## PRE-CLINICAL RESEARCH

# Antisense Oligonucleotide Lowers Plasma Levels of Apolipoprotein (a) and Lipoprotein (a) in Transgenic Mice

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**Objectives**     This study sought to assess whether an antisense oligonucleotide (ASO) directed to apolipoprotein (a) [apo(a)] reduces apo(a) and lipoprotein (a) [Lp(a)] levels in transgenic mouse models.

**Background**     Elevated Lp(a) is a causal, independent, genetic risk factor for cardiovascular disease and myocardial infarction. Effective therapies to specifically lower plasma Lp(a) levels are lacking.

**Methods**     Three transgenic mouse models were utilized: 8K-apo(a) mice expressing 8 kringle IV (KIV) repeats with a single copy of KIV-2; 8K-Lp(a) mice expressing both the 8K apo(a) plus human apolipoprotein B-100; and 12K-apo(a) mice expressing a 12K apo(a) with 3 KIV-2 repeats. The mice were treated intraperitoneally with saline, a control ASO, or ASO 144367 directed to KIV-2 for 4 to 6 weeks. Apo(a), Lp(a), and oxidized phospholipids present on human apoB (OxPL/h-apoB) or apo(a) [OxPL/apo(a)] were measured at baseline and on and off therapy.

**Results**     ASO 144367 significantly reduced Lp(a) by 24.8% in 8K-Lp(a) mice, and reduced apo(a) levels by 19.2% in 8K-Lp(a) mice, 30.0% in 8K-apo(a) mice, and 86% in 12K-apo(a) mice; ASO 144367 also significantly reduced OxPL/apoB 22.4% in 8K-Lp(a) mice, and OxPL/apo(a) levels by 19.9% in 8K-Lp(a) mice, 22.1% in 8K-apo(a) mice, and 92.5% in 12K-apo(a) mice (p < 0.004, or less, for all). No significant changes occurred in Lp(a), apo(a), OxPL/apoB, or OxPL/apo(a) levels with control ASO or saline.

**Conclusions**     This study documents the first specific therapy, to our knowledge, for lowering apo(a)/Lp(a) levels and their associated OxPL. A more potent effect was documented in mice expressing apo(a) with multiple KIV-2 repeats. Targeting liver expression of apo(a) with ASOs directed to KIV-2 repeats may provide an effective approach to lower elevated Lp(a) levels in humans.     (J Am Coll Cardiol 2011;57:1611–21) © 2011 by the American College of Cardiology Foundation

Lipoprotein (a) [Lp(a)] represents a unique class of lipoproteins that are composed of apolipoprotein (a) [apo(a)] covalently bound to apolipoprotein B-100 (apoB) by a

disulfide bond (1). Apo(a) expression leading to Lp(a) formation is restricted to humans, apes, and Old World monkeys and an unrelated version in European hedgehogs (2). Elevated Lp(a) levels (>25 to 30 mg/dl) are present in up to 30% of whites and 70% of blacks (3). Despite having been discovered nearly 50 years ago, a physiological function for Lp(a) has not been identified. Plasma Lp(a) concentrations are primarily determined by the *LPA* gene locus and are only minimally affected by environmental factors (4). The *LPA* gene encodes for repeating apo(a) subunits called kringles (K), and includes 10 unique copies of KIV, which additionally includes variable numbers of identical KIV-2 repeats (from 3 to >40), KV, and a protease-like domain that is catalytically inactive. Most subjects have 2 distinct apo(a) alleles, which vary in size due to kringle copy number, resulting in a marked size heterogeneity of the expressed apo(a) protein.

Lp(a) levels are generally inversely associated with apo(a) size, and the variation in kringle number results in a 1,000-fold (<0.1 to >250 mg/dl) range of plasma Lp(a) levels among individuals, unlike most proteins where normal levels may vary by <3- to 5-fold.

Lp(a) has proatherogenic properties due to its low-density lipoprotein (LDL) component, but the apo(a) moiety may confer additional proinflammatory and proatherogenic properties (1). In addition, Lp(a) may be prothrombotic by inhibiting fibrinolysis because of its high homology to plasminogen and its enhancement of platelet aggregation (5). After a large series of studies, our group has proposed the hypothesis that some of the atherogenic properties of Lp(a) may be mediated by its unique ability among lipoproteins to bind proinflammatory oxidized phospholipids (OxPL) (6,7). Elevated Lp(a) levels in humans are associated with increased risk of cardiac death, myocardial infarction, stroke, and peripheral arterial disease, particularly in subjects with small apo(a) isoforms (8–10). Recently, genetic association studies with Mendelian randomization approaches have clearly demonstrated that Lp(a) is a strong, causal, genetic risk factor in myocardial infarction and coronary artery disease (11). In fact, its association with coronary artery disease was the strongest in a study evaluating 48,742 single nucleotide polymorphisms (SNPs) in 2,100 candidate genes, including a higher odds ratio than the previously reported 9p21 SNP (12).

Apo(a) is synthesized primarily in the liver, where it is subsequently covalently linked to apoB-100 to form the Lp(a) lipoprotein. Effective therapeutic modalities to reduce Lp(a) levels in humans, and particularly drugs that specifically target only Lp(a), are lacking. Antisense oligonucleotides (ASO) are emerging as viable therapeutic agents to treat disorders where overexpression of proteins is associated with a disease process. In the current study, we hypothesized that an ASO targeted to apo(a) would reduce Lp(a) levels and their accompanying OxPL in apo(a)/Lp(a) transgenic mice.

## Methods

**Antisense oligonucleotides.** ASO 144367 is targeted to position 174 within KIV-2 of the apo(a) transcript and is a 5-10-5 MOE gapmer, a similar class ASO as mipomersen, which is directed to human apolipoprotein B-100 (apoB) (13). It is composed of 20-nucleotide phosphorothioate oligonucleotides with 5 2′-O-(2-methoxyethyl) modified ribonucleosides (2′-MOE) at the 3′ and 5′ ends with 10 2′-deoxynucleosides in-between. The sequence of ASO 144367 is 5′-GGCAGG-TCCTTCCTGTGACA-3′. ASO 144367 does not hybrid-

ize with plasminogen mRNA. ASO 141923 (5′-*CCTTC*-CCTGAAGGTT*CCTCC*-3′), which is the same chemical class as ASO 144367 but does not hybridize to apo(a) mRNA or to murine or human apoB-100, was used as a control.

**Transgenic mice.** We used 3 unique transgenic mouse models overexpressing apo(a), which are defined by the number of Kringle (K) IV repeats, to assess the safety and efficacy of ASO 144367.

**8K-APO(A) MICE.** This mini apo(a) construct contains the apoE promoter, 8 KIV repeats (KIV-1, a single copy of KIV-2, a fusion of KIV-3 and KIV-5, and KIV-6 to KIV-10), KV, and the protease-like domain. The generation of 8K-apo(a) mice on a C57BL6/SJL background was previously described (14,15).

**8K-LP(A) MICE.** The 8K-Lp(a) mice were generated by crossing hemizygous 8K-apo(a) mice with hemizygous mice overexpressing human apoB-100, as previously described (14,15).

**12K-APO(A) MICE.** The generation of 12K-apo(a) mice on an FVB background overexpressing a natural 12KIV apo(a) isoform was previously described (16). This construct contains a 270-kb yeast artificial chromosome with a human genomic DNA clone containing the intact *LPA* gene and a 70-kb *LPA*-like gene with extensive 60-kb flanking regions on both sides. 12K-Lp(a) mice were not available for this study.

All murine protocols used in this study were approved by the University California, San Diego Institutional Animal Care and Use Committee (IACUC).

**Treatment protocol.** EFFECT OF ASO 144367 ON LIVER MRNA APO(A) EXPRESSION. Liver mRNA apo(a) expression was evaluated in 8 8K-Lp(a) mice (4 received ASO 144367 and 4 saline), in 7 8K-apo(a) mice (4 received ASO 144367 and 3 saline) with a dose of 25 mg/kg intraperitoneally twice weekly for 4 weeks and in 9 12K-apo(a) mice (3 received ASO 144367, 3 received control ASO, and 3 saline) at 50 mg/kg intraperitoneally once weekly for 4 weeks.

EFFECT OF ASO 144367 ON LIVER AND KIDNEY FUNCTION TESTS AND HISTOLOGY. Laboratory tests including liver and kidney function tests using commercial assays, and histologic analysis of the liver and kidney with hematoxylin and eosin were performed on the 12K-apo(a) mice and controls.

EFFECT OF ASO 144367 ON APO(A) AND LP(A) LEVELS AND OXIDIZED PHOSPHOLIPIDS ON APOB AND APO(A). After establishing that ASO 144367 reduced liver apo(a) expression in all 3 transgenic models, a study was initiated to assess its effect on plasma apo(a) and Lp(a) levels. ASO 144367 was injected intraperitoneally (25 mg/kg in sterile PBS) twice weekly for 6 weeks into 8K-apo(a) mice and 8K-Lp(a) mice (n = 21 total, 11 and 10 in each group, respectively). Similarly, control ASO 141923 was injected into 8K-apo(a) mice and 8K-Lp(a) mice (n = 20 total, 10 in each group). Treatment was given for 6 weeks, and the mice were observed off treatment for another 6 weeks. Blood samples were collected at 0, 3 and 6, 9 and 12 weeks.

To assess whether the efficacy of ASO 144367 was dependent on the number of KIV repeats or the presence of a genomic versus a cDNA construct, we also performed a similar study in the 12K-apo(a) mice by injecting them intraperitoneally (50 mg/kg per week in sterile PBS) on weeks 0 to 3 and obtaining blood samples at weeks 0, 2, and 4. This dosing strategy was given for convenience as there are no pharmacokinetic or pharmacodynamic differences after 2 weeks of dosing with this regimen compared with 25 mg/kg twice weekly (data not shown).

**RNA and reverse transcription PCR analysis.** Total RNA was extracted from whole liver tissue and primary hepatocytes (Qiagen RNeasy, Valencia, California) as previously described (17). The apo(a) primers used were 5′-CCACAGTGGCCCCGGT-3′ and 5′-ACAGGG-CTTTTCTCAGGTGGT-3′ with the fluorescent probe 5′-CCAAGCACAGAGGCTCCTTCTGAACAAG-3′. The mouse apoB-100 primers were: 5′-CGTGGGCTC-CAGCATTCTA-3′ and 5′-AGTCATTTCTGCCTT-TGCGTC-3′ with fluorescent probe 5′-CCAATGGT-CGGGCACTGCTCAA-3′. Expression values were normalized to cyclophilin A. DNA was isolated using Qiagen DNeasy tissue kit.

**Determination of total cholesterol, triglycerides, Lp(a), and apo(a) levels.** Total cholesterol and triglycerides were determined by commercial enzymatic assays using the Roche Cobas Mira Plus Analyzer (Roche, Indianapolis, Indiana). Plasma Lp(a) levels were measured by a sandwich ELISA, as previously described (15). A detailed description of the methodology is included in the Online Appendix.

**Determination of OxPL on human apoB-100, on apo(a), and mouse apoB, and determination of apo(a) on mouse apoB.** Chemiluminescence ELISA was used to measure the amount of OxPL present on various lipoproteins, as previously described (15). A detailed description of the methodology is included in the Online Appendix.

**Statistical analysis.** Differences between the groups were assessed by Student *t* test. Analysis of quantitative parameters within groups of mice over the time course of the studies was performed with repeated measures analysis of variance (ANOVA). Parametric versus nonparametric tests were performed depending on whether the data were normally distributed or not normally distributed, respectively. Post-test analysis was performed for changes within individual time points with the Bonferroni or Dunn multiple comparison test. Data represent means $\pm$ SD in the text and as mean $\pm$ SEM in graphs. $p < 0.05$ was considered significant. Statistical analysis was performed with GraphPad InStat, version 3.02 (GraphPad Software, La Jolla, California).

## Results

**Baseline lipid and OxPL profiles of the 8K-apo(a) and 8K-Lp(a) transgenic mice.** The lipid profiles of these transgenic mouse lines were previously described (14,15). In the current study, the cholesterol, triglyceride, Lp(a), apo(a)

levels, and OxPL on human apoB (h-apoB) and mouse apoB and on apo(a), as well as apo(a) on mouse apoB, are shown in Table 1. It was noted that 8K-Lp(a) mice have higher total cholesterol and triglyceride levels than 8K-apo(a) mice, as previously described (14,15). The median (range) of Lp(a) levels in the 8K-Lp(a) mice chosen for this study was 35.5 mg/dl (5.1 to 111 mg/dl). There were no differences in apo(a) levels between the groups. The OxPL/h-apoB levels were in the lower range of what we described recently (15), as this group of mice had lower overall Lp(a) levels. OxPL/apo(a) levels were similar between groups, but there was more apo(a) and OxPL associated with the murine apoB in the 8K-apo(a) mice than in the 8K-Lp(a) mice, consistent with the fact that apo(a) binds noncovalently to mouse apoB (14,15). The lipid profiles of the 12K-apo(a) mice were previously described (18).

**The effect of ASO 144367 on liver mRNA apo(a) expression.** The effect of ASO 144367 on hepatic apo(a) mRNA expression in the 8K-Lp(a) mice and 8K-apo(a) mice was found to be similar, and therefore, the data were combined. Compared to saline-injected controls, ASO 144367 reduced hepatic apo(a) mRNA expression by 52% (p = 0.018) (Fig. 1A). In the 12K-apo(a) mice, there was a more potent effect with a 87% reduction (p = 0.012) compared with saline (Fig. 1B). The control ASO had no effect on apo(a) mRNA expression. There was no effect of ASO 144367 on hepatic mouse apoB mRNA expression or mouse plasma apoB levels (data not shown).

**Safety parameters.** There were no differences in body weight or weights of the liver, kidney, and spleen in the 12K-apo(a) mice treated with saline, ASO control, and ASO 144367 (data not shown). The Online Appendix describes laboratory parameters (Online Table 1) and histological analysis (Online Figure 1) of the liver and kidney, showing no signs of liver or kidney toxicity.
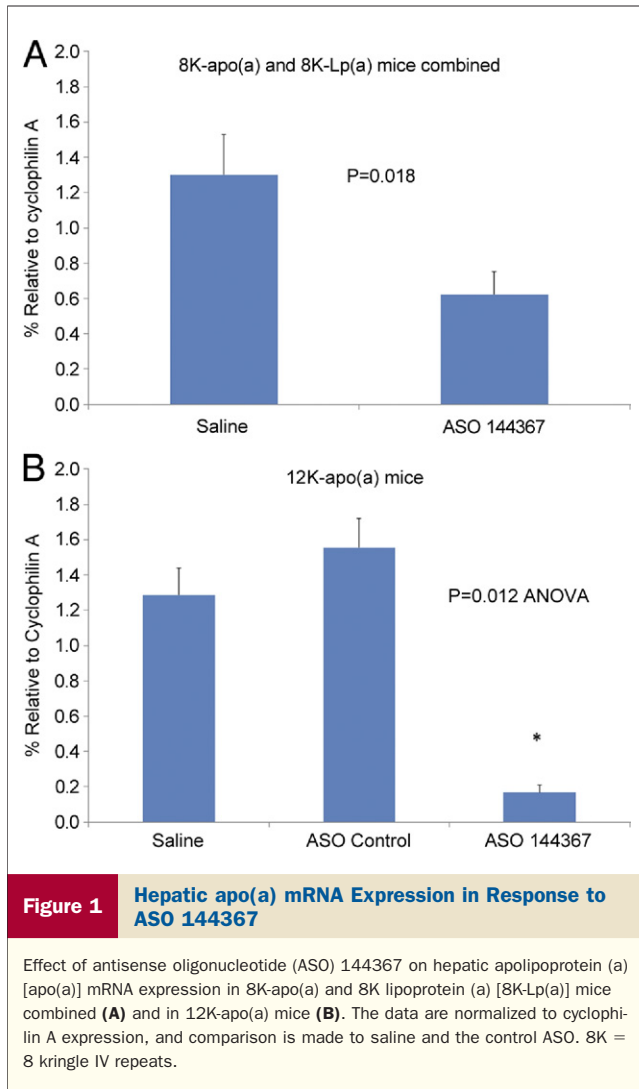
**The effect of ASO 144367 on plasma apo(a) and Lp(a) levels in 8K-Lp(a) mice and 8K-apo(a) mice.** Table 2 displays the changes in total cholesterol, triglycerides, Lp(a),

| Table 1 | Baseline Levels of Lipid and Oxidation Variables | | |
|---|---|---|---|
| | **8K-apo(a) Mice (n = 21)** | **8K-Lp(a) Mice (n = 20)** | **p Value** |
| Total cholesterol, mg/dl | 124.3 ± 27.2 | 179.2 ± 25.6 | <0.001 |
| Triglycerides, mg/dl | 101.9 ± 45.2 | 146.4 ± 33.9 | 0.001 |
| Lp(a), mg/dl | N/A | 43.2 ± 33.1 | N/A |
| Lp(a), mg/dl | N/A | 35.5 (5.1–111.0) | N/A |
| Apo(a), mg/dl | 303 ± 229 | 260 ± 250 | 0.70 |
| Apo(a), mg/dl | 178 (38–753) | 186 (36–1080) | 0.70 |
| OxPL/h-apoB, RLU | N/A | 82,209 ± 49,370 | N/A |
| OxPL/m-apoB, RLU | 21,257 ± 14,224 | 10,893 ± 15,573 | <0.001 |
| OxPL/apo(a), RLU | 75,555 ± 38,622 | 66,399 ± 40,452 | 0.41 |
| Apo(a)/m-apoB, RLU | 4,620 ± 2,723 | 1,273 ± 2,985 | <0.001 |
| Apo(a)/m-apoB, ratio | 1.72 ± 0.99 | 0.40 ± 0.86 | <0.001 |

Values are mean ± SD or median (range).

8K = 8 kringle IV repeats; apo(a) = apolipoprotein (a); h-apoB = human apolipoprotein B; Lp(a) = lipoprotein (a); m-apoB = mouse apolipoprotein B; N/A = not applicable; OxPL = oxidized phospholipids; RLU = relative light units.

Effect of antisense oligonucleotide (ASO) 144367 on hepatic apolipoprotein (a) [apo(a)] mRNA expression in 8K-apo(a) and 8K lipoprotein (a) [8K-Lp(a)] mice combined **(A)** and in 12K-apo(a) mice **(B)**. The data are normalized to cyclophilin A expression, and comparison is made to saline and the control ASO. 8K = 8 kringle IV repeats.

and apo(a) levels in 8K-Lp(a) mice and 8K-apo(a) mice in response to ASO 144367 or ASO control. Over the 6-week treatment period, total cholesterol levels were reduced modestly in both 8K-Lp(a) mice and 8K-apo(a) mice treated with ASO 144367, but not with ASO control. Small, but statistically significant reductions in total cholesterol were also noted in 8K-apo(a) mice treated with ASO control.

In 8K-Lp(a) mice, significant reductions in response to ASO 144367 were noted in both Lp(a) (p < 0.001 by ANOVA) and apo(a) levels (p = 0.027 by ANOVA) at 3 and 6 weeks, which then returned toward normal once therapy was stopped. In contrast, no significant changes were noted in response to ASO control. Similarly, in 8K-apo(a) mice, significant reductions were noted in apo(a) levels (p < 0.001 by ANOVA) at 3 and 6 weeks, which then returned to normal once therapy was stopped. No significant changes were noted in response to ASO control.

We also evaluated the data as the mean percentage change from baseline. By 6 weeks, ASO 144367 lowered Lp(a) levels by 24.8% (p < 0.0001) in 8K-Lp(a) mice (Fig. 2A), and apo(a) levels by 31.7% (p < 0.001) in

8K-Lp(a) mice and 30.0% (p = 0.001) in 8K-apo(a) mice (Fig. 2B). Lp(a) and apo(a) levels returned to baseline by 3 to 6 weeks off treatment.

**The effect of ASO 144367 on OxPL on h-apoB and apo(a) particles.** In 8K-Lp(a) mice, OxPL/h-apoB levels (as relative light units [RLU]) were reduced by 16.8% at 3 weeks and 22.4 % at 6 weeks, and then returned to baseline by 9 to 12 weeks (p = 0.002 by ANOVA) (Fig. 3A). Similarly, OxPL/apo(a) levels (as RLU) decreased 12.1% at 3 weeks and 19.9% at 6 weeks, and then returned to baseline by 9 to 12 weeks (p < 0.001 by ANOVA) (Fig. 3B). No significant changes were noted with ASO control.

In 8K-apo(a) mice, OxPL/h-apoB levels are not measurable as they do not have human apoB. OxPL/apo(a) levels (as RLU) decreased 22.1% at 3 weeks and 11.8 % at 6 weeks, and then returned to baseline by 9 to 12 weeks (p = 0.015 by ANOVA) (Fig. 3B). No significant changes were noted with ASO control.

**The effect of ASO 144367 on apo(a) and OxPL associated with mouse apoB in 8K-Lp(a) mice and 8K-apo(a) mice.** As noted in Table 1, the 8K-apo(a) mice have higher apo(a)/m-apoB ratios than the 8K-Lp(a) mice, as a greater proportion of apo(a) associates with mouse apoB, in contrast to the 8K-Lp(a) mice where it preferentially associates with human apoB as part of the Lp(a) lipoprotein. We evaluated the mean percentage change in apo(a)/m-apoB and OxPL/m-apoB in response to ASO 144367 or control ASO. Figure 4A demonstrates that apo(a)/m-apoB levels were significantly reduced in 8K-apo(a) mice and 8K-Lp(a) mice treated with ASO 144367 but not control ASO. In parallel with these changes, the OxPL/m-apoB levels also were significantly reduced in the 8K-apo(a) mice (Fig. 4B).

**The effect of ASO 144367 on apo(a), OxPL/apo(a), apo(a)/m-apoB, and OxPL/m-apoB levels in 12K-apo(a) mice.** Table 3 displays the data on changes in apo(a), OxPL/apo(a), apo(a)/m-apoB, and OxPL/m-apoB levels in the 12K-apo(a) mice and demonstrates significant and robust reductions in all parameters in response to ASO 144367, whereas no significant changes were noted with saline or ASO control. The data were also evaluated as mean percentage change from baseline (Fig. 5), demonstrating an 86% reduction in apo(a), 93% reduction in OxPL/apo(a), 82% reduction in apo(a)/m-apoB, and 64% reduction in OxPL/m-apoB (all statistically significant by ANOVA and with post-test analysis).

## Discussion

This is the first study, to our knowledge, to demonstrate a specific therapy for lowering apo(a) and Lp(a) levels using an ASO that prevents synthesis of apolipoprotein (a) by binding to a region on KIV-2 apo(a) mRNA. The findings were consistent across 3 unique apo(a)/Lp(a) transgenic mouse models expressing apo(a) with a variable number of KIV-2 repeats. More potent reduction in hepatic apo(a) mRNA (87%) and apo(a) plasma levels (86%) was noted in

| Table 2 | Lipoprotein Profiles of 8K-Lp(a) and 8K-apo(A) Mice Following Treatment With ASO 144367 | | | |
|---|---|---|---|---|
| **8K-Lp(a) Mice** | **TC** | **TG** | **Lp(a)** | **apo(a)** |
| ASO 144367 | | | | |
|   Baseline | 186.4 ± 20.3 | 159.4 ± 32.9 | 45.2 ± 38.9 | 297 ± 327 |
|   3 weeks | 156.2 ± 23.4* | 167.1 ± 56.8 | 34.1 ± 27.7† | 201 ± 157 |
|   6 weeks | 144.4 ± 20.7† | 179.3 ± 61.6 | 33.0 ± 27.6* | 191 ± 163 |
| Off therapy | | | | |
|   9 weeks | 150.3 ± 19.2† | 183.9 ± 100.1 | 41.3 ± 31.0 | 263 ± 226 |
|   12 weeks | 160.2 ± 21.6* | 190.2 ± 73.5 | 42.9 ± 32.5 | 263 ± 229 |
|   p-ANOVA | <0.001 | 0.28 | <0.001 | <0.05 |
| ASO control | | | | |
|   Baseline | 171.9 ± 29.3 | 133.3 ± 31.1‡ | 41.2 ± 28.2 | 226 ± 145 |
|   3 weeks | 175.2 ± 22.0 | 139.2 ± 47.2 | 43.1 ± 28.8 | 253 ± 161 |
|   6 weeks | 172.4 ± 16.7 | 138.0 ± 42.6 | 42.9 ± 26.5 | 242 ± 166 |
| Off therapy | | | | |
|   9 weeks | 166.9 ± 15.8 | 177.1 ± 56.3† | 44.6 ± 30.0 | 251 ± 184 |
|   12 weeks | 185.9 ± 38.9 | 161.6 ± 48.3 | 44.0 ± 31.3 | 223 ± 145 |
|   p-ANOVA | 0.010 | <0.001 | 0.76 | 0.57 |
| **8K-apo(a) mice** | **TC** | **TG** | | **apo(a)** |
| ASO 144367 | | | | |
|   Baseline | 140.4 ± 23.6 | 101.0 ± 31.5 | | 384 ± 242 |
|   3 weeks | 122.1 ± 20.2 | 119.6 ± 51.6 | | 276 ± 159† |
|   6 weeks | 116.9 ± 19.8* | 141.6 ± 46.4 | | 272 ± 189* |
| Off therapy | | | | |
|   9 weeks | 111.2 ± 16.8† | 141.6 ± 100.1 | | 323 ± 374 |
|   12 weeks | 114.5 ± 16.7* | 124.0 ± 53.7 | | 374 ± 224 |
|   p-ANOVA | <0.001 | 0.13 | | <0.001 |
| ASO control | | | | |
|   Baseline | 109.7 ± 22.0 | 102.7 ± 56.4 | | 230 ± 199 |
|   3 weeks | 106.7 ± 18.6 | 108.7 ± 41.4 | | 266 ± 241 |
|   6 weeks | 105.4 ± 17.7 | 91.5 ± 33.5 | | 260 ± 204 |
| Off therapy | | | | |
|   9 weeks | 97.4 ± 21.1† | 96.4 ± 34.0 | | 225 ± 169 |
|   12 weeks | 98.5 ± 18.0 | 109.3 ± 38.4 | | 163 ± 131 |
|   p-ANOVA | <0.01 | 0.59 | | 0.65 |

Values are in mg/dl and represent mean ± SD. p-ANOVA represents repeated measures analysis of variance (ANOVA) for the 5 time points for each variable. *p < 0.01, †p < 0.001, and ‡p < 0.05 compared with baseline values within each group with Bonferroni multiple comparisons test (10 comparisons).

ASO = antisense oligonucleotide; TC = total cholesterol; TG = triglycerides; other abbreviations as in Table 1.

mice overexpressing a human apo(a) genomic construct with 3 KIV-2 repeats compared with a mini apo(a) cDNA construct with only 1 KIV-2 repeat. Concomitantly with a reduction in apo(a) and Lp(a) levels, OxPLs associated with circulating apo(a)/Lp(a) were reduced in relative proportion to the lowering of apo(a)/Lp(a), consistent with prior observations that Lp(a) is a carrier of proinflammatory OxPLs (6,14,19).

There has been considerable controversy about the potential atherogenicity of Lp(a), which was driven by many factors, including nonstandardized methodologies for measuring Lp(a) levels, issues of sample storage, and inadequate study design. However, recent meta-analyses (8) and large clinical studies (9,11,12), some of which included Mendelian randomization approaches that allow analyses of risk based on genetic differences in lifelong Lp(a) levels, have clearly documented that Lp(a) is a causal, independent,

genetic risk factor for myocardial infarction and cardiovascular disease. The future challenge is to demonstrate that Lp(a) is a modifiable risk factor. To accomplish this, specific therapeutic agents that selectively lower plasma Lp(a) levels need to demonstrate clinical benefit.

Apolipoprotein (a) is primarily synthesized in the liver, and although all of the pathways are not fully delineated, it is thought to then bind extracellularly in the space of Disse to newly synthesized apoB-100 to create Lp(a) particles. Several of the synthetic steps in generation of Lp(a) have been previously delineated in cell culture (20). It is well appreciated that small apo(a) isoforms, which are more rapidly synthesized, are associated with higher Lp(a) particle number in plasma and therefore higher plasma Lp(a) levels. In contrast, larger apo(a) isoforms take longer to synthesize in the hepatocyte, and therefore, their production rates are lower, generally leading to lower plasma levels. In patients with elevated triglyceride levels,
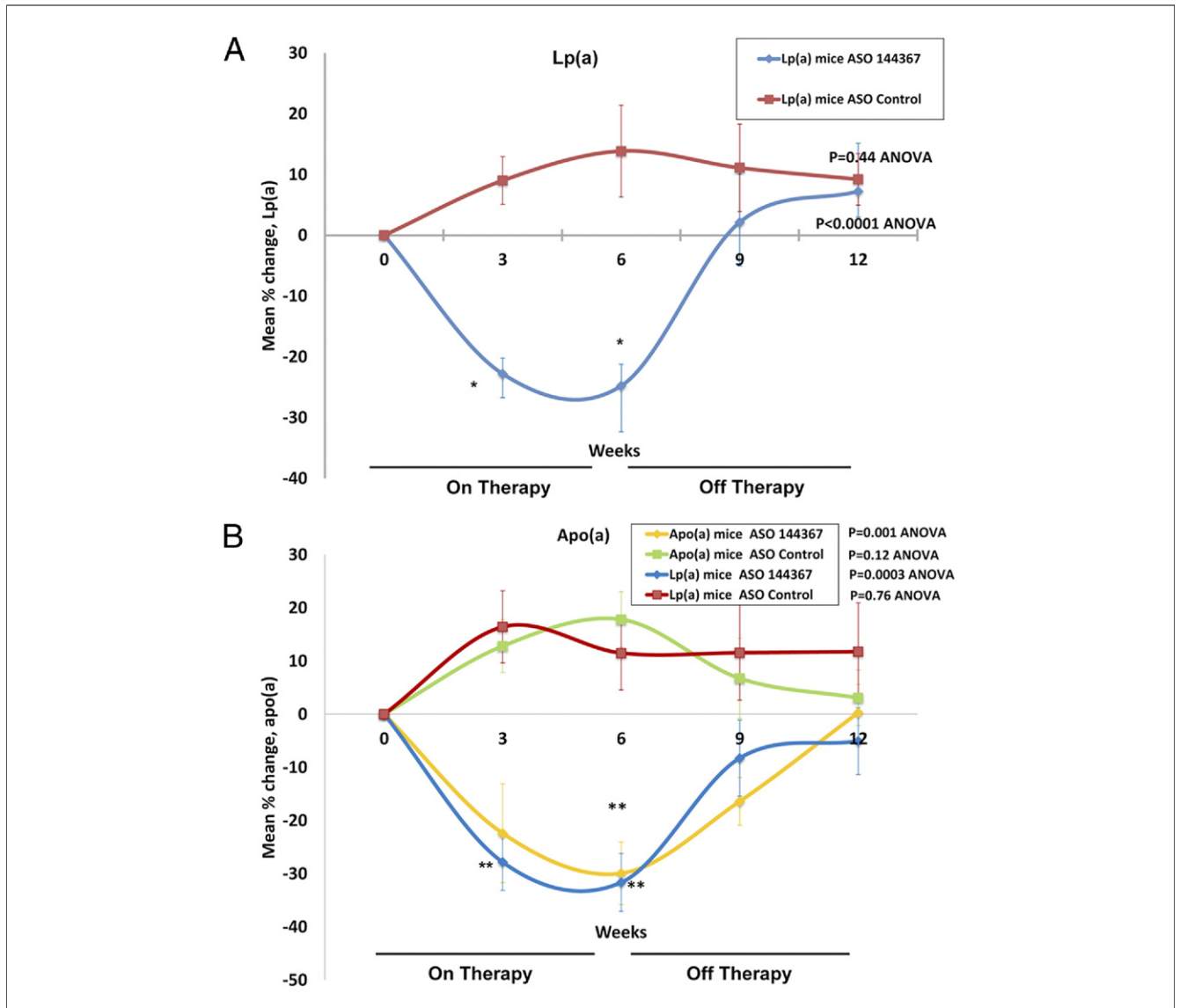
**Figure 2    Changes in apo(a) and Lp(a) Levels in Response to ASO 144367**

**(A)** Mean percentage change in Lp(a) levels in 8K-Lp(a) mice in response to ASO 144367 or ASO control. **(B)** Mean percentage change in total apo(a) levels in 8K-Lp(a) mice and 8K-apo(a) mice in response to ASO 144367 or ASO control. *p < 0.05 and **p < 0.01 for Bonferroni within-group post-test compared to baseline. Abbreviations as in Figure 1.

up to 5% of the apo(a) can be associated with large very-low-density lipoprotein particles. Humans generally have no free apo(a) circulating in plasma, as plasma apoB-100 synthesis is in excess of synthesis of apo(a).

The clearance of Lp(a) is not well understood, although it does not appear to involve the LDL receptor and is not dependent on kringle copy number (20). Murine studies have suggested the existence of a yet to be identified apo(a) receptor in the liver (21). In addition, impaired renal mechanisms may also play a role leading to elevated Lp(a) levels, but in these patients, there is accumulation of large but not small apo(a) isoforms. In such patients, a small proportion of free apo(a) (∼5% of total) can be found in the plasma. Furthermore, in some patients in whom Lp(a) is

not measurable in plasma, apo(a) degradation products generated by proteases and elastases may be found in the urine, suggesting that there is metabolism of apo(a) that mediates its clearance. Because Lp(a) is not normally present in small animal models, it has been difficult to fully evaluate the syntheses and clearance mechanisms of Lp(a), and further work in this area is needed to fully understand all the implications for development of therapeutic agents.

In addition to the size heterogeneity of apo(a) isoforms influencing plasma Lp(a) levels, it has been recently appreciated that additional genetic differences may play a role (12,22,23). For example, although it is well known that the size heterogeneity of the KIV-2 repeats is a direct mediator of Lp(a) levels, it explains <50% of the variability. Recent
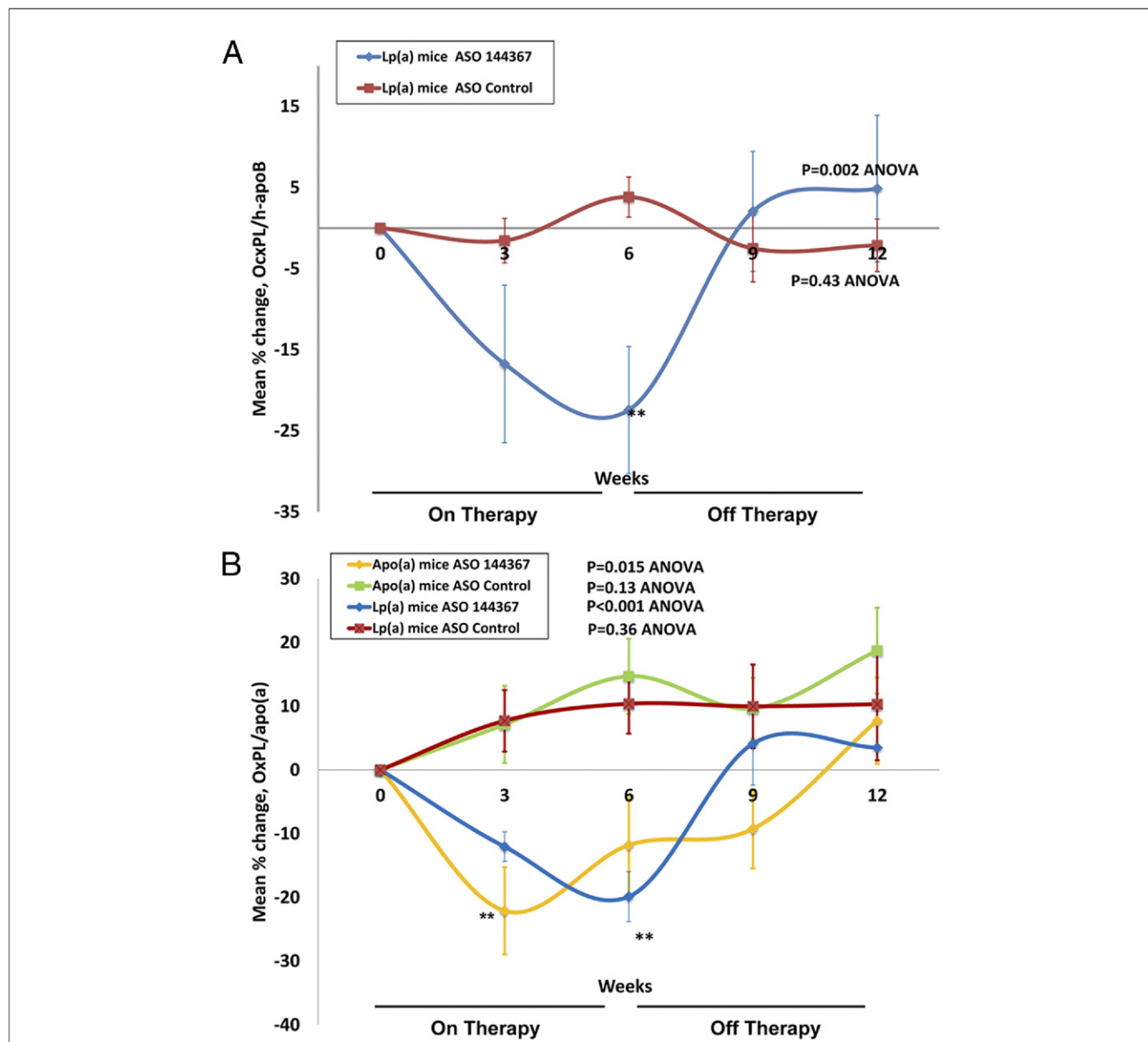
**Figure 3    Changes in OxPL/h-apoB and OxPL/apo(a) in Response to ASO 144367**

**(A)** Mean percentage change in OxPL/h-apoB levels in 8K-Lp(a) mice in response to ASO 144367 or ASO control. **(B)** Mean percentage change in OxPL/apo(a) levels in 8K-Lp(a) mice and 8K-apo(a) mice in response to ASO 144367 or ASO control. *p < 0.05 and **p < 0.01 for Bonferroni within-group post-test compared to baseline. H-apoB = human lipoprotein B; OxPL = oxidized phospholipids; other abbreviations as in Figure 1.

genetic studies have reported several SNPs that explain an additional 35% of the variability in plasma Lp(a) levels. In particular, SNPs rs3798220 and rs10455872 have been associated with both increased Lp(a) levels and an increased risk for coronary artery disease (12,22,23). There are also other SNPs that have been previously identified that can result in increased Lp(a) levels (4). However, the underlying mechanisms by which these SNPs mediate elevated Lp(a) levels are not known, and further research is needed to assess how they may impact increased synthesis or delayed catabolism of Lp(a).

This study advances the concept that ASOs directly targeting apo(a) mRNA synthesis are able to specifically lower plasma apo(a)/Lp(a) levels. Furthermore, it suggests that ASOs directed to the multiple identical KIV-2 repeats are more potent, possibly due to targeting multiple sites on apo(a) mRNA. Additionally, one may postulate that genomic apo(a) constructs, which more closely reflect human apo(a), appear to be optimal substrates for terminating mechanisms as they require post-transcriptional modification in the nucleus to remove noncoding regions. This editing requires longer nuclear residence time, which is
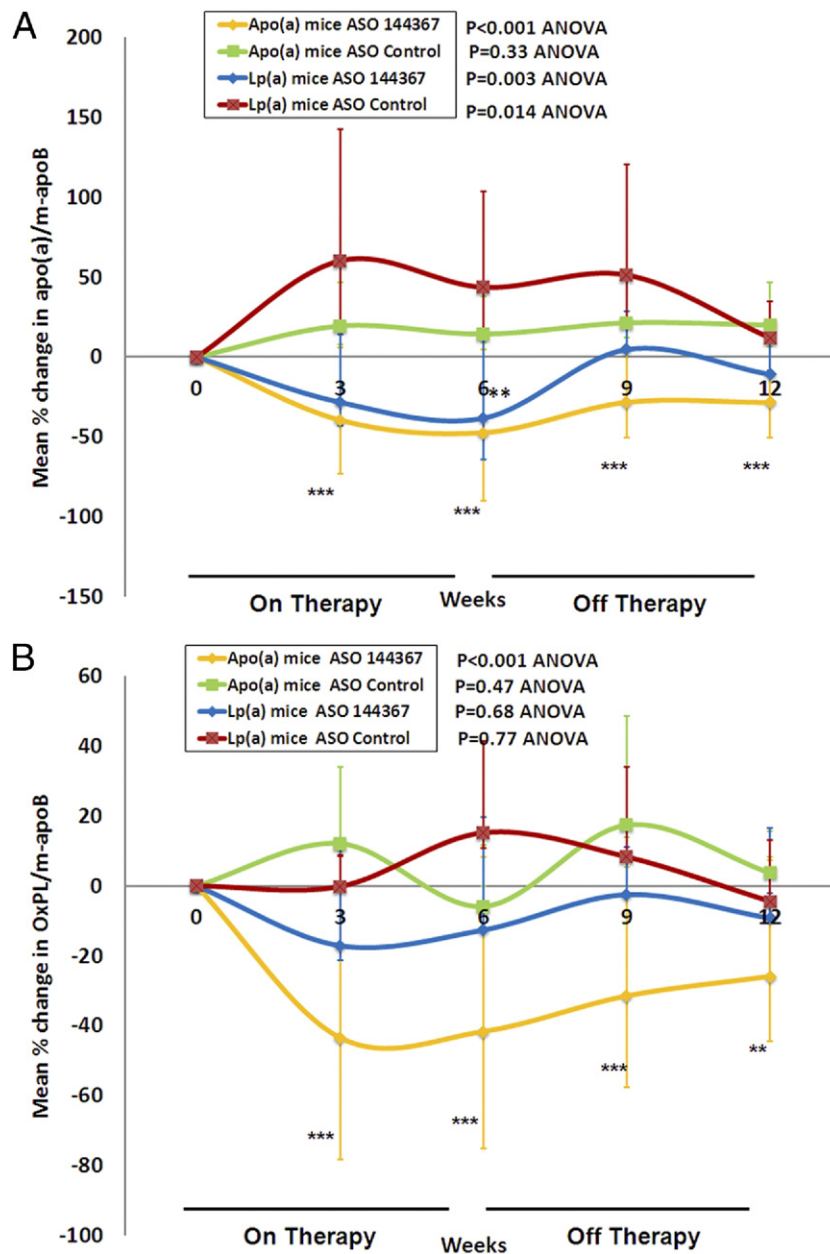
**Figure 4    Changes in apo(a)/m-apoB and OxPL/m-apoB in Response to ASO 144367**

**(A)** Mean percentage change in apo(a)/m-apoB levels in 8K-apo(a) mice and 8K-Lp(a) mice in response to ASO 144367 or ASO Control. **(B)** Mean percentage change in OxPL/m-apoB levels in 8K-apo(a) mice and 8K-Lp(a) mice in response to ASO 144367 or ASO control. **p < 0.01 and ***p < 0.001 for Bonferroni within-group post-test compared to baseline. Abbreviations as in Figures 1 and 3.

optimal for RNaseH activity. In contrast, cDNA constructs do not require this process and are much more rapidly exported in the cytoplasm, where RNaseH is not present (24).

This study complements a recent study where we demonstrated significant reductions in Lp(a) levels using mipomersen (15), an ASO directed to human apoB-100 that is currently being evaluated to lower LDL levels in human subjects. In that study (15), mipomersen resulted in a

~90% reduction in human apoB levels and a corresponding 75% reduction in Lp(a) levels in 8K-Lp(a) mice. This was mediated by a different mechanism, however, due exclusively to a marked reduction of human apoB-100 levels without affecting apo(a) mRNA synthesis or plasma levels.

In the current study, there was a modest reduction in total cholesterol levels in the mice treated with ASO 144367, but the mechanism of this lowering is not yet established. We

| Table 3 | Apo(a), OxPL/apo(a), apo(a)/m-apoB, and OxPL/m-apoB Levels in 12K-apo(a) Mice | | | |
|---|---|---|---|---|
| | Apo(a) | OxPL/apo(a) | apo(a)/m-apoB | OxPL/m-apoB |
| **Apo(a) mice saline** | | | | |
| Baseline | 91,112 ± 11,506 | 34,438 ± 3,520 | 61,243 ± 7,436 | 19,036 ± 1,841 |
| 2 weeks | 104,911 ± 10,483 | 48,159 ± 14,011 | 89,401 ± 13,915 | 31,478 ± 13,915 |
| 4 weeks | 72,173 ± 21,551 | 24,236 ± 11,212 | 65,093 ± 11,584 | 23,847 ± 7,462 |
| p-ANOVA | 0.08 | 0.19 | 0.11 | 0.36 |
| **Apo(a) mice ASO control** | | | | |
| Baseline | 74,935 ± 10,318 | 27,229 ± 2,735 | 75,403 ± 9,362 | 21,268 ± 2,621 |
| 2 weeks | 78,473 ± 8,854 | 33,229 ± 4,931 | 7,8141 ± 9,902 | 25,468 ± 2,616 |
| 4 weeks | 67,414 ± 42,663 | 25,867 ± 15,186 | 80,867 ± 6,028 | 24,093 ± 3,891 |
| p-ANOVA | 0.87 | 0.70 | 0.76 | 0.26 |
| **Apo(a) mice ASO 144367** | | | | |
| Baseline | 76,983 ± 14,598 | 32,736 ± 13,163 | 70,835 ± 7,423 | 28,003 ± 2,573 |
| 2 weeks | 17,580 ± 11,162* | 5,734 ± 3,633† | 16,189 ± 9,024* | 12,249 ± 2,616‡ |
| 4 weeks | 11,060 ± 7,137* | 2,556 ± 1,751† | 13,019 ± 9,911* | 9,965 ± 907‡ |
| p-ANOVA | 0.004 | 0.017 | 0.001 | 0.0001 |

Apo(a) values are in RLU and represent mean ± SD. p-ANOVA represents repeated measures ANOVA for the 3 time points for each variable.
*p < 0.01, †p < 0.05, and ‡p < 0.001 compared with baseline values within each group with Bonferroni multiple comparisons test (3 comparisons).
Abbreviations as in Tables 1 and 2.

also demonstrate that the OxPL content of both apoB-100 and Lp(a) particles was diminished following treatment with ASO 144367, a similar finding previously described with mipomersen (15). This is consistent with the fact that Lp(a) is particularly enriched in OxPL, which can be bound both covalently to apo(a) and in the lipid phase of Lp(a), although the relative proportions of these are not yet fully delineated (6). In agreement with this, the content of noncovalently bound apo(a) to mouse apoB, and its OxPL content, was also significantly reduced with ASO 144367.

Despite the fact that Lp(a) is a carrier of OxPL and that these 2 parameters correlate highly when measured in plasma in human populations as a whole, data from the EPIC (European Prospective Investigation into Cancer and Nutrition)-Norfolk population demonstrate that measuring both OxPL/apoB and Lp(a) in patients gives additive information regarding risk of future coronary events compared with measuring each alone (25). One potential explanation for this is derived from the Dallas Heart Study, where we showed that the correlation between OxPL/apoB and Lp(a) was strongest when Lp(a) levels were high in the setting of small apo(a) isoforms, but the correlation was weak in patients with low Lp(a) levels and large apo(a) isoforms (7). Because most patients (80%) have 2 distinct apo(a) isoforms and since the Lp(a) levels and the OxPL/apoB measures reflect the net effect of these factors, it is possible the OxPL/apoB measure may closely reflect the cumulative atherogenicity of small Lp(a) particles. This was also demonstrated in the Mayo Clinic study, where we showed that OxPL/apoB was an independent predictor of the presence of angiographically defined coronary artery disease in a multivariable adjusted model that also contained Lp(a) levels (26). Furthermore, subjects with

SNP rs3798220 have increased plasma levels of OxPL/apoB, in a setting of elevated Lp(a) levels and small isoforms (27). These relationships make a plausible pathophysiological model in which the oxidized phospholipid content of Lp(a) appears to be a key component of the atherogenic risk of Lp(a), along with other potential mechanisms already described (20). Mechanistic support of these clinical observations of the atherogenicity of Lp(a) and its associated OxPL was recently provided by the Tabas laboratory in collaboration with our group, where it was demonstrated that Lp(a) and the OxPL that it binds promote apoptosis in endoplasmic reticulum–stressed macrophages by activating an ERK-NADPH oxidase–oxidative stress pathway through a CD36 and toll-like receptor 2 (TLR2) pathway (28).

## Conclusions

We demonstrate the first specific therapy for lowering apo(a) mRNA expression in the liver and apo(a)/Lp(a) levels in plasma. These data also suggest that targeting a kringle repeat sequence may result in optimal apo(a) lowering in humans. Assuming that such ASOs are ultimately approved for clinical investigation, this study demonstrates in principle that such an approach could be used in patients to test the hypothesis that lowering plasma Lp(a) levels reduces cardiovascular events. For example, one could select patients with increased cardiovascular risk and elevated Lp(a) levels (i.e., >30 mg/dl [or >75 mmol/l]) and randomize them to ASO therapy versus placebo and evaluate cardiovascular imaging and clinical endpoints. This study may set the stage for future translational research for developing and testing novel therapeutic agents to lower the atherogenic potential of Lp(a).
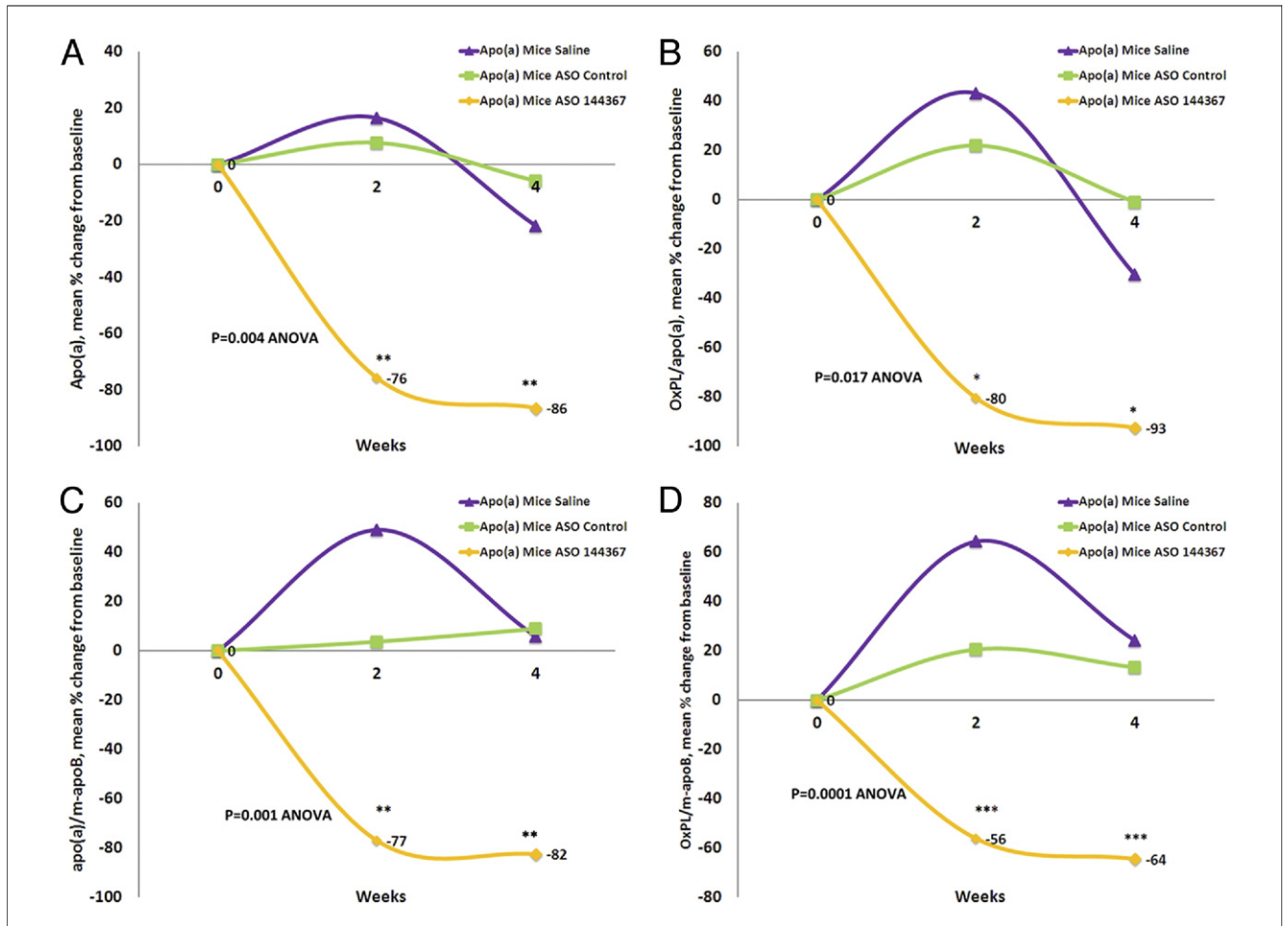
**Figure 5    Changes in apo(a), OxPL/apo(a), apo(a)/m-apoB, and OxPL/m-apoB Levels**

Mean percentage change in apo(a) levels **(A)**, OxPL/apo(a) **(B)**, apo(a)/m-apoB **(C)**, and OxPL/m-apoB **(D)** in 12K-apo(a) mice in response to ASO 144367, ASO control, or saline. *p < 0.05, **p < 0.01, and ***p < 0.001 for Bonferroni within-group post-test compared with baseline. Only the mice treated with ASO 144367 had significant changes over the 4-week time course. Abbreviations as in Figures 1 and 3.

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**Key Words:** antisense oligonucleotides ■ atherosclerosis ■ lipoproteins ■ lipoprotein (a) ■ oxidation ■ oxidized phospholipids.

▶ **APPENDIX**

**For a supplemental figure, methods, and table, please see the online version of this article.**