Lipoprotein Subclass Profiles of Hyperlipidemic Diabetic Mice Measured by Nuclear Magnetic Resonance Spectroscopy

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Dyslipidemia accelerates vascular complications of diabetes. Nuclear magnetic resonance (NMR) analysis of lipoprotein subclasses is used to evaluate a mouse model of human familial hypercholesterolemia +/- streptozotocin (STZ)-induced diabetes. A double knockout (DKO) mouse (low-density lipoprotein receptor [LDLr] -/-; apolipoprotein B [apoB] mRNA editing catalytic polypeptide-1 [Apobec1] -/-) was studied. Wild-type (WT) and DKO mice received sham or STZ injections at age 7 weeks, yielding control (WT-C, DKO-C) and diabetic (WT-D, DKO-D) groups. Fasting serum was collected when the mice were killed (age 40 weeks) for Cholestech analysis (Cholestech Corp, Hayward, CA) and NMR lipoprotein subclass profile. By Cholestech, fasting triglyceride and total cholesterol increased in DKO-C versus WT-C. Diabetes further increased total cholesterol in DKO. High-density lipoprotein cholesterol (HDL-C) was similar among all groups. NMR revealed that LDL in all groups was present in a subclass the size of large human LDL and was increased 48-fold in DKO-C versus WT-C animals, but was unaffected by diabetes. HDL was found in a subclass equivalent to large human HDL, and was similar among groups. In conclusion, NMR analysis reveals lipoprotein subclass distributions and the effects of genetic modification and diabetes in mice, but lack of particles the size of human small LDL and small HDL may limit the relevance of the present animal model to human disease.

IT IS WELL ESTABLISHED that diabetic patients experience vascular complications (accelerated atherosclerosis, retinopathy, nephropathy[1,2]). Dyslipoproteinemia has been implicated in the acceleration of atherosclerosis in diabetes, but the interaction of pre-existing dyslipoproteinemia with hyperglycemia, and the contribution of dyslipoproteinemia to microvascular complications of diabetes are not well understood.[3,4] Some studies have implicated qualitative modification of lipoproteins by glycation and oxidation in vascular injury.[5,6] Others have defined quantitative lipoprotein abnormalities, showing that increased levels of small dense low-density lipoprotein (LDL),[7-9] and of the small subfraction of high-density lipoprotein (HDL)[10] are associated with increased risk for atherosclerosis. A recent nuclear magnetic resonance (NMR) spectroscopy technique[11] provides a convenient and rapid method for characterizing serum lipoprotein size-based subclass profiles. Using this technique, we recently showed that in human type 1 diabetic patients, eye and kidney disease are associated with higher levels of small LDL and small HDL, and lower levels of large LDL and large HDL subclasses.[12,13]

Animal models are in constant use to investigate the pathogenesis of complications of diabetes, but typically, although hyperglycemia is achieved and documented, lipoprotein profiles are ignored. A mouse model of a common human dyslipidemia, hypercholesterolemia, could be useful in assessing the effects of dyslipoproteinemia on the vascular complications of diabetes. However, in the few reported mouse models combining hyperglycemia and hyperlipidemia,[14-17] no detailed characterization of lipoprotein subclass profiles has been undertaken. In the present study, we employed a double knockout (DKO) hyperlipidemic mouse, null for the LDL receptor (LDLr) and for the apolipoprotein (apoB) mRNA editing catalytic polypeptide1 (*Ldlr-/- Apobec1-/-*).[18] This animal expresses high concentrations of cholesterol in apoB100-containing LDL, develops spontaneous and severe atherosclerosis on a normal chow diet, and, as in humans, males develop more severe disease than age-matched females.[18,19] We considered this mouse model potentially useful to assess the interactive effects of hypercholesterolemia and diabetes on lipoprotein subclass profiles and vascular complication status. In the present study, we report results of NMR lipoprotein subclass analysis in this animal model.

MATERIALS AND METHODS

Animals

Hyperlipidemic DKO male mice and their wild-type (WT) (C57BL/6) counterparts were obtained from Genentech (South San Francisco, CA). The DKO mice had been crossed 6 times into the WT background. Half of the mice from each genotype were rendered diabetic with 3 daily doses of streptozotocin (STZ; 200 mg/kg body weight, intraperitoneally) at 7 weeks of age under an approved animal use protocol. Control mice received sham injections. The presence or absence of diabetes was confirmed 1 week after the first injection by determining nonfasting plasma glucose by tail stick. Approximately 10% of the mice required 2 additional STZ injections at the 1-week time point. To be categorized as having diabetes, animals had to have nonfasting plasma glucose levels greater than 275 mg/dL 1 week after STZ injection, or if repeat injections were needed, within 3 weeks of the first injection. The mice were divided into 4 experimental groups: WY nondiabetic control (WT-C, n = 13), DKO nondiabetic control (DKO-C, n = 18), WT diabetic (WT-D, n = 14), and DKO diabetic (DKO-D, n = 14). Diabetic animals were maintained without insulin injections, and all animals were kept on a normal chow diet. The study was terminated when the mice were 40 weeks old, since previous

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Table 1. Body Weight (g) of the Wild-Type and the *Ldlr−/− Apobec−/−* Double Knockout Mice With and Without STZ Injection Over the Duration of the Study

			Ldlr−/− Apobec−/−	
Age (wk)	WT-C (n = 13)	WT-D (n = 14)	DKO-C (n = 18)	DKO-D (n = 14)
7 (pre-STZ injection)	19.9 ± 2.4	20.1 ± 2.7	21.3 ± 1.8	20.9 ± 1.4
8	20.7 ± 1.9	19.0 ± 2.5	21.7 ± 1.9	20.0 ± 1.1†
10	23.3 ± 2.0	20.8 ± 3.0*	24.1 ± 2.4	21.02 ± 1.8‡
18	28.3 ± 2.1	24.7 ± 2.1‡	28.0 ± 2.7	24.6 ± 1.4‡
40 (at sacrifice)	30.8 ± 3.1	20.9 ± 2.9‡	30.8 ± 4.1	22.2 ± 2.6‡

NOTE. Weights are expressed as mean ± SD.

*Significant at $P < .05$, diabetic group *v* corresponding nondiabetic control group.

†Significant at $P < .01$, diabetic group *v* corresponding nondiabetic control group.

‡Significant at $P < .001$, diabetic group *v* corresponding nondiabetic control group.

experience suggested high mortality among DKO animals beyond this age.

Blood Glucose and Conventional Plasma Lipoprotein Profiles

To confirm presence or absence of diabetes, blood glucose (tail stick) was measured using a Glucometer Elite meter (Bayer, Elkhart, IN). To assess lipid profiles, plasma total cholesterol, HDL cholesterol (HDL-C), total triglycerides (TG) (and glucose) were determined by Cholestech L.D.X (Cholestech Corp, Hayward, CA) at age 7 weeks (ie, before STZ injection, nonfasting), at 18 weeks (nonfasting), and at 40 weeks (fasting, at sacrifice). Using these primary lipid measurements, estimates were made of very–low-density lipoprotein cholesterol (VLDL-C) and LDL cholesterol (LDL-C) based on the Friedewald equation[20]: VLDL-C = TG/5; and LDL-C = Total cholesterol − (VLDL-C + HDL-C).

NMR Lipoprotein Subclass Profiles (NMR LipoProfile)

At death (age 40 weeks) after a 4-hour fast (6 AM to 10 AM), 0.5 mL of jugular blood was collected in a 1.5-mL microtube containing glass beads to enhance clotting. Serum was then separated by centrifugation and stored frozen at −80°C until shipped to the laboratories of LipoScience, Inc (Raleigh, NC) for NMR analysis. NMR LipoProfile measurements were conducted using 400-MHz proton NMR analyzers as previously described.[21,22] In brief, the NMR method uses the characteristic signals broadcast by lipoprotein subclasses of different size as the basis of their quantification. Each subclass signal emanates from the aggregate number of terminal methyl groups on the lipids contained within the particle. Cholesterol esters and triglycerides in the particle core each contribute 3 methyl groups, and phospholipids and unesterified cholesterol in the surface shell each contribute 2 methyl groups. To a close approximation, the diameter of the particle determines the number of methyl groups present (and hence, the amplitude of the methyl NMR signal), irrespective of differences in lipid composition arising from, for example, variations in the relative amounts of cholesterol ester and triglyceride in the particle core, varying degrees of unsaturation of the lipid fatty acyl chains, or varying phospholipid composition. For this reason, the methyl NMR signal emitted by each subclass serves as a direct measure of the particle concentration of that subclass.

Mouse serum signals (from 250 μL serum) were acquired in duplicate at 47°C and the composite lipid methyl group signal envelope decomposed by computer analysis to produce the signal amplitudes of 16 subclasses (equivalent in size to human VLDL, intermediate-density lipoprotein [IDL], LDL, and HDL as defined below). Since the NMR properties of mouse and human lipoprotein subclasses are closely similar (unpublished data), the only modification of the human spectral decomposition model needed to analyze mouse spectra was the use of a protein background signal derived from the $d > 1.21$ g/mL bottom fraction of mouse, rather than human, serum. Conversion factors relating the subclass signal amplitudes to particle concentration units were obtained from NMR and chemical lipid analyses of a set of purified subclass standards. These standards were isolated from a diverse group of normo- and dyslipidemic subjects by a combination of ultracentrifugation and agarose gel filtration chromatography, and characterized for size distribution by electron microscopy (VLDL and LDL subclasses) or polyacrylamide gradient gel electrophoresis (HDL subclasses). Particle concentrations (nmol/L or μmol/L) were derived for each subclass standard by measuring the total concentration of core lipid (cholesterol ester plus triglyceride) and dividing the volume occupied by these lipids by the core volume per particle calculated from knowledge of the particle's diameter.[23]

To simplify analyses, the following 10 size-based lipoprotein subclass categories were used (human size equivalents are shown in parentheses): 60 to 200 nm (large VLDL); 35 to 60 nm (medium VLDL); 27 to 35 nm (small VLDL); 23 to 27 nm (IDL); 21.3 to 23 nm (large LDL); 19.8 to 21.2 nm (medium LDL); 18.3 to 19.7 nm (small LDL); 8.8 to 13 nm (large HDL); 8.2 to 8.8 nm (medium HDL); and 7.3 to 8.2 nm (small HDL). Average particle sizes (diameter, nm) of VLDL, LDL, and HDL were computed as the sum of the diameter of each subclass multiplied by its relative mass percentage, as estimated from the amplitude of its NMR signal.

Statistics

For comparisons of mean values between 2 groups, the unpaired *t* test was used. To compare values among the 4 experimental groups, 1-way analysis of variance (ANOVA) and a mean separation procedure was applied. The among-group differences were also evaluated by a 2-way ANOVA, the factors being the presence or absence of STZ-induced diabetes and the DKO (hyperlipidemia) genotype. The possibility of interaction between the 2 factors was tested, and in the absence of interaction, differences with respect to one factor were analyzed independent of the other. All values are reported as mean ± SD, and significance was defined as $P < .05$.

RESULTS

Characteristics of the 2 control groups (WT-C and DKO-C) and the 2 diabetic groups (WT-D and DKO-D), including body weight, mean blood glucose level, and conventional lipid profile as measured by Cholestech L.D.X, are shown in Tables 1 through 3. As early as 1 week after STZ injection, body weights of the diabetic animals were lower than weights of the corresponding nondiabetic control groups (Table 1). By definition,

Table 2. Plasma Glucose Level (mg/dL) in the Wild-Type and the *Ldlr−/− Apobec−/−* Mice With and Without STZ Injection Over the Duration of the Study

			Ldlr−/− Apobec−/−	
Age (wk)	WT-C (n = 13)	WT-D (n = 14)	DKO-C (n = 18)	DKO-D (n = 14)
7 (pre-STZ injection)	150 ± 39	161 ± 43	179 ± 33	164 ± 32
8	116 ± 19	233 ± 127*	140 ± 16	226 ± 62†
10	124 ± 19	342 ± 94†	136 ± 41	324 ± 93†
18	174 ± 32	479 ± 54**	173 ± 32	486 ± 145†
40 (at sacrifice)‡	157 ± 42	457 ± 102†	181 ± 37	484 ± 50†

NOTE. Glucose levels are expressed as mean ± SD.

*Significant at $P < .01$, diabetic group *v* corresponding nondiabetic control group.

†Significant at $P < .0001$, diabetic group *v* corresponding nondiabetic control group.

‡Plasma samples were collected after 4-hour fast.

glucose levels in the diabetic groups were significantly higher than levels in the corresponding control groups starting 1 week after STZ injection (Table 2).

Lipoprotein Profile in the WT-C Mouse

In WT-C animals (using Cholestech L.D.X), HDL-C concentration constituted about 75% of total cholesterol (Table 3). At death, NMR analysis of fasting serum samples collected revealed that the vast majority of the HDL in WT-C mice are in a subclass equivalent in size to large human HDL (Fig 1A-C).

Effect of DKO on the WT Background Lipoprotein Profile

At baseline and throughout the study, plasma total cholesterol, LDL-C, and TG levels were significantly higher in the DKO than in the WT mice, as expected, but there were no differences in HDL-C (Table 3). NMR analysis showed that the hypercholesterolemia of the DKO-C mouse resides mainly in particles of size equivalent to human large LDL (21.3 to 23.0 nm), which were increased 48-fold compared with WT-C mice (Fig 1B). Consistent with this, particles equivalent in size to human LDL (18.3 to 23 nm) were significantly larger in diameter in DKO-C than in WT-C mice (21.8 ± 0.24 nm *v* 20.6 ± 1.1, $P < .0001$) (Fig 2). Therefore in DKO-C versus WT-C animals, high LDL-C resulted from increases in both particle concentration and mean LDL particle size (Table 3).

Compared with WT-C mice, DKO-C mice also had higher levels of particles equivalent in size to human VLDL (27 to 200 nm) (Fig 1A), and human IDL (23 to 27 nm) (both $P < .0001$) (Fig 1B). The increase in concentration of the former was predominantly in the subclass equivalent in size to small human VLDL (27 to 35 nm) (Fig 1A).

In both WT-C and DKO-C mice, lipoproteins equivalent in size to human HDL (7.3 to 13 nm) were present almost entirely in a subclass equivalent in size to large human HDL (8.8 to 13 nm). The molar concentration of these HDL-sized particles did not differ between the 2 groups (Fig 1C), but overall, DKO-C mice had HDL-equivalent particles larger in diameter than WT mice (10.7 ± 0.36 *v* 9.8 ± 0.31 nm, $P < .0001$) (Fig 2).

Table 3. Plasma Lipid Levels in the Wild-Type and the *Ldlr−/− Apobec−/−* Mice With and Without STZ Injection Over the Duration of the Study Measured by Cholestech L.D.X.

				Ldlr−/− Apobec−/−	
Plasma Lipid (mg/dL)	Age§ (wk)	WT-C (n = 13)	WT-D (n = 14)	DKO-C (n = 18)	DKO-D (n = 14)
Total TG	7	66 ± 27	95 ± 37	257 ± 37	244 ± 34
	18	81 ± 24	156 ± 73†	213 ± 30	220 ± 33
	40	55 ± 21	77 ± 53	149 ± 53	140 ± 60
Total cholesterol	7	104 ± 7	105 ± 11	455 ± 84	420 ± 38
	18	102 ± 9	114 ± 17*	417 ± 67	458 ± 60
	40	115 ± 13	115 ± 16	283 ± 25	375 ± 70‡
LDL-C	7	ND	ND	366 ± 69	323 ± 37
	18	ND	ND	330 ± 68	368 ± 50
	40	57 ± 15	56 ± 27	219 ± 35	261 ± 41*
HDL-C	7	80 ± 28	85 ± 15	46 ± 11	48 ± 5
	18	76 ± 18	86 ± 17	39 ± 15	50 ± 10*
	40	47 ± 15	48 ± 21	35 ± 14	44 ± 24

NOTE. TG and cholesterol levels are expressed as mean ± SD.

Abbreviation: ND, not detectable.

*Significant at $P < .05$, diabetic group *v* corresponding nondiabetic control group.

†Significant at $P < .01$, diabetic group *v* corresponding nondiabetic control group.

‡Significant at $P < .001$, diabetic group *v* corresponding nondiabetic control group.

§7 wk: pre-STZ injection; 40 wk: at sacrifice when plasma samples were collected after 4-hour fast.

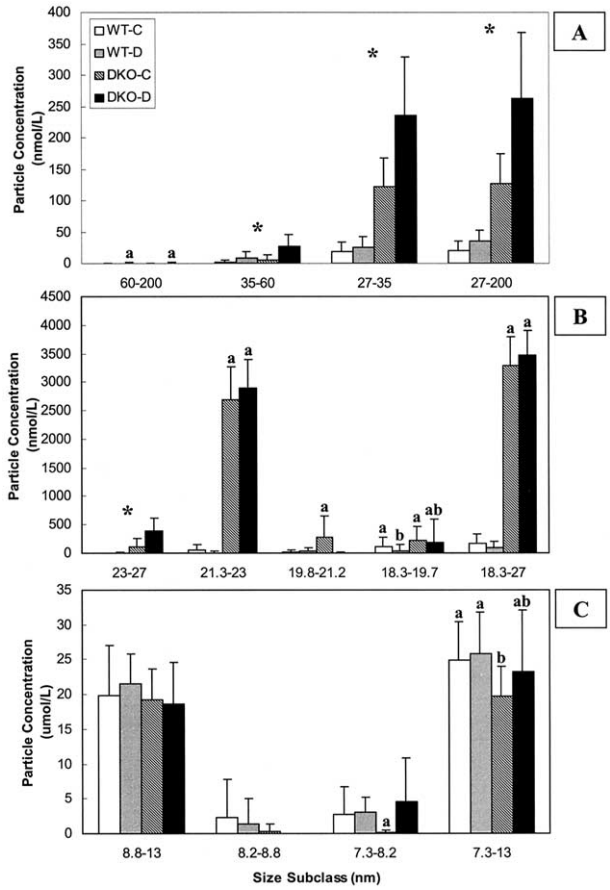

Fig 1. NMR analysis of (A) VLDL, (B) LDL and IDL, and (C) HDL subclass profile in WT and *Ldlr-/- Apobec1-/-* mice with and without STZ-induced hyperglycemia. Serum samples were obtained after 4-hour fast at age 40 weeks (at sacrifice). Values presented are mean ± SD. *Significant interaction among means within lipoprotein subclass ($P < .05$). Means within subclass having the same or no letters are not significantly different ($P < .05$). WT-C, wild-type nondiabetic control; WT-D, wild-type diabetic; DKO-C, *Ldlr-/- Apobec-/-* double knockout nondiabetic control; and DKO-D, *Ldlr-/- Apobec-/-* double knockout diabetic.

Effect of Diabetes on the Lipid Profiles in WT and DKO Mice

In nonfasting WT animals, using Cholestech L.D.X, diabetes increased total cholesterol and TG, but these increases were not observed in fasting WT animals (Table 3). In nonfasting DKO animals, diabetes had no effect on lipid profiles, but in fasting DKO animals, diabetes increased LDL-C and total cholesterol (Table 3).

As determined by NMR analysis, diabetes had no effect on the fasting concentrations of LDL- and HDL-equivalent particles in either WT or DKO animals (Figs 1B and C). Diabetes did, however, increase levels of the VLDL-equivalent particles (27 to 200 nm) ($P < .01$). All 3 VLDL-sized subclasses (27 to 35, 35 to 60, and 60 to 200 nm) were significantly affected by diabetes, but much the greatest increase was seen in concentrations of the smallest particles (27 to 35 nm) (Fig 1A). For particles between 27 and 60 nm in size, interactions between genetic background and diabetes were significant, indicating a further increase in particle concentration in the presence of both diabetes and hypercholesterolemia (Fig 1A).

DISCUSSION

There has been increasing interest in using mice as possible models of vascular complications in diabetes, amplified by the generation of gene knockout mouse models that develop atherosclerotic lesions similar to those of humans.[24,25] However, the contribution of dyslipoproteinemia to the accelerated atherosclerosis of diabetes has not been clearly defined in mouse models,[14-17] and lipoprotein subclass profiles of diabetic and genetically hyperlipidemic mice have not been defined in detail. We view such characterization as a key step in evaluating candidate animal models to address the effects of dyslipoproteinemia on vascular injury in diabetes. In the present study, we assessed the lipoprotein subclass distribution in a genetically hyperlipidemic mouse with and without STZ-induced diabetes using NMR analysis technology.

Conventional lipid assays, including the Cholestech method employed in this study, determine the total amounts of cholesterol and TG present in all circulating lipoproteins, and the amount of cholesterol carried by HDL. However, these measures are unable to provide any quantification of lipoprotein classes or subclasses, either in terms of composition or size distribution. The NMR technique provides an opportunity to gain important information about subclass size distribution rapidly, using small samples of serum or plasma without physical separation of lipoprotein subclasses.

In the present study, the NMR data show that there was a 2-fold increase in the concentration of VLDL-sized particles in the DKO-D versus DKO-C mice (Fig 1A), despite no accompanying increase in total TG levels (Table 3). This observation implies that, in the DKO-D mice, particles within the size range of small human VLDL are in fact similar in lipid composition to particles in the large LDL size range, ie, they are rich in cholesteryl esters, not TGs. Similarly, the data show that despite elevated levels of calculated "LDL-C" in the DKO-D versus DKO-C mice (Table 3), there was no significant diabe-

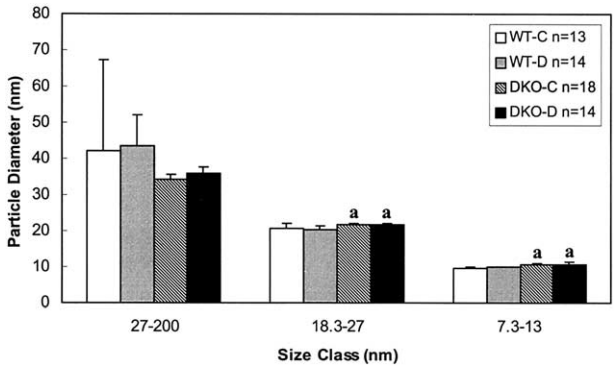

Fig 2. NMR analysis of lipoprotein particle diameter in WT and *Ldlr-/- Apobec1-/-* mice with and without STZ-induced hyperglycemia. Values presented are mean ± SD. Means within lipoprotein class having the same or no letters are not significantly different ($P < .05$).

tes-associated change in the level of LDL-size particles (Fig 2B). This apparent discrepancy may be explained in the same way: the additional cholesterol resides in particles the size of small human VLDL.

The NMR lipoprotein profiling method used in the present study supplied considerable useful information about mouse lipoprotein characteristics. It defined which size-based lipoprotein categories were affected by the genetic manipulation and/or by diabetes. When this new information was combined with data obtained from "conventional" measures of total cholesterol, TG, and HDL-C, further information could be inferred about lipoprotein distributions. Our data show that cholesterol-rich particles in the size range of human large LDL are responsible for most of the hypercholesterolemia in the DKO mouse, but as detailed above, particles in the size range of small human VLDL may also contribute significantly to the hypercholesterolemia. The data also show that none of the animal groups had measurable amounts of lipoproteins in the size ranges of human small LDL. Since small LDL is believed to have a significant influence on the development of human vascular disease, this lack may limit the relevance of the present DKO mouse model. Furthermore, in all groups of mice employed in this study, HDL was present in the large HDL subclass only, and its concentration was unaffected by either diabetes or hyperlipidemia. In mice, as in several other animal species, HDL is the dominant lipoprotein and the major cholesterol carrier.[26,27] The lack of the small, noncardioprotective form of HDL may limit the relevance of this animal as a model for human disease.

Knowledge gained by the NMR analysis may help guide the future development of animal models with lipoprotein profiles that simulate human profiles more closely. Such animals will be of great potential value for studying human dyslipidemia, including the role of diabetic dyslipidemia in accelerating the vascular complications of diabetes. Improved animal models may readily be envisaged; for example, further modification of the *Ldlr-/- Apobec1-/-* mouse model by transfection with the cholesteryl ester transfer protein (*CETP*) gene might induce a lipoprotein profile closer to the human profile. It has already been reported that transgenic mice expressing *CETP* display decreased plasma HDL-C[19,28] and have smaller HDL particle size.[29]

In an effort to define the role of lipoprotein particle size in susceptibility to atherosclerosis in the mouse, Veniant et al recently characterized the lipoproteins in apoE-deficient apoB100-only and LDLr-deficient apoB100-only mice, which have similar total plasma cholesterol levels.[30] They concluded that at a given cholesterol level, large numbers of small apoB-containing lipoproteins are more atherogenic than lower numbers of large ones.[30] In their study, lipoprotein particle diameters were determined by dynamic light scattering analysis with a Microtrac Series 150 Ultrafine particle analyzer (Clearwater, FL).[30] As a continuation of the present study, we are working to relate our lipoprotein data to the development and/or acceleration of vascular injury, both micro- and macrovascular, in diabetic mice.

In conclusion, we have shown that the NMR technique is a promising tool for assessing lipoprotein profiles in candidate mouse models, such as our *Ldlr-/- Apobec1-/-* mouse. It has potential for the assessment of interactions between hyperlipidemia and diabetes, and in refining animal models to simulate human lipoprotein profiles.

REFERENCES

1. Pyorala K, Laakso M, Uusitupa M: Diabetes and atherosclerosis: An epidemiologic view. Diabetes Metab Rev 3:463-524, 1987

2. Bierman EL: George Lyman Duff Memorial Lecture. Atherogenesis in diabetes. Arterioscler Thromb 12:647-656, 1992

3. Uusitupa MI, Niskanen LK, Siitonen O, et al: 5-Year incidence of atherosclerotic vascular disease in relation to general risk factors, insulin level, and abnormalities in lipoprotein composition in non-insulin-dependent diabetic and nondiabetic subjects. Circulation 82:27-36, 1990

4. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. N Engl J Med 329:977-986, 1993

5. Lyons TJ, Jenkins AJ: Glycation, oxidation and lipoxidation in the development of the complications of diabetes mellitus: A "carbonyl stress" hypothesis. Diabetes Rev 5:365-391, 1997

6. Baynes JW, Thorpe SR: Glycoxidation and lipoxidation in atherogenesis. Free Radic Biol Med 28:1708-1716, 2000

7. Gardner CD, Fortmann SP, Krauss RM: Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 276:875-881, 1996

8. Lamauche B, Tchernof A, Mourjani S, et al: Small, dense, low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men: Prospective results from the Quebec Cardiovascular Study. Circulation 95:69-75, 1997

9. Superko HR: New aspects of risk factors for the development of atherosclerosis, including small dense lipoprotein, homocyst(e)ine, and lipoprotein (a). Curr Opin Cardiol 10:347-354, 1995

10. Eckardstein A, Huang Y, Assmann G: Physiological role and clinical relevance of high-density lipoprotein subclasses. Curr Opin Lipidol 5:404-416, 1994

11. Otvos J, Jeyarajah E, Bennett D: A spectroscopic approach to lipoprotein subclass analysis. J Clin Ligand Assay 19:184-189, 1996

12. Lyons TJ, Jenkins AJ, Zheng D, et al: Serum lipoproteins in the DCCT/EDIC Cohort: Associations with diabetic retinopathy. Invest Ophthalmol Vis Sci (in revision)

13. Jenkins AJ, Lyons TJ, Zheng D, et al: Serum lipoproteins in the DCCT/EDIC Cohort: Associations with diabetic nephropathy. Kidney Int (in press)

14. Kunjathoor VV, Wilson DL, LeBoeuf RC: Increased atherosclerosis in streptozotocin-induced diabetic mice. J Clin Invest 97:1767-1773, 1996

15. Park L, Raman KG, Lee KJ, et al: Suppression of accelerated diabetic atherosclerosis by the soluble receptor for advanced glycation endproducts. Nat Med 4:1025-1031, 1998

16. Reaven P, Merat S, Casanada F, et al: Effect of streptozotocin-induced hyperglycemia on lipid profiles, formation of advanced glycation endproducts in lesions, and extent of atherosclerosis in LDL receptor-deficient mice. Arterioscler Thromb Vasc Biol 17:2250-2256, 1997

17. Keren P, George J, Shaish A, et al: Effect of hyperglycemia and hyperlipidemia on atherosclerosis in LDL receptor-deficient mice: Establishment of a combined model and association with heat shock protein 65 immunity. Diabetes 49:1064-1069, 2000

18. Powell-Braxton L, Veniant M, Latvala RD, et al: A mouse model of human familial hypercholesterolemia: markedly elevated low

density lipoprotein cholesterol levels and severe atherosclerosis on a low-fat chow diet. Nat Med 4:934-938, 1998

19. Nakamuta M, Taniguchi S, Ishida BY, et al: Phenotype interaction of apobec-1 and CETP, LDLR, and apoE gene expression in mice: Role of apoB mRNA editing in lipoprotein phenotype expression. Arterioscler Thromb Vasc Biol 18:747-755, 1998

20. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 8:499-502, 1972

21. Otvos JD, Jeyarajah EJ, Bennett DW, et al: Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. Clin Chem 38:1632-1638, 1992

22. Otvos JD: Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. Clin Lab 48:171-180, 2002

23. Redgrave TG, Carlson LA: Changes in plasma very low density and low density lipoprotein content, composition, and size after a fatty meal in normo- and hypertriglyceridemic man. J Lipid Res 20:217-229, 1979

24. Zhang SH, Reddick RL, Piedrahita JA, et al: Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 258:468-471, 1992

25. Ishibashi S, Brown MS, Goldstein JL, et al: Hypercholesterolemia in low density lipoprotein receptor knockout mice and its reversal by adenovirus-mediated gene delivery. J Clin Invest 92: 883-893, 1993

26. Jiao S, Cole TG, Kitchens RT, et al: Genetic heterogeneity of lipoproteins in inbred strains of mice: analysis by gel-permeation chromatography. Metabolism 39:155-160, 1990

27. Tsutsumi K, Hagi A, Inoue Y: The relationship between plasma high density lipoprotein cholesterol levels and cholesteryl ester transfer protein activity in six species of healthy experimental animals. Biol Pharm Bull 24:579-581, 2001

28. Hansen PR: Cholesterol ester transfer protein. A major player in cholesterol metabolism. Ugeskr Laeger 161:5295-5298, 1999

29. Francone OL, Royer L, Haghpassand M: Increased prebeta-HDL levels, cholesterol efflux, and LCAT-mediated esterification in mice expressing the human cholesteryl ester transfer protein (CETP) and human apolipoprotein A-I (apoA-I) transgenes. J Lipid Res 37: 1268-1277, 1996

30. Veniant MM, Sullivan MA, Kim SK, et al: Defining the atherogenicity of large and small lipoproteins containing apolipoprotein B100. J Clin Invest 106:1501-1510, 2000