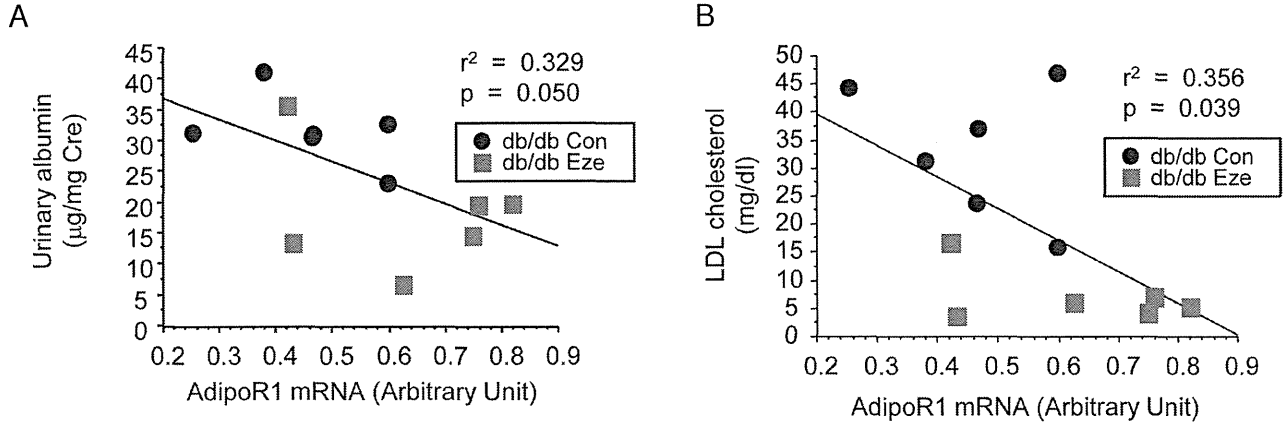


Supplementary Fig. 1. The correlation between the effect of ezetimibe on urinary albumin and LDL cholesterol (A), urinary albumin and small dense LDL cholesterol (B), glomerular size and LDL cholesterol (C), glomerular size and small dense LDL cholesterol (D) in db/db mice.



Supplementary Fig. 2. The correlation between the effect of ezetimibe on urinary albumin and AdipoR1 expression in the kidney (A), LDL cholesterol and AdipoR1 expression in the kidney (B) of db/db mice.

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ORIGINAL ARTICLE

Correlation of fasting serum apolipoprotein B-48 with coronary artery disease prevalence

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ABSTRACT

Background Postprandial hyperlipidemia partially refers to the postprandial accumulation of chylomicrons and chylomicron remnants (CM-R). Many *in vitro* studies have shown that CM-R has highly atherogenic properties, but consensus is lacking on whether CM-R accumulation correlates with the development of atherosclerotic cardiovascular diseases. We investigated the correlation between CM-R accumulation and the prevalence of coronary artery disease (CAD).

Design Subjects who received a coronary angiography and did not take any lipid-lowering drugs (n = 189) were enrolled. Subjects with coronary artery stenosis (≥ 75%) were diagnosed as CAD. Biochemical markers for glucose and lipid metabolism including fasting apolipoprotein (apo) B-48 concentration were compared between CAD patients (n = 96) and age-, sex-, and body mass index (BMI)-matched non-CAD subjects without overt coronary stenosis (< 75%) (n = 67). We tried to determine which metabolic parameters were correlated with the prevalence of CAD by multiple logistic regression analysis, and whether or not the combination of high apo B-48 and other coronary risk factors (high triglyceride, low HDL-C, high HbA1c or low adiponectin levels) increased the prevalence of CAD.

Results Fasting serum apo B-48 levels were significantly higher in CAD patients than in non-CAD subjects (3·9 ± 2·4 vs. 6·9 ± 2·6 μg/mL, P < 0·0001) and had the most significant correlation with the existence of CAD. The clustering of high fasting apo B-48 levels (> 4·34 μg/mL, the cut-off value) and other coronary risk factors were found to be associated with a stronger risk of CAD compared with single high fasting apo B-48 levels.

Conclusion Fasting serum apo B-48 levels significantly correlated with the prevalence of CAD.

Keywords Apolipoprotein B-48, chylomicrons, coronary artery disease, postprandial hyperlipidemia, remnant lipoproteins.

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Introduction

Fasting hypertriglyceridaemia and postprandial hyperlipidaemia (PH) are both closely related to the development of atherosclerotic cardiovascular diseases [1,2]. PH is characterized by postprandial accumulation of triglyceride (TG)-rich lipoproteins and their partially hydrolysed products, 'remnant lipoproteins', as suggested by Zilversmit [3] and supported by numerous subsequent studies [4,5]. Remnant lipoprotein cholesterol levels proved to be closely correlated with the prevalence of coronary artery disease (CAD) [6,7]. The atherogenicity of remnant lipoproteins has been the subject of numerous studies [5]. However, the atherogenicity of chylomicron remnants (CM-R) has been investigated less frequently than that of very-low-density lipoproteins (VLDL) remnants (VLDL-R) or intermediate-density lipoprotein (IDL). Investigators have developed an assay system for measuring serum apolipoprotein B-48 (apo B-48) concentration, which represents the

number of chylomicrons (CMs) and CM-R in the serum [8]. Fasting apo B-48 levels ranged from 0 to 25 μg/mL (the mean ± SD value was 5·2 ± 3·8 μg/mL) and were significantly higher in hyperlipidaemic patients with supposed accumulation of CMs and CM-R [8] as well as in patients with metabolic syndrome (MetS) [9] than in healthy subjects.

Several clinical studies have suggested a correlation between serum apo B-48 levels and atherosclerosis [10,11]. In our recent study, high levels of fasting apo B-48 significantly correlated with intima-media thickness (IMT) in subjects with normal but relatively high TG levels (100 < TG≤150 mg/dL) [12]. Emerging evidence from *in vivo* [13–15] and *in vitro* studies [5,16] suggests that CM-R might have atherogenic features and may be responsible for the initiation of atherogenesis. However, one report suggested that there was no significant correlation between fasting apo B-48 levels and CAD [17], emphasizing also that no consensus existed as to whether high levels of fasting apo B-48 were correlated with the prevalence of CAD. Moreover, it remained uncertain whether the prevalence of CAD in subjects with high levels of CAD would increase or not in combination with other metabolic disorders such as insulin resistance of MetS.

In this study, we attempted to investigate whether fasting serum levels of apo B-48 correlated with the prevalence of CAD and whether these correlations were stronger than other metabolic parameters recognized as coronary risk factors.

Subjects and methods

Subjects

A consecutive series of patients with suspected CAD were hospitalized in Osaka University Hospital and National Hospital Organization Kure Medical Center from January 2002 to December 2003. Patients who needed emergency care, had acute coronary syndrome or were already being treated with lipid-lowering drugs were eliminated. As a result, 189 subjects (120 men and 69 women) undergoing quantitative coronary angiography (CAG) were enrolled in this study. Height and weight were measured and (BMI, kg/m^2) was calculated. During hospitalization, all patients adhered to a standard diet which contained 25 kcal/kg standard body weight (BMI = 22 kg/m^2) per day (patients with hypertension took a sodium-restricted diet with the same calorie intake), and their blood pressure (BP) was measured in a supine position. The presence of hypertension was assessed by systolic BP ≥ 135 mmHg and/or diastolic BP ≥ 85 mmHg (based on the Guideline for the Management of Hypertension from the Japanese Society of Hypertension) or by intake of anti-hypertensive drugs (Ca blockers were mainly used, beta-blockers were used in only two patients in both CAD and non-CAD groups and no woman used contraceptives or received hormone replacement therapy). Angiographically significant coronary stenosis was defined as 75% or more luminal diameter stenosis by CAG. Those who had significant stenosis in the left anterior descending artery, left circumflex artery and/or right coronary artery were treated as CAD patients (n = 96, 71 men and 25 women). Age-, sex- and BMI-matched subjects who did not have significant stenosis were regarded as non-CAD subjects (n = 67, 49 men and 18 women).

Laboratory measurements and diagnosis of coronary risk factors

Immediately after blood samples were collected in the morning of CAG after an overnight fast, serum and plasma were separated by centrifugation (2000 g, 15 min, 4 °C) and stored at −80 °C until measurement. Serum total cholesterol (TC) and TG levels were determined by enzymatic methods, serum LDL-C and HDL-C levels by the direct method (Sekisui Medical Co., Ltd., Tokyo, Japan) and plasma adiponectin levels by ELISA (Otsuka Pharmaceuticals, Tokyo, Japan). The presence of dyslipidaemia was assessed by LDL-C ≥ 140 mg/dL, TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL [18]. Fasting plasma glucose (FPG) was measured by the enzymatic method, and HbA1c by ion-exchange high performance liquid chromatography (HPLC) (Sekisui Medical Co.). The presence of high fasting glucose was assessed by FPG ≥ 126 mg/dL (Japan Diabetes Society) or by intake of anti-diabetic drugs. The presence of MetS was diagnosed according to the criteria of the Japanese Society of Internal Medicine [19] and National Cholesterol Education Program-Adult Treatment Panel III (NCEP-ATP III), USA [20]. Serum apo B-48 levels were determined by the chemiluminescent enzyme immunoassay system (Fujirebio Inc., Tokyo, Japan) [21] which was modified from the sandwich ELISA system [8]. All samples were treated in accordance with the Helsinki Declaration.

Statistical analyses

Apo B-48, BMI and adiponectin levels were normalized by logarithmic transformation. The statistical significance of differences in TC, TG, HDL-C, LDL-C, systolic BP, diastolic BP, FPG, HbA1c, Log-apo B-48 and Log-adiponectin between CAD and non-CAD subjects was determined by Mann–Whitney U-test, by frequency of smoking, as well as by prevalence of dyslipidaemia, hypertension and high fasting glucose. The prevalence of Mets was then compared by chi-square test. The correlations between metabolic parameters and CAD were analysed by Pearson's correlation coefficients, and stepwise multiple logistic regression analysis was used to determine independent predictors of CAD. Age, sex, Log-BMI, smoking, TC, LDL-CADL-C, TG, systolic BP, diastolic BP, FPG, HbA1c, Log-apo B-48 and Log-adiponectin were included as explanatory variables in the

method. Receiver-operating characteristic (ROC) curves were used to examine the apo B-48 values for categorizing subjects on the basis of the presence of CAD, and the cut-off value was identified.

We compared the effect of different metabolic parameters of MetS (TG, HDL-C, HbA1c or plasma adiponectin; classified as low and high) on CAD prevalence in patients with low or high levels of apo B-48. The cut-off value of apo B-48 level in CAD patients was 4·34 μg/mL. We divided subjects (n = 163) into two groups according to their apo B-48 levels: low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL). Both groups were also divided into low (< 150 mg/dL) or high TG levels (≥ 150 mg/dL), high (≥ 40 mg/dL) or low HDL-C levels (< 40 mg/dL), low (< 5·8%) or high HbA1c levels (≥ 5·8%) and high (≥ 4·0 μg/mL) or low plasma adiponectin levels (< 4·0 μg/mL) [22]. The statistical significance of differences in the prevalence rates of CAD was determined among these four groups by chi-square test. The data were analysed with JMP8 software (SAS Institute, Cary, NC, USA). All statistical significance were accepted at $P < 0·05$.

Results

Comparison of clinical profiles between coronary artery disease patients and non- coronary artery disease subjects

Serum FPG and HbA1c levels were significantly higher, whereas serum levels of HDL-C and adiponectin were significantly lower in patients with CAD (n = 96) than in age-, sex- and BMI-matched non-CAD subjects (n = 67) (Table 1). Fasting apo B-48 and TG levels were significantly higher in CAD patients than in non-CAD subjects ($P < 0·0001$), and the statistical significance of difference was the highest for these parameters (Table 1). Fasting serum apo B-48 levels ranged from 0 to 13 μg/mL in non-CAD subjects, and from 0 to 19 μg/mL in patients with CAD (Fig. 1). The fasting apo B-48 concentration in a large majority of CAD patients and non-CAD subjects was 10 μg/mL or less, but the peak and average of fasting apo B-48 levels were higher in patients with CAD than in non-CAD subjects (Fig. 1 and Table 1). The ROC curve analysis showed that the AUC-ROC value was 0·79, and the cut-off value of apo B-48 was identified as 4·34 (overall sensitivity, 0·82; 1-specificity, 0·33; predictive positive value, 79; predictive negative value, 61).

Correlations between the existence of CAD and metabolic parameters

The correlations between the existence of CAD and metabolic parameters related to coronary risk were analysed by logistic regression analysis in these subjects (Table 2). By Pearson's correlation analysis, significant correlations with the existence of CAD were observed in smoking, HDL-C, TG, FPG, HbA1c, Log-apo B-48 and Log-adiponectin levels. Multiple regression analysis indicated that only log-apo B-48 was a significant determinant of the existence of CAD ($P < 0·0001$) among the various metabolic parameters related to coronary risk (Table 2).

Prevalence of coronary artery disease in subjects with high Apo B-48 levels and other metabolic parameters of abnormal levels

The clustering of metabolic parameters is a high risk state for CAD. We compared the prevalence of CAD in patients with low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL) levels of apo B-48 when their metabolic parameters of MetS (TG, HDL-C, HbA1c or plasma adiponectin levels) were in high risk status (detailed in *Subjects and Methods*). CAD was significantly more prevalent in subjects with high levels of apo B-48 than in subjects with low levels of apo B-48, irrespective of TG, HDL-C, HbA1c or plasma adiponectin levels (in Fig. 2). The prevalence of CAD was significantly higher in subjects with high levels of apo B-48 and high TG, low HDL-C, high HbA1c or low plasma adiponectin levels, compared with that in subjects with low levels of apo B-48 and normal TG, HDL-C, HbA1c or plasma adiponectin levels.

Discussion

This study demonstrated that fasting levels of apo B-48 were higher in patients with CAD than in those with non-CAD, and that high levels of fasting apo B-48 were definitely correlated with the prevalence of CAD among other metabolic biomarkers related to coronary risk. The combination of high fasting apo B-48 levels and other metabolic disorders represented a stronger risk state for CAD.

High fasting serum apo B-48 levels in patients with coronary artery disease compared with non-coronary artery disease subjects

The fasting apo B-48 concentration in a large majority of CAD patients and non-CAD subjects was 10 μg/mL or less, and the peak and average of fasting apo B-48 levels were higher in patients with CAD than in non-CAD subjects (Fig. 1 and Table 1). In CAD patients, MetS components, such as dyslipidaemia, hypertension, high fasting glucose and low adiponectin levels, were more clustered than in non-CAD subjects (Table 1), implying that CAD patients tended to have a pathophysiological background of MetS. The presence of insulin resistance leads to a deterioration of postprandial remnant metabolism [23]. Impaired clearance of lipoproteins is related to the accumulation of CM-R in the postprandial serum and the increase in fasting apo B-48 concentrations [24]. In this study, a high prevalence of CAD was observed in patients with high levels of apo B-48 (Fig. 2). This may indicate that the

Table 1 Clinical Profiles of the Non-CAD subjects and the patients with CAD

	non-CAD (n = 67)	CAD (n = 96)
Age (years)	62·7 ± 10·8	65·1 ± 9·9
Sex[†] (m vs. w)	49 vs. 18	71 vs. 25
Smoking (%)	48·2	60·4
BMI (kg/m^2)	24·1 ± 3·6	24·4 ± 2·8
Prevalence of Dyslipidaemia[‡] (%)	40·2	66·7
TC (mg/dL)	197·6 ± 37·1	199·5 ± 36·5
TG (mg/dL)	121·4 ± 37·1	163·1 ± 83·3**
HDL-C (mg/dL)	49·5 ± 13·3	43·8 ± 13·2*
LDL-C (mg/dL)	125·1 ± 34·3	125·5 ± 34·3
Prevalence of Hypertension[§] (%)	64·1	78·2
Systolic BP (mmHg)	130·0 ± 17·2	130·0 ± 22·9
Diastolic BP (mmHg)	74·6 ± 10·6	75·4 ± 12·2
Prevalence of drug-treated patients (%)	53·1	68·3
Prevalence of High fasting glucose[¶] (%)	19·3	40·0
FPG (mg/dL)	100·5 ± 25·1	116·7 ± 42·4*
HbA1c (%)	5·4 ± 1·0	6·3 ± 1·7*
Fasting apo B-48 μg/mL	3·9 ± 2·4	6·9 ± 2·6**
Adiponectin μg/mL	7·8 ± 4·3	6·4 ± 4·2*
Prevalence of the metabolic syndrome		
In Japanese criteria (%)	17·2	29·2*
In NCEP-ATPIII criteria (%)	22·6	53·1*

BMI, body mass index; BP, blood pressure; FPG, Fasting plasma glucose; TC, total cholesterol; TG, triglyceride; CAD, coronary artery disease.
[†]Number of men vs. women.
[‡]Ratio of subjects with TG ≥ 150 mg/dL and/or HDL-C < 40 mg/dL.
[§]Ratio of subjects with systolic BP ≥ 130 mmHg or diastolic BP ≥ 85 mmHg.
[¶]Ratio of subjects with FPG ≥ 126 mg/dL). The statistical significance of differences in TC, TG, HDL-C, LDL-C, systolic BP, diastolic BP, FPG, HbA1c, fasting apo B-48 and adiponectin were determined by Mann–Whitney's U-test, those in the prevalence of smoking, dyslipidaemia, hypertension, high FPG and the Mets were determined by the chi-square test. Significance was established at $P < 0·05$. *$P < 0·01$, **$P < 0·0001$.

atherogenicity of CM-R might be prolonged throughout the day in CAD patients with a pathophysiological background of MetS. We also found that a small number of CAD patients and non-CAD subjects had high fasting apo B-48 levels (> 10 μg/mL) (Fig. 1). In our former study, we found subjects with high apo B-48 levels (> 10 μg/mL) among patients with any type of hyperlipidaemia [8]. In that study, high fasting apo B-48 levels (> 10 μg/mL) were mainly observed in patients with type I, III, IV and V hyperlipidaemia [8], probably because of the characteristics of a genetic polymorphism (impaired lipoprotein lipase (LPL) activity, the existence of apo E2/E2 phenotype or apo A5) or the existence of PH. Although we did not diagnose the type of hyperlipidaemia in subjects with high fasting apo B-48 levels (> 10 μg/mL), high levels of fasting apo B-48 in CAD patients might be partly a result of impaired lipoprotein metabolism caused by a genetic disorder of apoproteins, enzymes and receptors, or the existence of MetS.

High fasting apo B-48 levels and atherosclerosis

A number of studies have suggested that CM-R had highly atherogenic properties, but there is still no consensus as to whether CM-R accumulation correlates with the development of atherosclerotic cardiovascular diseases. By multiple regression analysis, it was determined that log-apo B-48 was the only significant determinant of the existence of CAD ($P < 0·0001$) among other metabolic parameters related to coronary risk (Table 2).

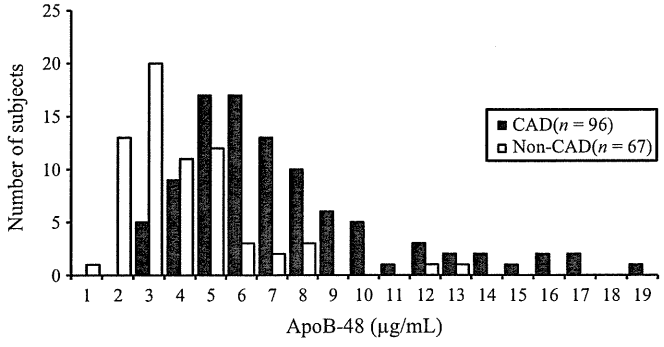

Figure 1 Distribution of Fasting Serum Apo B-48 Levels in non coronary artery disease (Non-CAD) Subjects and Patients with CAD. Fasting serum concentrations of apo B-48 in non-CAD subjects (open squares, n = 96) and patients with CAD (closed squares, n = 67). Serum apo B-48 level=1 represents concentrations between 0·0 and 1·0 μg/mL.

Table 2 Univariate and multivariate analyses of correlations between the existence of coronary artery disease and various metabolic parameters

	Univariate *P* value	Multivariate *P* value
Age	0·1581	–
Sex	0·3698	–
Log-BMI	0·4645	–
Smoking	0·0492	–
TC	0·7440	–
LDL-C	0·8508	–
HDL-C	0·0085	0·3721
Triglyceride	0·0017	0·1098
Systolic BP	0·9747	–
Diastolic BP	0·6757	–
FPG	0·0081	0·6110
HbA1c	0·0008	0·3036
Log-apo B-48	< 0·0001	< 0·0001
Log-APN	0·0239	0·6039

BMI, body mass index; TC, total cholesterol; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, haemoglobin A1c. APN, adiponectin. Univariate analysis was assessed using Pearson's correlation analysis. Multivariate analysis was assessed using stepwise multiple regression analysis.

Moreover, as shown in Fig. 2, the prevalence of CAD was significantly higher in subjects with high levels of apo B-48 than in subjects with low levels of apo B-48, irrespective of other coronary risk factors such as high TG, low HDL-C, high HbA1c or low plasma adiponectin levels. These results clearly show that the frequency of coronary stenosis was significantly and strongly correlated with fasting apo B-48 level, suggesting that the accumulation of CM-R was the strongest risk factor for CAD in these study subjects. Whereas the cut-off value of apo B-48 was 4·34 as determined by ROC curve analysis, the specificity was unfortunately low (1-specificity; 0·33) perhaps because of some degree of coronary stenosis in subjects who were diagnosed as non-CHD subjects. Therefore, high levels of apo B-48 might correlate with patients with more severe CAD than with patients with less severe CAD or non-CAD subjects.

Clustering of high apo B-48 level and other coronary risk factors

The prevalence of CAD gradually increased with higher levels of apo B-48 in association with high TG, low HDL-C, high HbA1c or low plasma adiponectin levels (Fig. 2). Whereas high TG, low HDL-C, high HbA1c and low plasma adiponectin levels are independent coronary risk factors, these metabolic disorders correlated with the accumulation of CM-R. The accumulation of CM-R was associated with insulin resistance and prevalence of type II diabetes mellitus [23]. Plasma adiponectin and leptin concentrations were inversely and directly associated with plasma apo B-48, whereas plasma apo B-48 level was significantly and positively associated with plasma insulin, HOMA and visceral fat areas [25]. The combination of high levels of apo B-48 and other metabolic disorders related to coronary risk may synergistically increase atherogenicity. However, high levels of fasting apo B-48 independently enhanced the prevalence of CAD, irrespective of TG, HDL-C, HbA1c or plasma adiponectin levels. This indicates that a high level of apo B-48, namely the accumulation of CM-R, was the strongest risk status for the prevalence of CAD of all metabolic disorders, as shown in Table 2. These results suggest that without the measurement of fasting apo B-48 level, we may underestimate the CAD risk by the assessment of metabolic disorders using TG, HDL-C, HbA1c or plasma adiponectin levels. For the assessment of CAD risk in subjects with MetS or subjects with little coronary risk, measuring apo B-48 levels remains useful. Subjects with high apo B-48 levels should be assessed carefully by a variety of pharmacological and physiological approaches [26]. Both atorvastatin and fenofibrate have been shown to improve the postprandial increase of CM-R markedly [27,28]. We have also recently reported that ezetimibe, an intestinal cholesterol transporter inhibitor, improves PH in patients with type IIb hyperlipidaemia [29] by reducing the intestinal production of CMs [30]. As fasting and postprandial levels of apo B-48 decrease by these physiological and pharmaceutical interventions [26–29], the

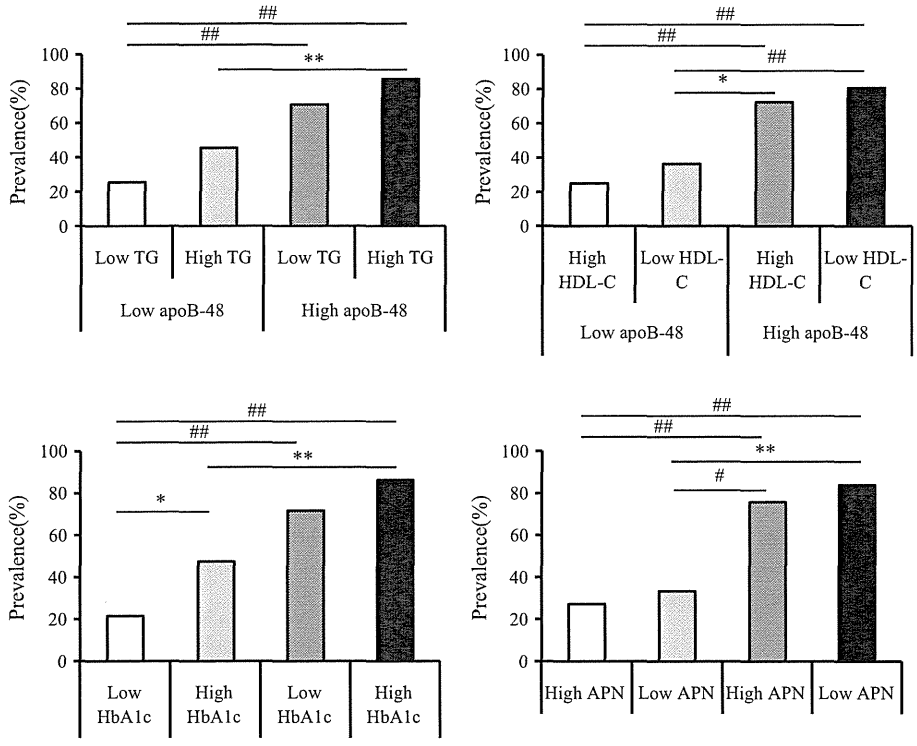

Figure 2 Prevalence Rate of coronary artery disease (CAD) in Subjects with a Combination of High Apo B-48 Levels and Other Coronary Risk Factors. We compared the effect of different metabolic parameters of metabolic syndrome (TG, HDL-C, HbA1c or plasma adiponectin; classified as low and high) on CAD prevalence in patients with low or high apo B-48 levels. We divided all subjects (n = 163) into low (≤ 4·34 μg/mL) and high (> 4·34 μg/mL) apo B-48 levels; these two groups were also divided according to low (< 150 mg/dL) or high (≥ 150 mg/dL) TG levels, high (≥ 40 mg/dL) or low (< 40 mg/dL) HDL-C levels, low (< 5·8%) or high (≥ 5·8%) HbA1c levels or high (≥ 4·0μg/mL) or low (< 4·0 μg/mL) plasma adiponectin levels. The prevalence rates of coronary artery disease were determined in each group, and the statistical significance of differences among these four groups was verified by chi-square test. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{\#}P < 0{\cdot}001$ and $^{\#\#}P < 0{\cdot}0001$

measurement of apo B-48 levels may be useful for managing CAD risk in subjects with MetS or PH.

Limitation of the study

In this study, the subjects were collected from outpatients who came to the cardiovascular department and were susceptible to having CAD. These subjects had already been treated with anti-diabetic drugs or anti-hypertension drugs, and the total number of patients was relatively small compared with that in other related studies.

Conclusion

In conclusion, fasting serum apo B-48 levels are significantly correlated with the existence of CAD and other metabolic disorders. The measurement of fasting apo B-48 is useful for detecting and managing CAD risk in subjects with MetS or low coronary risk.

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Original article

Clinical significance of the measurements of urinary liver-type fatty acid binding protein levels in patients with acute coronary syndrome

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ABSTRACT

Background: Recently, much attention has been focused on cardio-renal interaction. Urinary liver-type fatty acid binding protein (U-L-FABP), which is produced in the proximal tubule by renal hypoxia and oxidative stress, has been identified as a useful marker for diagnosis of acute kidney disease and a predictor of future events in chronic kidney disease. However, the clinical significance of U-L-FABP measurements in patients with acute coronary syndrome (ACS) has not been completely evaluated.

Methods and results: This study included 50 consecutive patients with ACS [37 with acute myocardial infarction (AMI) and 13 with unstable angina pectoris (UAP)] and 47 subjects without coronary artery disease (control group). U-L-FABP levels, urinary albumin (U-Alb), and other serum parameters were measured at admission and at 24 h after percutaneous coronary intervention.

Results: U-L-FABP levels in patients with AMI were significantly higher ($p = 0.0019$), than in control subjects, while patients with UAP did not exhibit such an increase. U-L-FABP levels at admission were positively correlated with brain natriuretic protein levels ($p = 0.001$) and duration of hospitalization ($p = 0.025$). At follow-up angiography, patients with restenosis had significantly higher U-L-FABP ($p = 0.047$) and U-Alb levels ($p < 0.0001$) than those without restenosis. After a median follow-up of 42 months, U-L-FABP levels at second measurement in patients with major adverse cardiocerebrovascular events (MACCEs) were significantly higher than those in patients without MACCEs ($p = 0.028$). After adjusting for confounding factors, high U-L-FABP levels at second measurement were found to be independent factors for MACCEs ($p = 0.019$).

Conclusions: These data suggest that patients with ACS, especially those with AMI, have high U-L-FABP levels, and that U-L-FABP measurements may be useful in identifying high-risk patients for future cardiovascular events after ACS.

Introduction

Observational studies and clinical trials have shown that acute and chronic kidney injuries initiate and accelerate cardiovascular diseases [1–5]. Decreased glomerular filtration rates (GFRs) are closely associated with hospitalization risk and mortality in patients with coronary artery disease (CAD) [3]. If cardiac and kidney dysfunctions co-exist, the risk of cardiovascular events increases by 1.5–3.5 fold [6]. Hence, much attention has been focused on the cardio-renal interaction.

Fatty acid binding proteins (FABPs) are 15 kDa proteins that belong to the lipocalin family. The following 9 different FABPs with tissue-specific distribution have been identified: L (liver), I (intestinal), H (muscle and heart), A (adipocyte), E (epidermal), Il (ileal), B (brain), M (myelin), and T (testis) [7]. In the human kidney, 2 types of FABP have been identified; the liver-type FABP (L-FABP) is produced in the proximal tubule and the heart-type FABP is produced in the distal tubule [8]. Urinary L-FABP (U-L-FABP) binds free FAs (FFAs) produced by proteinuria, oxidative stress, and toxic insults [9]. U-L-FABP could potentially prevent FFA-induced tubulointerstitial damage. Traditional markers of kidney dysfunction, such as albuminuria and creatinine clearance, are based on the distant production of an endogenous marker and its subsequent filtration,

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