Redox Index of Cys-Thiol Residues of Serum Apolipoprotein E and Its Diagnostic Potential

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Abstract

We explored the proper index for estimating the redox status of Cys-thiol of serum apolipoprotein E (apoE), named “redox-IDX-apoE,” which is necessary to understand the redox biology of age-related diseases, such as atherosclerosis. The fractions of reduced form (red-), reversible oxidized form (roxi-), and irreversibly oxidized form (oxi-) apoE were measured by a band-shift assay. Candidates of redox-IDX-apoE were determined by calculating the values of these fractions and total apoE concentration. The ratios of roxi-apoE to total-apoE (roxi/total), red-apoE to roxi-apoE (red/roxi), and [red-apoE + oxi-apoE] to roxi-apoE ([red + oxi]/roxi) were independent of age and sex. Roxi/total showed significant negative correlations with serum triglyceride (TG) and HbA1c levels, while red/roxi and [red + oxi]/roxi showed significant positive correlations with them. However, red/roxi and [red + oxi]/roxi in the patients with atherosclerosis were significantly lower than those in control subjects, although serum TG and HbA1c levels in the patients were significantly higher than those in controls. The redox status of serum apoE-Cys-thiol is closely involved in the metabolism of TG-rich lipoproteins and glucose and may vary depending on the difference in pathological conditions. The appropriate usage of these ratios could be helpful in the diagnosis and prognosis of age-related diseases.

Introduction

The establishment of prevention or early diagnosis and treatment of age-related diseases is an urgent issue in a rapidly aging society. It is well known that age-related diseases, such as atherosclerotic diseases and Alzheimer’s disease (AD), are caused by oxidative stress¹. Additionally, apolipoprotein E (apoE) has long been regarded as a key molecule contributing to the pathogenesis of several such diseases². Therefore, numerous studies focusing on these two points have been performed to clarify the pathophysiological mechanism specific to age-related diseases; however, the details remain obscure.

Apolipoprotein (apo) E participates in cholesterol transport and metabolism as the main constituent of plasma lipoproteins and a ligand for the low-density lipoprotein (LDL) receptor³. ApoE has three major isoforms (E2, Cys112/Cys158; E3, Cys112/Arg158; E4, Arg112/Arg158), which are derived from Cys-Arg interchanges at residues 112 and/or 158³. These interchanges provide the structural and functional basis for these three isoforms⁴ and make a noticeable difference in the existing form of plasma apoE among these three isoforms. Namely, apoE2 and apoE3 exist in the plasma not only in monomeric forms but also as disulfide-linked complexes, such as homodimer, apoE-AII complex, and apoAII-E2-AII complex, while apoE4 exists only in the monomeric form^{5,6}.

These properties, based on the Cys-Arg interchanges, have been considered to contribute to the development of variable age-related diseases^{1,4,7}. Specifically, apoE4, an isoform without a Cys residue, is an identified risk factor for various atherosclerotic diseases, such as cardiovascular disease and cerebral infarction^{2,8} and sporadic late-onset AD^{4,7}. On the other hand, apoE2, an isoform with two Cys residues,

is a causative factor of type III hyperlipidemia since apoE2 is defective in LDL receptor binding affinity owing to the effect of Cys158⁹.

Cys is one of the least abundant amino acids. The thiol group of Cys in proteins imparts functional sites with their specialized properties, such as nucleophilicity, high-affinity metal binding, and disulfide bond formation¹⁰. Thereby, Cys-thiol, as one of the main targets of post-translational redox-mediated modifications, has roles in various physiological functions (e.g., as regulatory switches in signal pathways, modulation of transcription and protein expression, maturation of proteins, and protection from oxidative stress)^{11–13}.

Redox-mediated modifications of Cys-thiol are roughly classified into reversible and irreversible oxidations. The irreversible oxidation of Cys-thiol is predicted to cause loss of function, leading to the degradation of the modified protein^{14, 15}. Conversely, the temporary alteration of a protein due to reversible oxidation protects the protein from irreversible detrimental changes as well as modulates the protein function¹⁶. Consequently, redox-mediated modifications of Cys-thiol participate in the pathogenesis of various diseases¹⁷.

Based on this evidence, there is no doubt that the redox status of Cys-thiol of apoE (apoE-Cys-thiol) also affects its pathophysiological functions. We believe that some redox modulations of Cys-thiol confer detrimental or beneficial properties on apoE2 or apoE3, which affects the development of various apoE-related diseases, especially in the apoE4 non-carrier patients.

In our previous study, we demonstrated that the apoE3-AII complex is beneficial for maintaining the apoE3 redox status by preventing changes to the irreversibly oxidized form¹⁸. We also recently reported that the interactions of apoE2 and apoE3, especially apoE2, with lipids are markedly enhanced by the formation of apoE-AII and apoAII-E2-AII complexes¹⁹. However, besides our studies, few studies focusing on the redox status of apoE-Cys-thiol have been conducted; thus, its precise physiological meaning is still obscure. Understanding whether or how the redox status of apoE-Cys-thiol is related to the development of age-related diseases is necessary to establish an adequate method of estimating it.

In the present study, we explored the proper index for estimating the redox status of apoE-Cys-thiol, named redox-IDX-apoE, and assessed their clinical availability by investigating its pathophysiological variance.

Materials And Methods

Materials

Horseradish peroxidase (HRP)-conjugated goat anti-apoE polyclonal antibody was supplied by the Academy Bio-medical Company, Inc. (Houston, TX, USA). Photocleavable maleimide-conjugated polyethylene glycol (PEG-PC-Mal) was purchased from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). All other chemicals used were of the highest grade.

Subjects

Two hundred eighty serum samples were obtained from outpatients with normal results on the following serum laboratory tests: creatinine (male, 0.65-1.07 mg/dL; female, 0.46-0.79 mg/dL), Uric acid (male, 3.7-7.8 mg/dL; female, 2.6-5.5 mg/dL), AST (13-30 U/L), ALT (male, 10-42 U/L; female, 7-23 U/L), LD (124-222 U/L), and gGT (male, 13-64 U/L; female, 9-32 U/L). All serum samples were stored at -80 °C until analysis. This study was conducted in accordance with the principles of the Declaration of Helsinki and approved by the Tsukuba University Ethics Committee (H28-075). Written informed consent was obtained from all patients.

ApoE phenotyping

The serum apoE phenotype was determined by isoelectric focusing and immunoblot analysis, as described previously²⁰.

Determination of candidates of redox-IDX-apoE

The redox status of apoE was analyzed with a band-shift assay using PEG-PC-Mal (PM), according to our previous study^{19,21}. The specific bands, probed with HRP-conjugated anti-apoE polyclonal antibody, were developed using an ECL detection kit (Nacalai Tesque, Inc., Kyoto, Japan) and were analyzed using ImageJ 1.45 software from the National Institutes of Health. A typical pattern of the present assay is shown in **Supplemental Figure**. As described previously^{19,21}, the 40-kDa band (apoE-(PM)₁) was defined as the reduced form of apoE (red-apoE), while the monomeric form (35-kDa unlabeled apoE) was termed as the irreversibly oxidized form of apoE (oxi-apoE). The concentrations of red- and oxi-apoE were determined by multiplying the fraction ratios by the total apoE concentration. In the present method, irreversibly oxidized apoE2 and apoE3 are indistinguishable from apoE4 since apoE4 is also detected at the position of monomeric apoE. Thus, we also determined the concentration of the reversible oxidized form of apoE (roxi-apoE) (i.e., the total concentration of homodimer, heterodimers, and PM conjugates of these disulfide-linked complexes) by subtracting the oxi-apoE concentration from the total apoE concentration. In addition, the following redox ratios of apoE were calculated from the concentrations of total-, red-, roxi-, and oxi-apoE: ratios of red-apoE to total apoE (red/total), roxi-apoE to total apoE (roxi/total), oxi-apoE to total apoE (oxi/total), red-apoE to oxi-apoE (red/oxi), red-apoE to roxi-apoE (red/roxi), roxi-apoE to oxi-apoE (roxi/oxi), red-apoE to [roxi-apoE + oxi-apoE] (red/[roxi + oxi]), [red-apoE + roxi-apoE] to oxi-apoE ([red+roxi]/oxi), and [red-apoE + oxi-apoE] to roxi-apoE ([red+oxi]/roxi).

Other assays

The concentrations of serum apolipoproteins (apoAI, apoAII, and apoE), lipids (total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], LDL-C, and TG), and CRP were determined by turbidimetric immunoassay, enzymatic assay, and latex turbidimetric immunoassay, respectively. The HbA1c values were measured by cation-exchange high performance liquid chromatography. The concentration of non-HDL-C was calculated as TC minus HDL-C.

Statistical methods

All laboratory parameters and ages of subjects are presented as the mean $\pm$ standard error (SE) and the mean $\pm$ standard deviation (SD), respectively. One-way ANOVA was used to compare the differences in each laboratory test result among apoE phenotype groups and the differences in each redox ratio among apoE phenotype groups or between male and female subject groups. Univariate and multivariate regression analyses were performed to assess the relationship between each redox-IDX-apoE and each laboratory test result and to determine the independent factors that affect each redox-IDX-apoE. A value of $p < 0.05$ was considered significant.

Results

Effect of apoE phenotype on the candidates of redox-IDX-apoE

We evaluated the effect of the apoE phenotype, namely the number of Cys residues per two apoE molecules, on various candidates of redox-IDX-apoE (**Table 1**). The roxi-apoE concentrations in serum with apoE2/E3 were significantly higher than those with apoE3/E3 or apoE3/E4 ($p < 0.001$), while the oxi-apoE concentrations in serum with apoE3/E4 were significantly lower than those in serum with apoE3/E3 ($p < 0.001$). However, it was not certain whether these differences were due to only the number of Cys residues in apoE since total-apoE concentration itself, although not statistically significant, was different among phenotype groups. Hence, we narrowed down the candidates of redox-IDX-apoE to the redox ratios of apoE, calculated from concentrations of total-, red-, roxi-, and oxi-apoE and assessed the effect of the number of Cys residues in apoE on these ratios. Serum results with apoE2/E4 were excluded from this evaluation because the sample size was small. Roxi/total, roxi/oxi, and [red+roxi]/oxi were clearly proportional to the number of Cys residues in apoE and varied in the order E2/E3 > E3/E3 > E3/E4 (**Fig. 1A, B, C**). Roxi/total, roxi/oxi, and [red+roxi]/oxi in the serum with apoE2/E3 were approximately 1.5-fold ($p < 0.001$), 2.5-fold ($p < 0.001$), and 2.2-fold ($p < 0.001$) higher than those in the serum with apoE3/E4, respectively. In contrast, oxi/total, red/roxi, and [red+oxi]/roxi decreased with increasing Cys number of apoE and were varied in the rank order E2/E3 < E3/E3 < E3/E4 (**Fig. 1D, E, F**). Oxi/total, red/roxi, and [red+oxi]/roxi in the serum with apoE3/E4 were approximately 1.7-fold ($p < 0.001$), 2.1-fold ($p < 0.01$), and 2.6-fold ($p < 0.001$) higher than those in the serum with apoE2/E3, respectively. Unlike the six ratios, red/total, red/oxi, and red/[roxi + oxi] were not affected by the number of Cys residues in apoE. These three ratios in the serum with apoE3/E3 were significantly higher than those in the serum with other phenotypes (**Fig. 1G, H, I**). Hence, to avoid the effect of the number of Cys residues in apoE, we conducted the following analysis with a focus on the subjects with apoE3/E3.

Effect of age on the candidates of redox-IDX-apoE

We assessed the effect of age on the candidates of redox-IDX-apoE in the serum with apoE3/E3 according to sex (**Table 2**). Oxi/total level was significantly increased by aging regardless of sex ($r = 0.368$, $p < 0.001$). In contrast, red/total, red/oxi, and [red + roxi]/oxi levels were significantly decreased with increasing age, regardless of sex ($r = -0.246$, $p < 0.001$ for red/total; $r = -0.358$, $p < 0.001$ for red/oxi; $r = -0.302$,

$p < 0.001$ for [red + roxi]/oxi). Red/[roxi + oxi] in males was also significantly decreased with increasing age ($r = -0.312$, $p < 0.005$), whereas those in females showed a decrease in inclination, but the difference was not statistically significant ($r = -0.113$). On the other hand, no significant correlation was observed between roxi/total, red/roxi, and [red + oxi]/roxi with age. We then compared each of these three ratios among male and female subjects, which were divided into three groups by age (≤ 40 years, 41-60 years, ≥ 61 years), respectively. No significant differences in roxi/total (**Fig. 2A**), red/roxi (except for the slight significant difference between the male subjects aged ≤ 40 years and female subjects aged ≥ 61 years (**Fig. 2B**), and [red + oxi]/roxi (**Fig. 2C**) were observed between male and female subjects. Based on these results, we selected roxi/total, red/roxi, and [red + oxi]/roxi as the redox-IDX-apoE.

Determination of the factors affecting redox-IDX-apoE

We statistically determined the factors that affect the redox-IDX-apoE. First, we assessed the relationship between each redox-IDX-apoE and each laboratory test result (apoAI, apoAII, HDL-C, LDL-C, non-HDL-C, TG, TG to HDL-C ratio (TG/HDL-C), CRP, and HbA1c) by simple linear regression analysis (**Supplemental Table 1**). Roxi/total showed positive correlation with HDL-C levels ($r = 0.236$, $p < 0.001$), whereas it showed negative correlations with non-HDL-C levels ($r = -0.275$, $p < 0.001$), TG levels ($r = -0.483$, $p < 0.001$), TG/HDL-C ($r = -0.384$, $p < 0.001$), and HbA1c levels ($r = -0.192$, $p < 0.005$). Both of red/roxi and [red + oxi]/roxi showed positive correlations with non-HDL-C levels ($r = 0.247$, $p < 0.001$ for red/roxi; $r = 0.237$, $p < 0.001$ for [red + oxi]/roxi), TG levels ($r = 0.542$, $p < 0.001$ for red/roxi; $r = 0.515$, $p < 0.001$ for [red + oxi]/roxi), TG/HDL-C ($r = 0.447$, $p < 0.001$ for red/roxi; $r = 0.425$, $p < 0.001$ for [red + oxi]/roxi), and HbA1c levels ($r = 0.182$, $p < 0.01$ for red/roxi; $r = 0.183$, $p < 0.01$ for [red + oxi]/roxi), whereas it showed negative correlation with HDL-C levels ($r = -0.277$, $p < 0.001$ for red/roxi; $r = -0.256$, $p < 0.001$ for [red + oxi]/roxi).

Next, we performed multiple regression analysis with each redox-IDX-apoE as the objective variable and with the factors, narrowed down by the above-mentioned linear regression analysis, as explanatory variables that affected each redox-IDX-apoE. However, non-HDL-C and TG/HDL-C were excluded from this analysis because they showed a linear combination with HDL-C and TG levels. On this analysis, HbA1c and serum TG levels were independently associated with redox-IDX-apoE (**Table 3**).

Effect of atherosclerosis on redox-IDX-apoE

To investigate the clinical significance and usefulness of roxi/total, red/roxi, and [red + oxi]/roxi as the redox-IDX-apoE, we selected 16 subjects with atherosclerosis combined with type 2 diabetes (56.8 ± 19.6 years) and 38 subjects with *normolipidemia* and no apparent disease (controls, 54.7 ± 17.0 years) from the above-mentioned 218 subjects with apoE3/E3. We then compared each of these ratios between the subjects with atherosclerosis and controls.

The effects of atherosclerosis on roxi/total, red/roxi, and [red + oxi]/roxi conflicted with the results described in Section 3.3. Red/roxi and [red + oxi]/roxi were 0.6-fold lower ($p < 0.05$) and 0.4-fold lower ($p < 0.001$) in subjects with atherosclerosis than in controls, respectively (**Fig. 3A, B**). On the other hand, roxi/total tended to be higher in subjects with atherosclerosis than in controls (**Fig. 3C**), even though

serum TG and HbA1c levels in subjects with atherosclerosis were significantly higher than those in controls ($p < 0.005$ for TG; $p < 0.01$ for HbA1c, **Supplemental Table 2**).

Discussion

Redox-IDX-apoE is considered the proper index for pathophysiological assessment of the redox status of serum apoE-Cys-thiol. In this study, we aimed to determine redox-IDX-apoE to establish its use as a novel biomarker for age-related diseases, such as atherosclerotic diseases and AD.

The concentrations of red-, roxi-, and oxi-apoE are not appropriate indices because they are directly affected by the total apoE concentration. However, when the redox ratios, calculated from those concentrations, were used as indices, the phenotype-specific effects on the redox status of serum apoE-Cys-thiol could be explicitly estimated. In particular, the subjects with apoE2/E3 showed the highest level of roxi/total and the lowest levels of oxi/total, red/total, red/roxi (which reflects the content ratio of reduced monomeric form to reversible oxidized or reducible polymeric form), and [red + oxi]/roxi (which reflects the content ratio of total monomeric form to reducible polymeric form) among phenotype groups. Therefore, serum apoE2 exists as a reducible polymeric molecule rather than as a reduced monomeric molecule, thereby preventing the change to the irreversibly oxidized monomeric molecule. These properties of the subjects with apoE2/E3 were completely opposite to those of the subjects with apoE3/E4. Recently, we reported that the formation of the reversible oxidized form of apoE, such as homodimer and apoE-All complex, is beneficial for maintaining the apoE3 redox status by preventing changes to the irreversibly oxidized form¹⁸. The present results could also be explained by this notion.

Previous studies have shown that the cyst-thiol group of a protein is susceptible to aging-associated oxidative stress, which consequently affects the pathophysiological function of the corresponding protein^{17,22}. In the analysis focusing on only subjects with apoE3/E3, regardless of sex, oxi/total positively correlated with age, whereas red/total, red/oxi, and [red + roxi]/oxi negatively correlated with age, indicating that the level of irreversibly oxidized monomeric apoE molecule increased, whereas that of the reduced monomeric molecule decreased with aging. These findings provide compelling evidence that the redox status of serum apoE-Cys-thiol must also be altered by aging. The subtle discordance in the effect of age on red/[roxi + oxi] between male and female subjects suggests the possibility that the regulatory mechanism of the redox status of apoE-Cys-thiol would be affected by the physiological variations based on sex, such as pre-, peri-, or post-menopause. However, we could not grasp the menopausal status of the subjects in the present study. A previous study has demonstrated that estrogen, an antiatherogenic hormone, regulates apoE expression²³. It seems that estrogen may also affect the redox status of apoE-Cys-thiol. Further studies will be necessary to confirm whether the redox status of apoE-Cys-thiol varies according to menopausal status.

Unlike the six ratios, roxi/total, [red + oxi]/roxi, and red/roxi were independent of age and sex. Considering these results, we eventually selected these three ratios as the potent redox-IDX-apoE.

Roxi/total, [red + ox]/roxi, and red/roxi showed significant correlations with serum lipid levels and TG/HDL-C, suggesting that the redox status of serum apoE-Cys thiol is closely involved in lipoprotein metabolism, especially in those of HDL and non-HDL, which are mainly composed of TG-rich lipoproteins. Previous studies have demonstrated that HbA1c levels are positively associated with circulating TG levels, reflecting hypertriglyceridemia led by type 2 diabetes²⁴. Each of the three ratios also showed a significant correlation with HbA1c level, strongly suggesting that the redox status of serum apoE-Cys thiol may also be involved in disorders of glucose metabolism or accompanying dyslipidemia. In addition to hypertriglyceridemia, hypo-HDL-cholesterolemia is a typical and common dyslipidemia evoked by insulin resistance in patients with type 2 diabetes²⁵. Thus, TG/HDL-C, an atherogenic index²⁶, is also useful as a surrogate marker for insulin resistance^{27,28}. In the present study, roxi/total showed a negative correlation with TG/HDL-C, whereas [red +ox]/roxi and red/roxi showed a positive correlation with TG/HDL-C, indicating that these ratios could also reflect the degree of insulin resistance, i.e., the reduction of roxi/total and the increment of [red +ox]/roxi or red/roxi may be indicative of exacerbation of insulin resistance.

The results of the multivariate analysis indicated that the redox status of serum apoE-Cys-thiol would be more strongly associated with the metabolism of TG-rich lipoproteins than that of HDL. TG-rich lipoproteins, such as chylomicrons and VLDL, are catabolized by lipoprotein lipase (LPL)^{25,29}. Subsequently, their remnants are rapidly removed from circulation via receptor-mediated clearance^{25,29}. LPL also enhances this process along with apoE, which is a critical ligand for the clearance of remnant lipoproteins²⁹. Additionally, the activity of LPL is regulated by insulin³⁰. Therefore, the exacerbation of insulin resistance causes the stagnation of remnant lipoproteins, followed by the formation of atherosclerotic lesions^{25,31}. On the other hand, previous studies have reported that insulin signaling is modulated by apoE and its genotype^{32,33}. Taken together, we speculate that the strong linkage between serum TG levels and roxi/total, [red +ox]/roxi, and red/roxi may reflect the fluctuations of TG-rich lipoproteins. Moreover, their remnants are affected by the pathophysiological redox changes of apoE-Cys-thiol, which is accompanied by the modulation of insulin signaling.

Remnant lipoproteins are highly atherogenic lipoprotein particles^{25,34}.

Based on this pathophysiological property, we predicted the possibility that the reduction of roxi/total and the increment of [red +ox]/roxi or red/roxi would exert atherogenic effect or reflect its consequences. Furthermore, we speculated that these findings would be observed in patients with atherosclerosis combined with type 2 diabetes. However, contrary to expectations, our study showed the opposite results, although the TG level in the patients was significantly higher than that in the controls. This discrepancy suggests that pathophysiological redox changes in the serum apoE-Cys-thiol by or for the development of atherosclerosis accompanied by dyslipidemia would be quite different from those by or for the development of dyslipidemia without atherosclerosis. Hence, the appropriate usage of these redox ratios of apoE according to the apoE phenotype would allow for the detection of insulin resistance, dyslipidemia, and their related pathological conditions, such as atherosclerosis. However, at this stage, it

is not certain whether the redox change of serum apoE-Cys-thiol is the cause or effect of these pathological changes. One limitation of the present study is the small sample size; thus, we could not perform a stratified analysis based on the type of dyslipidemia, even though various types of dyslipidemia are induced by type 2 diabetes³⁵. Further studies will be necessary to clarify the clinicopathological characteristics of redox changes in serum apoE-Cys-thiol using redox-IDX-apoE.

It is well known that Cys-thiol-based redox regulatory switches are closely involved in several signaling pathways¹². Based on this evidence, we speculate that apoE-Cys-thiol also functions as a redox switch in the apoE-regulated metabolic pathway, such as the clearance of remnant lipoprotein via insulin signaling, and consequently affects the pathogenesis of atherosclerosis. Similarly, the same is true for AD. Hence, we believe that the estimation of the redox status of serum apoE-Cys-thiol using a valid index could also help understand the pathology of these diseases, especially in the case of apoE4 non-carriers.

In conclusion, we propose that the three redox ratios of apoE, namely, roxi/total, [red +oxi]/roxi, and red/roxi, could be the proper redox-IDX-apoE, which could be used as a tool for molecular pathological analysis or as a novel biomarker for age-related diseases.

Abbreviations

AD, Alzheimer's disease; apoE, apolipoprotein E; HDL-C, high-density lipoprotein cholesterol; HRP, horseradish peroxidase; LDL-C, low-density lipoprotein cholesterol; LPL, lipoprotein lipase; oxi, irreversibly oxidized form; PM, photocleavable maleimide-conjugated polyethylene glycol; red, reduced form; roxi, reversible oxidized form; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein

Declarations

Conflict of interests: The authors declare no conflict of interest associated with this manuscript. The research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Author Contributions: KY has designed the present project and has carried out experiments. KY and YK analyzed experimental data. KY has written this manuscript, and YK has commented on drafts of the manuscript. All authors approved the final version of this manuscript.

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Tables

Due to technical limitations, table 1, 2 and 3 is only available as a download in the Supplemental Files section.

Figures

Figure 1

Comparisons of the candidates of redox-IDX-apoE among apoE phenotype groups. The differences in roxi/total (A), roxi/oxi (B), [red + roxi]/oxi (C), oxi/total (D), red/roxi (E), [red + oxi]/roxi (F), red/total (G), red/oxi (H), and red/[roxi + oxi] (I) among the apoE phenotype groups were compared using one-way ANOVA. Data are expressed as the mean $\pm$ SE. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$, compared with the group of subjects with apoE3/E3. #, $p<0.05$; ##, $p<0.01$; ###, $p<0.001$, compared with the group of subjects with E2/E3. †, $p<0.05$, compared with the group of subjects with apoE3/E4.

Figure 2

Comparisons of the candidates of redox-IDX-apoE among age-stratified male and female groups. The differences in roxi/total (A), red/roxi (B), and [red + oxi]/roxi (C) among the apoE3/E3 male subjects and the apoE3/E3 female subjects, which were divided into three groups by age (≤ 40 years, 41 to 60 years, ≥ 61 years), were compared using one-way ANOVA. Data are expressed as the mean $\pm$ SE. *, $p<0.05$.

Figure 3

Effect of atherosclerosis on the redox-IDX-apoE. The differences in roxi/total (A), red/roxi (B), and [red + oxi]/roxi (C) between the apoE3/E3 subjects with atherosclerosis combined with type 2 diabetes (n=16, 56.8 ± 19.6 years) and the apoE3/E3 subjects with normolipidemia and no apparent disease (n=38, 54.7 ± 17.0 years) were compared using the Mann-Whitney U test. Data are expressed as the mean ± SE. *, p<0.05; **, p<0.001.

Supplementary Files

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