Mechanisms mediating insulin resistance in transgenic mice overexpressing mouse apolipoprotein A-II

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Abstract **We previously demonstrated that transgenic mice overexpressing mouse apolipoprotein A-II (apoA-II) exhibit several traits associated with the insulin resistance (IR) syndrome, including increased atherosclerosis, hypertriglyceridemia, obesity, and IR. The skeletal muscle appeared to be the insulin-resistant tissue in the apoA-II transgenic mice. We now demonstrate a decrease in FA oxidation in skeletal muscle of apoA-II transgenic mice, consistent with reports that decreased skeletal muscle FA oxidation is associated with increased skeletal muscle triglyceride accumulation, skeletal muscle IR, and obesity. The decrease in FA oxidation is not due to decreased carnitine palmitoyltransferase 1 activity, because oxidation of palmitate and octanoate were similarly decreased. Quantitative RT-PCR analysis of gene expression demonstrated that the decrease in FA oxidation may be explained by a decrease in medium chain acyl-CoA dehydrogenase. We previously demonstrated that HDLs from apoA-II transgenic mice exhibit reduced binding to CD36, a scavenger receptor involved in FA metabolism. However, studies of combined apoA-II transgenic and CD36 knockout mice suggest that the major effects of apoA-II are independent of CD36. Rosiglitazone treatment significantly ameliorated IR in the apoA-II transgenic mice, suggesting that the underlying mechanisms of IR in this animal model may share common features with certain types of human IR.**—Castellani, L. W., P. Gargalovic, M. Febbraio, S. Charugundla, M.-L. Jien, and A. J. Lusis. **Mechanisms mediating insulin resistance in transgenic mice overexpressing mouse apolipoprotein A-II.** ***J. Lipid Res.*** **2004.** 45: **2377–2387.**

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Alterations in both glucose and lipid metabolism are two consistent features of the insulin resistance (IR) syndrome) (1, 2). Several studies have demonstrated that primary changes in FA metabolism can lead to IR (3–8). Although much has been learned about the insulin signaling pathways and the metabolism of both glucose and FAs, the underlying cause of most cases of IRS and type 2 diabetes is unknown (9, 10). Whether primary defects in FA metabolism or glucose metabolism underlie the majority of human cases of IRS and type 2 diabetes remains to be determined.

Studies using genetically altered mice have demonstrated that both the scavenger receptor CD36 and the HDL-associated protein apolipoprotein A-II (apoA-II) alter FA metabolism and IR (11–16). Furthermore, HDL is a ligand for the CD36 receptor, which raises the possibility of an HDL–CD36 interaction that affects FA metabolism (15). Mice with a null mutation for apoA-II exhibit increased sensitivity to insulin, with decreased plasma concentrations of triglycerides, FFA, and glucose (11). We previously demonstrated that increasing plasma concentrations of mouse apoA-II in transgenic mice produced several aspects of the IR syndrome including hypertriglyceridemia, obesity, and IR, as well as increased atherosclerotic lesion development (12, 17–19). The skeletal muscle appeared to be the insulin-resistant tissue in the apoA-II transgenic mice, whereas the liver and adipose tissue in the apoA-II trangenic mice appeared to respond normally (12). Compared with control mice, isolated soleus muscles from the apoA-II transgenic mice exhibited decreased glucose uptake and increased triglyceride accumulation (12). We hypothesize that primary alterations in FA metabolism in skeletal muscle initiate IR and promote the development of obesity. In the present study, we demonstrate that FA oxidation is reduced in the skeletal muscle of apoA-II transgenic mice, which could initiate the metabolic events that lead to skeletal muscle IR. We also placed the apoA-II transgene onto a CD36 knockout background and demonstrated that plasma triglyceride and insulin concentrations are not markedly different in the apoA-II transgenic mice, whether or

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Abbreviations: apoA-II, apolipoprotein A-II; CPT-1, carnitine palmitoyltransferase 1; FATP-1, fatty acid transport protein 1, (FATP-1H-FABP, heart type fatty acid-binding protein; IR, insulin resistance.

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not functional CD36 receptors are present. To determine whether the mechanism underlying IR in apoA-II transgenic mice resembles IR in humans, we were interested in determining if it would respond to thiazoledinediones, a class of drugs used in the treatment of IR in humans. We found that IR in apoA-II transgenic mice quickly responds to treatment with rosiglitazone.

MATERIALS AND METHODS

Animals

Transgenic mice with multiple copies of the mouse apoA-II gene and CD36 null mice were derived as described previously (13, 17). Homozygous apoA-II transgenic and CD36 null mice were bred to produce 83 combined apoA-II transgenic/CD36 null F2 progeny. All animals were housed 3 to 4 to a cage, maintained at 24°C on a 12-h light/dark cycle, and provided with Harlan-Teklad rodent chow (6% fat) and water ad libitum. The care of the mice, as well as all other procedures used in this study, complied with National Institutes of Health animal care guidelines. The animals were housed at the upper approved temperature limit (24°C), which appears to be more appropriate for mice (20, 21). With the exception of apoA-II transgenic mice generated in the cross to the CD36 null mice described above, all apoA-II transgenic animals used in the present study were males homozygous for the apoA-II transgene. Age-matched male C57BL/6 mice were used as controls.

Lipid analyses

Plasma was collected from mice that were fasted overnight and bled 2 to 3 h after the beginning of the light cycle from the retro-orbital plexus under isoflorane anesthesia. Total cholesterol, HDL cholesterol, triglycerides, and FFA concentrations were determined as described previously (17, 18). HDL was isolated via precipitation of VLDL and LDL with heparin and manganese chloride (22). Each lipid determination was measured in triplicate. An external control sample with known analyte concentration was run in each plate to ensure accuracy.

Hepatic and skeletal muscle glycogen content

Tissue samples were isolated 2 to 3 h after the beginning of the light cycle from animals that had been allowed ad libitum access to food and water. After homogenization in ddH_2O, aliquots were transferred to an acetate buffer solution containing 5×10^{-5} g α-glucosidase [Sigma (St. Louis, MO), cat. #A7420] and 2.7 μl α-amylase (Sigma, cat. #A4268) per ml of buffer solution. Aliquots of homogenates were also transferred to acetate buffer solution containing no enzymes, to serve as a sample blank. A standard curve was generated with a serial dilution of glucose. After addition to the buffer solution, the tubes were incubated at 37°C for 10 min and centrifuged in a microcentifuge for 5 min at 3,000 rpm, and 170 μl of the supernatant, in triplicate, was transferred to a 96-well plate and read at 490 nm in a Molecular Devices Spectramax plus microplate reader (Sunnyvale, CA).

Skeletal muscle FA oxidation

Following an overnight fast, mice were anesthetized with pentobarbital (50 mg/kg body wt) and the soleus muscle was isolated under a dissecting microscope. The muscles were weighed and immediately incubated for 40 min in Krebs-Henseleit buffer under 95% O_2:5% CO_2 that contained 5.5 mM glucose, 3% BSA, 1 mM palmitate and either [^{14}C-U]palmitic acid (2.5 uCi/ml) or [^{14}C-U]octanoic acid (2.5 uCi/ml). Rates of FA oxidation were assessed by determining radioactivity in $^{14}CO_2$ trapped in hyamine hydroxide as described previously (23). Palmitate (1 mM) was used as the unlabeled exogenous FA in experiments with both [^{14}C-U] palmitic acid and [^{14}C-U]octanoic acid tracers. Octanoate, which is not normally present in high concentrations, has markedly different effects on metabolism compared with long chain FAs (24–26). By using palmitic acid as the unlabeled FA substrate in both sets of experiments, the physiological effects of the exogenous FA substrate was the same in both groups allowing us to observe differences in metabolism of the tracer quantities of radiolabeled palmitate and octanoate under the same conditions.

Rosiglitazone treatment

One group of 14 apoA-II transgenic mice were fasted overnight and bled 2 to 3 h after the beginning of the light cycle from the retro-orbital plexus under isoflorane anesthesia. The mice were allowed to recover for 2 weeks and were then placed on a diet that contained rosiglitazone (4 g/kg diet). After 1 week of ad libitum access to the rosiglitazone diet, the mice were again fasted and bled. Plasma lipids, insulin, and glucose concentrations were determined before and after rosiglitazone treatment. To determine the effect of rosiglitazone treatment on skeletal muscle FA oxidation, another group of nine apoA-II transgenic mice were placed on the rosiglitazone diet for 1 week. The animals were fasted overnight, and rates of FA oxidation (^{14}C-palmitate) were determined in isolated soleus muscles as described above, and were compared with rates of oxidation in another group of age matched apoA-II transgenic male mice that received the identical diet without rosiglitazone.

Insulin and glucose assays

Plasma insulin concentrations were determined in duplicate via ELISA using kits from Crystal Chem Inc. (Downers Grove, IL; cat. #IVSKR020). The intra- and inter-assay precision for the insulin assays are 3.5% and 6.3%, respectively. The minimum detectable level of mouse insulin is 156 pg/ml. Plasma glucose concentrations were determined in triplicate using a commercially available kit (Sigma, cat. #315-100). In experiments in which glucose concentrations were determined, plasma was isolated within 15 min after bleeding to minimize glucose metabolism by erythrocytes.

Western blot analysis of CD36, apoA-II, and apoA-I

CD36 was extracted from skeletal muscles of control and apoA-II transgenic mice following an overnight fast. Briefly, aliquots of skeletal muscle were homogenized in ice-cold PBS containing 1% Igepal CA-630 (Sigma, cat. #I3021), 0.5% sodium deoxycholate (Sigma, cat. #D6750), 0.1% SDS (Sigma, cat # L4509), 1×10^{-4} g/ml PMSF (Sigma, cat. #P7626), 30 μl/ml aprotinin (Sigma, cat. #A6279)), and 1×10^{-4} M sodium orthovanadate (Sigma, cat. #S6508). Extracted tissue proteins were separated on 4-20%, 1-mm thick Novex Tris-glycine gels (Invitrogen, Carlsbad, CA). CD36 was identified via Western blotting using the Cascade Bioscience Primary CD36 antibody (Winchester, MA) (cat. #ABM5525) diluted 1:1000 in 5% milk-phosphate buffered saline with Tween and quantitated using chemiluminescent detection (Amersham, Piscataway, NJ) and densitometry. Plasma apoA-II and apoA-I concentrations were determined using the same type of gels and chemiluminescent detection followed by densitometry. Aliquots of a pooled mouse plasma sample were included on each gel to allow normalization of the results among samples from different gels. The anti-mouse apoA-II and apoA-I antibodies were obtained from Biodesign International (Camarillo, CA). Previously determined average apoA-I (119 mg/dl) and apoA-II (15 mg/dl) concentrations in plasma from C57BL/6 mice were used as the reference value to convert densitometric units to mg/dl (27).

Expression of heart type FA-binding protein (H-FABP), FA transport protein 1 (FATP-1), and medium-chain acyl-CoA dehydrogenase (MCAD) in skeletal muscle of apoA-II transgenic and control mice

Total mouse muscle RNA was isolated from four individual control and four apoA-II transgenic male mice using Trizol reagent (Invitrogen) according to the manufacturer's protocol. One μg of total RNA was reverse transcribed using Oligo dT and Superscript-III reverse transcriptase (Invitrogen).

Quantitative RT-PCR was performed using an ABI Prizm 7700 Sequence Detection System (Applied Biosystems, Foster City, CA) and SYBR Green detection (SYBR Green Taq ready mix, Sigma). cDNA sequences for the analyzed genes were obtained from the gene bank and primers were designed using the PrimerQuest software (Integrated DNA Technologies, Coralville, IA). The primer sequences were as follows: mFABP forward 5′-AACGGGCAGGAGACAACACTAACT-3′, reverse 5′-TCATAAGTCCGAGTGCTCACCACA-3′; mMCAD forward 5′-TCGGTGAAGGAGCGGTTTCAAGA-3′, reverse 5′-AAACTCCTTGGTGCTCCACTAGCA-3′; mFATP1 forward 5′-ACAGCCAGTTGGACCCTAACTCAA-3′, reverse 5′-TGGATCTTGAAGGTGCCTGTGGTA-3′. Primer sequences for the housekeeping gene β2 microglobulin have been described previously (28). Primers were designed for their product to span at least 2 exons and have a melting temperature of 60°C and were verified using BLAST search against the mouse genome database. Correct sizes of the PCR products (amplicons) were verified with agarose gel electrophoresis. Additional melting curve analysis of each PCR product was performed to confirm the presence of a single amplicon in the PCR reaction. Serial dilutions of the pooled samples were used to construct the standard curve and determine the real-time PCR efficiency for each primer pair using ABI Prizm 7700 software. Each individual mouse cDNA sample was analyzed separately by obtaining the relative expression values from the constructed standard curve and correcting for the β2 microglobulin expression. The final data are expressed as relative expression of a respective gene in the apoA-II group versus the control group (set as 100%).

RESULTS

We previously observed that mice overexpressing mouse apoA-II exhibited not only altered lipoprotein profiles but also IR (12). ApoA-II plasma concentrations differed between males and females and between mice heterozygous and homozygous for the transgene (**Table 1**). Clearly there is a dose-dependent effect of apoA-II expression on plasma concentrations of cholesterol, triglycerides, FFAs, glucose, and insulin (see Table 1). Our present studies have been conducted using male mice homozygous for the apoA-II transgene because of the higher levels of apoA-II, making it easier to detect apoA-II effects on traits relevant to glucose and FA metabolism.

We previously reported that skeletal muscle appears to be the insulin-resistant tissue in apoA-II transgenic mice (12). Those aspects of the phenotype that we had examined with respect to hepatic metabolism suggested that the livers in the apoA-II transgenic mice were responding normally to the increased plasma insulin concentrations. Because hepatic and skeletal muscle glycogen content is an indicator of insulin responsiveness (29), we determined the glycogen content of these tissues in apoA-II transgenic and control mice. Hepatic glycogen mass was significantly increased in the apoA-II transgenic mice, while skeletal muscle glycogen was significantly reduced (**Fig. 1A, 1B**). These results are consistent with a normal response of the liver to the elevated insulin levels in the apoA-II transgenic mice, and with skeletal muscle IR.

Because altered FA oxidation in skeletal muscle is believed to result in skeletal muscle IR, we determined rates of oxidation of U-^{14}C-palmitic acid in soleus muscles isolated from animals that had been fasted overnight. Oxidation of palmitic acid to CO_2 was reduced approximately 30% in the skeletal muscle from apoA-II transgenic mice compared with controls (**Fig. 2A**). Carnitine palmitoyltransferase 1 (CPT-1) is necessary for the transport of long-chain FAs into the mitochondria and is generally regarded as the rate-limiting step in FA oxidation (30). Decreased CPT-1 activity has been linked to skeletal muscle IR, increased skeletal muscle triglyceride content and the development of obesity, all phenotypes of the apoA-II transgenic mouse (31–33). To determine if changes in CPT-1 activity could account for the lower rates of FA oxidation in skeletal muscle from the apoA-II transgenic mice, the experiments were repeated using the short-chain FA octanoate. Octanoate does not require CPT-1 for transport into the mitochondria. The rates of oxidation of octanoate were also decreased approximately 25% in the skeletal

TABLE 1. Changes in plasma concentrations of total cholesterol, HDL cholesterol, triglycerides, FFAs, glucose, and insulin correlate with plasma apoA-II concentrations in transgenic mice

	Female C57 (n = 20)	Female het (n = 30)	Female homo (n = 30)	Male C57 (n = 37)	Male het (n = 30)	Male homo (n = 30)
Total cholesterol	90 ± 3	139 ± 3	216 ± 4[a,b]	101 ± 4	211 ± 4[a]	357 ± 19[a,b]
HDL cholesterol	71 ± 3	111 ± 2[a]	167 ± 4[a,b]	74 ± 3	165 ± 3[a]	240 ± 5[a,b]
Triglycerides	57 ± 10	97 ± 6[a]	156 ± 16[a,b]	59 ± 5	160 ± 13[a]	345 ± 24[a,b]
FFAs	54 ± 5	73 ± 2[a]	82 ± 4[a,b]	56 ± 2	83 ± 6[a]	107 ± 5[a,b]
Glucose	95 ± 6	105 ± 9	106 ± 8	101 ± 7	111 ± 9	139 ± 11[a]
Insulin	453 ± 105	522 ± 142	1018 ± 397[a,b]	537 ± 98	1090 ± 386[a]	1752 ± 476[a,b]
apoA-II	15 ± 1	39 ± 3[a]	66 ± 5[a,b]	17 ± 2	55 ± 4[a]	97 ± 7[a,b]

Five-month-old C57BL/6 (C57) controls, heterozygous apoA-II transgenics and homozygous apoA-II transgenics were fasted overnight before bleeding. All values are expressed as the mean ± SEM in mg/dl except for insulin concentrations, which are expressed in pg/ml.

het, heterozygous; homo, homozygous.

[a] Significantly different from C57BL/6 mice of the same sex ($P < 0.05$).

[b] Significantly different from heterozygous mice of the same sex ($P < 0.05$).

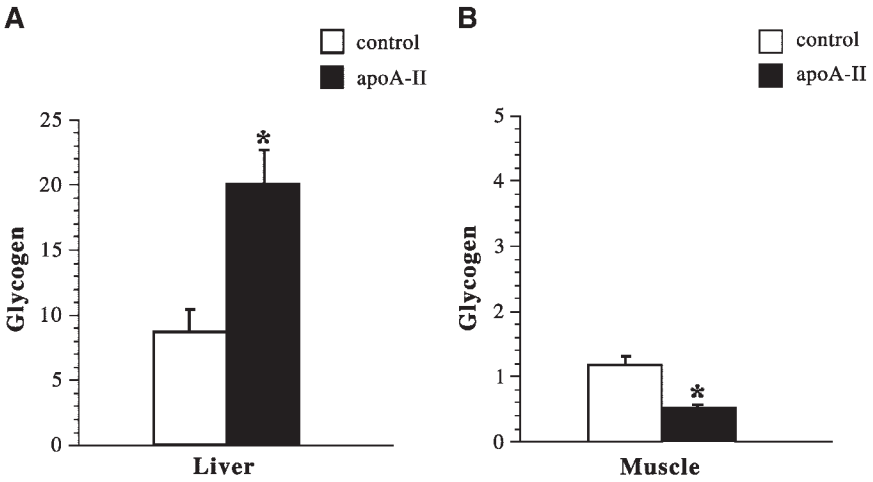

Fig. 1. Hepatic and skeletal muscle glycogen content. The concentration of glycogen in liver (A) and skeletal muscle (B) from apoA-II transgenic and control mice were determined. Animals were sacrificed 3 h after the beginning of the light cycle, having been allowed ad libitum access to food throughout the dark cycle. Data represent the mean ± SEM for four animals in each group. * Values significantly different from control mice ($P < 0.05$).

muscle of the apoA-II transgenic mice, suggesting that the lower rate of FA oxidation in skeletal muscle is not due to decreased activity of CPT-1 (Fig. 2B).

Although CPT-1 is generally regarded as the rate-limiting step in the β-oxidation of long chain FAs, β-FA oxidation is a complex process involving many different steps. We examined the expression of three genes whose products are known to affect the β-oxidation of FAs by acting at diverse points in the FA metabolic pathway. Medium-chain acyl-CoA dehydrogenase (MCAD) is a mitochondrial enzyme that catalyzes the initial reaction in the FA β-oxidation cycle (34, 35). Heart type FA-binding protein (H-FABP) is a cytosolic protein important in the intracellular transport of FAs with effects on triglyceride accumulation and oxidation (36–38). Fatty acid transport protein 1 (FATP-1) is an integral plasma membrane protein involved with FA uptake into the cell, and which also has been reported to have acyl-CoA synthase activity (39–41). Consistent with the decreased FA oxidation observed in skeletal muscle of the apoA-II transgenic mice, expression of MCAD was significantly decreased, and there was a trend toward decreased expression of both H-FABP and FATP-1 (**Fig. 3**).

As described above, CD36 appears to be a good candidate for mediating the effects of apoA-II on skeletal muscle FA metabolism. To determine if the content of CD36 in skeletal muscle was altered in the apoA-II transgenic mice, total protein was extracted from aliquots of skeletal muscle from fasted animals and separated via PAGE. Western blot analysis demonstrated that skeletal muscle CD36 content was not significantly different between control and apoA-II transgenic mice (**Fig. 4**).

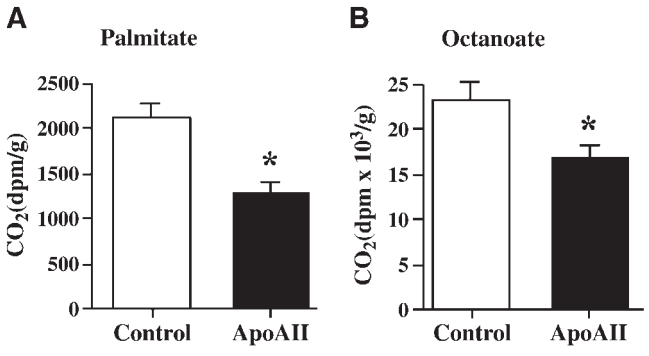

Fig. 2. Skeletal muscle FA oxidation. Oxidation of ^{14}C-labeled palmitate (A) and ^{14}C-octanoate (B) were determined in soleus muscles isolated from control and apoA-II transgenic mice that had been fasted overnight. Data represent the mean ± SEM for five animals in each group. * Values that are significantly different from control mice ($P < 0.05$).

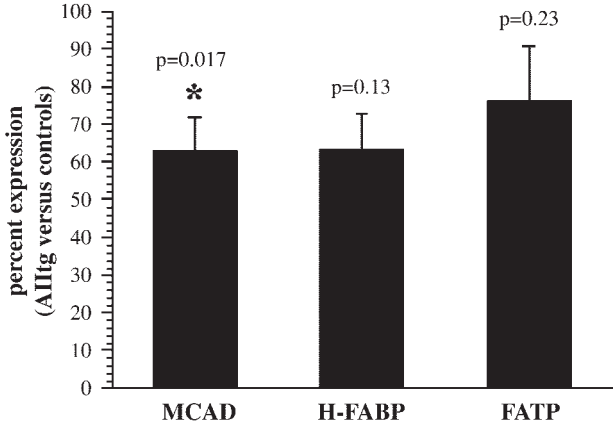

Fig. 3. Expression of medium-chain acyl-CoA dehydrogenase (MCAD), heart type FA-binding protein (H-FABP) and FA transport protein (FATP) in skeletal muscle. Expression of MCAD, H-FABP, and FATP was determined in skeletal muscle of control and apoA-II transgenic mice with quantitative real-time PCR using SYBR Green detection as described in Materials and Methods. Data are the mean ± SEM for four animals in each group, presented as the percentage of expression observed in the control mice. * Values that are significantly different from control ($P < 0.05$).

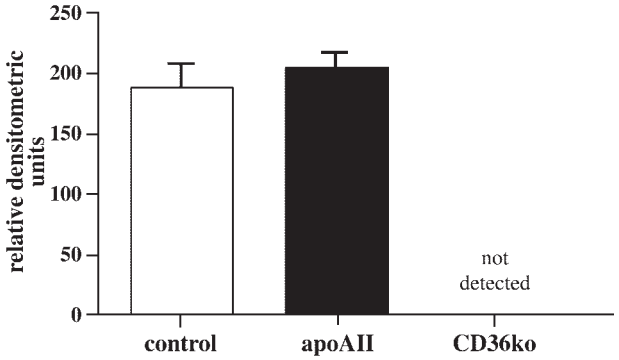

Fig. 4. Skeletal muscle CD36 content. Following an overnight fast, skeletal muscle from control, apoA-II transgenic (apoAII), and CD36 null (CD36ko) mice was dissected under a microscope. Total protein was isolated and subjected to PAGE followed by Western blot analysis and densitometric scanning. The relative concentrations of CD36 in each sample are expressed in relative densitometry units. The data represent the mean ± SEM for three animals in each group. CD36 null mice were included as a negative control. As expected, CD36 was not detected in this group.

To determine if CD36 alters plasma concentrations of apoA-II in mice, we determined plasma concentrations of apoA-II in the CD36 knockout and control mice. ApoA-II was increased in the plasma of CD36 null mice, while apoA-I concentrations were unchanged, providing support for an interaction between CD36 and apoA-II (**Fig. 5**). In in vitro HDL-binding studies using CD36-transfected COS cells, we demonstrated previously that HDLs from apoA-II transgenic mice exhibit significantly reduced binding to the CD36 receptor, and that this decreased binding is associated with a decrease in cholesterol exchange (42).

To investigate the in vivo effects of CD36 on the apoA-II transgenic phenotype, apoA-II transgenic mice on the CD36 null background were derived as described in Materials and Methods. The effects of the apoA-II transgene

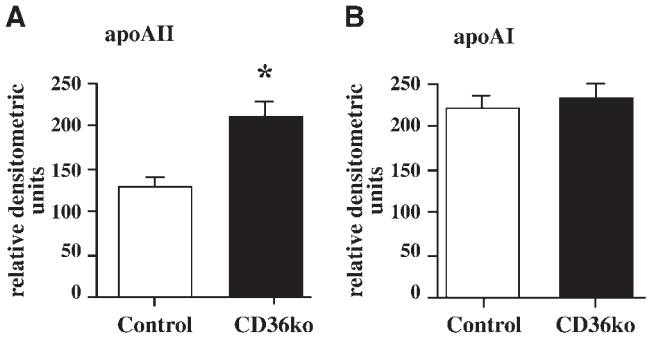

Fig. 5. Plasma apoA-II and apoA-I concentrations in CD36 null mice. Following an overnight fast, mice were anesthetized and blood was obtained from the retro-orbital plexus. Aliquots of plasma were subjected to PAGE followed by Western blot analysis for apoA-II and apoA-I. The relative concentrations of apoA-II (A) and apoA-I (B) were then determined via densitometric scanning. The relative concentrations of the apolipoproteins in each sample are expressed in relative densitometry units. The data represent the mean ± SEM for five animals in each group. * Values that are significantly different from control ($P < 0.05$).

were not markedly different, whether or not the CD36 receptor was present. Plasma concentrations of apoA-II correlated with total and HDL cholesterol (**Fig. 6**), free FAs and triglycerides (**Fig. 7**), and glucose and insulin (**Fig. 8**), in both CD36 wild-type and CD36 null mice. We conclude that the major effects of apoA-II on plasma lipids and IR in our mice are not mediated by CD36.

To determine if the IR in the apoA-II transgenic mice would respond to thiazolidinediones, a class of drugs used in the treatment of human IR, we treated the mice with rosiglitazone. After only 1 week of treatment, rosiglitazone significantly reduced plasma concentrations of triglycerides, FFAs, glucose, and insulin (**Table 2**), effects similar to those observed in humans (43). There was also a decrease in HDL cholesterol after rositglitazone treatment; however, no significant changes in plasma concentrations of apoA-I or apoA-II were observed (see Table 2).

To determine if skeletal muscle FA oxidation was altered by rosiglitazone treatment, we placed another group of apoA-II transgenic mice on rosiglitazone for 1 week and compared rates of palmitate oxidation in isolated soleus muscles from these animals to rates of oxidation in muscles from a separate group of age-matched apoA-II transgenic mice that did not receive rosiglitazone. Following rosiglitazone treatment, there was a trend toward increased FA oxidation (**Fig. 9**).

DISCUSSION

We previously demonstrated that transgenic mice overexpressing the HDL apolipoprotein, apoA-II, exhibit several traits associated with the IR syndrome (12). In examining the basis for IR in the apoA-II transgenic mice, we had determined that the skeletal muscle was the insulin-resistant tissue (12). In the present study, we demonstrate that FA oxidation is decreased in the skeletal muscle of the apoA-II transgenic mice (see Fig. 2), and that this decrease in oxidation is unlikely due to changes in CPT-1, but rather to the step in oxidation catalyzed by MCAD (see Fig. 3). We previously demonstrated that HDL enriched in apoA-II binds less efficiently to CD36, a scavenger receptor with known effects on FA and glucose metabolism (42). In the present study, we demonstrate that overexpression of apoA-II does not alter CD36 protein concentrations in skeletal muscle (see Fig. 4); however, knocking out CD36 does increase plasma concentrations of apoA-II (see Fig. 5). To further evaluate the role of CD36 in mediating the effects of apoA-II overexpression, we generated 83 progeny from an intercross of CD36 null and apoA-II transgenic mice. The apoA-II transgenic phenotype was not markedly different whether or not CD36 was expressed (see Figs. 6-8). We also demonstrate that the IR phenotype in the apoA-II transgenic mice is markedly ameliorated by only 1 week of treatment with rosiglitazone (see Table 2).

Plasma insulin concentrations are 2.5 to 3 times higher in the apoA-II transgenic mice. Insulin is a regulator of the glycogen content in both liver and skeletal muscle (29). Insulin stimulates the conversion of glucose to glu-

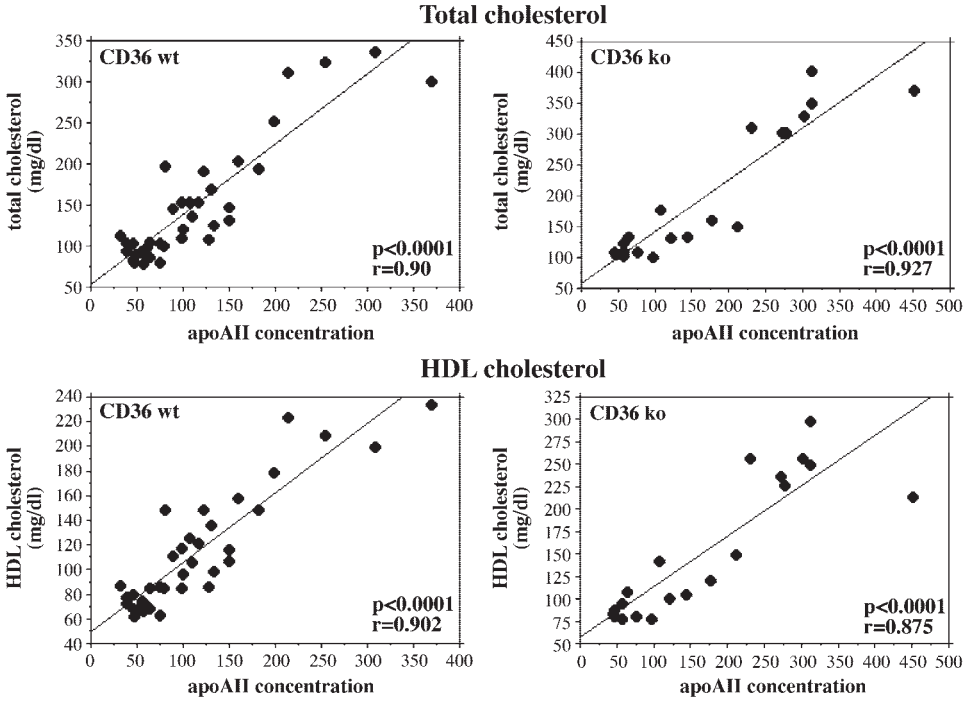

Fig. 6. Effects of plasma apoA-II concentrations on total and HDL cholesterol in control and CD36 null mice. The apoA-II transgene was bred onto the CD36 null background, producing 83 F2 progeny. From the F2 progeny, 21 mice (11 females, 10 males) were wild-type for CD36 (CD36 wt) and 38 (23 females, 15 males) were homozygous for the CD36 null transgene (CD36ko). Following an overnight fast, mice were bled from the retro-orbital plexus under isoflorane anesthesia, and plasma concentrations of total cholesterol, HDL cholesterol, and apoA-II were determined as described in Materials and Methods. Plasma total cholesterol is plotted against plasma apoA-II concentrations in the upper section of the figure for both CD36 wt (left panel) and CD36 null (right panel) mice. Plasma HDL is plotted against apoA-II concentrations in the lower portion of the figure. Cholesterol concentrations are expressed in mg/dl; apoA-II concentrations are expressed in relative densitometry units. Linear regression analysis was performed using Statview statistical analysis software (SAS Institute, Cary, NC).

cose-6-phosphatase, which then undergoes isomerization to glucose-1-phosphate and is incorporated into glycogen by the enzyme glycogen synthase, the activity of which is also stimulated by insulin. Insulin also inhibits phosphorylase, which decreases glycogen breakdown. The increased concentration of glycogen in the livers of the apoA-II transgenic mice is consistent with a normal response by the liver to the increased plasma insulin concentrations (see Fig. 1). In contrast, the decreased skeletal muscle glycogen content in the apoA-II transgenic mice (see Fig. 1), even in the presence of the increased plasma insulin concentrations in these animals, is consistent with skeletal muscle IR.

Increased plasma FFA concentrations have been hypothesized to contribute to IR by increasing use of FAs by muscle as a consequence of the concentration-dependent uptake of FFAs (4, 44). Increased FA oxidation is then believed to inhibit glucose oxidation and glycolysis, with subsequent inhibition of glucose uptake. On the other hand, some evidence suggests that the basis of skeletal muscle IR in obesity may actually be an impaired capacity for use of plasma FFAs (45–48). Use of FFAs for energy by skeletal muscle has been reported to be reduced in obesity. There is evidence that impaired oxidation of fat may be a contributing factor to weight gain (32, 33). The activity of CPT-1 in muscle has been negatively correlated with visceral fat mass, and rates of FFA uptake have been correlated with CPT-1 activity (31). These studies suggest that decreased oxidative capacity and use of FFAs by skeletal muscle can result in skeletal muscle IR and increased adiposity. Skeletal muscle FA oxidation is reduced in apoA-II transgenic mice (see Fig. 2), consistent with studies that have linked decreased FA oxidation to the development of skeletal muscle IR and obesity, the phenotype we observed in apoA-II transgenic mice (12). However, the reduced rates of oxidation in apoA-II transgenic mice do not appear to be due to lower CPT-1 activity, because the oxidation of octanoate, which does not require CPT-1 for transport into mitochondria, was also reduced (see Fig. 2). We used quantitative RT-PCR to examine the expression of three genes that are important in FA metabolism and act at diverse points in the FA metabolic pathway: MCAD, H-FABP, and FATP-1. The expression of MCAD was significantly decreased in skeletal muscle of apoA-II transgenic mice compared with controls (see Fig. 3). MCAD is a member of a family of mitochondrial dehydrogenases that catalyze the initial reaction in the FA β-oxidation cycle (34, 35). MCAD shows greatest activity for FA

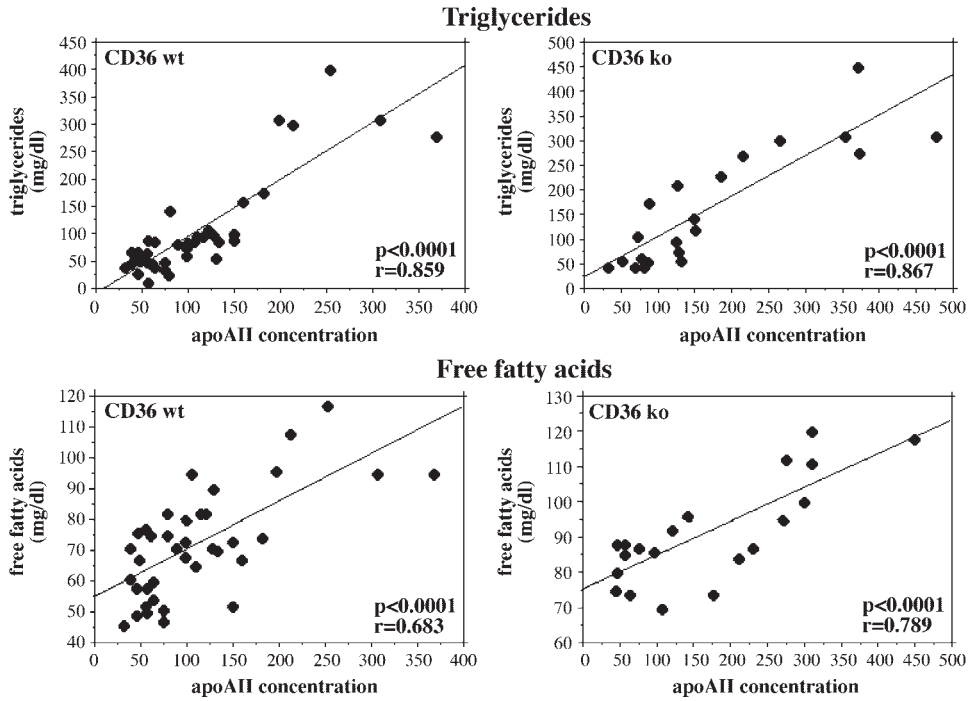

Fig. 7. Effects of plasma apoA-II concentrations on triglycerides and FFAs in control and CD36 null mice. The apoA-II transgene was bred onto the CD36 null background, producing 83 F2 progeny. From the F2 progeny, 21 mice (11 females, 10 males) were wild-type for CD36 (CD36 wt) and 38 (23 females, 15 males) were homozygous for the CD36 null transgene (CD36 ko). Following an overnight fast, mice were bled from the retro-orbital plexus under isoflorane anesthesia and plasma concentrations of triglycerides, free fatty acids, and apoA-II were determined as described in Materials and Methods. Plasma triglyceride concentrations are plotted against plasma apoA-II concentrations in the upper section of the figure for both CD36 wt (left panel) and CD36 ko (right panel) mice. Plasma FFAs are plotted against apoA-II concentrations in the lower portion of the figure. Triglyceride and FFA concentrations are expressed in mg/dl; apoA-II concentrations are expressed in relative densitometry units. Linear regression analysis was performed using Statview statistical analysis software (Abacus Concepts).

substrates that are from 8 to 12 carbons in chain length. Decreased activity of MCAD would affect the oxidation of the 8-carbon FA octanoate, as well as the oxidation of the 16-carbon palmitate, after the length of the original palmitate was shortened through successive cycles of β-oxidation. We also observed a trend toward decreased expression of both H-FABP and FATP-1 (see Fig. 3). H-FABP is a cytosolic protein that is important in the intracellular transport of FAs, with effects on triglyceride accumulation and oxidation (36–38). FATP-1 is an integral plasma membrane protein that is involved with FA uptake into the cell and also has been reported to have acyl-CoA synthase activity (39–41). Decreased expression of MCAD is sufficient to explain the results of our FA oxidation experiments. Whether decreased expression of H-FABP and FATP-1 contribute to reduced FA oxidation or merely represent a response to the reduced rates of oxidation as FAs and their metabolites accumulate in the cell has yet to be determined.

HDL is a ligand for CD36, a scavenger receptor that has been demonstrated to be involved in FA and glucose metabolism (13, 14). We have previously demonstrated that HDL from apoA-II transgenic mice exhibits reduced binding to the CD36 receptor, decreasing cholesteryl ester exchange as a result (42). Also, genetic analysis of a Mexican American study population indicated a locus on chromosome 7 (lod acore 3.0) over the CD36 gene that was linked to plasma concentrations of apoA-II (data not shown). Therefore, CD36 appears to be a reasonable candidate for mediating the effects of altered HDL upon skeletal muscle FA metabolism. In the present study, there were no significant differences in the CD36 content of skeletal muscle from apoA-II transgenic mice compared with controls (see Fig. 4); however, plasma concentrations of apoA-II were significantly increased in the plasma of the CD36 knockout mice (see Fig. 5). This effect of CD36 on apoA-II concentrations is supported by the association of the CD36 gene locus with plasma apoA-II concentrations in the Mexican American study described above. An earlier study also demonstrated that CD36 knockout mice had a significant increase in plasma HDL cholesterol, as well as a shift to larger HDL particles, which is consistent with the changes in HDL observed with increasing apoA-II concentrations in our transgenic mice (13). The mechanism through which CD36 increases plasma HDL cholesterol and apoA-II concentrations in these mice is not known, but it may involve changes in normal HDL metabolism related to cholesteryl ester exchange mediated by

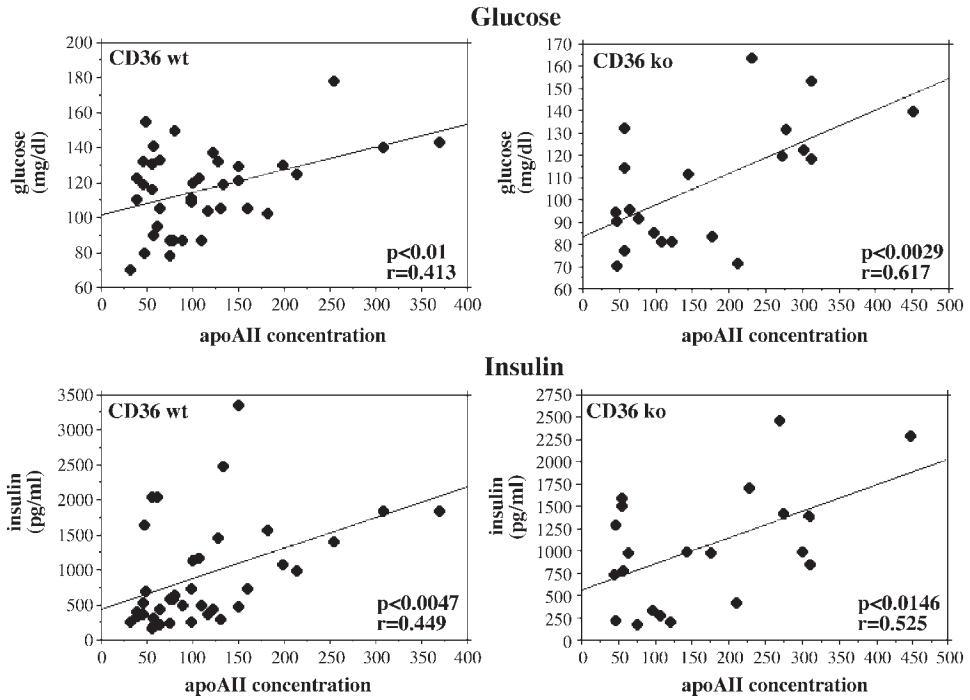

Fig. 8. Effects of plasma apoA-II concentrations on glucose and insulin in control and CD36 null mice. The apoA-II transgene was bred onto the CD36 null background, producing 83 F2 progeny. From the F2 progeny, 21 mice (11 females, 10 males) were wild-type for CD36 (CD36 wt) and 38 (23 females, 15 males) were homozygous for the CD36 null transgene (CD36 ko). Following an overnight fast, mice were bled from the retro-orbital plexus under isoflorane anesthesia, and plasma concentrations of glucose, insulin, and apoA-II were determined as described in Materials and Methods. Plasma glucose concentrations are plotted against plasma apoA-II concentrations in the upper section of the figure for both CD36 wt (left panel) and CD36 null (right panel) mice. Plasma insulin concentrations are plotted against apoA-II concentrations in the lower portion of the figure. Glucose concentrations are expressed in mg/dl, insulin concentrations are expressed in pg/ml, and apoA-II concentrations are expressed in relative densitometry units. Linear regression analysis was performed using Statview statistical analysis software (Abacus Concepts).

the CD36 receptor, which ultimately affects clearance and metabolism of apoA-II-enriched HDL. To examine the role of CD36 in mediating the apoA-II transgenic phenotype in vivo, we derived combined apoA-II transgenic/CD36 null mice. The effects of apoA-II on plasma lipids, cholesterol, insulin, and glucose were similar whether or not CD36 was present (see Figs. 6–8). Overall, these results suggest that there is an HDL–CD36 interaction that has effects on HDL metabolism; however, the primary effects of apoA-II in the transgenic mice do not appear to be mediated by CD36.

TABLE 2. Treatment with rosiglitazone ameliorates IR in apoA-II transgenic mice

	Pretreatment (n = 14)	Posttreatment (n = 14)
Total cholesterol (mg/dl)	252 ± 20	216 ± 17
HDL cholesterol (mg/dl)	176 ± 12	137 ± 9[a]
Triglycerides (mg/dl)	212 ± 28	98 ± 14[a]
FFAs (mg/dl)	41 ± 2	32 ± 2[a]
Glucose (mg/dl)	142 ± 9	109 ± 8[a]
Insulin (pg/ml)	1880 ± 318	718 ± 110[a]
apoA-II (mg/dl)	93 ± 8	85 ± 7
apoA-I (mg/dl)	140 ± 13	127 ± 8

Fasting plasma lipids, glucose, insulin, and apoA-I and apoA-II concentrations were determined in a group of 14 apoA-II transgenic mice before (pretreatment) and after (posttreatment) administration of rosiglitazone in the diet (4 mg/kg) for 1 week.

[a] Significantly different from pretreatment values ($P < 0.05$).

To further assess the similarity between the IR phenotype in the apoA-II transgenic mice and certain types of human IR, we treated the mice with the insulin-sensitizing thiazolidinedione rosiglitazone. After only 1 week of treatment, rosiglitazone significantly reduced plasma concentrations of triglycerides, FFAs, glucose, and insulin (see Table 1), consistent with effects observed in humans (43). We also observed a significant reduction in HDL cholesterol; however, plasma concentrations of apoA-I and apoA-II were not significantly changed (see Table 1). In humans, by contrast, most studies report an increase in HDL cholesterol in response to thiazolidinedione treatment (49, 50). The effect we observed with rosiglitazone on HDL cholesterol levels in apoA-II transgenic mice is consistent with a previous study that demonstrated a decrease in HDL cholesterol when diabetic LDL receptor-deficient mice were treated with troglitazone (51). Although the mechanism responsible for the different effects of thiazolidinediones on plasma HDL concentrations between mice and humans is not known, it most likely reflects differences in HDL metabolism between the two species (as discussed below). In a separate series of experiments, rates of

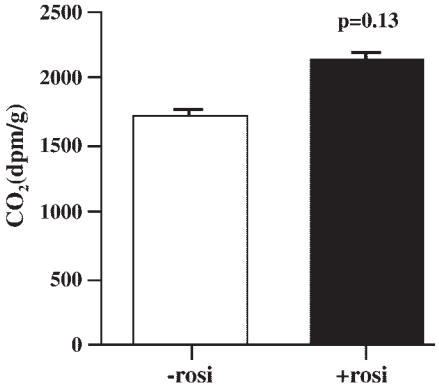

Fig. 9. Effects of rosiglitazone treatment on skeletal muscle FA oxidation. Rosiglitazone was administered in the diet (4 mg/kg) to a group of apoA-II transgenic male mice for 1 week (+rosi). A control group of age-matched apoA-II transgenic male mice received the identical diet without rosiglitazone (−rosi). Oxidation of ^{14}C-labeled palmitate to CO_2 was determined in soleus muscles isolated from mice that had been fasted overnight. Data represent the mean ± SEM for nine animals in each group.

FA oxidation in soleus muscles isolated from mice that were treated with rosiglitazone were compared with rates of oxidation in another group of apoA-II transgenic mice that did not receive the drug. There was a trend toward increased FA oxidation after rosiglitazone treatment (see Fig. 9). The failure to reach statistical significance could reflect differences in basal rates of oxidation between the two groups of mice used in the study. In these experiments we could not use the same group of mice to compare pre- and post-drug effects, as we were able to do in examining the changes in plasma lipids, glucose, and insulin in the initial experiment.

The effects of overexpressing mouse apoA-II on FA and glucose metabolism does not appear to be a nonphysiological response that is observed only with markedly elevated plasma apoA-II concentrations. Earlier genetic studies from our laboratory demonstrated a link between the apoA-II gene locus and plasma concentrations of apoA-II and free FAs in common inbred strains of mice (52). In our apoA-II transgenic mice, the effects of apoA-II in both sexes, and between mice heterozygous and homozygous for the apoA-II transgene, demonstrate a dose–dependent relationship with plasma apoA-II concentrations (see Table 1). This dose–dependent effect of apoA-II was also observed in the progeny from the apoA-II transgenic/CD36 null cross (see Figs. 6-8) Furthermore, mice that completely lack apoA-II exhibit increased insulin sensitivity, with decreased plasma concentrations of triglycerides, FFAs, and glucose (11). Thus, the effects of mouse apoA-II on plasma lipids and IR have been demonstrated over the entire range of plasma apoA-II concentrations.

The present study was performed using mice that express a transgene for mouse apoA-II. Results from studies in mice that express a transgene for human apoA-II have shown similarities as well as differences with respect to the effects of apoA-II in the mouse (53). Most (54–61) but not all (62–66) of these studies have demonstrated that increasing human apoA-II in the mouse resulted in an increase in plasma triglycerides and non-HDL cholesterol, consistent with the effects observed in the present study. However, unlike the transgene for mouse apoA-II, human apoA-II does not increase HDL cholesterol or appear to affect IR or obesity in these mouse models (67). Sequence differences between mouse and human apoA-II are likely to account for some of these differences in HDL metabolism (53). Although human apoA-II does not affect IR and obesity in mice, several studies have demonstrated an effect of apoA-II on IR and increased fat mass in humans (68–70, 72). Therefore, failure to observe these effects with human apoA-II in mice, may be due to species-specific interactions with other apolipoproteins, receptors, enzymes, and lipid transfer proteins that are involved in HDL metabolism.

Many studies have demonstrated the usefulness of the mouse as an experimental model to investigate aspects of lipoprotein metabolism relevant to human physiology. Still, care must be exercised when extrapolating the results of any animal study to humans. The phenotype in apoA-II transgenic mice resembles many aspects of the IR syndrome in humans, including increased adiposity, IR, and hypertriglyceridemia. However, the IR syndrome in humans is associated with decreased plasma HDL cholesterol, while apoA-II transgenic mice have increased HDL cholesterol. Part of this difference in HDL cholesterol probably relates to differences in human versus mouse apoA-II, as discussed above. When a transgene for human apoA-II was expressed in mice, plasma HDL cholesterol concentrations were reduced and the HDL became smaller in size (57, 64). Mouse apoA-II appears to have the opposite effect, because increasing mouse apoA-II in our studies results in increased HDL cholesterol and larger particles, while apoA-II null mice have HDL particles that are smaller in size. Although overexpression of mouse apoA-II had the opposite effect on total HDL cholesterol compared with human apoA-II, both increased triglycerides and non-HDL cholesterol. A similar situation apparently exists with thiazolidinedione treatment, which appears to ameliorate IR in both mice and humans with a decrease in plasma concentrations of triglycerides, FFAs, glucose and insulin, while total HDL cholesterol is generally increased in humans and decreased in mice. Thus, because of differences in HDL metabolism between mice and humans, effects on total HDL cholesterol can be different, while other effects on lipoprotein metabolism and IR may be similar. Another explanation for this apparent difference in effects on HDL cholesterol between human metabolic syndrome and our mouse model relates to the use of total HDL cholesterol as an indication of metabolically relevant changes in HDL. In both mice and humans, HDLs are not a single homogeneous type of lipoprotein, but consist of several types of particles that differ in size and composition and have been demonstrated to have different metabolic effects. Changes in total HDL cholesterol may not reflect important changes in various subfractions of HDL, which may be more relevant to the traits under investigation. This is supported by the observation that HDLs from

apoA-II transgenic mice are not as protective as HDLs from control mice in preventing the oxidation of LDLs in a cell culture model of the artery wall (19). Evidently, the subfraction of HDLs important for this protective effect is reduced in apoA-II transgenic mice, even though total HDL cholesterol is increased.

Studies in humans also have demonstrated links between apoA-II and increased atherosclerosis, type 2 diabetes, plasma concentrations of triglycerides and FFAs, and visceral obesity (52, 68–72). The similar effects of apoA-II in our transgenic mice and in humans, and the observation that IR in apoA-II transgenic mice responds to treatment with rosiglitazone, suggest similarities to some types of human IR and type 2 diabetes. Thus, the apoA-II transgenic mouse appears to be an interesting animal model for further research.

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