Lipoprotein Profile Characterization of the KKA'y Mouse, a Rodent Model of Type II Diabetes, Before and After Treatment With the Insulin-Sensitizing Agent Pioglitazone

Christine K. Castle, Jerry R. Colca, and George W. Melchior

The purpose of this study was to characterize the lipoprotein profile in the KKA'y mouse, a rodent model of type II diabetes, before and after treatment with the insulin-sensitizing drug pioglitazone. Analysis of the plasma from untreated KKA'y mice showed that they were severely hyperglycemic, severely hypertriglyceridemic, and moderately hypercholesterolemic. Agarose column chromatographic analysis showed that essentially all of the triglyceride eluted with very low density lipoprotein, and the majority of the cholesterol eluted with high density lipoprotein. Thus, both the very low density lipoprotein and high density lipoprotein levels were markedly elevated in KKA'y mice. Analysis of the lipoproteins by agarose electrophoresis-immunoblotting showed that apoprotein A-I and apoprotein B had aberrant electrophoretic behavior, typical of apoproteins that have been modified by nonenzymatic glycosylation. Treatment of KKA'y mice with pioglitazone for 8 days caused a marked reduction in blood glucose and plasma triglyceride concentrations but had no significant effect on plasma cholesterol concentration or distribution. The aberrant electrophoretic behavior of the apoproteins was corrected to normal by drug treatment. These data show that the KKA'y mouse has a severe dyslipoproteinemia that is probably secondary to its insulin resistance, but that its lipoprotein profile differs significantly from that of the insulin-resistant human in that the majority of the plasma cholesterol is carried in high density lipoprotein, and those high density lipoprotein levels are very high. (Arteriosclerosis and Thrombosis 1993;13:302-309)

KEY WORDS • yellow KK mouse • KKA'y mouse • non-insulin-dependent diabetes mellitus • pioglitazone • lipoproteins • apo A-I • apo B • apo E • LDL • HDL

Premature atherosclerosis is a principal cause of death in individuals with non-insulin-dependent diabetes mellitus (NIDDM). Although the reason(s) for the increased rate at which vascular disease progresses in these individuals has not been determined, one common feature of NIDDM is an altered lipoprotein profile. These individuals frequently have severe hypertriglyceridemia and reduced high density lipoprotein (HDL) cholesterol levels. Both have been implicated in the development of atherosclerosis, and both appear to be related to insulin resistance, although the metabolic link between insulin resistance and the altered lipoprotein profile is unknown.

There are few animal models of NIDDM, and little information is available on the lipoprotein profiles in such animals. One animal that does spontaneously develop NIDDM is the KKA'y mouse. Although this strain was developed for use as a model of non-insulin dependent diabetes mellitus, the KKA'y mouse has been found to be a useful model for studying the role of insulin resistance in the development of atherosclerosis. Kondo et al. from Japanese native mice. The KK mouse carries the diabetes gene and is hyperinsulinemic but does not develop overt hyperglycemia. The yellow obese mouse, originally described by Cuénot, is insulin resistant, and a large percentage of the males have abnormal glucose tolerances. The KKA'y mouse (the yellow offspring obtained from a cross of black KK females with obese yellow A'y males) are obese, hyperglycemic, hyperinsulinemic, and insulin resistant. In addition, they are severely hypertriglyceridemic and mildly hypercholesterolemic.

The primary aim of this study was to characterize the lipoprotein profile of the KKA'y mouse to determine whether it is a good model with which to study the hyperlipidemia that develops in humans with NIDDM. We report here the aberrations in the plasma lipoprotein distribution and electrophoretic behavior of the KKA'y mouse. In addition, we show that these abnormalities can be corrected to near normal with the insulin-sensitizing drug pioglitazone. (Pioglitazone is one of the thiazolidinediones, a class of drug that has previously been shown to alleviate, to a large extent, the insulin insensitivity in animal models with that disorder.) These data provide support for the proposition that the dyslipoproteinemia in the KKA'y mouse is, in fact, secondary to its insulin resistance.

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Kondo et al. from Japanese native mice. The KK mouse carries the diabetes gene and is hyperinsulinemic but does not develop overt hyperglycemia. The yellow obese mouse, originally described by Cuénot, is insulin resistant, and a large percentage of the males have abnormal glucose tolerances. The KKA'y mouse (the yellow offspring obtained from a cross of black KK females with obese yellow A'y males) are obese, hyperglycemic, hyperinsulinemic, and insulin resistant. In addition, they are severely hypertriglyceridemic and mildly hypercholesterolemic.
Animals and Diets

Male KKA' mice approximately 3 months old and obtained from the Upjohn Laboratories colony were used for these experiments. The original breeding stock, consisting of yellow KKA' males and black KK females, were procured from Takeda Chemical Industries, Osaka, Japan. Information relative to the genetic background of these mice has been previously reported.5 C57BL/6 mice were used as control animals and were considered to be "normal" mice for this study. The C57BL/6 breeding stock was obtained from Jackson Laboratories, Bar Harbor, Me. All procedures were reviewed and approved by the institutional Animal Care and Use Committee.

The mice were randomly assigned to a drug-treated or placebo-treated group. Each group consisted of eight mice. The mice were housed individually and fed ground Purina mouse chow (No. 50-15). Pioglitazone (5-[(4-[2-(5-ethyl-2-pyridinyl)ethoxy]phenyl)methylene]thiazolidine-2,4-dione monohydrochloride; U-72107A) was added to the diet of the drug-treated mice at a concentration of 0.2 mg/g of diet (i.e., an approximate dose of 20 mg/kg body wt). The placebo group received the chow diet devoid of the drug.

Blood samples were taken at times t=0, just before starting the medication regimen, and again at t=8 days. In both instances, blood was obtained from the periorbital sinus and collected into heparinized tubes. Approximately 200 µL was taken at t=0, and approximately 1 mL was taken at t=8 days. The plasma was obtained by centrifugation, diluted 1/2 or 1:1 (vol/vol) with phosphate-buffered saline containing 0.01% EDTA and 0.02% NaN3, and stored at 4°C until analyzed.

Lipid and Apolipoprotein Analyses

The cholesterol concentration of the plasma was determined by enzymatic assay. Thirty microliters of sample or standard (0–50 mg/dL cholesterol calibrator, Sigma Chemical Co., St. Louis, Mo.) was added to the wells of an ordinary microtiter plate followed by 150 µL cholesterol reagent (Boehringer Mannheim, Indianapolis, Ind.). The mixture was incubated for 15 minutes at room temperature, and the absorbance at 490 nm of the material in each well was measured using a Vmax kinetic microplate reader ( Molecular Devices Corp., Palo Alto, Calif.). To measure HDL cholesterol levels, the apoprotein (apo) B–containing lipoproteins were precipitated by polyethylene glycol (PEG)–8000 in glycine buffer. Equal volumes (40 µL) of the diluted plasma sample and the PEG-8000 solution were used for precipitation. The very low density lipoprotein plus low density lipoprotein (VLDL+LDL) cholesterol concentration of the samples was determined by subtraction. The cholesterol concentration of agarose column chromatography fractions (see below) was determined using essentially the same procedure as described for plasma, except that larger volumes (200 µL of the column fraction and 1 mL cholesterol reagent) were used and standards ranging from 0 to 200 mg/dL were required.

Triglyceride concentrations in plasma and column fractions were also measured using an enzymatic assay (Trace Scientific, Baulkam Hills, Australia). Twenty microliters of the diluted plasma or 600 µL of the column fraction was mixed with 1 mL triglyceride reagent, the samples incubated at 37°C for 10 minutes, and the absorbance at 500 nm measured using a spectrophotometer. Triglyceride aqueous standard (0–300 mg/dL, Sigma) was run simultaneously and was used as the calibration standard.

The apo A-I, apo B, and apo E concentrations in the plasma samples were determined by electroimmunoassay essentially as described previously.10,11 The apo A-I and apo B concentrations in column fractions were determined by slot-blot immunoassay, also as described previously.12 Apo A-I and apo B were purified from mouse plasma, and antibodies to these proteins were produced as described previously.10,13 The antibody to apo E (generously provided by Dr. Paul Roheim, Louisiana State University Medical Center, New Orleans, La.) was made against rat apo E but was found to strongly cross-react with the apo E in mouse plasma. The conditions for the apo E electroimmunoassay were similar to those reported previously,14 except that the dextran concentration in the gels was 4% by volume and the antisemum concentration 0.6%.

The size distribution of the apo A-I and apo B–containing lipoproteins was determined by A-15M agarose column chromatography (Bio-Rad Laboratories, Richmond, Calif.) as described previously.10,12 Plasma (300 µL from each mouse taken at t=8 days) was pooled by treatment group, and 1 mL from each pool was passed through the column. The process was then repeated with a second 1-mL aliquot from each pool. Thus, the size distribution of each pool was analyzed in duplicate. The same column was used for all analyses and the elution volume of apoproteins expressed as a fraction of the volume at which the albumin in that same sample eluted (Vv/Veb). The albumin elution volume (mean±SD) was 401±2 mL. Agarose electrophoresis–immunoblotting analyses were performed exactly as described previously.15

Statistics

Differences between C57BL/6 mice and KKA' mice were tested for significance with an independent sample t test. The mean of the t=0- and the t=8-day values for a given measurement was taken as the best estimate of the actual value for a given mouse. The mean±SD of these means was then used in the t test. In instances in which the variances of the two groups were significantly different, the values were transformed by one of several methods (logarithm, square root, reciprocal, etc.), and the t test was conducted using the transformed values.

Changes in the various measurements after pioglitazone treatment were tested for significance by comparing the differences (t=8-day value−t=0-day value) in the pioglitazone-treated KKA' mice with the differences in untreated KKA' mice. The null hypothesis was that the mean difference for the treated group was equal to that of the untreated group, as tested with an independent sample t test. In all instances the null hypothesis was not rejected unless p<0.1.

Results

Table 1 contains the mean body weights and blood glucose levels and Table 2 the mean plasma cholesterol concentrations and distributions; the plasma triglyceride concentrations; and the apo B, apo A-I, and apo E levels in the placebo- and pioglitazone-treated KKA' mice. The mean glucose, lipid, and apolipoprotein con-
centrations and distributions of eight C57BL/6 mice, run in parallel and considered as normal mice for this study, are shown in each table for comparison. Statistical analysis of the data in Tables 1 and 2 indicated that with the exception of apo B, the plasma levels of every component measured were significantly higher in KKA'y than in C57BL/6 mice. Thus, relative to the normal mouse, the KKA'y mouse is a spontaneously hyperglycemic, hypercholesterolemic, hypertriglyceridemic animal. Treatment of such mice with pioglitazone caused a significant increase in their body weight and a significant decrease in plasma glucose, triglyceride, apo B, and VLDL+LDL cholesterol levels. Pioglitazone treatment had no effect on total plasma cholesterol concentrations (Tables 1 and 2).

The total plasma apo B levels were not significantly higher in KKA'y than in C57BL/6 mice, even though the triglyceride and cholesterol levels were markedly higher (Table 2). Furthermore, neither the main triglyceride peak nor the main cholesterol peak eluted from the agarose column with the main apo B peak (Figure 1). The majority of the triglycerides eluted in the column void volume (V_e/V_\text{void}=0.4), which is where human VLDLs elute. The majority of the plasma cholesterol appeared to elute in the region between the void volume and the LDL peak (V_e/V_\text{void} between 0.4 and 0.7) which was much higher in the diabetic mice than in the normal mouse. This is the region where small VLDL, intermediate density lipoprotein, and chylomicron remnants would be expected to elute if they were present in the plasma of these animals. Thus, these data suggest that diabetic mice have more "remnant"-sized particles in their plasma, and it was these particles that appeared to be most affected by pioglitazone treatment; i.e., reduction of apo B-containing particles in the remnant size range appeared to be a major factor in the reduction in total plasma apo B levels in the pioglitazone-treated animals (Table 2 and Figure 1).

The majority of the plasma cholesterol appeared to be associated with HDL. This is evident from both the cholesterol distribution data in Table 2 (the majority of the cholesterol did not precipitate with the VLDL+LDL fraction) and the cholesterol elution profile in Figure 1 (which shows that the majority of the

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Treatment</th>
<th>Body weight (g)</th>
<th>Plasma glucose (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t=0</td>
<td>t=8</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>None</td>
<td>28.5±0.8</td>
<td>29.2±1.0</td>
</tr>
<tr>
<td>KKA'y</td>
<td>None</td>
<td>40.9±1.3</td>
<td>40.0±1.2</td>
</tr>
<tr>
<td>KKA'y</td>
<td>Pioglit.</td>
<td>38.9±1.3</td>
<td>45.1±1.3</td>
</tr>
</tbody>
</table>

Probability >t

C57BL/6 vs. untreated KKA'y <0.001  <0.001  <0.001  <0.001  <0.001  <0.001
KKA'y (treated vs. untreated) 0.994  0.058  0.355  0.001  0.001  0.001  0.005

VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; Pioglit., pioglitazone. Lipid and apoprotein values are in milligrams per deciliter (mean±SEM, n=8 mice per treatment group) except those for apoprotein (apo) E, which are in arbitrary units.
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FIGURE 1. Chromatograms showing the size distribution of triglyceride-, cholesterol-, apoprotein (apo) B- and apo A-I-containing lipoproteins from KKA<sup>+</sup> mice before and after treatment with pioglitazone. Plasma samples taken from the mice at t=8 days were pooled by treatment group, and 1 mL from each pool was passed through a 2.6×100 cm column of BioGel A-15M agarose and fractionated as previously described. The procedure was then repeated with a second 1-mL aliquot from each pool. The elution patterns shown represent the mean of duplicate analyses. Triglyceride- and cholesterol-containing lipoproteins were identified by enzymatic analysis, and the apo B- and apo A-I-containing lipoproteins were identified by immunoassay. Actual elution volume (V<sub>e</sub>) is expressed as a fraction of the volume at which albumin in that sample eluted (V<sub>alb</sub>). Mean±SD albumin elution volume was 401±2 mL. □, Plasma from untreated mice; ■, plasma from pioglitazone-treated mice.

cholesterol coeluted with apo A-I, indicating that it was eluting with HDL). The fact that plasma apo A-I levels were also significantly higher in KKA<sup>+</sup> mice than in C57BL/6 control mice indicates that the high HDL levels in the former were probably due to an increase in the number of HDL particles in the circulation, rather than simply to an increase in the cholesterol content of HDL. Nonetheless, the KKA<sup>+</sup> HDLs were apparently enriched with cholesterol relative to the C57BL/6 HDLs; the cholesterol/apo A-I ratio in KKA<sup>+</sup> mice was 0.97±0.11 (mean±SD), whereas in the C57BL/6 mice this ratio was 0.78±0.15 (p=0.01). Pioglitazone treatment had no effect on HDL cholesterol levels; however, from a statistical standpoint, there appeared to be a drug-induced decrease in plasma apo A-I levels; i.e., there was an apparently spontaneous increase in plasma apo A-I levels in the untreated KKA<sup>+</sup> mice, a phenomenon that did not occur in the treated group. It is not clear whether pioglitazone did in fact prevent what would have been an increase of apo A-I in the absence of the drug, or if the change evident in untreated KKA<sup>+</sup> mice was simply an idiosyncratic fluctuation in the plasma apo A-I levels in that group.

Plasma apo E levels are often increased in humans with NIDDM, and the same was true of the untreated KKA<sup>+</sup> mice; i.e., the plasma apo E concentrations in these mice were significantly higher than those of the C57BL/6 control mice (Table 2). We were unable to determine the apo E size distribution. Therefore, we could not determine which lipoproteins the apo E was associated with nor why it was increased in KKA<sup>+</sup> mice. The plasma apo E levels were further increased in the pioglitazone-treated mice, and the basis of this response is unknown.

In addition to analyzing the size distribution of lipoproteins in KKA<sup>+</sup> mice, we also analyzed the lipoprotein agarose electrophoresis migration patterns and the effect of pioglitazone treatment on these patterns. These
data are shown in Figure 2. Note that in pretreatment samples, the lipids, apo B-containing lipoproteins, and apo A-I-containing lipoproteins showed a very rapid rate of migration through the gel. All three components migrated faster than bovine serum albumin run on the same plate (not shown). However, treatment with pioglitazone corrected these agarose electrophoresis patterns to those typical of the C57BL/6 mouse. These data not only indicate that the lipoproteins in untreated KKAy mice have a higher-than-normal charge/mass ratio but also that the altered ratio can be corrected by treatment with pioglitazone. The basis of the altered charge/mass ratio is presently unknown; however, the migration patterns shown in Figure 2 are very similar to those of lipoproteins that have been subjected to non-enzymatic glycosylation. In addition, it is noteworthy that the normalization of electrophoretic behavior coincided with the pioglitazone-induced reduction in blood glucose concentrations.

Although the majority of lipoproteins in untreated KKAy mice had faster migration rates, the autoradiograms showed that there was also a slow-migrating apo A-I fraction in the plasma of untreated KKAy mice (Figure 2, second panel from the right). This apo A-I band migrated even more slowly than the apo B-containing lipoproteins. From the autoradiograms, all of the untreated KKAy mice appeared to have substantial quantities of the slow-migrating apo A-I in their plasma, but this fraction was absent or barely detectable in the samples from C57BL/6 mice and the pioglitazone-treated KKAy mice. It is noteworthy that the slow-migrating apo A-I is not typical of the "pre-β"-migrating apo A-I found in humans and cynomolgus monkeys but appears to correspond to the "pre-β-HDL" fraction reported by Ishida et al. Why this fraction is present at elevated levels in KKAy mice is unclear.

Discussion

The KKAy mouse is obese, hyperglycemic, hyperinsulinemic, and insulin resistant and is gaining recognition as a rodent model of human NIDDM. Although several aspects of carbohydrate metabolism in these animals have been studied, there is little information available regarding their lipoprotein profile. The principal objective of this study was to characterize the lipoprotein profile in these mice so that it could be compared and contrasted with the lipoprotein profile typical of humans with NIDDM. A second objective was to determine how the lipoprotein profile would be affected by reducing insulin resistance in these mice with the experimental drug pioglitazone.

The results of lipoprotein characterization show that the lipoprotein profile of the KKAy mouse is similar in some respects to that of humans with NIDDM but is different in other respects. For example, the hypertriglyceridemia evident in these mice is characteristic of that seen in human NIDDM with regard to both the severity and lipoprotein distribution (i.e., an increase in VLDL-associated triglyceride); however, in human NIDDM, HDL cholesterol levels are frequently reduced (<35 mg/dL), and such reductions are principally due to a reduction in the larger HDLs (HDL{3}). This clearly is not the case with the KKAy mouse. Rather, HDL levels were markedly increased in these mice, regardless of their method of measurement (as HDL cholesterol or as plasma apo A-I), and these HDLs were quite large (as large or larger than human HDLs). Therefore, the mechanism by which insulin resistance leads to depressed HDL levels in humans is apparently not operative in the KKAy mouse.

The processes that regulate plasma HDL levels in humans have not been completely defined, and as a result, the metabolic basis of low HDL levels in humans with NIDDM is still somewhat obscure. However, one reasonably consistent finding in humans and nonhuman primates is that small HDLs tend to have shorter plasma residence times than do the larger particles. It is not known how these small HDLs are cleared, nor is it understood how or why the particle size affects its half-life. Nonetheless, conditions that tend to decrease the size of the HDL particle are frequently associated with shorter plasma HDL residence times and consequently lower plasma concentrations. The fact that large HDLs predominate in the plasma of the insulin-resistant mouse and that small HDLs predominate in the plasma of the insulin-resistant human probably accounts in part for the marked differences in plasma HDL concentrations when the two species are compared; however, the metabolic basis of the size differences in HDL is unknown.

HDLs originate as small, disk-shaped particles containing phospholipid, apo A-I, and unesterified cholesterol, which are secreted by the liver and intestine. Once in the plasma, these nascent HDLs grow in volume as a result of cholesterol uptake and esterification and in surface area as a result of phospholipid uptake (for a comprehensive review of HDL metabolism, see Reference).
ence 29). One key aspect of HDL metabolism and the one in which humans and mice appear to differ to the greatest extent is the exchange of core lipids (triglycerides and cholesteryl esters) between HDL and other lipoproteins. This process is mediated by cholesteryl ester transfer protein (CETP; also called lipid transfer protein-1) in humans. CETP is capable of transferring triglycerides, cholesteryl esters, phospholipids, and possibly other hydrophobic compounds in lipoproteins; however, under normal conditions, CETP mediates a net movement of cholesterol esters from HDL to VLDL in exchange for triglyceride. Thus, the VLDLs become enriched in cholesterol esters and the HDLs gain triglyceride. The VLDL-associated cholesterol is returned to the liver when the VLDL and its daughter products are cleared from the plasma by the hepatic LDL receptors. The triglyceride contained in HDL is thought to be removed by hepatic triglyceride lipase, without destruction of the HDL particle itself.

It has been postulated that in hypertriglyceridemic humans, HDL becomes depleted of cholesterol because of exchange of cholesterol esters for triglyceride; then as that triglyceride is hydrolyzed by hepatic lipase, the HDL particle shrinks. If, as suggested above, small HDLs have a shorter plasma residence time than do large HDLs, a CETP–hepatic lipase–induced reduction in HDL size could account for the reduced plasma concentration seen in NIDDM.

Rats, dogs, and mice (and possibly other species) do not have detectable CETP activity in their plasma. Therefore, the cholesterol esters in the HDL of these animals are essentially trapped there until the entire particle is cleared. Apparently, these HDLs increase their cholesterol content until they become relatively large, at which time they begin to accumulate apo E. As the apo E content of the particle increases, so also does its affinity for the hepatic lipoprotein receptors (LDL receptors and hepatic LDL-receptor–like proteins), and they are cleared intact by the liver.

With these considerations, we propose that HDL levels are high in the insulin-resistant KKA' mouse because these animals lack detectable CETP activity in their plasma. In the human, the abundance of triglyceride-rich lipoproteins in the plasma can act as a “sink,” taking up cholesterol esters that are transferred from HDL by CETP, but because this process is inoperative in the KKA' mouse, these cholesterol esters remain trapped in HDL and the HDL particle increases in size and concentration. If this hypothesis is correct, one would predict that HDL levels would be high in other hypertriglyceridemic animals that lack CETP and that introduction of CETP into the plasma of these animals would reduce the size and concentration of HDL. With regard to the former, it is noteworthy that obese Zucker rats have lipoprotein profiles very similar to those of the KKA' mouse (severe hypertriglyceridemia and very high levels of large, cholesteryl-rich HDL) and no detectable CETP activity in their plasma (C.H. Spilman and G.W. Melchior, unpublished observations). With regard to the latter, it has been reported that C57BL/6 transgenic mice that express high levels of primate CETP had indeed significantly lower plasma HDL levels than did nonexpressing control animals, and these HDLs were significantly smaller than the HDLs of mice with no CETP activity in their plasma. The C57BL/6 transgenic mice were not diabetic and were not hyperlipidemic. One wonders how much further those HDL levels might have been reduced had excessive levels of triglyceride-rich VLDLs been present in their plasma. In any case, taken together, these data are compatible with the concept that CETP plays a significant role in the processes responsible for the reduced HDL levels in human NIDDM.

A second major aim of this study was to evaluate the effect of the insulin-sensitizing agent pioglitazone on the lipoprotein profile of the KKA' mouse. Pioglitazone is one of the thiazolidinediones, a class of drugs that has been previously shown to alleviate to a large extent the insulin insensitivity in animal models with that disorder. In this study, treatment of KKA' mice with the drug for 8 days had a marked effect on two aspects of their lipoprotein profile: their VLDL levels were significantly reduced, and the abnormal electrophoretic behavior of their lipoproteins was corrected.

The basis of the hypertriglyceridemia evident in insulin-resistant humans has not been determined with absolute certainty. It appears to be due in part to an overproduction of VLDL in response to the increased mobilization of free fatty acids in NIDDM and also in part to inhibited lipoprotein lipase activity. No information is available regarding the origin of the hypertriglyceridemia in the KKA' mouse, but alleviation of this hypertriglyceridemia by treatment with pioglitazone tends to support the proposition that, as in humans, it is secondary to their insulin insensitivity. In this regard it is noteworthy that in streptozocin-induced diabetic rats, a severe hypertriglyceridemia comparable to that seen in KKA' mice is produced within 4 days by feeding them a high-fat diet. In the absence of insulin, treatment of the animals with pioglitazone had minimal effect on their plasma triglyceride concentrations. Treatment with low doses of insulin also had relatively small effects. It was only when pioglitazone was given in combination with low doses of insulin that triglyceride levels were normalized. Since the actions of the drug were dependent on the addition of insulin, the effects of pioglitazone on plasma triglyceride levels in the streptozocin-induced diabetic model were probably secondary to augmented insulin activity. It seems reasonable that the same is true in the KKA' mouse.

Interestingly, the hypercholesterolemia evident in the KKA' mouse was not reduced by pioglitazone treatment. These data may indicate that pioglitazone's (or insulin's) principal effects are on apo B–containing lipoproteins and that when the cholesterol is carried in other hypertriglyceridemic animals that lack CETP and that introduction of CETP into the plasma of these animals would reduce the size and concentration of HDL. With regard to the former, it is noteworthy that obese Zucker rats have lipoprotein profiles very similar to those of the KKA' mouse (severe hypertriglyceridemia and very high levels of large, cholesteryl-rich HDL) and no detectable CETP activity in their plasma (C.H. Spilman and G.W. Melchior, unpublished observations). With regard to the latter, it has been reported that C57BL/6 transgenic mice that express high levels of primate CETP had indeed significantly lower plasma HDL levels than did nonexpressing control animals, and these HDLs were significantly smaller than the HDLs of mice with no CETP activity in their plasma. The C57BL/6 transgenic mice were not diabetic and were not hyperlipidemic. One wonders how much further those HDL levels might have been reduced had excessive levels of triglyceride-rich VLDLs been present in their plasma. In any case, taken together, these data are compatible with the concept that CETP plays a significant role in the processes responsible for the reduced HDL levels in human NIDDM.
have a potent effect on plasma cholesterol levels if that cholesterol is carried by apo B-containing lipoproteins, but its effect on cholesterol carried in HDL appears to be minimal in these models.

In addition to the high plasma levels of some lipoproteins in these insulin-resistant mice, there was an additional abnormality that was significant, and that was the increased rate of migration of lipoproteins during agarose electrophoresis. This increased rate of migration indicates that lipoproteins from untreated KKAy mice have a significantly higher-than-normal charge/mass ratio. Since in general the lipoproteins in the untreated KKAy mouse had masses as large or larger than those of the C57BL/6 mouse, it seems probable that the more rapid electrophoretic mobility of the KKAy lipoproteins was due primarily to an increase in net negative charge. This could have occurred by one of two methods: the addition of negative charges to the particle (as might occur if small, negatively charged proteins such as the C-apolipoproteins were adsorbed to the surface of the lipoproteins) or the neutralization of positive charges that are normally expressed in the lipoprotein.

With regard to the addition of negative charges to the particle, it has been well established that the C-apolipoproteins (apo C-I, apo C-II, and apo C-III) adsorb to the surface of chylomicrons and VLDLs shortly after they enter the peripheral circulation, and it is conceivable that in the untreated KKAy mouse the lipoproteins are more enriched in these peptides than are the VLDLs in the normal mouse. With regard to the neutralization of positive charges, it has been shown that the major apolipoproteins do undergo a nonenzymatic glycosylation in hyperglycemic humans and that glycosylation results in neutralization of positive charges (i.e., an increase in net negative charge) in these lipoproteins. Although amino acid analyses were not conducted in the studies reported here, it is noteworthy that the electrophoretic behavior of lipoproteins from untreated KKAy mice was similar to that of glycosylated human lipoproteins. Finally, the fact that alleviation of insulin resistance (and the accompanying hyperglycemia) by pioglitazone essentially normalized the electrophoretic behavior suggests that, whatever the primary cause of the altered electrophoretic behavior, it is secondary to insulin resistance.

Given these marked alterations in lipoprotein levels and electrophoretic behavior, the question arises as to the development of atherosclerosis in these mice. Paigen et al have shown, for example, that experimental atherosclerosis can be produced in some mouse strains by feeding them a high-fat, high-cholesterol diet containing cholic acid. That diet had minimal effect on plasma triglyceride levels and produced a moderate hypercholesterolemia. The fact that KKAy mice develop hyperlipidemia at least as severe as that seen in the diet-induced disease and in addition are hyperinsulinemic and probably have significant quantities of modified lipoproteins in their circulation suggests that they should be prime candidates for the development of atherosclerotic lesions; yet, no atherosclerosis was detected at necropsy. In fact, we have maintained some KKAy mice for as long as 3 years, during which time all of the aforementioned risk factors were present, and no vascular lesions could be detected.

The reason(s) that KKAy mice fail to develop arterial lesions is not known, and several factors may play a role; however, as discussed above, one striking aspect of the lipoprotein profile in KKAy mice is their high HDL level. Paigen et al reported that different inbred strains of mice differ significantly in their susceptibility to diet-induced atherosclerosis, and the key difference noted in those studies between the “susceptible” and “resistant” strains was the degree to which HDL levels were reduced in response to the atherogenic diet; i.e., those mice able to maintain their plasma levels of HDL tended to be resistant to lesion development, whereas those whose plasma HDL levels decreased developed relatively complex lesions. None of the differences in frequency or severity could be explained by differences in VLDL or LDL levels. Subsequent studies using apo A-I transgenic mice have tended to confirm this potent influence of HDL on lesion progression. Thus, HDL is strongly implicated as a factor in experimental atherosclerosis, and the fact that KKAy mice fail to develop the disease, despite the presence of some key risk factors, is probably due to a large extent to their ability to maintain high HDL levels.

In summary, these studies show that KKAy mice have a severe dyslipoproteinemia that appears to be secondary to their insulin resistance but that their lipoprotein profiles differ significantly from those in insulin-resistant humans in that the majority of the plasma cholesterol is carried in HDL, and those HDL levels are very high. Therefore, the KKAy mouse may not be an ideal model with which to study the dyslipoproteinemia of type II diabetes. The fact that KKAy mice lack CETP may account in large part for the differences noted between the lipoprotein profiles of humans with NIDDM and mouse NIDDM. If that is correct, a transgenic KKAy mouse that expresses primate CETP might be a more appropriate model of the human disorder.

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