Overexpressing Human Lipoprotein Lipase in Mouse Skeletal Muscle Is Associated With Insulin Resistance

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Lipoprotein lipase (LPL) plays a rate-limiting role in triglyceride-rich lipoprotein metabolism and is expressed in most tissues. Overexpression of LPL in skeletal muscle has been linked with higher plasma glucose levels suggesting insulin resistance (Jensen et al., *Am J Physiol* 273:R683–R689, 1997). The aim of our study was to ascertain whether the overexpression of human LPL in skeletal muscle leads to insulin resistance and to investigate the mechanism. Respiratory quotient measurements in both transgenic (MCKhLPL) and nontransgenic mice on a high-carbohydrate diet were conducted and showed a shift in fuel usage in transgenic mice when fasting but not when actively feeding. An increase in citrate and glucose 6-phosphate levels in fasted MCKhLPL mice further supports this preferential use of lipids. When challenged with an intraperitoneal injection of glucose (1 g/kg), MCKhLPL mice had a higher plasma glycemic excursion than nontransgenic mice. No differences in insulin response were observed between the two groups. Further investigation using hyperinsulinemic-euglycemic clamps revealed insulin resistance in MCKhLPL mice. Despite signs of insulin resistance, there was no associated increase in free fatty acids, hypertriglyceridemia, or hyperinsulinemia in MCKhLPL mice. In conclusion, MCKhLPL mice are insulin resistant, presumably due to increased delivery of lipoprotein-derived fatty acids to muscle. *Diabetes* 50:1064–1068, 2001

Lipoprotein lipase (LPL; EC 3.1.1.34) plays a rate-limiting role in triglyceride-rich lipoprotein metabolism and has been shown to be expressed in a variety of tissues (1,2). LPL migrates to the surface of endothelial cells of the capillary bed of most tissues after synthesis, where it is activated to hydrolyze chylomicron and VLDL triglycerides to monoglycerides and free fatty acids (FFAs) and to produce remnant lipoproteins (1). The highest levels of LPL expression have been shown to occur in adipose tissue and muscle, where FFA is either metabolized or reesterified and stored as triglycerides (3).

Several lines of transgenic mice that overexpress human LPL have been created, and although the methodology and site of overexpression might differ, all result in decreased plasma triglyceride levels (4–6). Work conducted previously in our laboratory showed that mice overexpressing human LPL (MCKhLPL) in skeletal muscle displayed higher plasma glucose levels than wild-type controls (6). This suggests that overexpression of human LPL in skeletal muscle might cause insulin resistance.

More than 35 years ago, Randle et al. (7) suggested that elevated plasma FFA concentrations are associated with and play a key role in the development of insulin resistance. Although plasma FFA levels in the MCKhLPL mice are reduced compared with controls (6), it is possible that the overexpression and, therefore, elevated activity of LPL in the muscle might lead to greater use of lipid by muscle as a fuel source. This preferential use of lipid in skeletal muscle might, in turn, account for the apparent insulin resistance.

The collective aim of these studies was to ascertain whether the overexpression of human LPL in skeletal muscle leads to an insulin-resistant state and to further investigate the mechanism.

RESEARCH DESIGN AND METHODS

Adult male MCKhLPL and nontransgenic FVB mice (6) were maintained at ~20°C on a 12:12-h light/dark photoperiod and given unrestricted access to water and standard laboratory diet. On the day of the experiment, the animals were fasted for 4 h, except for the respiratory quotient (RQ) measurements, during which the animals were given unrestricted access to food. Mice used in the RQ measurement were maintained on a synthetic high-carbohydrate diet (Research Diets, New Brunswick, NJ) for 13 weeks. Mice used in all other experiments were fed standard laboratory diet (Diet 8640; Harlan Teklad, Madison, WI). Both the standard laboratory diet and the synthetic diet were designed to have comparable compositions. The animals involved in the measurement of muscle metabolites were fasted for 4 h before being anesthetized with Avertin (2,2,2-tribromoethanol, 32 mg; Aldrich, Milwaukee, WI) and sampling of the quadriceps muscle. The quadriceps muscle was chosen because it is a muscle of mixed fiber type and, as such, would represent all skeletal muscle.

RQ measurement. The indirect calorimetry system consists of cages, pumps, flow controllers, valves, and analyzers and is calibrated with O_2 (1%) and CO_2 (0.8%) primary gas standards (Air Liquide, Houston, TX). The system is computer controlled and designed to sequentially measure the O_2 and CO_2 concentrations in four separate cages on a continual basis. The system operates as follows: air taken from a constant air source is pulled through four metabolic chambers (Metabowl; Jencons Scientific, Bridgeville, PA), a blank cage, and a reference line connected via separate but identical air sampling pathways. Mice are placed in four metabolic chambers, and the blank cage is used as a reference cage for periodic monitoring of ambient O_2 and CO_2 concentrations. The condensation in the air exiting the chambers is removed with electronic sample coolers (Universal Analyzers, Carson City, NV) and then pushed through mass flow controllers (Teledyne Hasting-Raydist, Hampton, VA) that maintain constant air flow (0.75 l/min). The air then travels to a manifold with five valves that the computer closes and opens at predefined intervals, thereby shunting air from a cage through the O_2 and CO_2 analyzers (Oxymat/Ultramat 6; Siemens, Roswell, GA). The analyzers compare the O_2 and CO_2 levels in the air stream in the cage and in a reference line. The differential O_2 and CO_2 concentrations, flow rate, RQ, and metabolic rate (Weir equation) are calculated and stored in a computer configured with data

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Received for publication 18 July 2000 and accepted in revised form 12 January 2001.

FFA, free fatty acid; GIR, glucose infusion rate; LPL, lipoprotein lipase; RQ, respiratory quotient.

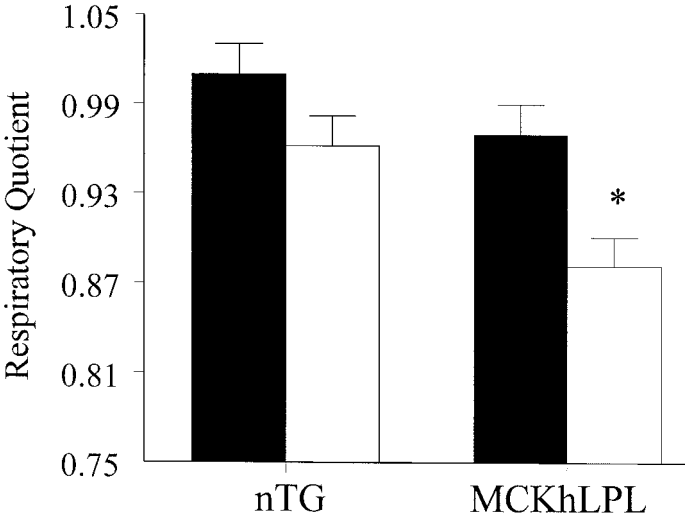

FIG. 1. RQ measurements in MCKhLPL and nontransgenic (nTG) mice during a 24-h period. In MCKhLPL and nTG mice, RQ ratios were measured over a 12-h light period (white bars) and a 12-h dark period (black bars). RQ values for MCKhLPL mice decreased during light hours, suggesting that the overexpression of LPL causes a preferential use of lipid when fasting. No such decrease was seen in the nTG mice (n = 7 and 6 for MCKhLPL and nTG mice, respectively). *P = 0.027 compared with nTG mice during the same period.

acquisition hardware (Analogic, Wakefield, MA) and software (Labtech, Wilmington, MA).

Intraperitoneal glucose challenge. Blood samples were collected from 14- to 17-week-old MCKhLPL mice and weight- and age-matched nontransgenic mice after a 4-h fast to measure basal blood glucose and plasma insulin levels. After the initial blood sampling, both groups of mice received an intraperitoneal injection of 1 g/kg glucose. Blood glucose and plasma insulin levels were monitored at 10, 20, 30, 60, and 120 min after glucose injection by sampling from tail bleeds. Because it was impossible to extract enough blood from the tail to measure both blood glucose and plasma insulin levels, these parameters were measured using two groups of mice. In mice used to measure insulin response, blood glucose was also checked at 0 and 20 min to ensure a blood glucose response comparable with that of the first group.

Hyperinsulinemic-euglycemic clamp. MCKhLPL and nontransgenic animals underwent a hyperinsulinemic-euglycemic clamp study as described by Shen et al. (8). Briefly, a catheter was inserted in the jugular vein and the animals were allowed to recover from the surgery for 2–3 days. Mice were fasted for 12 h before the start of the clamp to deplete the liver of glycogen stores. The 12-h fast was deemed necessary because the insulin rate chosen was not sufficient to completely inhibit hepatic glucose output (8). Both insulin (Novolin-R; Novo Nordisk, Princeton, NJ) and glucose (50% dextrose; Abbott Laboratories, North Chicago, IL) were dissolved in 0.9% saline to form respective infusates. After obtaining the basal plasma glucose concentration, a venous catheter was connected to infusion syringes, and both glucose and insulin infusions were initiated at time 0. Insulin was infused at a constant rate of 24.4 ml/h, whereas glucose, at a concentration of 20% dextrose, was infused at a variable rate. Mice were clamped at a blood glucose concentration of 6.67 mmol/l ± 10%, and measurements were conducted at 5-min intervals. Blood glucose concentrations were determined via the glucose oxidase method with a One Touch profile blood glucose meter (Lifespan, Milpitas, CA). Clamp glucose infusion rates (GIRs) were calculated from the means of the GIRs obtained during the last 30 min of the clamp.

Measurement of metabolites. Muscle samples were immediately freeze-clamped in liquid nitrogen after excision and stored at −80°C for later analysis, when they were ground using a mortar and pestle in liquid nitrogen. The resulting powder was homogenized with 10 vol 6% (wt/vol) perchloric acid. A portion of the homogenate (700 μl) was centrifuged, the pellet was reextracted with ice-cold 6% (wt/vol) perchloric acid, and the combined supernatants were neutralized with 2 mol/l K_2CO_3 before centrifugation and storage of the supernatant at −80°C. These samples were used later for the measurement of citrate and glucose-6-phosphate using the spectrophotometric assays of Lowry and Passonneau (9) and Bergmeyer (10), respectively. All enzymes and cofactors were purchased from Roche Molecular Biochemicals (Indianapolis, IN).

The other quadriceps muscle (~0.1 g) was also excised and immediately placed in a 2:1 solution (~0.4 ml) of ethanol and KOH (30%) (11). After saponification at 60°C for 48 h, an aliquot (0.2 ml) was taken and 1 mol/l $MgCl_2$ (0.216 ml) was added. After centrifugation, muscle lipid content was determined by enzymatic measurement of glycerol using a commercially available kit (Sigma, St. Louis, MO).

Plasma insulin was measured by radioimmunoassay (Linco Research, St. Louis, MO) with a sensitivity of 0.02 ng/ml. Plasma triglycerides and FFAs were measured using a Roche Cobas Mira Plus (Roche Diagnostics, Indianapolis, IN).

Statistics. Data were analyzed using either a two-way analysis of variance or Student's t test (Sigmastat for Windows 2.0; Jandel Scientific Software, San Rafael, CA) to compare results among groups. When data were not normally distributed, nonparametric tests were used. $P < 0.05$ was considered statistically significant.

RESULTS

RQ. Both MCKhLPL and nontransgenic mice fed a high-carbohydrate diet used carbohydrate preferentially during the night (Fig. 1). However, during fasting (daytime), transgenic mice demonstrated a decrease in RQ, suggesting a greater use of lipids (0.881 ± 0.02 vs. 0.97 ± 0.02). Nontransgenic mice, however, did not exhibit any such shift in fuel usage (0.962 ± 0.02 vs. 1.01 ± 0.02). The RQ values for the MCKhLPL mice during light hours were significantly lower than those for the nontransgenic mice (P = 0.027).

Intraperitoneal glucose tolerance tests. After the intraperitoneal administration of glucose, blood glucose levels in both MCKhLPL and nontransgenic mice increased; the MCKhLPL mice demonstrated a greater increase, espe-

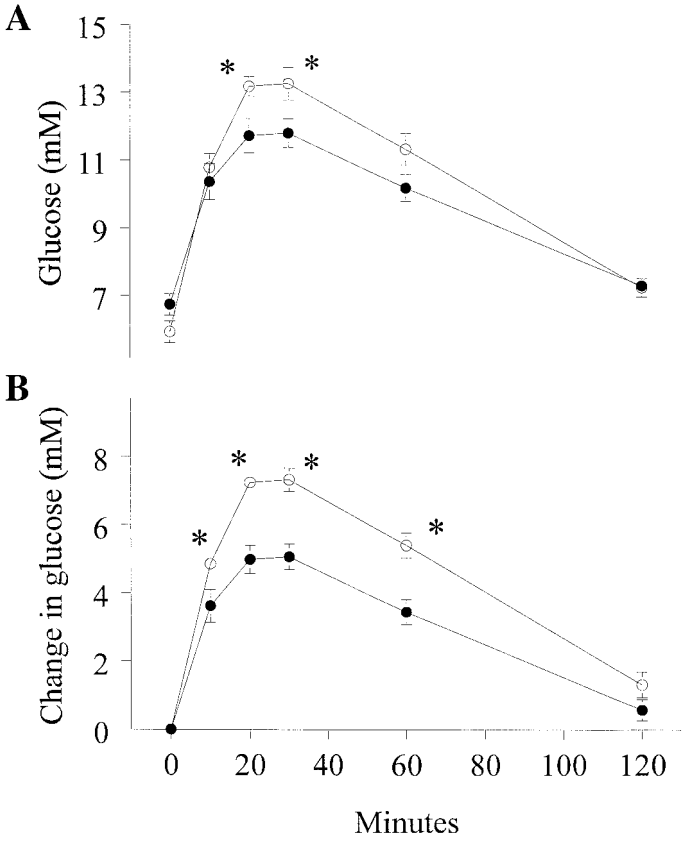

FIG. 2. Blood glucose levels in 14- to 17-week-old mice during intraperitoneal glucose bolus. Blood was collected from the tail vein of MCKhLPL (○) or nontransgenic mice (●) and blood glucose levels were measured after intraperitoneal administration of a glucose bolus. Blood glucose (mmol/l) suggests insulin resistance in the MCKhLPL mice, because levels are higher in the transgenic mice. *A*: Total change for MCKhLPL and nontransgenic mice. *B*: Difference from baseline (0 min) in response to bolus (n = 6 and 9 for MCKhLPL and nontransgenic mice, respectively). *P < 0.05.

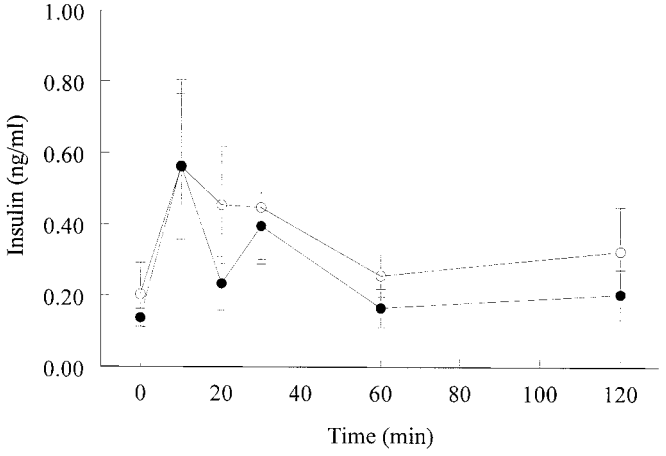

FIG. 3. Plasma insulin levels in 14- to 17-week-old mice during intraperitoneal glucose bolus. Blood was collected from the tail vein of MCKhLPL (○) or nontransgenic mice (●) and plasma insulin levels were measured. No differences were seen in the response of insulin to the intraperitoneal glucose bolus between the two groups (n = 6).

cially when normalizing for the starting plasma glucose concentration (Fig. 2*A* and *B*). A peak glucose concentration was achieved at 30 min and returned to basal by 120 min. Interestingly, the insulin response to the glucose bolus was similar in both MCKhLPL and nontransgenic animals. A peak insulin response was observed in both groups 10 min after intraperitoneal glucose injection (Fig. 3), and no statistical differences between groups were noted.

Hyperinsulinemic-euglycemic clamps. To further investigate the presence of insulin resistance in MCKhLPL mice, hyperinsulinemic-euglycemic clamps were performed at a blood glucose concentration of 6.67 mmol/l and an insulin infusion rate of 5 mU · kg^{-1} · min^{-1} after a 12-h fast (Fig. 4). MCKhLPL mice were clamped at a mean GIR of 50.1 ± 4.2 mg · kg^{-1} · min^{-1}, whereas the nontransgenic mice were clamped with a mean GIR of 70.5 ± 3.1 mg · kg^{-1} · min^{-1} (P = 0.015).

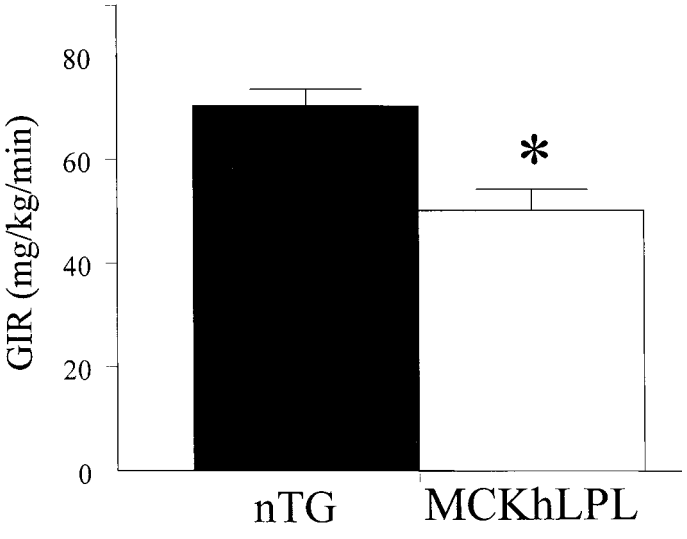

FIG. 4. Mean GIRs after hyperinsulinemic-euglycemic clamps. MCKhLPL and nontransgenic (nTG) mice (14–17 weeks of age) were fasted for 12 h and had undergone blood glucose clamping at 6.67 mmol/l under a constant insulin infusion rate of 5 mU · kg^{-1} · min^{-1}. Mean GIRs for muscle in MCKhLPL mice (black bar) were significantly lower than in their nTG counterparts (white bar) (n = 4 and 3 for MCKhLPL and nTG mice, respectively). *P = 0.015.

TABLE 1
Levels of plasma metabolites in both MCKhLPL and nontransgenic animals after a 4-h fast

	Nontransgenic	MCKhLPL
Glucose (mmol/l)	11.6 ± 0.4	12.2 ± 0.3
Insulin (ng/ml)	0.34 ± 0.04	0.29 ± 0.04
Triglycerides (mmol/l)	0.69 ± 0.07	0.49 ± 0.05*
FFA (μmol/l)	808 ± 56	728 ± 41

Data are means ± SE; n = 26. *P < 0.001 compared with nontransgenic mice.

Plasma metabolites. In agreement with previous findings, MCKhLPL mice were found to have lower plasma triglyceride levels (Table 1). After a 4-h fast, transgenic mice had a plasma triglyceride level of 0.49 ± 0.05 mmol/l, compared with 0.69 ± 0.07 mmol/l in the nontransgenic mice (P < 0.001). MCKhLPL and nontransgenic mice were found to have comparable fasting plasma glucose levels (12.2 ± 0.3 and 11.6 ± 0.4 mmol/l, respectively) and insulin levels (0.29 ± 0.04 and 0.34 ± 0.04 ng/ml, respectively) (Table 1). Unlike previous reports (6), however, no statistical differences were observed in plasma FFAs between MCKhLPL and nontransgenic mice (Table 1), with levels of 869 ± 75 and 882 ± 64 μmol/l, respectively.

Muscle metabolites. After a 4-h fast, higher levels of citrate were observed in the quadriceps muscle of the MCKhLPL mice than of the nontransgenic mice (0.15 ± 0.01 vs. 0.085 ± 0.02 μmol/g wet wt, respectively; P = 0.027) (Table 2). Similarly, glucose-6-phosphate levels were significantly higher in the MCKhLPL mice than in the nontransgenic mice (0.81 ± 0.04 vs. 1.35 ± 0.16 μmol/g wet wt, respectively; P = 0.013) (Table 2).

No statistical difference was found in the amount of triglyceride per gram of muscle between MCKhLPL and nontransgenic mice (8.8 ± 1.8 and 7.9 ± 0.7 mg/g, respectively) (Table 2).

DISCUSSION

For more than 35 years, it has been common dogma that the increased availability and oxidation of FFA is associated with insulin resistance (7). Insulin resistance has been proposed as the metabolic basis of atherogenesis and is viewed as the primary abnormality that gives rise to dyslipidemia, essential hypertension, impaired glucose tolerance, and type 2 diabetes.

A potential role of LPL in muscle is to provide fatty acids for energy metabolism (12). The overexpression of LPL has been shown to reduce hypertriglyceridemia in mice (4) and may therefore be a potential candidate for gene therapy (13). This might raise an interesting dilemma, because the overexpression of LPL in the skeletal muscle could potentially cause insulin resistance by "forcing" skeletal muscle to preferentially use lipids as an energy source.

RQ values are commonly used to determine the type of fuel being preferentially metabolized (14). In mice fed a high-carbohydrate diet, it was shown that during the dark period when the mice were actively feeding, carbohydrates were preferentially oxidized (Fig. 1). During light hours, however, when the animals were mostly inactive and presumably feeding less (15), the RQ values for the MCKhLPL mice decreased from 0.960 to ~0.875 (P = 0.027), indicating that the MCKhLPL mice were oxidizing

TABLE 2
Levels of quadriceps muscle metabolites in both MCKhLPL and nontransgenic animals after a 4-h fast

	Nontransgenic	MCKhLPL
Citrate ($\mu mol \cdot l^{-1} \cdot g^{-1}$ wet wt)	0.09 ± 0.02	0.15 ± 0.01*
Glucose-6-phosphate ($\mu mol \cdot l^{-1} \cdot g^{-1}$ wet wt)	0.81 ± 0.04	1.35 ± 0.16†
Triglycerides (mg/g)	7.91 ± 0.7	8.84 ± 1.8

Data are means ± SE; $n = 6$. *$P = 0.027$ compared with nontransgenic mice; †$P = 0.013$ compared with nontransgenic mice.

more lipids. The nontransgenic mice, on the other hand, showed a very small decrease from 1.00 to 0.96, indicating that they were using carbohydrate as the main fuel source, even when fasting. This decrease in RQ values suggests that overexpression of human LPL in skeletal muscle causes skeletal muscle to use more lipids when mice are fasted. The increased availability of FFAs in tissues predicts their uptake and subsequent metabolism, i.e., in muscle storage versus oxidation (16).

If the MCKhLPL mice were truly oxidizing more FFAs, as the RQ data suggest, elevated levels of skeletal muscle citrate would be expected. The MCKhLPL mice were observed to have higher levels of citrate, which is a potent inhibitor of phosphofructokinase (17). This rate-limiting enzyme for the glycolytic pathway is associated with insulin resistance. Work conducted recently has shown that inhibition of phosphofructokinase has been linked to insulin resistance, presumably a consequence of the accumulation of products from the hexosamine pathway (18, 19). Furthermore, the high levels of glucose-6-phosphate observed in the MCKhLPL mice could also contribute to the insulin resistance (Table 2). Recent work provides evidence that insulin decreases the inhibition of hexokinase by glucose-6-phosphate in insulin-sensitive but not in insulin-resistant muscle (20).

To ascertain whether the elevated plasma glucose levels observed in earlier work (6) was, in fact, a consequence of insulin resistance, an intraperitoneal bolus of glucose was administered and blood glucose and insulin levels were measured for 120 min. The MCKhLPL animals demonstrated a greater increase in blood glucose in response to the glucose bolus, strongly suggesting glucose intolerance. This was especially accentuated when the differences in initial plasma glucose levels were taken into account (Fig. 2*B*). The similarity in insulin response to the intraperitoneal glucose bolus (Fig. 3) between the two groups again suggests that the pattern of glucose disposal was due to glucose intolerance. To investigate this further, hyperinsulinemic-euglycemic clamps were conducted on both MCKhLPL and nontransgenic mice (Fig. 4). The results showed a reduction in the glucose requirements needed to maintain euglycemia, a likely result of the preferential use of lipid as a fuel in the MCKhLPL mice.

The magnitude of insulin resistance induced in fat-fed rats has been positively correlated with the triglyceride levels in skeletal muscle (21–23). Accordingly, work conducted in vitro demonstrated that insulin-stimulated glucose uptake is decreased in cells preloaded with triacylglycerol (24). The results of the present study, however, demonstrate that transgenic mice show signs of insulin resistance without higher levels of muscle triglyceride (Table 2). This is consistent with another transgenic model in which an increased use of lipid in muscle is likely (25). The MCK-CD 36 mouse is one with muscle-specific overexpression of the membrane protein CD 36. Despite implication of CD 36 in the binding and transport of long-chain fatty acids, no differences were detected in muscle lipid deposits. This model is similar to MCKhLPL mice, in that plasma triglyceride levels were reduced and FFA levels were not elevated. Moreover, the MCK-CD 36 model suggests insulin resistance, in that higher levels of plasma glucose and insulin were noted.

Insulin resistance has been proposed as the metabolic basis of atherogenesis and is viewed as the primary aberration that gives rise to a plethora of metabolic sequelae. Despite showing signs of insulin resistance, however, MCKhLPL mice do not have the expected and associated increase in FFAs, hypertriglyceridemia, or hyperinsulinemia. Whether this modest modification of glucose metabolism per se is deleterious to the vasculature will be important to examine.

ACKNOWLEDGMENTS

This work was supported by a National Institutes of Health Grant DK-26356 to R.H.E.

We thank the staff of the Core Laboratory of the General Clinical Research Center at the University of Colorado Health Sciences Center (RR-00051) for measurement of plasma glucose, FFA, and triglyceride levels.

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