J Nutr Sci Vitaminol, **54**, 196–202, 2008

Myo-Inositol Treatment Increases Serum Plasmalogens and Decreases Small Dense LDL, Particularly in Hyperlipidemic Subjects with Metabolic Syndrome

Ryouta MAEBA[1], Hiroshi HARA[2], Hiroshi ISHIKAWA[3], Shigeru HAYASHI[4], Nakayuki YOSHIMURA[5], Jyun KUSANO[5], Yoko TAKEOKA[5], Daijiro YASUDA[5], Tomoki OKAZAKI[1], Makoto KINOSHITA[5] and Tamio TERAMOTO[5]

[1]*Department of Biochemistry, and* [5]*Department of Internal Medicine, Teikyo University School of Medicine, Itabashi-ku, Tokyo 173–8605, Japan*
[2]*Graduate School of Agriculture, Hokkaido University, Sapporo, Japan*
[3]*Hokkaido Sugar Co., Ltd., Ishikari, Japan*
[4]*Hayashi Clinic, 4–28–14 Komone, Itabashi-ku, Tokyo, Japan*

(Received June 20, 2007)

Summary Background and aim: We have previously shown that serum plasmalogen levels positively correlate with HDL, and significantly decrease with aging, and may be related to LDL particle size. The objective of the present study was to investigate the effects of increased serum plasmalogens on lipidosis, particularly the appearance of atherogenic small dense LDL (sdLDL), of subjects with hyperlipidemia and metabolic syndrome (MetS). Methods and results: The effects of increased serum plasmalogen levels, induced by 2 wk of *myo*-inositol treatment, on several clinical and biochemical parameters were examined in 17 hyperlipidemic subjects including some with MetS. After *myo*-inositol treatment, significant increases in plasmalogen-related parameters, particularly ChoPlas, and significant decreases in atherogenic cholesterols including sdLDL, were observed. Among the hyperlipidemic subjects treated with *myo*-inositol, compared to subjects without MetS, subjects with MetS had a significant increase in plasmalogens and a tendency towards reduced sdLDL, high sensitivity C-reactive protein (hsCRP), and blood glucose levels. Correlation analyses between the measured parameters showed that plasmalogens, as well as HDL, function as beneficial factors, and that sdLDL is a very important risk factor that shows positive correlations with many other risk factors. Conclusion: These results suggest that increased plasmalogen biosynthesis and/or serum levels are especially effective in improving MetS among hyperlipidemic subjects with MetS.

Key Words plasmalogens, metabolic syndrome, small dense low-density lipoprotein, *myo*-inositol

Plasmalogens (1-alkenyl-2-acyl-*sn*-glycero-3-phospholipids) in serum are biosynthesized in the liver and secreted into circulating blood as lipoprotein components. Several studies have shown that plasmalogen levels in the plasma or red blood cell membranes decrease with aging or in the presence of hyperlipidemia (*1*); plasmalogen levels are decreased in the low-density lipoprotein (LDL) of familial hypercholesterolemia patients (*2*). However, large scale clinical studies have not yet been done, because the conventional methods used to determine plasmalogens are laborious and unsuitable for highly sensitive quantification.

Recently, we established a highly sensitive and convenient method for quantifying choline plasmalogen (ChoPlas) and ethanolamine plasmalogen (EtnPlas) separately (*3*). Using this new method, we quantified the serum plasmalogen levels of approximately 300 subjects, and analyzed the correlations of serum plasmalogens with clinical markers and serum biochemical markers. The following results were obtained: i) serum plasmalogens decrease markedly with aging; ii) serum plasmalogen levels are positively correlated with high-density lipoprotein (HDL); and iii) the ratio of ChoPlas to EtnPlas is positively correlated with LDL particle size (*4*). These results suggest that serum plasmalogens, as well as HDL, function as beneficial factors, and furthermore that plasmalogen biosynthesis and/or serum levels are related to the appearance of atherogenic small dense LDL (sdLDL).

Recently, we found a significant increase in rat serum plasmalogen levels after *myo*-inositol treatment (unpublished data). *Myo*-inositol is a type of vitamin B that is present in a variety of foods, such as wheat germ, fruits, and vegetables. The mean human dietary intake of *myo*-inositol is estimated to be 500–2,000 mg/d. *Myo*-inositol is also synthesized in vivo from glucose 6-phosphate. It is well known that *myo*-inositol exerts various physiological effects, such as preventing fatty liver, improving hypercholesterolemia, and facilitating calcium absorption (*5*). In the current study, *myo*-inositol was used to

E-mail: maeba@med.teikyo-u.ac.jp

investigate the effects of increased serum plasmalogens. The lipidosis of metabolic syndrome (MetS) is characterized by high TG and low HDL levels, and the consequent appearance of sdLDL. Therefore, the investigations of the effects of increased serum plasmalogens, induced by *myo*-inositol treatment, on lipidosis of hyperlipidemia and MetS may provide the clinical evidence that serum plasmalogens are involved in the appearance of sdLDL.

METHODS AND MATERIALS

Study subjects and design. Seventeen subjects, who were referred for a medical examination to the Hayashi Clinic and who were diagnosed with lifestyle-related diseases such as obesity and hyperlipidemia, but without taking medications, were recruited; written informed consent was obtained from all subjects prior to enrollment. The study was approved by the ethics committee of the Hayashi Clinic. All subjects took *myo*-inositol (Hokkaido Sugar Co., Ltd, Ishikari, Japan) 5 g daily for 1 wk, followed by *myo*-inositol 10 g daily for 1 wk. The subjects' diets were not restricted, though special nutrient supplements were prohibited during the treatment period. Blood was collected, and measurements of weight, stature, and waist size were recorded before and after the first week and second weeks of *myo*-inositol treatment. Questionnaires to obtain information about their medical history, medication status, and current cigarette smoking status were administered when the subjects visited the Hayashi Clinic. After at least a 12-h fast, venous blood samples for the measurement of biochemical parameters were collected. Serum was separated by centrifugation. The lipids and lipoprotein levels were determined immediately, and part of the serum was stored at $-80^{\circ}C$ until further analysis.

Measurements of plasmalogens and phospholipids. Serum plasmalogen levels were measured with high-performance liquid chromatography (HPLC) using radioactive iodine ($^{125}I_3^-$), as previously described (*3*). In brief, serum lipids were extracted according to the Bligh and Dyer method (*6*) and redissolved in methanol. The methanol-soluble lipids were reacted with a radioactive iodine reagent containing $^{125}I_3^-$, which specifically and quantitatively binds to the vinyl ether bonds of plasmalogens. Aliquots of the reaction mixture were applied to the HPLC apparatus equipped with a Diol column (Merck, Darmstadt, Germany), and eluted with acetonitrile/water. The elution was fractionated. To measure the amount of ChoPlas and EtnPlas, the radioactivity of each fraction was counted with an auto-well γ-counter. The phospholipid level was determined by measuring the phosphorus content according to the procedure described by Chalvardjian and Rudnicki (*7*).

Measurements of other biochemical parameters. Serum triglyceride (TG), total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), and small dense low-density lipoprotein-cholesterol (sdLDL-C) levels were measured enzymatically using the appropriate commercial kits (Denka Seiken, Tokyo, Japan). Small dense LDL-C was measured by a direct homogenous LDL-C assay in the supernatant that remained after heparin-magnesium precipitation with density <1.044 lipoproteins (*8*). Apolipoproteins (apo A-I, B, and E) and high sensitivity C-reactive protein (hsCRP) were measured using turbidimetric immunoassays. Blood glucose was determined with a commercial enzyme kit (Wako Pure Chemical Industries, Ltd., Tokyo, Japan).

Statistical analyses. The statistical analyses were performed using the SPSS 10.0J software (SPSS Japan Inc., Tokyo, Japan). All values are expressed as the mean$\pm$SD. Differences between the means of before and after *myo*-inositol treatment were compared with the paired *t*-test. Differences between the means of MetS and non-MetS groups of before/after treatment were compared with the unpaired *t*-test. Correlations among the measured parameters were assessed with Pearson's product-moment correlation coefficient and *p*-value.

RESULTS

The clinical background and serum biochemical parameters of the study subjects ($n=17$) before *myo*-inositol treatment are shown in Tables 1 and 2. Sixteen of the subjects were hyperlipidemic; the rates of hypertriglyceridemia, hypercholesterolemia, and hyper LDL cholesterolemia were 65, 76, and 76% respectively. Half of the subjects were diagnosed with MetS according to Japanese guidelines. A waist size greater than 85 cm is one of the criteria for MetS in Japanese men; 13 of the 15 males had a waist size greater than 85 cm. All the two females had a waist size less than 90 cm, which is one of the criteria for MetS in Japanese women. Therefore, a sex difference was not considered to distinguish MetS from non-MetS group in the present study. The mean small dense LDL (sdLDL-C) level was 46.4 mg/dL

Table 1. Clinical background of study subjects ($n=17$).

Sex, Male/Female	15/2
Age, years	43.6$\pm$6.6
Rate of obesity (BMI$\geqq$25)	11/17 (65%)
Rate of hyperlipidemia (TG$\geqq$150 and/or TC$\geqq$220 mg/dL)	16/17 (94%)
Rate of diabetes mellitus	3/17 (18%)
Rate of hypertension (systolic$\geqq$140 and/or diastolic pressure$\geqq$ 90 mmHg)	5/17 (29%)
Rate of metabolic syndrome (according to Japanese guideline*)	8/17 (47%)
Rate of current cigarette smoking	12/17 (71%)

BMI, body mass index; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.
*Japanese criteria for metabolic syndrome (MetS).
1) Waist size: Men$\geqq$85 cm, Women$\geqq$90 cm.
2) TG$\geqq$150 mg/dL and/or HDL-C $<$40 mg/dL.
3) Systolic pressure$\geqq$130 mmHg and/or Diastolic pressure$\geqq$85 mmHg.
4) Fasting glucose$\geqq$110 mg/dL.
The diagnosis of MetS is made when 1)+more than two of the risk determinants 2) – 4) mentioned above.

Table 2. Effect of *myo*-inositol treatment on clinical and serum biochemical parameters of the hyperlipidemic subjects (n=17).

	Before	After	*t*-test, *p*
BMI, kg/m^2	27.3±2.5	27.3±2.8 (100.0)	0.560
Waist size, cm	91.9±7.3	90.9±8.5 (98.9)	0.220
Systolic pressure, mmHg	134±9	132±10 (98.5)	0.660
Diastolic pressure, mmHg	81±6	87±7 (107.4)	0.008**
Blood glucose, mg/dL	99.9±14.0	92.9±11.7 (93.0)	0.117
hsCRP, mg/dL	0.18±0.17	0.32±0.38 (177.8)	0.487
apo A-I, mg/dL	135.2±17.4	134.9±15.1 (99.8)	0.945
apo B, mg/dL	132.7±30.4	124.9±33.0 (94.1)	0.038*
apo E, mg/dL	5.1±1.3	4.9±1.5 (96.1)	0.352
TG, mg/dL	188.6±71.1	184.4±94.4 (97.8)	0.766
TC, mg/dL	258.9±45.2	247.8±53.3 (95.7)	0.090
HDL-C, mg/dL	51.9±8.8	52.4±8.7 (101.0)	0.749
LDL-C, mg/dL	179.1±46.3	165.8±51.0 (92.6)	0.032*
sdLDL-C, mg/dL	46.4±18.5	35.6±17.4 (76.7)	0.010*
PL, mM	2.84±0.38	2.80±0.47 (98.6)	0.762
ChoPlas, μM	46.1±9.4	54.6±9.6 (118.4)	0.011*
EtnPlas, μM	51.1±14.1	57.1±10.9 (111.7)	0.122
ChoPlas+EtnPlas, μM	97.1±22.5	111.7±20.4 (115.0)	0.036*
ChoPlas/PL ($\times 10^3$)	16.2±2.5	19.8±2.9 (122.2)	0.0003***
EtnPlas/PL ($\times 10^3$)	18.3±4.9	20.9±4.4 (114.2)	0.045*
(ChoPlas+EtnPlas)/PL ($\times 10^3$)	34.7±7.4	40.4±7.3 (116.4)	0.008**
ChoPlas/EtnPlas ratio	0.94±0.17	0.98±0.12 (104.3)	0.455

Values are means±SDs, and values in parentheses are relative percentage to each value before treatment.
*p<0.05, **p<0.01, ***p<0.001.
BMI, body mass index; hsCRP, high sensitive C-reactive protein; apo A-I, B, E, apolipoprotein A-I, B, E; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; sdLDL-C, small dense low-density lipoprotein cholesterol; PL, phospholipid; ChoPlas, choline plasmalogen; EtnPlas, ethanolamine plasmalogen; Plas/PL, ratio of plasmalogen to phospholipid.

(range, 15.0–83.9 mg/dL). The mean value was greater than that of normal subjects (31±12 mg/dL) and was nearly equal to that seen in hyper LDL cholesterolemia (53±17 mg/dL) and hypertriglyceridemia (52±19 mg/dL), as reported previously by Ito et al. (*8*). Half of the subjects had an hsCRP level greater than the normal hsCRP level (0.1 mg/dL) proposed by the Centers for Disease Control and Prevention, which is an agency of the United States Department of Health and Human Services. The mean serum phospholipid level was normal, whereas the mean plasmalogen (ChoPlas+EtnPlas) level was less than the normal range (100–300 μM). The mean ratio of choline plasmalogen to ethanolamine plasmalogen (ChoPlas/EtnPlas ratio) was nearly equal to that of elderly subjects (0.83±0.17) that we previously reported (*4*).

Changes in clinical and serum biochemical parameters before and after 2 wk of *myo*-inositol treatment are shown in Table 2. *Myo*-inositol treatment significantly increased plasmalogen-related parameters and diastolic pressure; it furthermore significantly decreased LDL-related parameters, especially the mean sdLDL level. Blood glucose tended to decline after treatment. The largest changes included 11–22% increases in plasmalogen-related parameters and a 23% reduction in sdLDL levels. The mean hsCRP level after 2 wk was substantially elevated, probably due to the onset of an inflammation in one subject. *Myo*-inositol ingestion had no effect on HDL-related parameters, such as HDL-C and apo A-I, nor on TG level.

Since the presence of sdLDL is one of characteristics of lipidosis of MetS (*9*), we investigated whether *myo*-inositol treatment improves the status of MetS. The hyperlipidemic subjects treated with *myo*-inositol were divided into two groups: MetS and non-MetS. The MetS group was characterized by significantly higher BMI, waist size, and apo E and lower HDL-related parameters. Furthermore, MetS group showed high levels of hsCRP, TG, and sdLDL and low levels of plasmalogen-related parameters (Table 3). Comparison of the efficacy of *myo*-inositol treatment on the MetS and non-MetS groups of hyperlipidemic subjects revealed that the plasmalogen-related parameters significantly increased and blood glucose decreased only in the MetS group. The levels of hsCRP and sdLDL also tended to decrease, particularly in the MetS group. However, *myo*-inositol treatment had no effect on HDL or TG levels (Table 3).

Correlation analyses of the parameters before and after *myo*-inositol treatment were done. The parameters with statistically significant correlations with notable items are shown in Tables 4–1 and 4–2. Prior to *myo*-inositol treatment, HDL-C was positively correlated only with plasmalogen-related parameters, and was negatively correlated with many risk factors, such as TG, apo

Table 3. Effect of *myo*-inositol treatment on clinical and serum biochemical parameters of the hyperlipidemic subjects with (MetS) and without metabolic syndrome (non-MetS).

	MetS (n=8)		non-MetS (n=9)	
	Before	After	Before	After
BMI, kg/m^2	29.3±3.0§	29.7±3.7 (101.4) §	25.3±2.3	25.2±2.6 (99.6)
Waist size, cm	99.6±6.3§§§	99.9±9.1 (100.3) §§§	85.0±4.6	82.9±4.0 (97.5)
Systolic pressure, mmHg	136±13	140±26 (102.9)	132±11	125±9 (94.7)
Diastolic pressure, mmHg	85±12	93±16 (109.4)	78±7	81±5 (103.8)
Blood glucose, mg/dL	104.1±16.6	87.5±11.6 (84.1)*	96.2±18.6	97.7±17.4 (101.6)
hsCRP, mg/dL	0.316±0.315	0.171±0.137 (54.1)	0.056±0.029	0.453±0.995 (808.9)
apo A-I, mg/dL	121.3±21.6§§	122.5±15.2 (101.0) §§	147.6±14.7	146.0±16.4 (98.9)
apo B, mg/dL	142.6±43.4	130.6±40.4 (91.6)	123.9±41.2	119.8±48.9 (96.7)
apo E, mg/dL	6.0±1.2§	5.8±1.3 (96.7)	4.3±1.5	4.2±2.0 (97.7)
TG, mg/dL	230.1±89.9	239.5±142.4 (104.1)	151.8±87.4	135.3±100.8 (89.1)
TC, mg/dL	261.9±69.7	246.1±62.6 (94.0)	256.3±66.1	249.3±85.2 (97.3)
HDL-C, mg/dL	44.9±11.1§§	45.5±11.3 (100.9) §§	58.2±7.1	58.6±6.7 (100.7)
LDL-C, mg/dL	185.5±71.4	166.1±68.0 (89.5)	174.4±58.0	164.4±70.8 (94.3)
sdLDL-C, mg/dL	56.4±25.0	39.2±19.7 (69.5)	37.5±21.4	32.5±23.3 (86.7)
PL, mM	2.8±0.5	2.8±0.5 (100.0)	2.9±0.5	2.8±0.8 (96.6)
ChoPlas, μM	40.9±9.0	51.5±3.6 (125.9)*	50.7±12.5	57.3±18.5 (113.0)
EtnPlas, μM	44.9±13.1	53.9±6.7 (120.0)	56.6±20.6	60.0±20.3 (106.0)
ChoPlas+EtnPlas, μM	85.8±20.3	105.4±8.6 (122.8)*	107.2±30.3	117.3±37.4 (109.4)
ChoPlas/PL ($\times 10^3$)	14.5±1.7	18.9±2.3 (130.3)***	17.7±4.1	20.5±5.0 (115.8)
EtnPlas/PL ($\times 10^3$)	16.0±3.8	19.9±3.9 (124.4)	20.4±9.4	21.7±7.5 (106.4)
(ChoPlas+EtnPlas)/PL ($\times 10^3$)	30.5±4.9	38.8±5.8 (127.2)**	38.1±13.1	42.3±12.1 (111.0)
ChoPlas/EtnPlas ratio	0.94±0.23	0.97±0.12 (103.2)	0.94±0.21	0.98±0.17 (104.3)

Values are means±SDs, and values in parentheses are relative percentage to each value before treatment.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared with before treatment.
§ $p<0.05$, §§ $p<0.01$, §§§ $p<0.001$ compared with non-MetS.

Table 4–1. The parameters with statistically significant correlations with HDL-C or plasmalogen-related parameters before and after *myo*-inositol treatment.

HDL-C	Before	After
apo A-I	0.951***	0.922***
TG	−0.747***	−0.694***
apo E	−0.655**	−0.411
hsCRP	−0.563*	−0.086
EtnPlas	0.548*	0.381
ChoPlas+EtnPlas	0.540*	0.373
(ChoPlas+EtnPlas)/PL	0.504*	0.291
ChoPlas/PL	0.502*	0.274
Waist size	−0.498*	−0.497*
ChoPlas	0.490*	0.330
EtnPlas/PL	0.479*	0.284
sdLDL	−0.452*	−0.243
BMI	−0.444*	−0.409

ChoPlas+EtnPlas	Before	After
HDL-C	0.540*	0.373
apo A-I	0.532*	0.412
Waist size	−0.416*	−0.270
hsCRP	−0.393	−0.652***
PL	0.323	0.589**
TC	0.213	0.529**
LDL-C	0.177	0.456*
apo B	0.083	0.450*

(ChoPlas+EtnPlas)/PL	Before	After
sdLDL	−0.520*	−0.330
HDL-C	0.504*	0.291
apo A-I	0.487*	0.351
Waist size	−0.463*	−0.374
apo E	−0.458*	−0.228

Values are correlative coefficients. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

E, hsCRP, waist size, sdLDL, and BMI. Plasmalogen-related parameters were also positively correlated only with HDL-related parameters, and were negatively correlated with risk factors, such as waist size, sdLDL, and apo E (Table 4–1). On the other hand, sdLDL was positively correlated with a variety of risk factors, in addition to LDL-related parameters; sdLDL was negatively correlated with HDL- and plasmalogen-related parameters. C-reactive protein (hsCRP) was positively correlated with waist size and negatively with HDL-C. Blood glucose was negatively correlated with PL, ChoPlas, and ChoPlas/EtnPlas ratio (Table 4–2). After *myo*-inosi-

Table 4–2. The parameters with statistically significant correlations with each sdLDL-C, LDL-C, blood glucose, and hsCRP before and after *myo*-inositol treatment.

sdLDL-C	Before	After
apo B	0.900***	0.806***
apo E	0.804***	0.817***
TC	0.804***	0.758***
LDL-C	0.773***	0.688**
PL	0.683***	0.710**
Diastolic pressure	0.673**	0.414*
BMI	0.603*	0.224
TG	0.583**	0.639**
apo A-I	−0.580*	−0.304
EtnPlas/PL	−0.528*	−0.344
(ChoPlas+EtnPlas)/PL	−0.520*	−0.330
HDL-C	−0.452*	−0.243
Systolic pressure	0.446*	0.227
ChoPlas/PL	−0.444*	−0.280
Waist size	0.440*	0.128

Blood glucose	Before	After
PL	−0.576*	−0.110
ChoPlas	−0.456*	−0.173
ChoPlas/EtnPlas ratio	−0.434*	−0.550*

LDL-C	Before	After
TC	0.985***	0.984***
apo B	0.961***	0.971***
PL	0.744***	0.677***
sdLDL	0.773***	0.688**
Diastolic pressure	0.719**	0.447*
Systolic pressure	0.542*	0.447*
apo E	0.486*	0.600*
BMI	0.414*	0.377
ChoPlas	0.368	0.572**
ChoPlas+EtnPlas	0.177	0.456*

hsCRP	Before	After
HDL-C	−0.563*	−0.086
Waist size	0.403*	−0.030
ChoPlas+EtnPlas	−0.383	−0.652***
EtnPlas	−0.339	−0.653**
ChoPlas	−0.266	−0.593**

Values are correlative coefficients. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

tol treatment, the correlation of plasmalogens with LDL-related parameters and those with hsCRP became stronger.

DISCUSSION

This clinical study was conducted to investigate the effects of increased serum plasmalogens, induced by *myo*-inositol ingestion, on several clinical and biochemical parameters in hyperlipidemic subjects including those with metabolic syndrome (MetS). Since this study was not a placebo-controlled design, the effect was assessed using the paired *t*-test to compare values before and after treatment.

This study is the first to report that *myo*-inositol ingestion significantly increases human serum plasmalogen levels, which was previously documented in rat liver and plasma (unpublished data). The increased ratio of plasmalogens to phospholipids (Plas/PL) after *myo*-inositol treatment was greater than that of the actual plasmalogen level (Table 2), which means a specific enhancement of plasmalogen biosynthesis in glycerophospholipids. *Myo*-inositol is reported to increase the brain plasmalogen content in rats (*10*), and this effect may contribute to improve affective disorders, such as major depression and panic disorder (*11*). Although the mechanism by which *myo*-inositol enhances plasmalogen biosynthesis remains unknown, *myo*-inositol intake possibly facilitates plasmalogen biosynthesis in the liver, and this may lead to the secretion of plasmalogen-rich lipoproteins.

Plasmalogen biosyntheses are strictly regulated and the plasmalogen levels in cells or tissues are physiologically kept constant (*12–15*). Our result appears to support these facts. The increase in the plasmalogen levels after *myo*-inositol treatment tended to be inversely proportional to the serum plasmalogen levels before treatment (Fig. 1). Serum plasmalogens reached a plateau level of around 150 μM with *myo*-inositol treatment, which corresponds to the average level observed in elderly subjects reported by a previous study (*4*).

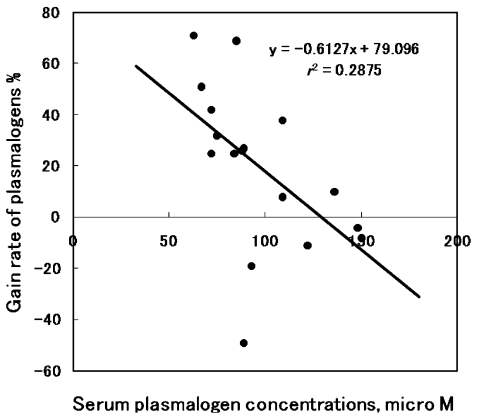

Fig. 1. Relationship between the gain rate of plasmalogens after *myo*-inositol treatment and serum plasmalogen levels before treatment.

Myo-inositol significantly reduced serum atherogenic cholesterol levels, including LDL-C and sdLDL-C levels (Table 2). Although dietary *myo*-inositol, as well as phytate (*myo*-inositol hexaphosphate, IP6), is reported to have a cholesterol-lowering effect (*16*, *17*), this is the first paper to document a reduction in sdLDL with *myo*-inositol treatment. Our previous study suggests that serum plasmalogen may be involved in the appearance

of sdLDL; the lower the ratio of choline plasmalogen to ethanolamine plasmalogen (ChoPlas/EtnPlas ratio), the greater the sdLDL level (*4*). *Myo*-inositol particularly increased the ChoPlas level (Table 2), which resulted in an increase in the ChoPlas/EtnPlas ratio. This may be related to the reduction in the sdLDL level, although, in the present study, sdLDL was determined using a precipitation method (*18*), rather than by measuring particle size as in a previous study (*19*). The ChoPlas/EtnPlas ratio reflects the transfer rate from EtnPlas to ChoPlas, because EtnPlas is a precursor of ChoPlas biosynthesis (*20*). Therefore, the declining rate of transfer from EtnPlas to ChoPlas was inferred to be potentially related to the appearance of atherogenic sdLDL. However, the mechanism by which plasmalogens are involved in the modification of lipoproteins metabolism is unknown.

A significant positive correlation between serum plasmalogens and HDL (Table 4–1) was reported by Engelmann et al. (*21*), and this finding has been supported by our previous study (*4*). We speculate that plasmalogen biosynthesis may be closely related to the production of apo A-I through peroxisomal function. However, *myo*-inositol treatment did not change the HDL-C or apo A-I levels, despite substantial increase in serum plasmalogen levels. This result suggests that the acceleration of plasmalogen biosynthesis caused by water-soluble *myo*-inositol is mediated via an alternate pathway, which is different from the activation of peroxisomal function. Therefore, *myo*-inositol treatment could be used to achieve the beneficial effect of plasmalogens alone without HDL.

Based on the correlation analyses, sdLDL was confirmed to be a very important risk factor (Table 4–2). Because the presence of sdLDL is one of characteristics of lipidosis for MetS (*9*), we investigated whether *myo*-inositol treatment improves the status of MetS. Notable reduction of sdLDL, blood glucose, and hsCRP levels were observed particularly in the MetS group of the hyperlipidemic subjects treated with *myo*-inositol (Table 3). The blood glucose level had a significant negative correlation with the ChoPlas level (Table 4–2). Moreover, our previous study has shown that plasmalogens have a significant negative correlation with HbA_{1c} (*4*), an increased level of which indicates diabetes mellitus. Therefore, the facilitation of plasmalogen biosynthesis may contribute to improving glucose metabolism. The antioxidative effect of plasmalogens may be supported by the reduced level of hsCRP, an indicator of chronic vascular inflammation, which reflects the oxidative damage that occurs due to atherosclerosis (*22*). The marked reduction in the hsCRP level with *myo*-inositol treatment in MetS group (Table 3) suggests the physiological importance of serum plasmalogens in protecting blood vessels from oxidative stress. Interestingly, however, *myo*-inositol treatment had no effect on low HDL and high TG status, which is a typical feature of lipidosis for MetS. This finding suggests that the status of low HDL and high TG may not directly cause the production of sdLDL, but only that of accompanying phenomena.

In conclusion, the present study suggests that the increases in plasmalogen biosynthesis and/or in serum levels improve the status of MetS among hyperlipidemic subjects with MetS by the reduction of sdLDL, hsCRP, and blood glucose levels, and that serum plasmalogens are closely related to the appearance of sdLDL.

Acknowledgments

We especially thank the subjects who participated in this study. We thank Mr. Yamazaki of the Food Department Laboratory, ADEKA Co. (7–2–34 Higashiogu, Arakawa-ku, Tokyo, Japan), for plasmalogen and phospholipid measurements. Part of this work was enabled by a Grant-in-Aid for the Technology Development Project for the Creation of Collective Private Agribusiness (H15–7) from the Ministry of Agriculture, Forestry and Fisheries, Japan.

REFERENCES

1) Brosche T. 2001. Plasmalogen levels in serum from patients with impaired carbohydrate or lipid metabolism and in elderly subjects with normal metabolic values. *Arch Gerontol Geriatr* **32**: 283–294.

2) Brautigam C, Engelmann B, Reiss D, Reinhardt U, Thiery J, Richter WO, Brosche T. 1996. Plasmalogen phospholipids in plasma lipoproteins of normolipidemic donors and patients with hypercholesterolemia treated by LDL apheresis. *Atherosclerosis* **119**: 77–88.

3) Maeba R, Ueta N. 2004. Determination of choline and ethanolamine plasmalogens in human plasma by HPLC using radioactive triiodide (1-) ion ($^{125}I_3^-$). *Anal Biochem* **331**: 169–176.

4) Maeba R, Maeda T, Kinoshita M, Takao K, Takenaka H, Kusana J, Yoshimura N, Takeoka Y, Yasuda D, Okazaki T, Teramoto T. 2007. Plasmalogens in human serum positively correlate with high-density lipoprotein and decrease with aging. *J Atheroscler Thromb* **14**: 12–18.

5) Pallauf J, Rimbach G. 1997. Nutritional significance of phytic acid and phytase. *Arch Tierernahr* **50**: 301–319.

6) Bligh EG, Dyer WJ. 1959. A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**: 911–917.

7) Chalvardjian A, Rudnicki E. 1970. Determination of lipid phosphorus in the nanomolar range. *Anal Biochem* **36**: 225–226.

8) Ito Y, Hirano T, Yamazaki J, Yoshino G. 2004. New simple method for small, dense LDL: development of a new quantification method and clinical significance. *Rinsho Byori* **52**: 406–413 (in Japanese).

9) Nozue T, Michishita I, Ishibashi Y, Ito S, Iwaki T, Mizuguchi I, Miura M, Ito Y, Hirano T. 2007. Small dense low-density lipoprotein cholesterol is a useful marker of metabolic syndrome in patients with coronary artery disease. *J Atheroscler Thromb* **14**: 202–207.

10) Hoffman-Kuczynski B, Reo NV. 2004. Studies of *myo*-inositol and plasmalogen metabolism in rat brain. *Neurochem Res* **29**: 843–855.

11) Levine J, Barak Y, Gonzalves M, Szor H, Elizur A, Kofman O, Belmaker RH. 1995. Double-blind, controlled trial of inositol treatment of depression. *Am J Psychiatry* **152**: 792–794.

12) Das AK, Holmes RD, Wilson GN, Hajra AK. 1992. Dietary ether lipid incorporation into tissue plasmalogens of humans and rodents. *Lipids* **27**: 401–405.

13) Das AK, Hajra AK. 1988. High incorporation of dietary

1-*O*-heptadecyl glycerol into tissue plasmalogens of young rats. *FEBS Lett* **227**: 187–190.

14) Cabot MC, Snyder F. 1980. Manipulation of alkylglycerolipid levels in cultured cells. Fatty alcohol versus alkylglycerol supplements. *Biochim Biophys Acta* **617**: 410–418.

15) Liu D, Nagan N, Just WW, Rodemer C, Thai TP, Zoeller RA. 2005. Roles of dihydroxyacetonephosphate acyltransferase in the biosynthesis of plasmalogens and nonether glycerolipids. *J Lipid Res* **46**: 727–735.

16) Okazaki Y, Katayama T. 2003. Effects of dietary carbohydrate and *myo*-inositol on metabolic changes in rats fed 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT). *J Nutr Biochem* **14**: 81–89.

17) Onomi S, Okazaki Y, Katayama T. 2004. Effect of dietary level of phytic acid on hepatic and serum lipid status in rats fed a high-sucrose diet. *Biosci Biotechnol Biochem* **68**: 1379–1381.

18) Hirano T, Ito Y, Koba S, Toyoda M, Ikejiri A, Saegusa H, Yamazaki J, Yoshino G. 2004. Clinical significance of small dense low-density lipoprotein cholesterol levels determined by the simple precipitation method. *Arterioscler Thromb Vasc Biol* **24**: 558–563.

19) Nichols AV, Krauss RM, Musliner TA. 1986. Nondenaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol* **128**: 417–431.

20) Nagan N, Zoeller RA. 2001. Plasmalogens: biosynthesis and functions. *Prog Lipid Res* **40**: 199–229.

21) Engelmann B, Streich S, Schonthier UM, Richter WO, Duhm J. 1992. Changes of membrane phospholipid composition of human erythrocytes in hyperlipidemias. I. Increased phosphatidylcholine and reduced sphingomyelin in patients with elevated levels of triacylglycerol-rich lipoproteins. *Biochim Biophys Acta* **1165**: 32–37.

22) Ridker PM, Glynn RJ, Hennekens CH. 1998. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation* **97**: 2007–2011.