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Decrease in serum levels of advanced glycation end-products by short-term lifestyle modification in non-diabetic middle-aged females

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Advanced glycation end products (AGEs) play an important role in development of atherosclerosis in diabetes and uremic diseases. However, there is currently little information available on the effects of lifestyle modification on circulating AGEs in subjects without these diseases.

Material/Methods: Serum levels of N^{ε}-(carboxymethyl) lysine (CML), pentosidine, derivatives of reactive oxygen metabolites (d-ROMs), adipokines, and renal function were determined in forty-seven middle-aged females. Among them, seventeen participated in a 12-week lifestyle modification (LM) program and twelve age-matched subjects were assigned to a control group. The LM program consisted of an initial educational session and encouragement.

Results: At baseline, serum HDL-cholesterol was correlated with CML level (P=0.003), whereas body fat mass (P=0.012) and fat consumption (P=0.007) were correlated with pentosidine levels. After intervention, significant reductions were observed in the LM group in body fat, serum HDL-cholesterol, CML, and pentosidine. Of note, in the LM group, average number of steps in daily walking was significantly correlated with decrease in CML level (P=0.044). Decrease in pentosidine level exhibited significant positive correlations with reduction in body weight (P=0.007) and body fat mass (P=0.038).

Conclusions: The present findings suggest lifestyle modification as a promising approach to reducing circulating AGE levels even in healthy middle-aged females with neither overt diabetes nor renal dysfunction.

key words: advanced glycation end-products • physical activity and diet • healthy middle-aged females

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BACKGROUND

Abundant interest in the pathophysiological roles of the advanced glycation end products (AGEs) family in development of atherosclerotic vascular changes has led to a growing literature on the subject, mainly in diabetes [1–3] and advanced renal diseases [4,5]. These products are a heterogeneous class of compounds that form with increasing age and under diverse circumstances. Nε-(carboxymethyl) lysine (CML) adducts of proteins, the most prevalent AGEs detected *in vivo* [6,7], and pentosidine is a fluorescent crosslink and one of the major AGE markers in proteins [8,9], both of which may form in milieus characterized by hyperglycemia and oxidative stress [10]. While these products have been associated with various diseases, there is currently limited information available as to how much AGE exist and whether these products are associated with anthropometric and metabolic parameters in subjects without overt diabetic and renal diseases [11-13].

While these products are inevitably formed *in vivo,* they are also formed externally and partially absorbed into the circulation [14]. In fact, dietary AGE restriction resulted in decreases in circulating AGE levels and disease progression in animal models of atherosclerosis [15] and diabetes [16] as well as in patients with diabetes [17]. In general, it is believed that lifestyle modification based on increased physical activity and a healthy diet is of great importance in preventing future development of atherosclerotic disease not only in patients with diseases of high risk for atherosclerosis but in subjects without overt diseases [18]. However, information on the effects of such lifestyle modification on circulating AGE components in low-risk subjects is currently lacking.

In the present study, we aimed to examine the association of AGEs with metabolic abnormalities and oxidative stress parameters and to examine prospectively whether serum AGE levels can be reduced by short-term intervention with lifestyle modification in healthy middle-aged females with neither overt diabetes nor renal disease.

MATERIAL AND METHODS

Subjects

Forty-seven middle-aged females (35–70 yrs) were recruited using local newspaper advertisements for health promotion in Osaka city. On the first visit, all subjects participated in initial screening tests including anthropometric measurements and blood tests. After explanation of the entire program, 17 of 47 subjects participated in the 3-month intervention study and were eligible for analysis [lifestyle modification (LM) group], while twelve age-matched subjects agreed to be assigned to a control group during the study period. All of the 47 subjects were healthy, non-smoking, and free of overt metabolic, cardiovascular, renal, or inflammatory disease as assessed by medical history and a comprehensive medical examination at initial screening. All subjects were sedentary (less than 30 minutes, 2 days/week, of structured physical activity) before the beginning of the present study. None of the subjects were currently using prescribed medications or nutritional supplements. Each subject provided informed written consent in adherence with the Ethics Committee of Osaka City University before participation.

Exercise training

On the second visit, LM group participants (n=17) received an educational session for reinforcement of daily physical activities. In the educational session, the participants performed a single walking exercise targeting at 60% of the maximal heart rate of each participant, and were taught how to measure their heart rate during walking by checking the radial arterial pulse. The participants in this group were encouraged to walk daily at the same pace as described above, but we did not set the target time and distance of daily walking for individual participant. After the initial session, participants in the LM group underwent a series of 90-min exercise sessions once a week for 12 weeks. The exercise training program consisted of a 15-min warm-up (stretching and walking), 60-min main exercise period (aerobics, rhythmic exercises, and exercises with dumbbells or balls), and a 15-min cool-down. The program included assessment of daily physical activities by reading of a pedometer worn by each LM participant during the entire day, which provided an indirect estimate of overall daily activity. All training sessions were supervised by a member of the research team and a certified strength and conditioning specialist. Twelve age-matched controls subjects did not participate in the exercise training program. The control subjects were also asked to keep their daily activity unchanged and to refrain from any regular exercise.

Dietary assessment

Subjects in both groups were asked to record dietary intake during the preceding 3 days at the beginning and end of the study period using a simple questionnaire to assess changes in dietary intake before and after the study period. The dietary records covered both quantitative and qualitative aspects of food consumed daily. Based on the records, daily total intakes of energy, protein, fat, and carbohydrate were calculated according to the fifth revised edition of the Standard Tables of Food Composition in Japan [19]. No intensive dietary restriction, education, or counseling was performed during the study period.

Anthropometric measurements

At the initial screening, body weight and height were obtained for all 47 subjects. Body composition, including percentages of body fat, fat mass, and body muscle mass, was also measured by bioelectrical impedance analysis (In body, Biospace, Tokyo).

Laboratory procedures

Blood samples were taken from all recruited subjects (n=47) at initial screening and from the LM group participants (n=17) and control subjects (n=12) at the end of the 3-month study. Blood were drawn from an antecubital vein in the morning between 9:00 and 10:00 after an overnight fast, and subsequently centrifuged at 4°C and immediately frozen at –80°C for biochemical analysis performed later.

Assays for serum triglyceride (TG), total cholesterol (t-cho), high-density lipoprotein cholesterol (HDL-cholesterol), and plasma glucose levels were performed in the hospital's chemistry laboratory with a SP-4420 (ARKRAY, Kyoto, Japan).

Table 1. Clinical and laboratory characteristics and simple regression analysis with serum AGE levels

Middle-aged females	Values	CML		Pentosidine	
		Single regression		Single regression	
		r	P	r	P
n	47				
Age (years)	56±8	0.230	NS	–0.079	NS
Height (cm)	157±6.7	–			
Weight (kg)	61.0±10.7	0.114	NS	–0.323	0.027
BMI (kg/m^2)	24.7±4.0	0.244	NS	–0.257	NS
Percentage of fat (kg)	33.0±6.7	0.347	0.017	–0.319	0.029
Fat mass (kg)	20.6±7.3	0.218	NS	–0.316	0.031
Muscle mass (kg)	38.0±4.9	–0.070	NS	–0.226	NS
Blood Pressure (mmHg)					
(Systolic)	136.1±17.5	0.292	0.046	0.028	NS
(Diastolic)	78.1±7.8	0.273	NS	0.102	NS
BUN (mg/dl)	15.1±3.3	0.177	NS	–0.119	NS
Creatinine (mg/dl)	0.58±0.12	–0.052	NS	0.215	NS
T-cho (mg/dl)	227.5±36.8	0.252	NS	–0.229	NS
HDL-cho (mg/dl)	66.1±13.6	–0.429	0.003	0.268	NS
TG (mg/dl)	95.7±58.7	0.353	0.015	–0.300	0.040
Glucose (mg/dl)	95.4±10.3	0.221	NS	–0.049	NS
Insulin (μU/ml)	5.6±2.8	0.146	NS	–0.201	NS
HOMA	1.3±0.8	0.157	NS	–0.152	NS
Adiponectin (μg/ml)	12.4±5.4	–0.296	0.043	0.162	NS
Leptin (ng/ml)	9.6±5.9	0.224	NS	–0.308	0.035
d-ROM (U.CARR)	289.9±58.4	0.207	NS	–0.196	NS
CML (μg/ml)	4.7±0.8	–	–	–	–
Pentosidine (pmol/ml)	118.9±32.1	–0.182	NS	–	–
Daily consumption					
Energy intake (kcal/day)	1847±286	–0.078	NS	–0.055	NS
Fat (g/day)	48.4±15.3	0.062	NS	–0.341	0.019
Carbohydrate (g/day)	248.5±47.8	0.003	NS	0.173	NS
Protein (g/day)	69.5±11.8	0.120	NS	–0.116	NS

Data are presented as mean ± SD. Correlations between serum AGE levels (CML and pentosidine) and other parameters were determined by simple correlation with determination of Pearson's correlation coefficients. BUN; blood urea nitrogen, T-cho; total cholesterol, TG; triglyceride, HDL-cho; high-density cholesterol, HOMA; homeostasis model assessment, d-ROM; derivatives of reactive oxygen metabolites, CML; carboxymethyllysine

Plasma levels of insulin (Insulin Riabeads II, Abbot Japan, Tokyo, Japan) and leptin (Leptin RIA Kit, Cosmic, Tokyo, Japan) were determined by radioimmunoassay. Plasma adiponectin levels were assessed by ELISA using a Human adiponectin ELISA kit (Otsuka Pharmaceutical, Tokyo, Japan) [20]. The intra- and inter-assay coefficients of variation (CV) of the ELISA for adiponectin were 3.3% and 7.4%, respectively. Insulin resistance in the fasting state was examined by homeostasis model assessment (HOMA) and calculated using the following formula: fasting plasma glucose (mg/dl) × fasting plasma insulin (μU/ml)/405. High HOMA scores denote insulin resistance [21].

Serum CML was measured by competitive ELISA according to methods described previously [22]. CML-bovine serum albumin (BSA) antigen was used for coating on 96-

Table 2. Changes in each measurement parameter in subjects of the present study.

	Control		Life-style modification (LM)	
	Pre (*n*=12)	**Post (*n*=12)**	**Pre (*n*=17)**	**Post (*n*=17)**
Age (years)	50±8		54±7	
Height (cm)	157±6.7	–	158±6.3	–
Weight (kg)	63.0±5.0	62.5±5.5	65.7±10.7	64.6±10.6[a]
BMI (kg/m^2)	24.8±1.7	24.4±1.6	26.3±5.2	25.7±5.0[b]
Percentage of fat (kg)	32.8±4.1	31.7±3.4	34.6±8.0	33.0±7.7[a]
Fat mass (kg)	20.6±2.6	19.8±2.5	23.4±8.8	21.9±8.3[a]
Muscle mass (kg)	40.0±4.6	40.3±4.4	39.8±3.9	40.2±4.0
Blood Pressure (mmHg)				
(Systolic)	134.8±16.6	125.8±16.4	135.9±20.4	129.8±15.4
(Diastolic)	76.9±7.6	73.1±5.6[b]	78.9±9.0	74.7±9.2[b]
BUN (mg/dl)	12.7±2.5	13.1±2.5	13.9±2.1	14.6±2.4
Creatinine (mg/dl)	0.56±0.13	0.56±0.10	0.58±0.11	0.60±0.14
T-cho (mg/dl)	206.1±27.6	202.8±32.0	230.9±37.4	224.4±32.3
HDL-cho (mg/dl)	65.4±11.6	64.2±15.3	67.2±11.8	62.0±13.7[a]
TG (mg/dl)	79.6±23.4	92.6±31.1	94.2±40.5	119.4±59.1[a]
Glucose (mg/dl)	90.3±6.6	88.1±5.9	99.4±12.5	98.9±11.6
Insulin (μU/ml)	4.8±1.1	4.7±1.5	6.3±3.6	6.1±3.3
HOMA	1.1±0.2	1.0±0.3	1.6±1.1	1.5±0.9
Adiponectin (μg/ml)	10.4±3.3	9.2±3.3[b]	11.7±3.9	10.9±3.7
Leptin (ng/ml)	8.5±2.1	12.1±1.8[a]	12.0±7.7	14.3±8.6
d-ROM (U.CARR)	280.3±22.4	172.1±20.4[a]	301.4±69.4	217.0±68.4[a]
CML (μg/ml)	4.5±0.7	5.2±1.3	4.9±0.5	4.6±0.8[c]
Pentosidine (pmol/ml)	132.6±29.6	116.2±21.6	109.7±32.9	88.0±20.2[b]
Daily consumption				
Energy intake (kcal/day)	1924±169	1901±195	1915±348	1744±426
Fat (g/day)	29.6±5.7	28.3±4.8	59.1±11.4*	46.1±17.4[b]
Carbohydrate (g/day)	249.5±27.5	247.8±28.8	253.8±67.25	216.8±79.3
Protein (g/day)	70.3±10.3	69.3±10.0	71.5±14.0	64.3±15.0

Data are presented as mean ± SD. Differences between the life-style modification (LM) (*n*=17) and control (*n*=12) groups in baseline characteristics were assessed by the unpaired *t*-test. Differences in variables before to after the 12-wk study period in each subject group were examined by paired *t*-test. Letters in table express significant differences before to after intervention; [a] $P<0.001$, [b] $P<0.01$; [c] $P<0.05$. * Significant difference in daily fat consumption was observed before intervention (control vs. LM group). BUN – blood urea nitrogen; T-cho – total cholesterol; TG – triglyceride; HDL-cho – high-density cholesterol; HOMA – homeostasis model assessment; d-ROM – derivatives of reactive oxygen metabolites; CML – carboxymethyllysine.

well plates in advance. The intra- and inter-assay CVs of the ELISA for CML were 5.0% and 11.4%, respectively. Serum pentosidine was also measured by competitive ELISA (FSK Pentosidine kit, FUSHIMI Pharmaceutical Co., Ltd. Kagawa, Japan) using methods similar to those for serum CML [23]. The intra- and inter-assay CVs of the ELISA for pentosidine were 8.0% and 4.2%, respectively. The sensitivities (minimum limits of detection) for CML and pentosidine were 0.43 μg/ml and 5.28 pmol/ml, respectively. All sample measurements were performed in duplicate according to the manufacturer's instructions.

Estimates of oxidative stress were obtained by quantifying plasma levels of derivatives of reactive oxygen metabolites (d-ROMs) with a Free Radical Analytical System 4 (FRAS4, H&D srl, Parma, Italy) [24,25]. In brief, plasma samples were dissolved in an acidic buffer, in which hydroperoxides react with transitional metal ions liberated from the proteins

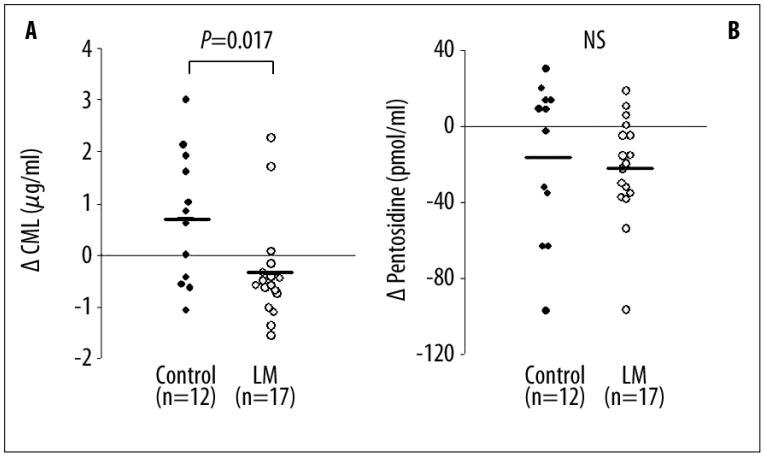

Figure 1. Changes in serum levels of CML (**A**) and pentosidine (**B**) in the control group and lifestyle modification (LM) group during the intervention period. Each Δ value was calculated as the post-intervention value minus the baseline value. The mean decrease in serum CML level was significantly larger in the LM group than in the control group, whereas no significant difference between groups was observed in mean decrease in pentosidine level. Mean values are indicated by horizontal bars.

and are converted to alkoxy and peroxy radicals. These newly formed radicals are able to oxidize an additive (*N, N*-diethyl-para-phenylendiamine) to the corresponding radical cation. The concentration of this persistent species can be easily determined through spectrophotometric procedures (with absorption at 505 nm). The normal values of the test are between 250 and 300 U.CARR (Carratelli Units), where 1 U.CARR is a conventional arbitrary unit and corresponds to the color development caused by a H_2O_2 (hydrogen peroxide) solution at a concentration of 0.8 mg/L. Values >300 U.CARR indicate the condition of oxidative stress.

Statistical analyses

All statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). All data were normally distributed, and are presented as means ±S.D. For 47 subjects at initial screening, correlations between serum AGE levels (CML and pentosidine) and other parameters were determined by simple correlation with determination of Pearson's correlation coefficients. In addition, multiple stepwise regression (backward elimination procedure) was performed to examine whether serum CML or pentosidine levels were related to anthropometric and biochemical parameters and daily nutritional intake examined in the present study. In the 12-wk intervention study, differences between the LM (n=17) and control (n=12) groups in baseline characteristics of the subjects were assessed by the unpaired t-test. The differences in variables before to after the 12-wk study period in each subject group were examined by paired t-test. Changes in CML and pentosidine levels during the 12-wk study period (ΔCML and Δpentosidine) were calculated and compared between the LM and control groups using the unpaired t-test. Pearson's correlation analysis was used to examine the correlations between changes in serum AGE levels (ΔCML or Δpentosidine) and other parameters. Each Δ value was calculated as the post-intervention value minus the baseline value. P-values less than 0.05 were considered significant.

Results

Independent variables affecting serum CML and pentosidine levels

The relationships between serum AGE levels (CML and pentosidine) and each variable measured before intervention are shown in Table 1. The serum CML and pentosidine levels for the group of all subjects in the present study (n=47) were 4.7±0.8 μg/ml and 118.9±32.1 pmol/ml (0.045±0.012 μg/ml), respectively. Serum CML level exhibited significant positive correlations with percentage body fat, systolic blood pressure, and serum triglyceride level and negative correlations with serum HDL cholesterol and plasma adiponectin levels, whereas serum pentosidine level was negatively correlated with body weight, percentage body fat, body fat mass, serum triglyceride, plasma leptin level, and daily fat consumption. By multiple stepwise regression analysis, serum HDL-cholesterol level was selected as a predictive variable for serum CML (r^2=0.411, P=0.003), whereas body fat mass (r^2=0.347, P=0.012) and fat consumption (r^2=0.371, P=0.007) were selected as predictive variables for serum pentosidine.

Effects of 3-month exercise intervention on serum CML and pentosidine levels

Variables measured before and after intervention are shown in Table 2. There were no differences in subject age or anthropometric or hematological parameters between the two subject groups before intervention. The mean daily number of walking steps in the LM group was 8418±2702. In the LM group, significant reduction was observed in body weight, BMI, percentage body fat, body fat mass, diastolic blood pressure, and serum levels of HDL-cholesterol, CML, and pentosidine, whereas plasma glucose, insulin level, and HOMA index were unchanged. There was a significant difference between the control and LM groups in change in serum CML level (ΔCML) (0.710 μg/ml vs. – 0.358 μg/ml; P=0.017) but not in serum pentosidine level (Δpentosidine) (–16.38pmol/ml vs. –21.73pmol/ml; P=0.675) (Figure 1A,B). In the LM group, mean daily number of walking steps was significantly correlated with decrease in serum CML level (r=–0.494, P=0.044) (Figure 2), but not with that in pentosidine level. In all 29 subjects, decreases in pentosidine level exhibited significant positive correlations with reduction in body weight (r=0.488, P=0.007) and body fat mass (r=0.387, P=0.038) (Figure 3A,B). No significant correlations were observed among changes in the serum level of pentosidine and those in dietary content during the study period.

Discussion

This is the first prospective study to examine the effects of lifestyle modification on changes in serum CML and pen-

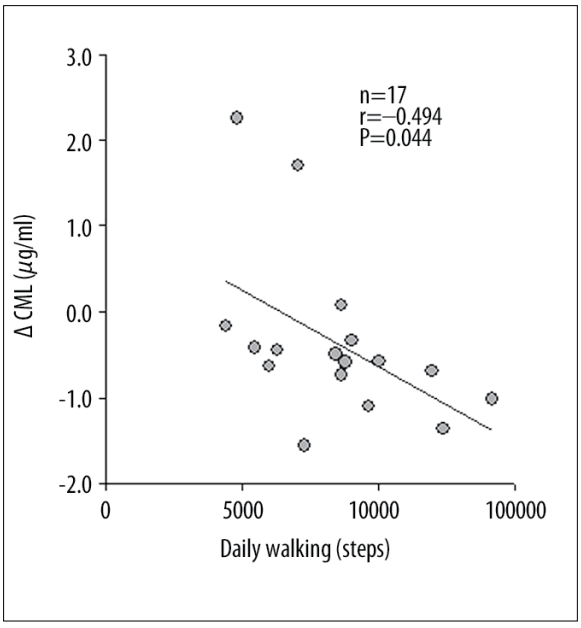

Figure 2. Correlation between mean daily number of walking steps and decrease in serum CML level (ΔCML) in the LM group (n=17), determined by Pearson's correlation analysis. Δ CML was calculated as the post-intervention CML level minus the baseline CML level.

tosidine levels and to investigate the association of changes in AGE parameters with those in anthropometric and metabolic markers and daily activities in middle-aged females without overt diabetic disease. The results of the present study included the following: 1) In middle-aged females without overt diseases, serum level of CML exhibited a significant correlation with those of blood fat components, especially HDL-cholesterol, while serum level of pentosidine was negatively correlated with body fat composition and dietary fat consumption at baseline. 2) Serum levels of CML and pentosidine were reduced by 12-wk lifestyle modification with a prescribed home-based walking exercise and weekly reinforcement sessions. 3) In addition, changes in serum CML were significantly related to number of daily walking steps during the study period, whereas decrease in serum pentosidine was significantly correlated with those in body fat composition.

Various AGE products have been shown to play important roles in the pathogenesis of various disease processes, including atherosclerosis [26,27], diabetic complications [28,29], chronic renal failure [30], osteoporosis [31], and inflammatory diseases [32]. Serum levels of CML and pentosidine have been measured to assess cumulative glycemic and glycoxidative injury, and can be used as diagnostic markers or quantitative predictors for some of these diseases [33–36]. However, only a limited number of studies have examined the correlations between serum markers of glycoxidation products and metabolic parameters in healthy subjects [11–13]. Maza and colleagues recently reported that serum level of total AGEs products was related to renal function and CRP, but not to body composition or lipoprotein, insulin, and glucose levels in middle-aged males without diabetes [12]. In contrast, the present study focused on the circulating concentrations of specific AGEs (CML and pentosidine) and demonstrated that serum concentration of CML was negatively correlated with serum HDL-cholesterol level while that of pentosidine was negatively correlated with body composition and daily fat consumption in healthy middle-aged females without overt diabetes.

A number of epidemiological studies have revealed negative correlations between HDL-cholesterol level and the incidence of atherosclerotic diseases. HDL-cholesterol has been shown to retard the progression of atherosclerosis by absorbing excess cholesterol from peripheral cells and by transporting lipid to the liver for excretion in the reverse cholesterol transport (RCT) system [37,38]. In addition, impairment of lipid efflux from cells, such as loss of ATP-binding cassette transporter A1 (ABCA-1) in Tangier patients, has been related to cholesterol deposition in arterial walls and to very low plasma HDL concentration [39]. In general, cholesterol is absorbed from cells by various acceptors, not only lipoprotein (ApoA-I) containing HDL cholesterol, but other plasma proteins as well, such as albumin [40] and globulin [41]. In particular, plasma albumin plays a role in mediating cholesterol equilibration between cells and lipoproteins [40]. A recent study revealed that AGE-adduct albumin could efficiently trap cholesterol and impair function of the HDL-mediated RCT system, resulting in the development of premature atherosclerosis [42]. In addition, AGE inhibitors, aminoguanidine and metformin, protected against the production of CML-adduct albumin and improved HDL-mediated cholesterol ef-

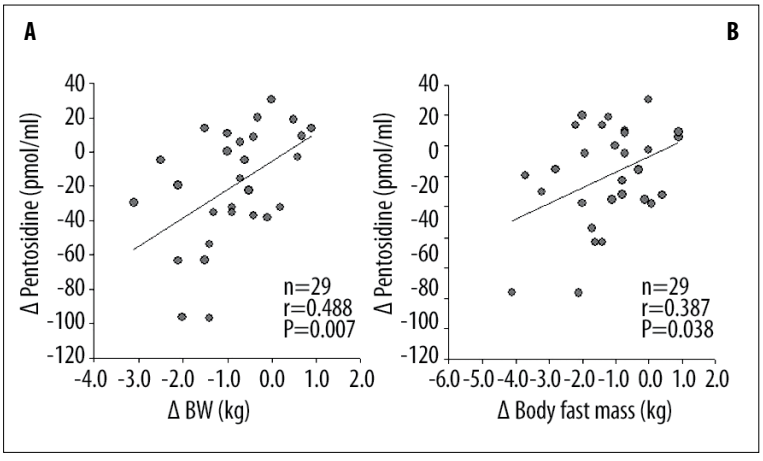

Figure 3. (**A**) Correlations between decreases in body weight (ΔBW) (**A**) and body fat mass (Δ body fat mass) (**B**) and those in serum pentosidine level (Δpentosidine) in both groups (n=29), determined by Pearson's correlation analysis. Each Δ value was calculated as the post-intervention value minus the baseline value.

flux from cells. Collectively, the present findings raise the possibility that serum level of CML is negatively correlated with that of HDL-cholesterol. Although the roles of serum CML in endothelial activation are less important than those of other metabolic and endothelial parameters [11], serum CML products appear to be associated with HDL-cholesterol and to play a role in cholesterol removal from arterial walls in healthy middle-aged subjects.

Contrary to our expectations, serum pentosidine level was negatively correlated with body fat parameters. Sebekova and colleagues have reported that childhood and adolescent obesity is characterized by lower plasma AGE levels, despite lower insulin sensitivity and enhanced oxidative stress [13]. Although levels of pentosidine were not directly measured in the study by Sebekova, their findings appear similar to our own with respect to the association of plasma AGE levels with obesity. In the present study, while no significant correlation was found between serum CML level and amount of daily intake of each food component, a significant association was found between the serum levels of pentosidine and mean daily fat intake at the baseline examination. In previous surveys of food derived-AGEs, mainly CML, AGE content was measured in commonly consumed meals, and fatty foods exhibited the highest AGE content, especially butter, cheese, and mayonnaise [43], whereas no correlation was found between serum AGE levels and dietary protein, fat, or carbohydrate intake in patients with renal failure [44]. Differences in time and temperature of cooking among studies might account, in part, for the differences in their results.

Some prospective studies have demonstrated effects of glycemic control on serum or skin AGE levels in patients with diabetes [45,46]. The present study first focused on the changes in serum CML and pentosidine levels produced by short-term lifestyle modification in non-diabetic subjects. Of note, serum CML level was decreased by intervention in the LM group, compared with the control group, which did not receive an educational session (Figure 1A). In addition, while there were no significant correlations between changes in CML levels and those in any anthropometric or metabolic parameters, a significant correlation was found between daily number of walking steps and decrease in serum CML level during the study period (Figure 2), suggesting the possibility that change in lifestyle habits, including daily walking, can decrease serum CML. It is well documented that physical inactivity is a strong risk factor for the development of atherosclerotic diseases [18,47]. Our findings suggest that daily physical activity might reduce exposure to CML, decreasing the risk of development of atherosclerosis in arterial walls. While no significant difference was observed in the change in serum pentosidine between the LM and control groups (Figure 1B), positive correlations were found between reduction of pentosidine level and those of body fat parameters (Figure 3A,B), suggesting that serum levels of pentosidine can be reduced with decrease in body fat composition, regardless of dietary and exercise intervention.

There are several potential limitations to the present study. First, we could not clearly distinguish between effects of dietary and physical activity on the changes in serum AGEs. Because the primary aim of the study was to investigate whether serum AGEs can be reduced by a simple intervention which modifies both dietary habits and physical activity, we did not intend to discriminate the responses to exercise intervention from those to dietary modification, and instead examined overall effects of lifestyle intervention, such as those performed in various health-promotion programs, on serum levels of AGEs. Although the main source of circulating and tissue AGEs is endogenous production, AGEs can be also formed in foods during heat treatment [48,49], and such products are another important source of AGEs [50], which are partially absorbed and retained in the body or eliminated in the urine [14]. Thus, the reduction in serum AGE parameters in the present study might have been due in part to unintended dietary changes, such as decrease in daily consumption of food AGEs, although participants were not instructed to avoid eating foods with AGEs. It will be necessary to separately examine the effects of each type of intervention (dietary and exercise) on changes in serum AGE levels. Second, after the present intervention, serum levels of TG were significantly increased and those of HDL decreased. Previous studies to determine whether circulating HDL levels can be altered by increase in physical activity have yielded inconsistent findings [51,52]. Some of these studies have reported increase [53] while others found decrease [54] or no change [55] in HDL-cholesterol levels. Differences in study design and concomitant changes in diet, lipoprotein profile, smoking, and alcohol intake may explain these conflicting results [51]. Specifically, previous studies have shown that a low-fat, high fiber diet is likely to reduce HDL-cholesterol and to raise TG levels in healthy pre-menopausal females [56]. In the present study, daily consumption of fat was decreased in AE subjects during the study period. It is possible that such dietary changes affected serum lipid profiles during the present intervention. Third, we recruited a small number of subjects in the present study. Despite the small sample size, however, the present findings have clearly demonstrated that lifestyle modification can significantly reduce circulating AGE levels even in middle aged females without overt diabetes. Study of a large population will be needed to confirm the present findings. In addition, it will also be necessary to examine prospectively whether changes in serum AGE levels by lifestyle modification actually affect the future development of atherosclerotic diseases in these non-diabetic subjects as well as patients with diabetes and renal diseases.

Conclusions

In summary, the findings of the present study suggest lifestyle modification as a promising approach to reducing circulating AGE levels even in healthy middle-aged females with neither overt diabetes nor renal dysfunction. Although attention has thus far been devoted by and large to the biochemical and nutritional processes of AGE formation and the pathophysiological involvement of AGE products in diabetic and renal diseases, our findings suggest that it is also important to consider how physical activity combined with dietary change can modify AGE accumulation in developing more effective programs for the prevention of the atherosclerotic diseases.

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