Evidence for the In Vivo Generation of Oxidatively Modified Epitopes in Patients With Atherosclerotic Endothelium

Ngoc-Anh Le, Xianzhou Li, Sung Kyung, and W. Virgil Brown

There is increasing evidence that autoantibodies (AAbs) against oxidatively modified low-density lipoprotein (LDL) are present in humans and may be detected in fasting plasma. Using a standardized immunoassay for the detection of circulating levels of AAbs against malondialdehyde (MDA)-modified LDL, we examined the acute changes in AAb levels during postprandial lipemia in a group of men and women without (n = 28) and with (n = 17) normal endothelium. The presence of atherosclerotic vessel is documented by clinical evidence of coronary artery disease (CAD). In response to the oxidative stress associated with postprandial lipemia, statistically significant reductions in AAb levels were demonstrated at 2 and 4 hours postprandially by paired *t* test. In patients with atherosclerotic arterial wall, the mean AAb level was reduced to 90.8% of fasting levels ($P < .001$) after 2 hours and to 90% after 4 hours ($P < .01$). This acute reduction in AAbs against MDA-LDL appears to be unique to atherosclerotic patients and could not be demonstrated in young controls with healthy blood vessels. In nonatherosclerotic controls, the mean normalized levels during postprandial lipemia were not statistically different from baseline (104.5% at 2 hours and 104.6% at 4 hours). The transient reduction in AAb levels with postprandial lipemia in atherosclerotic patients could be reproduced in a subset of the CAD patients after significant improvement in the lipid profile with weight loss. In patients with atherosclerotic disease, the transient reduction in the level of circulating AAbs reflects either an increased propensity to generate oxidatively modified epitopes or a reduced capacity to remove excess modified epitopes. These data are the first in vivo demonstration of an acute change in the oxidative process during postprandial lipemia.

WHILE THE PRESENCE OF oxidatively modified low-density lipoprotein (LDL) in atherosclerotic plaques has been repeatedly demonstrated in animal models[1-3] and in humans,[4,5] it is still not clear how plasma LDL could be modified in vivo. This is believed to stimulate the formation of autoantibodies (AAbs) with the task of removing these damaged LDLs from the circulation. In fact, immune complexes of modified LDL have been isolated from human lesions.[6-7]

The pathogenesis of oxidatively modified LDL in vivo and the subsequent generation of AAbs against these proteins are not well understood. AAbs developed against Cu^{2+} modified LDL have been shown to recognize a wide variety of chemically modified proteins, including proteins that have been modified by malondialdehyde (MDA) treatment. Using MDA-modified LDL, several studies have measured the circulating levels of AAbs against oxidatively modified LDL in controls without coronary artery disease (CAD) and patients with documented CAD.[8-12] In some reports, patients with CAD have been demonstrated to have higher AAb levels than healthy controls.[8-10] However, other investigators reported either lower or similar AAb levels in patients with CAD compared with controls.[11-12]

All of these reports used a direct enzyme-linked immunosorbent assay (ELISA) system that related the reactivity of the unknown plasma against native LDL to that against MDA-LDL. The difference in reactivity was expressed either as a ratio of the two optical densities (ODs) or as a difference between the two ODs. In the present report, we describe the standardization of an ELISA system that is based on a linear standard curve for the quantitation of AAb levels in whole plasma. Using this standardized immunoassay for the detection of AAb levels in plasma, we want to assess whether the circulating levels of these antibodies can be affected by interventions known to cause oxidative stress. The system we have selected is the oxidative stress associated with the generation of free fatty acids during postprandial lipemia. In a group of obese individuals with atherosclerotic endothelium as documented by clinical CAD, we have demonstrated acute reductions in AAb levels following the consumption of a standardized liquid meal containing polyunsaturated fat and cholesterol. This acute reduction in AAb levels can be reproduced in these individuals after a 6-month program of caloric restriction and medically supervised aerobic exercise that corrects many of the lipid abnormalities in this group of CAD patients. Similar changes in AAb levels could not be demonstrated in a group of young healthy controls with normal endothelium when they received the same test meal.

SUBJECTS AND METHODS

Subjects

Table 1 presents the clinical characteristics of the study subjects. Twenty-eight individuals with a history of bypass surgery or angioplasty were recruited for a study of the metabolism of intestinal lipoproteins. Eighteen of these individuals with a body mass index greater than 30 kg/m^2 participated in a 6-month rehabilitation program including supervised sessions of aerobic exercise and strictly controlled caloric restriction.[13] There were significant reductions in all lipid parameters (Table 1). Seventeen young individuals (18 to 37 years) with normal plasma lipids and no family history of heart disease were recruited as non-CAD controls. Eleven of the CAD patients and 10 of the non-CAD controls are African-Americans. Ethnic background did not affect the overall results and was not used to distinguish the subjects in subsequent analyses. All participants provided informed consent

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Table 1. Clinical Characteristics of the Subjects (mean ± SD)

Characteristic	CAD	CAD-Postrehabilitation	Non-CAD
No. of subjects (males/females)	28 (20/8)	17 (12/5)	17 (9/8)
Age (yr)	57 ± 6	57 ± 6	22 ± 4
Cholesterol (mg/dL)	248 ± 64.7	207 ± 47.5	157 ± 25.9
TG (mg/dL)	319 ± 214.1	195 ± 75.2	81.3 ± 33.7
HDL-C (mg/dL)	29 ± 5.4	37 ± 4.6	41 ± 4.8
AAb (mg/dL)	89.5 ± 77.9	87.3 ± 70.5	105.7 ± 64.7
IC (mg/dL)	75.1 ± 55.2	74.2 ± 47.9	82.5 ± 53.5

NOTE. There were statistical differences in all parameters between the CAD and non-CAD control groups ($P < .01$ by 2-sample t test).

according to the guidelines of the Human Investigations Committee at Emory University.

Preparation of MDA-LDL

A stock MDA solution was prepared by mixing 0.6 mL MDA-acetal with 1.9 mL 0.2-mol/L HCl. The mixture was allowed to incubate for 10 minutes at 37°C. The reaction was stopped by the addition of 1.4 mL 1-mol/L KOH, and the mixture was adjusted to pH 7.4 before adjustment to a final vol of 5 mL with distilled H_2O. This stock solution was prepared fresh and used on the same day. Pooled plasma was used to isolate LDL at density 1.020 to 1.063 g/mL by sequential ultracentrifugation using a fixed-angle rotor (Beckman 50.4Ti; Beckman Instruments, Fullerton, CA). The isolated LDL was dialyzed against 0.15 mol/L NaCl containing 0.01% EDTA (pH 7.4). Freshly prepared MDA solution was added to LDL at 120 µL/mg LDL protein, and the mixture was allowed to incubate at 37°C for 2.5 hours. The mixture was subsequently extensively dialyzed against phosphate-buffered saline (PBS) containing 1 mmol/L EDTA and subjected to centrifugation at 12,000 rpm for 10 minutes to remove any aggregate. The modified LDL was used to coat 96-well ELISA plates that can be stored at −20°C for up to 8 weeks.

Calibration of the ELISA System

A plasma pool that was prepared from fresh plasma obtained in a group of 100 free-living individuals participating in a worksite cholesterol screening program was assigned an arbitrary level of 100 AU/dL and designated as the primary calibrator. Three separate plasma pools were prepared according to the ODs obtained from the application of a 1:500 dilution in wells previously coated with MDA-LDL.

ELISA for AAbs Against MDA-LDL

The plates were coated with 100 µL MDA-LDL (100 µg/mL) diluted in 50 mmol/L PBS (containing 0.1 mmol/L NaCl, and 0.001 mol/L EDTA, pH 7.4) for 16 hours at 4°C. After extensive washing (borate buffer with 0.1% Tween-20), the plates were blocked with a solution of human serum albumin (3 mg/mL) diluted in PBS (16 hours at 4°C). Dilutions of plasma (typically 1:500) were applied to the plate (100 µL) in triplicate and allowed to incubate for 16 hours at 4°C. Detection was achieved with a 1:2,000 dilution of a commercially available goat anti-human immunoglobulin G conjugated with alkaline phosphatase (BioRad Laboratories, Richmond, CA).

Using the primary pool with an assigned concentration of 100 AU/dL as a calibrator, the AAb levels (mean ± SD) in the standard, high control (QC1), and low control (QC2) are 167 ± 10.5, 73.4 ± 9.8, and 43.7 ± 3.9 AU/dL, respectively. In all subsequent analyses, dilutions of the standard pool were used to construct a standard curve and the QC1 and QC2 pools were included as quality controls. The standard, QC1, and QC2 pools were stored at −80°C in small aliquots and thawed once for each assay and discarded. When a new pool had to be used for standard, AAb levels in this pooled sample were defined against the standard currently in use and the primary calibrator to ensure comparability of the assays.

ELISA for Soluble Immune Complexes

For this assay, we were interested specifically in the LDL-IgG immune complexes (ICs). The capture antibody is a specific goat IgG isolated by immunoaffinity chromatography against human LDL, and the detection antibody is a commercially available alkaline phosphatase–coupled goat IgG against human IgG. For this assay, a solution of milk protein (2 mg/mL) was used as the blocking agent. Standardization of this assay is similar to the process described for the AAb assay using the same plasma pools as standard and quality controls. The mean concentration in the standard, QC1, and QC2 pools were 91.3 ± 12.8, 106.4 ± 15.0, and 49.5 ± 6.4 AU/dL.

Oral Fat Load Protocol

Patients with documented CAD were admitted to the General Clinical Research Center (GCRC) on the afternoon before the study was scheduled. They received a standardized low-fat dinner at 6 PM and remained fasted until 8 AM the next morning. Control participants were asked to consume their usual dinner before 6 PM and report to the GCRC by 7 AM on the day of the study. The standardized test meal consisted of a fruitshake prepared with frozen orange juice, nonfat yogurt, sugar, Lipomul (Upjohn, Kalamazoo, MI), and the yolks from 2 medium hard-boiled eggs.[14] Blood samples were collected in EDTA every hour after ingestion of the meal. The plasma was isolated within 1 hour and several aliquots were made. All plasma samples were stored at −70°C in 0.5-mL aliquots until analysis (3 to 6 months). Only the fasting, 2-hour, 4-hour, and 6-hour samples that were never thawed and refrozen were available for analysis in the present study.

Data Analysis

All statistical analyses were performed using SigmaStat (Jandel Scientific, San Rafael, CA). A simple paired t test was used to compare changes in AAb levels at different times following the fat load and baseline levels. Theoretically, only the baseline and 4-hour sample would require analysis to demonstrate the difference in the postprandial-induced oxidative response between CAD and non-CAD subjects. ANOVA (2-factor without replication) was also used to relate the change in AAb levels with time in different subjects.

RESULTS

Validation of the ELISA for AAb and IC Determinations

Figure 1A illustrates the linearity for the standard curve available for the AAb assay. The interassay and intraassay coefficients of variation were 3.5% and 5.1%, respectively. Figure 1B illustrates the reproducibility of circulating plasma levels of AAb in a group of non-CAD individuals in whom 2 separate blood collections were available within a 2-week period. These individuals were asked to maintain their normal routine between the two visits. The slope of the linear regression was 0.93 with an intercept of 6.42 AU/dL ($R^2 = .992$).

Since fresh MDA-LDL was required every 2 to 3 weeks, it was necessary to demonstrate that the current procedure provides the same AAb levels for the QC samples independent of the batch of pooled LDL and MDA solution. Three separate preparations of MDA-LDL using different pooled plasma for LDL isolation and freshly prepared MDA solution were analyzed for AAb levels over a 9-week period. Each preparation of MDA-LDL was used in 15 different plates during this period.

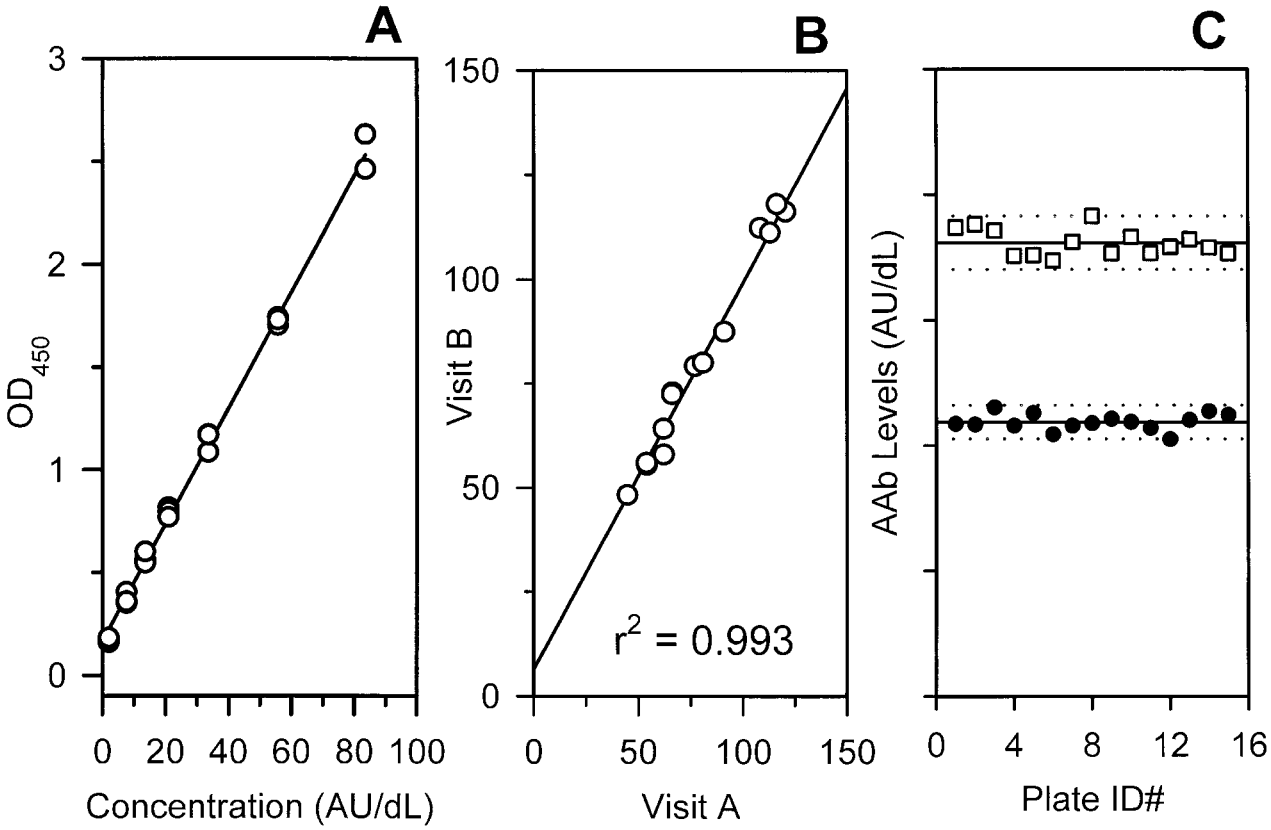

Fig 1. Determination of AAb levels by ELISA. (A) Standard curve for the level of AAbs against MDA-LDL in the control plasma with a concentration of 167 mg/dL. (B) AAb levels for a group of healthy non-CAD controls as determined from frozen plasma collected on 2 separate occasions exactly 2 weeks apart. (C) Reproducibility of the low (●) and high (□) QCs for AAb levels. Each point represents the mean of triplicate determinations on the same plate. Approximately 3-5 plates were analyzed each week. Solid lines represent the mean values for all analyses using a given preparation of MDA-LDL, and dotted lines represent the ±2 SD reference lines. Each batch of MDA-LDL was sufficient to coat 15-20 plates to be used within 60 days.

Figure 1C illustrates the reproducibility of the medium (QC1) and low (QC2) controls for a number of consecutive assays using a single preparation of MDA-LDL. The mean AAb levels for the three preparations of MDA-LDL were 74.1 ± 4.75, 73.0 ± 1.77, and 72.2 ± 2.14 AU/dL for QC1. For QC2, the mean AAb levels were 44.0 ± 3.3, 43.9 ± 3.24, and 43.5 ± 1.47 AU/dL.

In view of the wide range of triglyceride (TG) levels in the samples, it was necessary to demonstrate that the degree of lipemia did not affect the determination of AAb levels. Intralipid was added to the medium QC (QC1) to increase TGs from 97 mg/dL to 825 mg/dL, and AAb levels were subsequently determined in the different samples. AAb levels were not affected by TG levels; the mean AAb level was 74.1 ± 4.75 with low TG as compared with 72.6 ± 3.82. Using data from individual participants with TG levels of 35 to 1,107 mg/dL, the mean values for the four quartiles based on TG were as follows: Q1, 62 mg/dL and 90 AU/dL; Q2, 133 mg/dL and 96 AU/dL; Q3, 226 mg/dL and 76 AU/dL; and Q4, 518 mg/dL and 133 AU/dL (TG and AAb, respectively). There was no difference in AAb levels among TG quartiles based on the nonparametric test.

Figure 2 illustrates the effect of storage at −80°C for the determinations of AAb and IC in whole plasma. For this experiment, EDTA plasma samples were obtained from healthy subjects participating in a worksite screening and were analyzed within 24 hours of collection. Aliquots were stored at −80°C for 3 months and reanalyzed using freshly prepared plates. The slope for the linear regression was 0.783 with an intercept of 26.30 AU/dL (R^2 = .763) for AAb measurements (Fig 2A). When the ratio of fresh to frozen values was calculated, the mean ratio was 0.89 ± 0.18 for AAb (Fig 2B), or a mean difference of 11%. From 70 pairs of observations, 3 (4.3%) were beyond 2 SD for AAb levels. The greater difference between fresh and frozen samples is typically observed with samples having higher AAb levels (>150 AU/dL; Fig 2B). Repeated freeze-thaw cycles can also result in greater variability in AAb levels, while a longer storage period (up to 12 months) appears to have no effect in a subset of these samples (data not shown).

In contrast to AAb levels, a single freeze-thaw cycle appears to have a greater effect on the values obtained for IC. The slope of the linear regression between the two determinations was 0.633 with an intercept of 8.16 AU/dL (R^2 = .741; Fig 2C). The mean ratio for IC levels between fresh and frozen samples was 1.37 ± 0.43, or a difference of 37%. Thus, after a 3-month storage at −80°C, the level of LDL-IgG ICs was reduced by 37% as compared with the level in freshly collected plasma. While only 2 of the 70 pairs of observations were beyond 2 SD

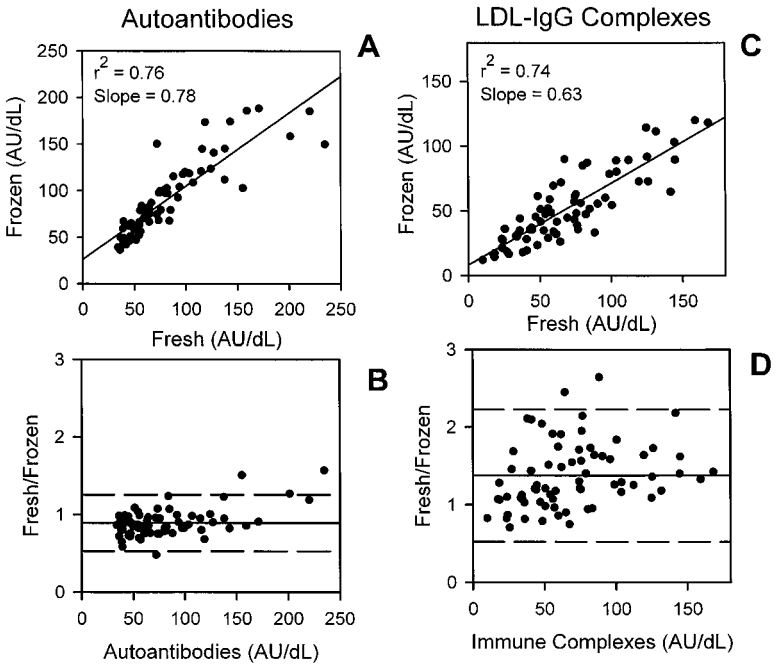

Fig 2. Effect of a single freeze-thaw cycle on AAb and IC levels. For this experiment, freshly collected plasma was obtained over a 4-day period as part of a worksite lipid screening. Aliquots were immediately stored at −80°C. The initial determinations were performed within 24 hours of blood collection, and the frozen samples were thawed after 3 months of storage for reanalysis. (A) Linear regression for AAb levels between the fresh and frozen samples (R^2 = .76). (B) On average, AAb levels in fresh plasma were only 89% of the values obtained in frozen plasma, and the effect of sample storage was small and independent of the actual AAb levels. (C) Linear regression for IC levels between the fresh and frozen plasma samples (R^2 = .74). (D) IC levels were reduced by 73% after 3 months of storage at −80°C and the reproducibility of IC levels in frozen plasma was poor.

for IC, the standard deviation was significantly larger (43%; Fig 2D). The large variability in IC determinations between fresh and frozen plasma would suggest that this assay should not be used with samples that have been previously stored at −80°C. Similar variability was observed with storage at −20°C, and the variability is even greater following repeated freeze-thaw cycles and a longer duration of storage. The present report focuses primarily on the changes in AAb levels.

Acute Changes in AAb Levels During Postprandial Lipemia

Figure 3 illustrates the changes in plasma TGs following consumption of the oral fat load for a group of young healthy controls with normal endothelium (n = 17) and a group of patients with atherosclerotic endothelium (n = 28). In view of the difference in fasting TGs between the two groups, postprandial TGs were normalized to fasting levels for each group. In the subset of 18 CAD patients who were part of the weight loss program, postprandial lipemia was significantly reduced after the intervention[13] and was more comparable to that observed in the control group. Analysis of the area under the curve indicates that the postprandial TG area was significantly greater in CAD patients and was reduced after weight loss.[13]

Table 2 illustrates the mean AAb levels determined in plasma samples collected prior to administration of the fat load for patients with documented CAD and the healthy non-CAD controls. The mean AAb level was lower in CAD patients and was acutely reduced during the postprandial period. Statistical significance was demonstrated at 2 hours ($P < .001$) and 4 hours ($P < .03$) by Wilcoxon signed-rank test (nonparametric). By 6 hours after the meal, the AAb levels tended to return to fasting values, with 10 of 27 subjects reaching pre–fat load levels. To adjust for the large variability in baseline AAb levels among the participants, postprandial changes were also expressed in terms of the normalized ratio using the individual's own AAb level prior to the oral fat load. Figure 4A illustrates

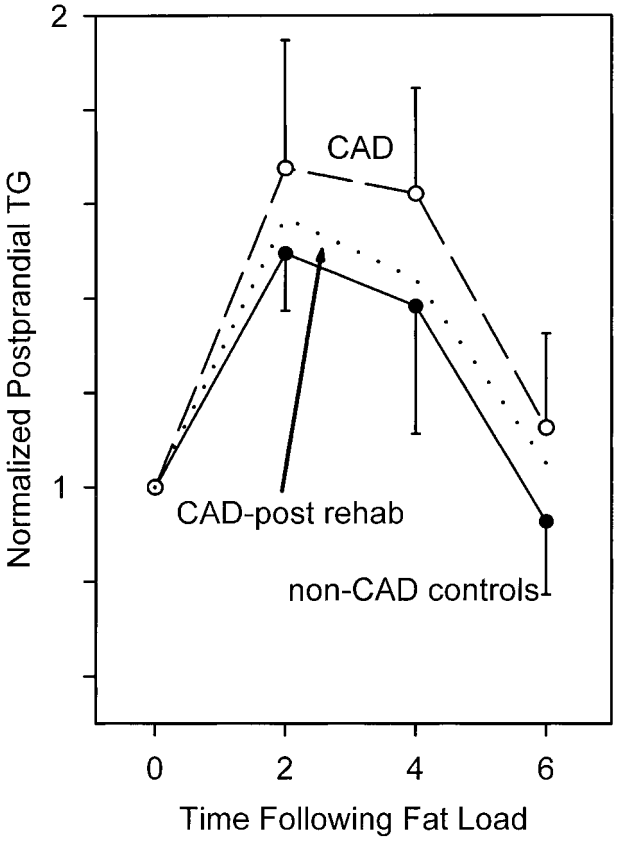

Fig 3. Changes in plasma TG during postprandial lipemia. Plasma TGs at different time points during postprandial lipemia were normalized to fasting levels for non-CAD controls (●) and patients with documented CAD (○). The dotted line represents the normalized postprandial response in a subset of CAD patients who were restudied after the intervention. The mean values and SD at 2, 4, and 6 hours are presented. The maximum change in TG was observed at 2 hours.

Table 2. Postprandial Changes in Mean AAb Levels (mean ± SD)

	CAD	CAD-Postrehabilitation	Non-CAD
No. of subjects (males/females)	28 (20/8)	18 (10/8)	17 (9/8)
Time			
0 h	89.9 ± 77.9	87.3 ± 70.5	105.7 ± 64.7
2 h	81.5 ± 69.9*	76.9 ± 69.9*	111.9 ± 71.6‡
4 h	80.3 ± 65.1†	78.8 ± 79.0†	111.0 ± 69.4
6 h	82.7 ± 66.6	80.2 ± 72.9	114.0 ± 75.7

*$P < .001$, †$P < .01$, ‡$P < .051$: compared with the fasting sample ($t = 0$).

the transient reduction in normalized AAb levels for the group of patients with documented CAD. The mean normalized levels were 0.91 ± 0.06, 0.90 ± 0.08, and 0.94 ± 0.09 at 2, 4, and 6 hours after the fat load, respectively. Using normalized data, statistical significance was noted at 2 hours ($P < .001$) and 4 hours ($P < .01$) as compared with the fasting levels. At 6 hours postprandially, the AAb levels were not statistically different versus the fasting levels. In a subset of these patients (n = 18), we had the opportunity to challenge them again with the same oral fat load after a 6-month program of rehabilitation involving aerobic exercise and diet.[13] Figure 4B demonstrates that the comparable transient reduction in AAb levels during postprandial lipemia was observed after the intervention in these CAD patients. ANOVA for 2 factors (subject and time) without replication also indicated a statistical difference from baseline for both study periods.

Table 2 also presents the mean AAb levels at different times during the postprandial period for the 18 non-CAD controls. AAb levels obtained at 2, 4, and 6 hours were slightly higher than the fasting level, but the difference between fasting levels and 2-hour values did not reach statistical significance ($P < .051$) by Wilcoxon signed-rank test (Fig 5). In a subset of these individuals (n = 6), a second oral fat challenge was available using a test meal that included 4 eggs instead of 2, resulting in greater increases in postprandial TG; postprandial AAb levels were not transiently reduced (data not shown).

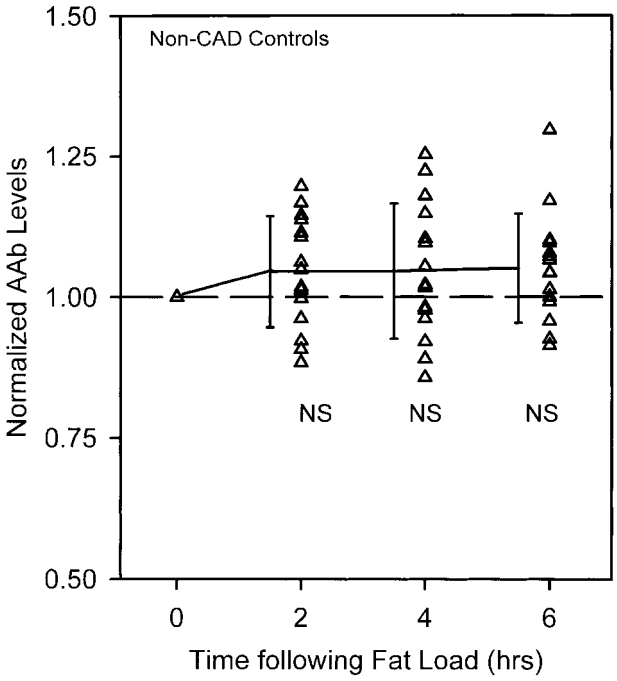

Fig 5. Postprandial changes in AAb levels in non-CAD controls. AAbs against MDA-LDL at different time points during postprandial lipemia were normalized to the level measured in the fasted state. Individual normalized values for all subjects are presented (△), as well as the mean value (–––) for all subjects and the SD at each time point. A statistically significant difference *v* fasting samples could not be demonstrated at any time point throughout postprandial lipemia.

DISCUSSION

While it is believed that LDL can undergo oxidative modification in vivo, the metabolic response to this process is not clear. It is possible that the presence of these modifications may elicit the formation of AAbs for the binding and removal of these damaged proteins via the scavenger pathway. Using glycosylated LDL in the guinea pig, Witztum et al[15] have elegantly demonstrated such a response. This would be consis-

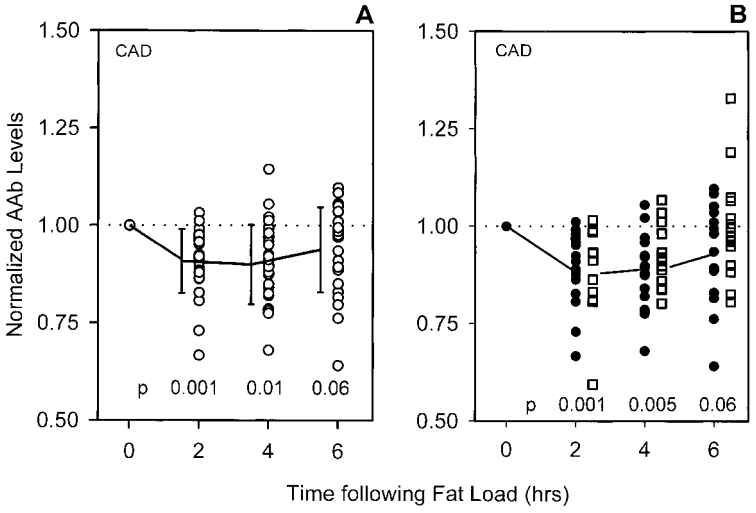

Fig 4. Postprandial changes in AAb levels in CAD. AAbs against MDA-LDL at different time points during postprandial lipemia were normalized to the level measured in the fasted state. (A) Individual normalized values for all patients with documented CAD (○) and mean values (–––) at each time point and the SD. Statistical significance was demonstrated at both 2 hours ($P < .001$) and 4 hours ($P < .01$). (B) Normalized values for a subset of 17 patients with documented CAD who participated in a second postprandial study. Comparable changes in normalized values were obtained before (●) and after a 6-month weight loss program (□) at 2, 4, and 6 hours postprandially.

tent with a protective role of the immune system. Data that demonstrate significant reductions in lesion areas in cholesterol-fed rabbits previously immunized with oxidatively modified LDL also support the protective role of AAbs.[16,17]

The presence of IgG-LDL complexes both in the circulation[10,18,19] and in the atherosclerotic plaques[1,6] would suggest that impaired removal of these ICs in some clinical states may actually contribute to the disease process. It is not clear whether the oxidatively modified lipoproteins[3-6] or IgG against these modified epitopes[6] are the first to be deposited in atherosclerotic lesions. The ICs may also accumulate in the lesions after antigen-antibody binding has occurred in the circulation. Independent of the actual sequence of the immune processes, it is clear that soluble AAbs against oxidatively modified epitopes are present and the level of circulating AAbs would be expected to change in response to the oxidative modifications that occur in the vascular space. Preliminary data from our laboratory would suggest that the levels of AAbs were not affected by interventions that decreased either fasting cholesterol[20] or fasting TG.[13,21] The protective value of soluble AAb against oxidative stress may not be the steady-state level measured at any given time of the day, but instead how the daily processes can modulate it.

To address this possibility, we used the acute oxidative stress associated with postprandial lipemia to examine the dynamics between oxidatively modified epitopes and circulating AAbs against MDA-LDL in subjects with normal and atherosclerotic endothelium. One group included patients with severely atherosclerotic vessels as evidenced by previous myocardial infarction and/or coronary bypass surgery. The control group consisted of healthy young normal subjects with no family history of heart disease and was selected to be the group most likely to have normal endothelium. This is the first demonstration of transient changes in the circulating level of AAbs triggered by a specific physiologic intervention. All participants received a single test meal that provided 26 g polyunsaturated fat and remained fasted for the subsequent 6 hours in the present study. By providing a large influx of polyunsaturated fatty acids, we expect to enhance the formation of oxidatively modified epitopes within the vascular space. This acute burst of free radicals and oxidized products, in turn, should affect the levels of circulating AAbs and ICs. Following consumption of the fat-containing meal, plasma TG levels reached a maximum at approximately 2 hours postprandially in both groups (Fig 3). In patients with documented CAD, the 9.8% reduction in AAb levels also occurred at 2 hours. For the same fat intake, healthy non-CAD controls had no change in AAb levels during postprandial lipemia (Fig 5). We hypothesize that the transient reduction in the level of soluble AAb against oxidatively modified epitopes in CAD subjects is due to an excess generation of oxidized products from the interaction of TG-rich intestinal lipoproteins with the diseased endothelium. In non-CAD controls, it is possible that with the same amount of dietary fat, there were fewer oxidatively modified products generated. This transient reduction in the level of soluble AAb was not related to lipid levels in fasting plasma or postprandial plasma. Following a 6-month intervention based on lifestyle modification, significant changes in lipid parameters toward the normal range were achieved, including reductions in cholesterol by 15%, in TG by 40%, and in postprandial lipemia by 30% and a 9% increase in HDL cholesterol. Comparable reductions in the level of AAb against MDA-LDL were observed transiently during the repeat postprandial study in these patients with documented CAD.

In both groups of subjects, we failed to demonstrate any changes in the levels of soluble ICs. However, additional studies in freshly collected plasma would be required in view of the fact that our data indicate that the levels of soluble ICs are not stable in samples stored at −80°C for extended periods.

While the CAD patients and non-CAD controls included in the present study were not matched for lipid levels, the available data would indicate that these factors do not affect the level of AAb in fasting plasma.[20,21] In a group of hypercholesterolemic patients with CAD in a multicenter trial with pravastatin, there was no change in AAb levels over a 3-year follow-up period despite a 27% reduction in LDL cholesterol.[20] In a subset of the CAD patients, repeated postprandial studies following significant reductions in TG (40%) and LDL (15%) with caloric restriction and aerobic exercise demonstrated comparable transient reductions in AAb levels (Fig 4B). This is the first report demonstrating that AAb levels can be affected acutely during food consumption. These observations were reproducible in the same subjects with either the same fat load after a lipid-lowering intervention or a fat load that provides a larger amount of fat and cholesterol (data not shown). It is our hypothesis that the transient reduction in AAb levels observed in CAD patients is associated with excess generation of oxidized epitopes by the diseased endothelium.

We cannot rule out the possibility that the difference in the AAb response to the fat load is associated with age. The control group represents primarily young individuals aged 18 to 24 years with 2 individuals who are slightly older, 31 and 32 years. The mean age of the CAD group was 59 years, with a range of 52 to 67 years. However, it is our hypothesis that the key difference in the two groups is the presence of an atherosclerotic endothelium and a healthy normal endothelium. In view of the recent report from Fukumoto et al,[22] the inclusion of an older group of non-CAD individuals as controls may have an impact on our finding. In this later study of 446 healthy men and women, the intima-media thickness of the carotid artery was correlated with age, suggesting that there might be impaired endothelium in some older subjects.[22]

The present data would support the transfer of reactive oxygen species or other oxidizing agents to TG-rich lipoproteins as these particles come in close contact with the endothelium for interactions with lipolytic enzymes. As these TG-rich lipoproteins are sequentially converted to LDL, oxidative modifications may spread to the apolipoprotein moiety, resulting in the formation of permanent protein epitopes. Clearly, the residence time of 2 to 4 hours required for the conversion of TG-rich lipoproteins to LDL may be adequate to propagate the oxidative process once the oxidation-initiating element has been transferred to the lipoprotein. In the atherosclerotic vessel wall, one may find an excess of these oxidizing agents, resulting in the transfer of multiple modified epitopes to the lipoprotein particles. Unless antioxidants are available to scavenge these oxidation-initiating elements and protect the lipoproteins, the net result will be the irreversible modification of the protein moiety.

Since polyunsaturated fatty acids are more susceptible to lipid peroxidation,[22] the beneficial effect of this dietary recommendation may be associated with the enhanced removal of oxidatively modified products by soluble AAb. This would be similar to the protective role of exercise, which is beneficial despite exposing the body to a high level of oxidative stress.[23] This interpretation would also be consistent with data in animal studies regarding the protective effect of immunization with oxidized LDL.[16,17] Furthermore, the capacity of the body's immune system to remove intermittently appearing oxidatively modified epitopes may be more important in predicting risk than the steady-state levels of AAbs against modified lipoproteins. Additional studies would be required to confirm this hypothesis.

Another issue raised by the present short-term study is the importance of fasting plasma in the determination of the level of AAbs against oxidatively modified LDL. The findings from previous studies have been contradictory. Some studies reported higher AAb levels in high-risk populations such as patients with documented CAD and patients with diabetes mellitus compared with healthy controls,[8-10] but other studies found lower levels in the patients.[10-11] If the blood collection protocol does not account for the type of meal consumed prior to sample collection, differences in the postprandial response could affect the actual measured AAb levels. Many of these high-risk patient populations have been demonstrated to have abnormal postprandial lipemia.[24-26] The difference in the level of AAbs against oxidatively modified LDL may thus be explained, in part, by the postprandial status of the participants with documented CAD.[26]

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