Studies With Apolipoprotein A-II Transgenic Mice Indicate a Role for HDLs in Adiposity and Insulin Resistance

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Apolipoprotein A-II (apoA-II) is the second most abundant protein in HDLs. Genetic studies in humans have provided evidence of linkage of the apoA-II gene locus to plasma free fatty acid (FFA) levels and to type 2 diabetes, and transgenic mice overexpressing mouse apoA-II have elevated levels of both FFA and triglycerides. We now show that apoA-II promotes insulin resistance and has diverse effects on fat homeostasis. ApoA-II transgenic mice have increased adipose mass and higher plasma leptin levels than C57BL/6J control mice. Fasting glucose levels were similar between apoA-II transgenic and control mice, but plasma insulin levels were elevated approximately twofold in the apoA-II transgenic mice. Compared with control mice, apoA-II transgenic mice exhibited a delay in plasma clearance of a glucose bolus. Adipose tissue isolated from fasted apoA-II transgenic mice exhibited a 50% decrease in triglyceride hydrolysis compared with adipose tissue from control mice. This is consistent with a normal response of adipose tissue to the increased insulin levels in the apoA-II transgenic mice and may partially explain the increased fat deposition. Skeletal muscle isolated from fasted apoA-II transgenic mice exhibited reduced uptake of 2-deoxyglucose compared with muscles isolated from control mice. Our observations indicate that a primary disturbance in lipoprotein metabolism can result in several traits associated with insulin resistance, consistent with the hypothesis that insulin resistance and type 2 diabetes can, under certain circumstances, be related primarily to altered lipid metabolism rather than glucose metabolism. *Diabetes* 50:643–651, 2001

The HDLs protect against the development of atherosclerosis, the major cause of heart disease and stroke, but the precise mechanisms involved are unclear. Most studies have focused on the role of HDL in reverse cholesterol transport and its antioxidant functions, two pathways by which HDLs are believed to protect against atherosclerosis (1,2). The major protein components of HDL are apolipoprotein (apo)A-I and apoA-II. ApoA-I clearly functions in cholesterol transport and is required for HDL assembly, consistent with the observation that increased levels of apoA-I protect against the development of atherosclerosis (3–5). The role of apoA-II, however, is unknown. ApoA-II appears to impair the reverse cholesterol transport and antioxidant functions of HDL, consistent with the observation that increased apoA-II levels promote the development of atherosclerosis (3,6–9). Also, genetic studies in both mice and humans have shown that the levels of plasma free fatty acids (FFAs) segregate with the apoA-II locus (10).

Insulin resistance is associated with a cluster of metabolic abnormalities, termed the "insulin resistance syndrome," including increased adiposity, elevated insulin levels, elevated plasma triglyceride levels, low HDL levels, and hypertension (11,12). These traits are strongly associated with several chronic diseases including atherosclerosis and type 2 diabetes. The interrelationships among these altered metabolic traits are poorly understood, although shared genetic factors are clearly involved. Human genetic studies have resulted in the identification of certain loci contributing to common forms of type 2 diabetes, adiposity, leptin levels, and plasma triglyceride levels, but the underlying genes are largely unknown (13,14). Biochemical approaches have helped to define disturbances in processes such as insulin signaling and glucose transport, but the primary defects responsible for common forms of insulin resistance have not been revealed. A major obstacle to the analysis of the insulin resistance syndrome is the considerable genetic heterogeneity, compounded by important environmental influences.

We have used genetic studies of animal models to address the problem. Animal models reduce the complications of genetic heterogeneity and environmental influences, and genetic crosses with animal models have much greater power to detect linkage compared with genetic studies in humans (15). In genetic crosses among common inbred strains of mice, we identified the apoA-II gene as an important genetic factor contributing to plasma lipoprotein levels, FFA levels, and atherosclerosis (10,16). Subsequently, we observed, by nonparametric linkage analysis, that variations of the apoA-II gene in humans contribute to the levels of apoA-II, FFAs (10), and plasma apoA-I (17). Transgenic mice overexpressing mouse apoA-II (6,7) or human apoA-II (8) were subsequently constructed and studied. Consistent with mouse genetic studies, the mouse

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Received for publication 25 April 2000 and accepted in revised form 27 November 2000.

apo, apolipoprotein; AUC, area under the curve; BSA, bovine serum albumin; CPT, carnitine palmitoyl transferase; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; IGT, impaired glucose tolerance.

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TABLE 1
Concentration of plasma lipids and lipase activities in apoA-II transgenic and control mice

	Nontransgenic mice	Transgenic mice
Total cholesterol (mg/dl)	101 ± 18 (35)	247 ± 18* (35)
HDL cholesterol (mg/dl)	65 ± 4 (35)	127 ± 8* (35)
VLDL + LDL cholesterol (mg/dl)	36 ± 2 (35)	120 ± 9* (35)
Triglycerides (mg/dl)	47 ± 6 (35)	426 ± 24* (35)
FFA (mmol/l)	0.9 ± 0.1 (35)	4.5 ± 0.4* (35)
β-Hydroxybutyrate (mg/dl)	18 ± 2 (12)	17 ± 2 (12)
Lactate (mg/dl)	17 ± 1 (12)	19 ± 2 (12)
LPL activity (dpm FFA · h^{-1} · ml^{-1} plasma)	11,200 ± 950 (11)	10,750 ± 780 (11)
HTGL activity (dpm FFA · h^{-1} · ml^{-1} plasma)	5,325 ± 350 (11)	4,950 ± 475 (11)
Skeletal muscle triglycerides (mg/g tissue)	12.98 ± 0.45 (3)	18.58 ± 1.79* (3)

Data are means ± SE (*n*). Control and apoA-II transgenic mice were bled after an overnight fast. Plasma concentrations of total cholesterol, HDL cholesterol, triglycerides, FFA, lactate, and β-hydroxybutyrate, as well as activities of lipoprotein lipase (LPL) and hepatic triglyceride lipase (HTGL) were determined as described in RESEARCH DESIGN AND METHODS. *Significantly different from controls ($P < 0.05$), determined by the Student's *t* test.

apoA-II transgene resulted in elevated HDL cholesterol levels, whereas the human apoA-II transgene resulted in variable changes in HDL levels. Mice expressing the transgene for either human or mouse apoA-II exhibited increased susceptibility to atherosclerosis (6,8). The transgenic mice overexpressing mouse apoA-II also exhibited elevated levels of triglycerides, and the plasma lipoproteins promoted accumulation of lipid hydroperoxides (2,7), consistent with the accelerated atherogenesis. Subsequently, mice lacking apoA-II were constructed by gene targeting (18). Consistent with previous genetic studies, the mice showed reduced HDL cholesterol levels and accelerated clearance of triglyceride-rich lipoproteins. The apoA-II-null mice also showed evidence of increased sensitivity to insulin, suggesting a role of apoA-II in insulin metabolism (18). Based on these results, and the hypertriglyceridemia and elevated FFA levels observed in apoA-II transgenic mice, we have further explored the possible influence of apoA-II expression on traits associated with insulin resistance.

We now report that increased expression of apoA-II can contribute to insulin resistance and altered body fat homeostasis. These data provide evidence for the concept that primary alterations in plasma lipid metabolism may contribute to various metabolic syndromes associated with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Animals. Transgenic mice containing multiple copies of the mouse apoA-II gene were derived as described previously (6). To eliminate genetic variation, the apoA-II transgene was isolated on a C57BL/6J background by backcrossing for >10 generations. Male mice homozygous for the transgene and age-matched controls (4–6 months) were used in all experiments. ApoA-II levels in transgenic mice were elevated about fivefold, whereas apoA-I levels were similar to those of controls (7) (data not shown). Animals were housed four to five to a cage, maintained at 25°C on a 12-h light-dark cycle, and provided Harlan-Teklad rodent diet (6% fat) and water ad libitum. The care of the mice, as well as all procedures used in this study, were done in accordance with National Institutes of Health animal care guidelines.

Whole body fat determinations. The percentage of total body fat was determined as described previously (19). Briefly, the animals were killed, and the carcasses were dried to remove all water. They were then homogenized, and the lipid was extracted from an aliquot of the homogenate using a soxhlet apparatus. The lipid content of the homogenate was determined by gravimetric analysis.

Lipid analyses. Plasma was collected from mice that were fasted overnight and bled 2–3 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 (2,20). HDL was isolated by precipitation of VLDL and LDL with heparin and manganese chloride (21). Each lipid determination was measured in triplicate. An external control sample with known analyte concentration was run in each plate to assure accuracy.

Skeletal muscle triglyceride content. The hindquarters of anesthetized (50 mg/kg Nembutal) mice were flushed thoroughly with 25 ml heparinized phosphate-buffered saline that was administered via the abdominal aorta at a flow rate of 6 ml/min. All visible adipose tissue was removed from sections of the soleus and gastrocnemius muscles under a dissecting microscope. Aliquots of muscle tissue were then homogenized, and total lipids were extracted by the method of Folch et al. (22). Triglyceride mass was determined as described above.

Skeletal muscle 2-deoxy-[U-^{14}C]glucose uptake. After an overnight fast, mice were anesthetized with pentobarbital (50 mg/kg body wt), and the soleus muscle was isolated under a dissecting microscope as described previously (23). In one set of experiments, the muscles were weighed and immediately incubated for 20 min in Krebs-Henseleit buffer under 95% O_2:5% CO_2, which contained 5.5 mmol/l glucose, 1% bovine serum albumin (BSA), 2-deoxy-[U-^{14}C]glucose (2.5 μCi/ml), and no exogenous insulin added. At the end of the experiment, the tissue was immediately immersed in an ice-cold saline (0.9%) rinse and then washed in ice-cold saline for 1 h to remove the radioactive label that was in the extracellular space of the muscle, as described previously (23). The uptake studies were repeated in a second set of experiments in which exogenous insulin was added to the incubation media. In these experiments, the soleus muscles were preincubated for 20 min in the same buffer used in the previous set of experiments, except that porcine insulin was present at a concentration of 2 mU/ml. This concentration was reported to provide maximal stimulation of glucose uptake in rat soleus muscle (24). After the preincubation, the tissue was transferred to another vessel that contained fresh media (also containing 2 mU/ml insulin) and 2-deoxy-[U-^{14}C]glucose, and the rates of uptake were determined as described previously.

Plasma apoB determinations. ApoB48 and apoB100 concentrations were determined by a combination of SDS-PAGE and immunoblotting in animals that had been fasted overnight. Plasma samples and molecular weight markers were run on 4–20% gradient gels (Novex). Plasma samples were diluted 1:25 in sample buffer (10% 2-mercaptoethanol, 0.25 mol/l Tris-HCl, pH 6.8, 0.2% SDS, 20% glycerol, and 0.025% bromothymol blue), and 20 μl of each sample preparation was loaded. The gels were electrophoresed at 25 mA for 2 h. The proteins were then transferred to nitrocellulose using a wet blotter, probed with rabbit anti-rat apoB antibody, visualized by chemiluminescence (Amersham), and quantitated by densitometry. Data are expressed in relative densitometry units.

Determination of plasma B-hydroxybutyrate and lactic acid. Plasma concentrations of β-hydroxybutyrate and lactic acid were determined in mice that had been fasted overnight. Blood was collected as described above. To obtain accurate plasma lactate concentrations, the blood collection tubes were prechilled on ice, and the samples were centrifuged within 5 min to remove the erythrocytes. Plasma β-hydroxybutyrate and lactate concentrations were both determined in duplicate measurements (kits 310-A and 735-10 for β-hydroxybutyrate and lactate, respectively; Sigma, St. Louis, MO).

Lipoprotein lipase and hepatic lipase activities. The activities of lipoprotein lipase and hepatic triglyceride lipase were measured in plasma from mice that had been fasted overnight. The animals were bled from the retro-orbital

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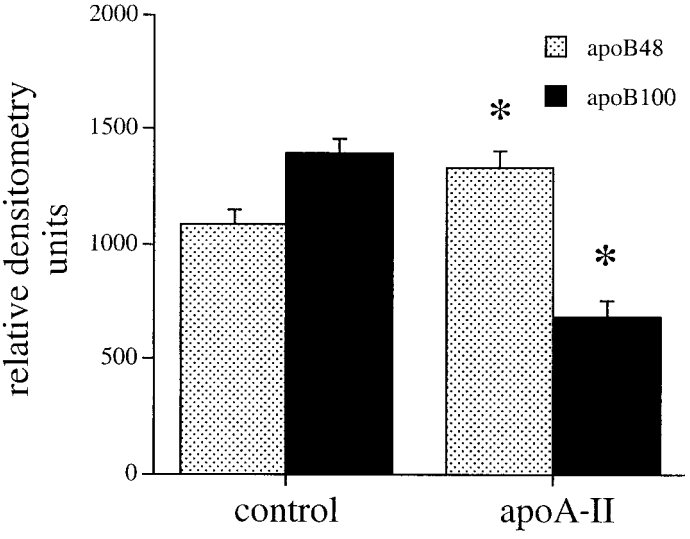

FIG. 1. The ratio of apoB48 to apo100 is increased in the plasma of apoA-II transgenic mice. ApoB48 and apoB100 concentrations were determined in plasma from apoA-II transgenic and control mice that had been fasted overnight. The apolipoproteins were isolated by SDS-PAGE followed by immunoblotting, visualized by chemiluminescence, and quantitated by densitometry. Data are expressed in relative densitometry units. n = 4 for both apoA-II and control groups. The Student's t test was used to test for differences between control and apoA-II transgenic mice. *Significantly different from control (P < 0.05).

plexus under isoflorane anesthesia 10 min after injection of heparin via the tail vein. Assays were done using the tri-[1-^{14}C]oleoylglycerol substrate as described (25).

Leptin, insulin, and glucose assays. Plasma leptin and insulin levels were determined in duplicate by enzyme-linked immunosorbent assay (ELISA) using kits from R&D Systems (catalog no. MOBOO) and Crystal Chemical (catalog no. IVSKR020), respectively. The intra-assay precision for the insulin assay is 3.5% and 3.8% for the leptin assay. The interassay precision for the insulin assay is 6.3% and 5.8% for the leptin assay. The minimum detectable level of mouse leptin is <22 pg/ml and is 156 pg/ml for mouse insulin. Plasma glucose concentrations were determined in triplicate using a commercially available kit (Sigma, no. 315-100). In experiments where glucose concentrations were determined, blood collection and plasma isolation was carried out as described for the lactate determinations to minimize glucose metabolism by erythrocytes.

Intraperitoneal glucose tolerance tests. Glucose tolerance tests were done essentially as previously described (26). Animals were fasted overnight and 50 μl of blood was drawn from the retro-orbital plexus under isoflorane anesthesia. A bolus of glucose (20% wt/vol in sterile lipoprotein lipase–free H_2O) was then injected into the peritoneal cavity (2 mg/g body wt). Blood samples were drawn from the retro-orbital plexus at 10, 20, 30, and 90 min after injection, and plasma glucose and insulin concentrations were determined as described above. To reduce the stress effects of repeated sampling, each animal underwent only one postinjection bleed, with different animals bled at each time point. Two-way analysis of variance, after logarithmic transformation to equalize variances among the groups, was used to compare the glucose and insulin curves between the control and apoA-II transgenic groups. The Tukey studentized range method was used for comparison of individual points. Each area under the curve (AUC) from time 0 to 90 min was estimated by the trapezoidal rule. Percentage differences of AUCs between control and transgenic groups are expressed as the estimate ± asymptotic SE.

Adipose tissue metabolism. Aliquots of epididymal and retroperitoneal fat pads from overnight fasted apoA-II transgenic and control mice were incubated in William's Media E, containing 5% fetal bovine serum, 200 μg/ml gentamicin, and 10 mmol/l HEPES, pH 7.4, for 1 h at 37°C in a shaking water bath. Aliquots of the media were then centrifuged to remove debris, and the glycerol concentration was determined. The difference in glycerol concentration between the media blank and the adipose tissue incubations represented glycerol released from hydrolysis of triglycerides. The data are expressed as milligram of triglyceride released per hour per gram of tissue.

RESULTS

We previously reported the development of transgenic mice overexpressing mouse apoA-II on the genetic background of strain C57BL/6J (6,7). The mice exhibited elevated levels of HDL cholesterol, as well as triglyceride-rich lipoproteins, compared with C57BL/6J mice (Table 1). Consistent with the mouse and human studies, which demonstrated a correlation between apoA-II and plasma FFA, we observed about a fivefold increase in the levels of FFA in the apoA-II transgenic mice relative to controls (Table 1). Despite the markedly increased plasma FFA levels, plasma concentrations of β-hydroxybutyrate and lactate were similar between apoA-II transgenic and control mice (Table 1).

The hypertriglyceridemia in the apoA-II transgenic mice does not appear to be due to differences in lipoprotein lipase or hepatic lipase because the activities of both were similar between control and transgenic mice (Table 1). We determined the ratio of apoB48 to apoB100 in the plasma from fasted animals and found the proportion of apoB48 to be significantly increased in the apoA-II transgenic mice compared with controls (Fig. 1).

Although plasma glucose levels after fasting were not significantly elevated in the apoA-II transgenic mice, fasting insulin levels were elevated approximately twofold (Fig. 2). To further test whether the apoA-II transgenic

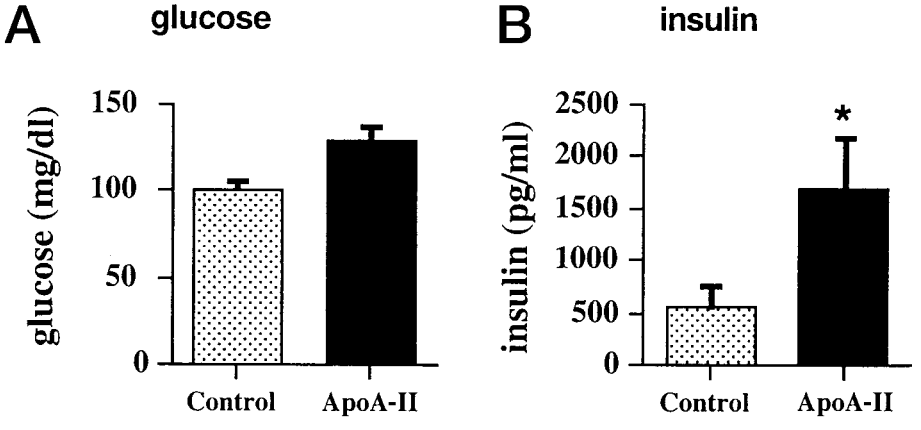

FIG. 2. Plasma insulin concentrations are increased in the apoA-II transgenic mice. ApoA-II transgenic and control mice that had been fasted overnight were bled, and plasma glucose and insulin levels were determined. *A*: Glucose levels expressed in milligrams per deciliter. *B*: Insulin levels expressed in picograms per milliliter. All values are given as the mean ± SE. n = 15 for both control and apoA-II transgenic groups. The Student's t test was used to test for differences between control and apoA-II transgenic mice. *Significantly different from control (P < 0.05).

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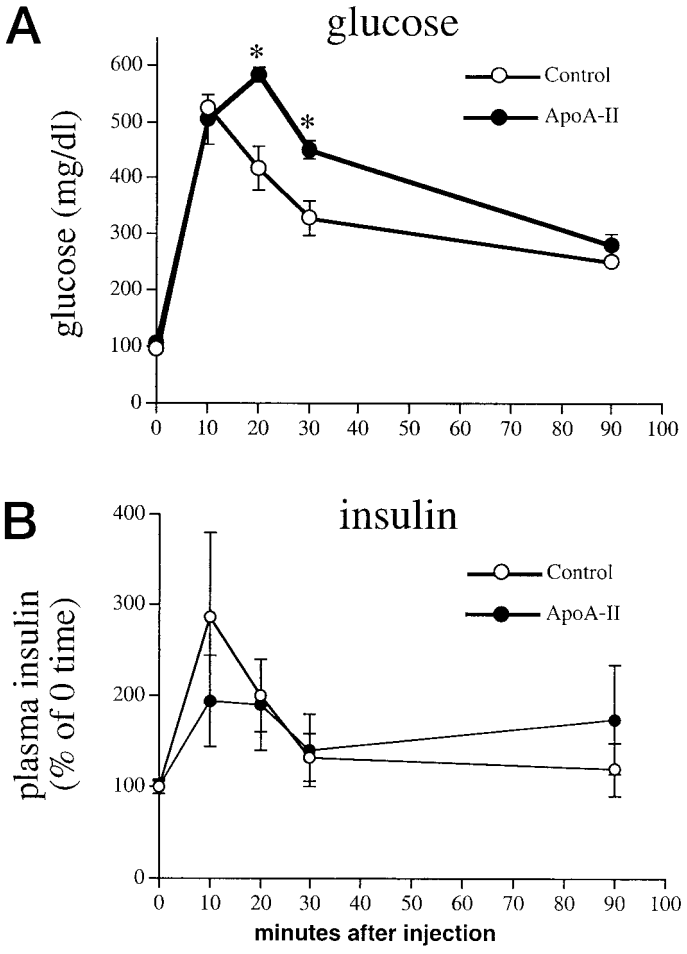

FIG. 3. Glucose clearance is delayed in apoA-II transgenic mice. After an overnight fast, plasma clearance of a bolus of glucose was determined in apoA-II transgenic and control mice. To reduce the stress effects of repeated bleeding, each animal was bled only once. Each data point represents the mean ± SE of nine different animals that were bled only at that time point. *A*: Glucose levels expressed in milligram per deciliter. *B*: Insulin levels expressed in picograms per milliliter. The Student's *t* test was used to test for differences between control and apoA-II transgenic mice. *Significantly different from control ($P < 0.05$).

mice exhibit insulin resistance, we measured the kinetics of glucose clearance after intraperitoneal injection of a bolus of glucose. Figure 3 shows a representative experiment in which a significant and reproducible delay in the clearance of glucose was observed in the apoA-II transgenic mice compared with controls. The area under the glucose curve was increased 28 ± 7% ($P < 0.001$) in the apoA-II transgenic group compared with controls. The AUC above baseline glucose levels was also higher in the transgenic group (increased 36 ± 13% over controls, $P < 0.001$). Plasma insulin concentrations were also determined throughout the glucose clearance studies and demonstrated that insulin secretion was not impaired in the apoA-II transgenic mice (Fig. 3). The AUC for the unadjusted insulin values was increased 123 ± 34% ($P = 0.001$) in the apoA-II transgenic mice compared with control mice. The AUC above baseline insulin levels for the transgenic group was 118 ± 183% higher than that of controls, but this was not statistically significant. The insulin-to-glucose ratios were also significantly increased in the apoA-II transgenic mice compared with controls at all time points (0 time: 5.72 ± 0.55 vs. 11.87 ± 1.35, $P = 0.0007$; 10 min: 2.78 ± 0.60 vs. 5.46 ± 0.99, $P = 0.0342$; 20 min: 2.91 ± 0.29 vs. 4.60 ± 0.67, $P = 0.0311$; 30 min: 2.60 ± 0.16 vs. 4.13 ± 0.623, $P = 0.0308$; 90 min: 2.75 ± 0.18 vs. 6.96 ± 1.06, $P = 0.0012$).

The strong association between fat stores and insulin resistance prompted us to examine fat metabolism in the apoA-II transgenic mice. Although body weights were only slightly increased in the apoA-II transgenic mice compared with age-matched controls, the apoA-II transgenic mice had a striking increase in fat pad mass, including both the retroperitoneal and epididymal fat (Fig. 4). The masses of the omental and subcutaneous fat pads were also markedly increased in the apoA-II transgenic mice compared with controls (data not shown); however, both of these fat depots, and in particular, the omental fat depots, were frequently too small in the control animals to allow accurate quantitation. Whole body fat content was increased ~45% in the apoA-II transgenic mice, similar to the percent increase observed in both the retroperitoneal and epididymal fat pads (Fig. 4). Thus, all fat depots in the apoA-II transgenic mice appeared to be increased to a similar extent compared with their counterparts in the control animals with no change in the regionalization of the fat deposits. Consistent with the increase in adipose tissue, leptin levels were elevated about twofold in the plasma of apoA-II transgenic mice compared with control mice (Fig. 4).

Because intracellular accumulation of triglycerides in muscle is frequently associated with the development of insulin resistance, the triglyceride content of skeletal muscle was also determined. The triglyceride mass in skeletal muscle was significantly increased in the apoA-II transgenic mice compared with control mice (Table 1).

Because the apoA-II transgenic mice exhibited increased plasma levels of triglycerides and FFA, as well as increased fat pad mass and insulin resistance, we expected that some of the metabolic consequences of overexpressing apoA-II were likely to be manifested as altered adipocyte metabolism. We examined adipose tissue metabolism by comparing the rates of triglyceride hydrolysis from epididymal and retroperitoneal fat pads removed from fasted apoA-II transgenic and control mice. As shown in Fig. 5, glycerol released from the hydrolysis of triglycerides was significantly reduced in both the epididymal and retroperitoneal fat pads from apoA-II transgenic mice compared with control mice. These experiments were repeated four times with totally consistent results.

Because the fasting insulin levels and glucose clearance studies suggested insulin resistance in the apoA-II transgenic mice, we examined uptake of 2-deoxy-[U-^{14}C]glucose in isolated soleus muscles of animals that had been fasted overnight. In experiments in which 2-deoxy-[U-^{14}C]glucose uptake was determined immediately after removal of the tissue from the animal without the addition of exogenous insulin, rates of uptake were similar between muscles obtained from apoA-II transgenic and control mice (Fig. 6). However, when uptake was again determined in experiments where exogenous insulin (2 mU/ml) was added to the incubation media, the rate of 2-deoxy-[U-^{14}C]glucose uptake was significantly increased in control animals compared with the apoA-II transgenic mice (Fig. 6).

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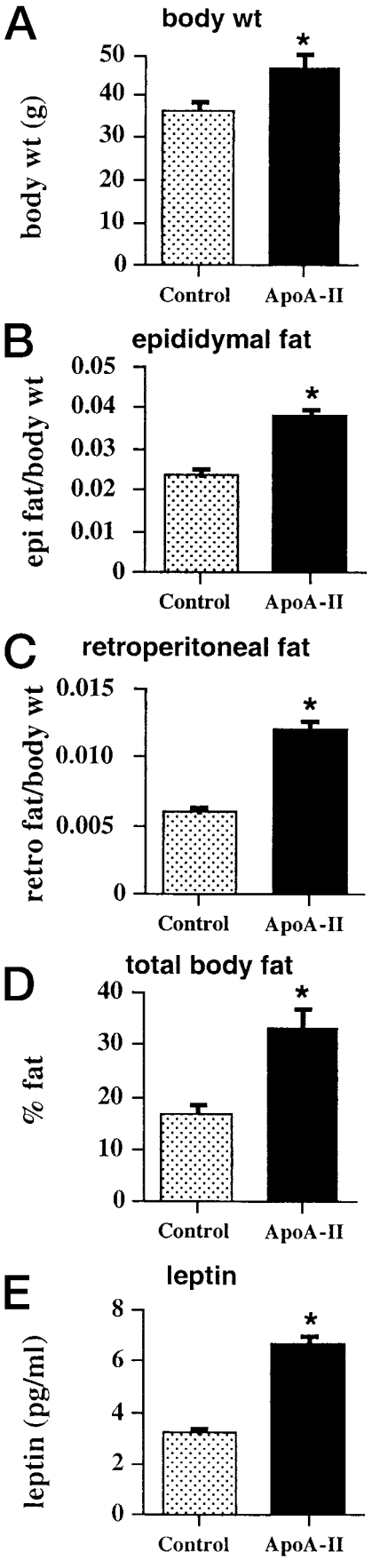

FIG. 4. Fat mass is increased in the apoA-II transgenic mice. Epididymal (epi) and retroperitoneal (retro) fat pad masses, as well as whole body fat content, were determined in apoA-II transgenic and control mice that had been maintained on standard Purina lab chow. Plasma leptin levels were also determined by ELISA. *A*: Body weight in grams. *B*: Retroperitoneal fat pad weight per body weight. *C*: Epididymal fat pad weight per body weight. *D*: Whole body fat mass expressed as percent of dry body weight. *E*: Plasma leptin levels in picograms per milliliter. All values are given as the mean ± SE. $n = 15$ for both control and apoA-II transgenic groups. The Student's t test was used to test for differences between control and apoA-II transgenic mice. *Significantly different from control ($P < 0.05$).

DISCUSSION

The apoA-II transgenic mice are insulin resistant, as demonstrated by higher fasting plasma levels of insulin and a delayed clearance of glucose bolus (Figs. 2 and 3). The delay in glucose clearance appears to be primarily a consequence of insulin resistance and not impaired insulin secretion because the plasma insulin–to–glucose ratios during the glucose clearance studies were significantly increased in the apoA-II transgenic mice compared with controls. Adipose tissue in the apoA-II transgenic mice, however, may not be resistant to insulin action. In incubations of adipose tissue explants obtained from animals that had been fasted overnight, rates of triglyceride hydrolysis were reduced by ~50% in tissue from apoA-II transgenic mice compared with controls (Fig. 5). This diminished triglyceride hydrolysis most likely reflects a normal response of the adipose tissue to the elevated fasting insulin levels. Furthermore, it is likely that the response of adipose tissue to the elevated plasma insulin levels contributes to the increased fat deposition in the apoA-II transgenic mice. The increased adiposity in the apoA-II transgenic mice is evident in mice of all ages. In animals as young as 6–8 weeks, there is a significant difference in fat pad mass between apoA-II transgenic and control mice, even though there is no significant difference in body weight (data not shown). As the animals mature, the difference in adiposity between the apoA-II transgenic and control mice increases, until 6 months of age when the difference is so great that the total body weight between the two strains becomes significantly different (Fig. 4). Several studies have demonstrated a positive correlation between adipose mass and plasma leptin levels. The elevated plasma leptin levels in the apoA-II transgenic mice are most likely a consequence of the increased adipose mass rather than altered rates of secretion by the adipose tissue. This evidence is supported by the observation that the ratio of leptin to percent total body fat is similar between the control and apoA-II transgenic mice (0.18 ± 0.03 and 0.20 ± 0.04 for control and apoA-II transgenic mice, respectively).

When we initially observed that the apoA-II transgenic mice were insulin resistant, we hypothesized that the increase in plasma FFA levels was a consequence of increased rates of triglyceride hydrolysis from adipose tissue, a hypothesis disproved by the fat pad incubation studies (Fig. 5). Plasma triglycerides are hydrolyzed in peripheral vascular beds, primarily in adipose tissue and skeletal muscle, and the majority of the fatty acids released into the plasma are taken up locally by these tissues. However, ~15% of the FFAs released from hydrolysis of triglycerides in the peripheral capillary beds escape local uptake and return to the liver. Because plasma triglycerides are elevated ~10-fold in the apoA-II transgenic mice, the increase in plasma FFAs may be due, primarily, to the greater mass of FFAs "escaping" local uptake.

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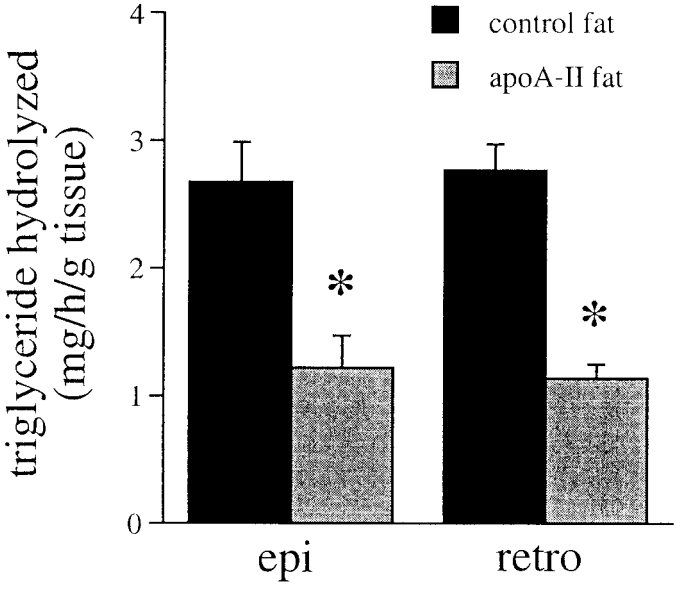

FIG. 5. Adipose tissue from apoA-II transgenic mice exhibits significantly less triglyceride hydrolysis than adipose tissue from control mice. Adipose tissue from epididymal (epi) and retroperitoneal (retro) fat pads was obtained from apoA-II transgenic (apoA-II) and control (control) mice that had been fasted overnight. Aliquots of the adipose tissue were incubated for 1 h at 37°C, and the glycerol released from hydrolysis of triglycerides was determined. Data are the mean ± SE of triglycerides hydrolyzed expressed in milligrams per hour per gram of tissue. n = 10 for both control and apoA-II transgenic groups. The Student's t test was used to test for differences between control and apoA-II transgenic mice. *Significantly different from control (P < 0.05).

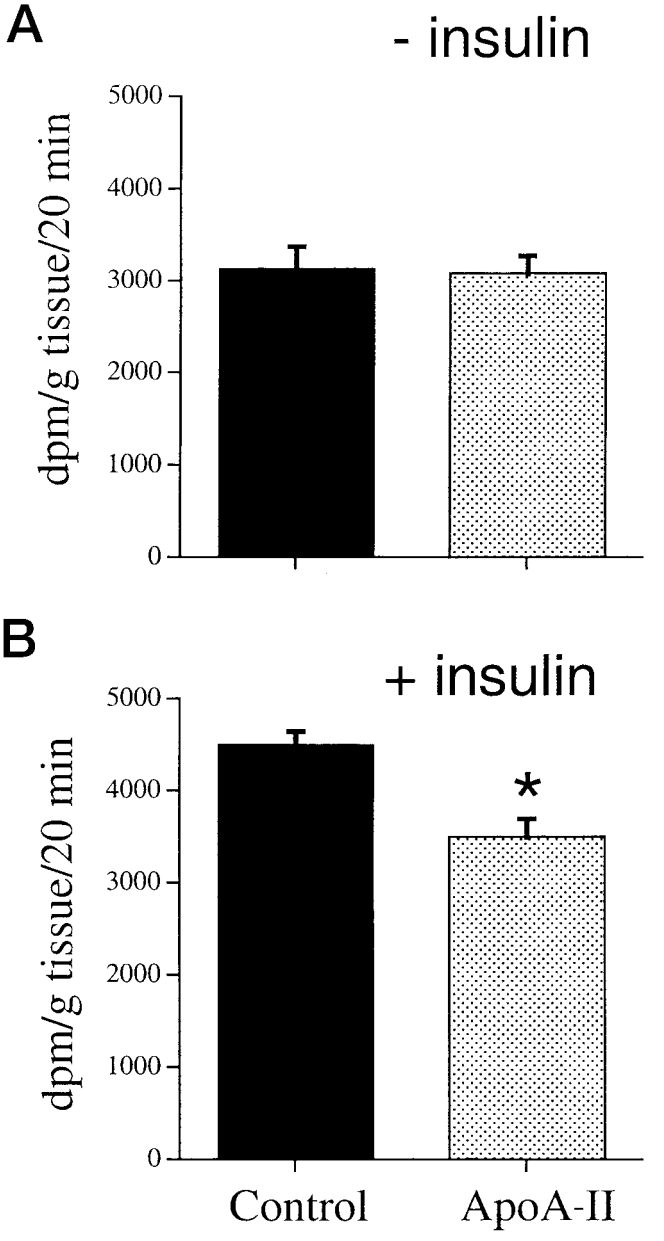

FIG. 6. Skeletal muscle from apoA-II transgenic mice exhibits significantly less uptake of 2-deoxy-[U-^{14}C]glucose. The soleus muscles were isolated from apoA-II transgenic and control mice that had been fasted overnight, as described in RESEARCH DESIGN AND METHODS. Uptake of 2-deoxy-[U-^{14}C]glucose (2.5 μCi/ml) was determined by incubating the muscles for 20 min in Krebs-Henseleit buffer that contained 5.5 mmol/l glucose and 1% BSA, with or without exogenous insulin added. *A*: Upon removal, the muscles were immediately incubated as described above without the addition of exogenous insulin. *B*: Upon removal, the muscles underwent a 20-min preincubation in Krebs-Henseleit buffer (without 2-deoxy-[U-^{14}C]glucose) that contained porcine insulin (2 mU/ml). At the end of the preincubation period, the tissue was transferred to new vials and incubated in Krebs-Henseleit buffer that contained insulin (2 mU/ml) as well as 2-deoxy-[U-^{14}C]glucose (2.5 μCi/ml). Data are the mean ± SE of radioactivity incorporated, expressed as dpm per gram of tissue per 20 min. n = 7 for both control and apoA-II transgenic groups in each set of experiments. The Student's t test was used to test for differences between control and apoA-II transgenic mice. *Significantly different from control (P < 0.05).

Although fasting plasma FFA levels are elevated fivefold in the apoA-II transgenic mice, ketone body production does not appear to be increased (Table 1). As a consequence of decreased glucose utilization by peripheral tissues, as well as increased plasma insulin levels, hepatic lipogenesis and triglyceride production may be increased in the apoA-II transgenic mice, with a corresponding decrease in rates of fatty acid oxidation. The normal plasma ketone body concentration in the apoA-II transgenic mice is consistent with the observation that in human insulin resistance and type 2 diabetes, plasma ketone body concentrations are usually not elevated (27). The increased ratio of apoB48 to apoB100 in the apoA-II transgenic mice is consistent with a normal response of hepatic apoB production to the elevated insulin levels (Fig. 1). Unlike humans, mouse liver can synthesize both apoB48 and apoB100. The editing of the apoB message has been shown to be regulated by insulin, which increases the proportion of apoB48 secreted (28).

The apoA-II transgenic mice are clearly insulin resistant; however, as described above, adipose tissue and liver appear to respond normally to the elevated plasma insulin levels. Our data are consistent with skeletal muscle being the insulin-resistant tissue in the apoA-II transgenic mice. Whereas there are clearly cases of adipose tissue insulin resistance, reduced utilization of glucose by skeletal muscle largely accounts for systemic insulin resistance (29).

To investigate if skeletal muscle in the apoA-II transgenic mice is insulin resistant, we determined rates of 2-deoxy-[U-^{14}C]glucose uptake in the isolated soleus muscle. In one set of experiments, the rate of uptake of 2-deoxy-[U-^{14}C]glucose was begun immediately upon removal of the muscle from the animal. In these experiments, there was no significant difference in rates of uptake (Fig. 6). We interpreted these results to indicate insulin resistance in skeletal muscle from the apoA-II

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transgenic mice because the skeletal muscle had been exposed in vivo to insulin levels that were approximately twofold higher in the apoA-II transgenic mice, but uptake was not significantly increased. The higher fasting insulin levels had, in effect, normalized uptake of glucose by the skeletal muscle in the apoA-II transgenic mice. We then repeated the uptake studies in a second set of experiments in which exogenous insulin was added to the incubation media at a concentration of 2 mU/ml, as described in RESEARCH DESIGN AND METHODS. This concentration of insulin was reported to promote maximal stimulation of glucose uptake in rat soleus muscle (24). After incubation in the presence of the high insulin concentration, rates of 2-deoxy-[U-^{14}C]glucose uptake by soleus muscle from control mice were significantly increased compared with muscles from the apoA-II transgenic mice, consistent with insulin resistance in the apoA-II transgenic mice (Fig. 6).

The link between increased adipose mass and insulin resistance has been recognized for many years, but the etiology of obesity-related insulin resistance is unclear. Increased plasma FFA concentrations have been hypothesized to contribute to insulin resistance by increasing utilization of fatty acid by muscle as a consequence of the concentration-dependent uptake of FFA (30–32). Increased fatty acid 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 insulin resistance in obesity may actually be an impaired capacity for use of plasma FFA (33–35). Use of FFA for energy by skeletal muscle is reported to be reduced in obesity. There is evidence that impaired oxidation of fat may be a contributing factor to weight gain (36,37). The activity of carnitine palmitoyl transferase (CPT) in muscle has also been negatively correlated with visceral fat mass, and rates of FFA uptake have been correlated with CPT activity (38). These studies suggest that decreased oxidative capacity and use of FFA by skeletal muscle can result in skeletal muscle insulin resistance and increased adiposity, consistent with the phenotype in the apoA-II transgenic mice. Data from both animal and human studies have demonstrated that the accumulation of triglyceride within skeletal muscle is associated with skeletal muscle insulin resistance (39,40). The triglyceride content of skeletal muscle was significantly increased in the apoA-II transgenic mice (Table 1). Thus, these studies suggest that decreased use of FFA by skeletal muscle could initiate a metabolic chain of events leading to the observed hypertriglyceridemic phenotype in apoA-II transgenic mice.

But how can an alteration in an apolipoprotein on HDL affect FFA use by skeletal muscle? One potential mechanism could involve CD36, a member of the scavenger receptor class B family of receptors (41), which is known to bind HDL and to be expressed on adipocytes, skeletal muscle, and other cells types that are involved in the rapid turnover of fatty acids (42). The identification of CD36 as a gene involved in FFA metabolism and glucose utilization supports this conclusion. Evidence suggests that CD36 may be involved in mediating an intracellular signaling pathway in response to ligand binding (43). Such a pathway could support an additional mechanism for CD36-mediated regulation of FFA and triglyceride metabolism in addition to a role in FFA uptake. Mice with a null mutation for CD36 had increased plasma HDL cholesterol, triglycerides, and FFA similar to the phenotype in the apoA-II transgenic mice (44). Furthermore, muscle-specific overexpression of CD36 in mice increased muscle fatty acid oxidation, reduced plasma triglycerides and FFA, and increased plasma glucose and insulin (45). Interestingly, these mice also had significantly reduced adipose mass. These data demonstrate that CD36 has effects on virtually every aspect of the phenotype observed in the apoA-II transgenic mice. We hypothesize that overexpression of apoA-II alters HDL composition such that HDL interaction with skeletal muscle CD36 is impaired, resulting in reduced use of FFAs for energy by skeletal muscle and the subsequent effects on skeletal muscle insulin resistance and increased fat mass as discussed above. Consistent with this hypothesis is the additional observation that ultracentrifugally isolated HDL from apoA-II transgenic mice exhibit markedly reduced binding to the CD36 receptor in cell culture (F.C. deBeer, personal communication).

Whereas the apoA-II transgenic mice are not diabetic, they are insulin resistant and mimic the human impaired glucose tolerance (IGT) phenotype. Individuals with IGT have an increased susceptibility to atherosclerotic disease associated with known risk factors including hypertension, hyperlipidemia, and adiposity (46–48). Moreover, individuals with IGT are at higher risk than the general population for the development of type 2 diabetes. Thus, overexpression of apoA-II, by causing insulin resistance and impaired tolerance to a glucose load, may predispose an individual to the development of frank type 2 diabetes, which may manifest itself under the appropriate dietary challenges, with aging, or on appropriate genetic backgrounds.

Insulin secretion does not appear to be significantly impaired in the apoA-II transgenic mice under the present conditions. Because impaired insulin secretion is a key component in the development of type 2 diabetes, this is the most likely reason why the apoA-II transgenic mice did not become diabetic. Aging is associated with a diminished insulin secretory response (49). Also, age has been demonstrated to be an important factor in the development of diabetes in another mouse model (50). The mice in the present study were tested at a relatively young age (6 months). Thus, the phenotype in older apoA-II transgenic mice could be altered in such a manner that diabetes would develop. In addition, the insulin resistance in the apoA-II transgenic mice appears to be localized to muscle and not adipose tissue under the present conditions. Tissue-specific muscle insulin resistance does not appear to cause diabetes in mice (51). Therefore, the apoA-II transgenic mice may also require additional genetic factors to develop type 2 diabetes.

Several lines of evidence suggest that the effects of apoA-II overexpression observed in the present study are not a nonphysiological response to high levels of apoA-II but, indeed, also occur with lower plasma apoA-II levels. The effects of apoA-II on plasma FFA levels were observed in genetic studies with common inbred strains of mice as well as with human families enriched for heart disease (10). Also, mice lacking apoA-II had decreased plasma FFA levels and increased insulin sensitivity (18).

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Our results suggest a novel mechanism for the cluster of traits referred to as "syndrome X" or "insulin resistance syndrome," which includes increased adiposity, elevated insulin levels, elevated plasma triglyceride and FFA levels, reduced HDL levels, and hypertension (11,12). These traits are remarkably similar to those aspects of the phenotype that we have thus far examined in the apoA-II transgenic mice, with the exception of the HDL levels. Plasma HDL cholesterol is increased rather than decreased in the apoA-II transgenic mice. Many studies have demonstrated the usefulness of the mouse as an experimental model to investigate aspects of lipoprotein metabolism relevant to human physiology. However, as with any animal model, some differences will be observed when applying the results to other species. Although species differences may contribute to the fact that the apoA-II transgenic model of the insulin resistance syndrome does not have low HDL, we believe that another factor is as likely to explain this difference. Numerous studies in humans and various animal models, including the mouse, have demonstrated that HDLs are a heterogeneous group of particles that differ in structure and function. Although total HDL cholesterol is elevated in the transgenic mice overexpressing mouse apoA-II, there are also differences in the size and composition of the HDLs that make up the different subclasses (6). This is supported by the observation that HDLs from the apoA-II transgenic mice are not as protective as HDLs from control mice in preventing the oxidation of LDL in a cell culture model of the artery wall (2). Evidently, the subfraction of HDLs important for this protective effect is reduced in the apoA-II transgenic mice even though total HDL is increased. Although total HDL cholesterol is elevated in the apoA-II transgenic mice, we cannot rule out the possibility that certain HDL subfractions, which may be metabolically important to the insulin resistance syndrome, are actually decreased.

Alterations in plasma lipoprotein metabolism have usually been assumed to be secondary to insulin resistance and adiposity (52,53). Our observations now indicate that a primary disturbance in lipoprotein metabolism can result in several traits associated with insulin resistance, consistent with the hypothesis (54) that insulin resistance and type 2 diabetes can, under certain circumstances, be related primarily to altered lipid metabolism rather than glucose metabolism. Our data suggest that variations in the expression and structure of apoA-II may contribute to insulin resistance and related traits in humans. The possibility is supported by the finding that the apoA-II gene segregates with plasma FFA levels in human pedigrees enriched for coronary artery disease (10). Furthermore, two recent family studies, involving the Pima Indian (55) and Utah Caucasian (56) pedigrees, observed linkage of type 2 diabetes with the apoA-II locus on human chromosome 1. Our results indicate that apoA-II is an excellent candidate gene for these linkage findings.

ACKNOWLEDGMENTS

This work was supported in part by U.S. Public Health Service Grants HL28481 and and HL60030 by the Laubisch Fund, UCLA.

We appreciate the help of Mark Cuevas in the fat pad metabolism studies and body composition analysis, Sarada Charugundla in the lipid analyses, and Dr. Howard Wong in the lipase assays. We would also like to thank Dr. Eli Ipp and Tat Rithaporn for help with the glucose clearance studies and Dr. Elliot Landaw for advice on statistical analyses. We are grateful to Jackie Bodnar, Hooman Allayee, and Drs. Richard Davis, Diana Shih, and Eli Ipp for their help in reviewing the manuscript.

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