Hypertriglyceridemic mice transgenic for the human apolipoprotein C-III gene are neither insulin resistant nor hyperinsulinemic

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Abstract Plasma glucose and insulin concentrations and in vivo and in vitro estimates of insulin action were compared in hypertriglyceridemic apolipoprotein C-III transgenic mice (mean ± SE triglyceride concentration = 11.8 ± 0.9 mmol/l) and their normotriglyceridemic (1.1 ± 0.1 mmol/l) littermates. There were no differences in the glucose (8.9 ± 0.2 vs. 9.3 ± 0.5 mmol/l) or insulin (172 ± 21 vs. 203 ± 17 pmol/l) concentrations of the transgenic and control mice, respectively. Steady-state plasma glucose concentrations at the end of a 150-min period of physiological hyperinsulinemia were also similar in transgenic (6.2 ± 0.5 mmol/l) and control mice (6.7 ± 0.5 mmol/l). As the steady-state plasma insulin levels were essentially identical in the two groups (~1000 pmol/l), these results show that whole body insulin-mediated glucose disposal was unchanged in the transgenic mice. Finally, values for isoproterenol-stimulated lipolysis, insulin-inhibition of lipolysis, and insulin-stimulated glucose disposal were similar in adipocytes isolated from transgenic and control mice. It can be concluded from these data that insulin resistance does not develop in hypertriglyceridemic mice transgenic for the human apolipoprotein C-III gene.—**Reaven, G. M., C. E. Mondon, Y-D. I. Chen, and J. L. Breslow.** Hypertriglyceridemic mice transgenic for the human apolipoprotein C-III gene are neither insulin resistant nor hyperinsulinemic. *J. Lipid Res.* 1994. **35:** 820–824.

Supplementary key words steady-state plasma glucose (SSPG) • transgenic mice • hypertriglyceridemia • insulin-mediated glucose transport • adipocytes • isoproterenol-stimulated lipolysis • insulin inhibition of lipolysis • insulin resistance

Results of previous case-control studies have shown that resistance to insulin-mediated glucose uptake, ambient plasma insulin levels, hepatic very low density lipoprotein (VLDL)-triglyceride (TG) production, and plasma TG concentrations are significantly correlated in normal individuals and patients with endogenous hypertriglyceridemia (1–5). Based upon these data, it has been proposed that insulin resistance is the primary abnormality, and the increase in plasma TG is a consequence of this defect and the resultant compensatory hyperinsulinemia (1–5). On the other hand, it is impossible to come to definitive conclusions concerning causality based upon the presence of correlation coefficients between variables. More direct evidence that hyperinsulinemia causes increased hepatic VLDL-TG secretion can be derived from studies of perfused and perifused rat liver (6, 7), but results of experiments using isolated hepatocytes (8, 9) have not supported the view that insulin stimulates hepatic VLDL-TG secretion. Thus, questions remain as to whether insulin resistance and compensatory hyperinsulinemia cause hypertriglyceridemia. Indeed, based upon data showing that insulin resistance improves, and plasma insulin levels decrease, in association with a fall in plasma TG concentration in gemfibrozil-treated subjects, Steiner (10) has suggested that hypertriglyceridemia causes insulin resistance, not vice versa. To further confound this issue, Vuorinen-Markkola, Yki-Järvinen, and Taskinen (11) were unable to demonstrate any change in insulin resistance or plasma insulin levels in patients whose plasma TG concentrations were lowered with gemfibrozil treatment.

It is obvious from the experimental data reviewed above that questions remain as to the causal relationship between insulin resistance, hyperinsulinemia, and hypertriglyceridemia. In an effort to gain new insight into this issue, we have used a recently described mouse model of primary hypertriglyceridemia (12, 13). If resistance to insulin-mediated glucose disposal and compensatory hyperinsulinemia result from a primary increase in plasma TG concentration, we should be able to discern

Abbreviations: TG, triglycerides; VLDL, very low density lipoproteins; SSPG, steady-state plasma glucose; SSPI, steady-state plasma insulin.

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differences in the values of these variables when comparing normal control mice with transgenic mice with severe hypertriglyceridemia. The data to be presented demonstrate that this was not the case, and that resistance to insulin-mediated glucose disposal and hyperinsulinemia were not present in transgenic mice with severe hypertriglyceridemia.

MATERIALS AND METHODS

Animals

Human apoC-III transgenic mice were created as previously described (12, 13). The fertilized eggs injected with foreign DNA were F1 (C57BL/6J × CBA/J). Transgenic mice were confirmed by tail-tip DNA analysis and mated to F1s of the same genetic background. Comparisons in metabolic studies were with nontransgenic litter mates. One transgenic line, 3707, was used in these studies. The 3707 line was created with the same human apoC-III gene construct used to make the previously reported transgenic lines 2674 and 2721 (12, 13). In the 3707 line the apoC-III transgene was expressed primarily in the liver (>90%), with some intestinal expression. The mice were shipped from Rockefeller University to the animal care facility at the Palo Alto Department of Veterans Affairs Medical Center at 6–7 months of age, and fed Purina laboratory chow #5012. Animals were monitored for positive body weight gain and were selected for study at 7–8 months of age. Basal blood samples were drawn 6 h after removal of food at 6:30 AM. Mice were held in a soft terry cloth towel and blood was collected from the tip of the tail into capillary tubes coated with 5% EDTA. Two capillary tubes of blood (150 μl) were collected from each animal and plasma was recovered for subsequent microdetermination of glucose (14), triglyceride (15), cholesterol (16), and insulin (17).

Assessment of insulin-mediated glucose uptake in vivo

The protocol for measurement of insulin-stimulated glucose uptake was as described previously (18). After obtaining the initial basal blood sample, mice were anesthetized with sodium pentobarbital and the right jugular vein was cannulated for administration of a solution of glucose and porcine insulin, diluted in saline containing added potassium chloride (5 mM) and bovine serum albumin (BSA) at a concentration of 0.1%. Glucose and insulin were infused at rates of 14.5 mg/min per g body weight and 0.2 ng/min per g body weight, respectively. Blood samples were obtained from the tip of the tail at 0, 60, 120, 140, 160, and 180 min for measurement of plasma glucose and insulin, and the values of the last four samples collected (120–180 min) were averaged to calculate steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations.

Assessment of insulin-mediated glucose transport and lipolysis in vitro

These studies were performed as described previously for experiments using rat adipocytes (19). Briefly, adipocytes were prepared from epididymal fat pads according to the method of Rodbell (20) with minor modifications. The fat pads were minced with scissors and placed in plastic flasks in Krebs-bicarbonate buffer, pH 7.4, with 4% bovine serum albumin, 2.5 mM glucose, and 1 mg collagenase/ml. Collagenase digestion was carried out at 37°C in a gyratory water-bath shaker for 75 min. Cells were washed three times in fresh Krebs–4% albumin–2.5 mM glucose buffer and allowed to separate from the infranatant by flotation. A 100-μl aliquot of diluted cells was fixed in a solution of 2% osmium tetroxide in collidine buffer and counted in a Coulter counter (Coulter Electronics Inc., Hialeah, FL) to determine cell number.

To quantify glucose transport, isolated adipocytes (2% lipocrit) were incubated in 500 μl of 3.5% albumin–Krebs–bicarbonate buffer, pH 7.4, in the presence of different concentrations of insulin (25 pM–80,000 pM) and tracer (300 nM) amounts of D-[U-^{14}C]glucose. The cell suspension was incubated at 37°C for 1 h with continuous shaking at 40 cycles/min. The incubation was terminated by centrifuging a 400-μl aliquot in a 550-μl microfuge tube, and the amount of activity associated with the adipocytes (as well as the total radioactivity in the incubation medium) was determined by liquid scintillation counting. Glucose transport at maximal insulin concentration (V_{max}) was expressed as FL/cell · sec and the concentration of insulin resulting in half-maximal transport rate (ED_{50}) was determined from linear regression of transport rate versus the log of the insulin concentrations at 25, 50, 100, 200, and 800 (if <80% of value at 8,000) pM insulin.

For measurement of lipolysis, adipocytes were diluted in Krebs–4% albumin–2.5 mM glucose buffer. Aliquots of diluted cells were placed into plastic vials (1×10^5 cells/ml), and incubated for 1 h at 37°C in the presence of variable amounts of isoproterenol in an atmosphere of O_2–CO_2 95%:5%. At the end of incubation an aliquot of infranatant was removed from each incubation mixture for measurement of glycerol concentration. Insulin inhibition of isoproterenol-stimulated lipolysis was estimated in a parallel series of vials containing a fixed amount of isoproterenol (3×10^{-8} M) and variable amounts of insulin.

Data are expressed as mean ± SE; the statistical significance of differences was evaluated by Student's nonpaired *t*-test, and/or two-way analysis of variance when multiple time points were involved.

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TABLE 1. Glucose and insulin concentrations

Variable	Control (n = 10)	Transgenic (n = 10)	*P*
TG (mmol/l)	1.1 ± 0.1	11.8 ± 0.2	<0.001
Cholesterol (mmol/l)	2.4 ± 0.2	3.5 ± 0.2	<0.001
Glucose (mmol/l)	9.2 ± 0.4	8.9 ± 0.2	NS
Insulin (pmol/l)	203 ± 17	172 ± 21	NS
SSPG (mmol/l)	6.8 ± 0.5	6.2 ± 0.5	NS
SSPI (pmol/l)	1065 ± 88	967 ± 92	NS

RESULTS

The results in **Table 1** demonstrate that mean (± SEM) plasma TG (11.8 ± 0.9 vs. 1.1 ± 0.1 mmol/l) and cholesterol (3.5 ± 0.2 vs. 2.4 ± 0.1 mmol/l) concentrations were significantly higher ($P < 0.001$) in the transgenic mice, consistent with previously published data in mice of similar origin (5, 6).

Plasma glucose and insulin concentrations measured approximately 6 h after food removal are also listed in Table 1, and it can be seen that the values were essentially identical in the control and transgenic mice. Furthermore, the steady-state plasma glucose (SSPG) concentrations attained during the infusion study were similar in the two groups. As the steady-state plasma insulin (SSPI) concentrations were also similar in the two groups, there were no differences in insulin-mediated glucose uptake between control and transgenic mice. The ability of increasing amounts of insulin to stimulate glucose transport by isolated adipocytes from the two groups is compared in **Fig. 1.** The data in the insert indicate that both maximal insulin-stimulated glucose uptake (95 ± 15 vs. 112 ± 17 FL/cell · sec) and the concentration of insulin resulting in half-maximal insulin stimulation of glucose transport (86 ± 5 vs. 98 ± 16 pmol/l) were similar in the transgenic and control rats, respectively. The results in **Fig. 2** demonstrate that isoproterenol-stimulated glycerol release was also essentially identical in the two groups over the dose range of 10^{-9}–10^{-6} M. Insulin inhibition of isoproterenol-induced lipolysis is also illustrated in Fig. 2 (see insert), and it can be seen that the anti-lipolytic effects of insulin were similar in control and transgenic mice.

DISCUSSION

The current experiments were initiated to gain insight into previously described relationships between resistance to insulin-mediated glucose uptake, compensatory hyperinsulinemia, and hypertriglyceridemia (1–11). The hypothesis to be tested was that a primary increase in plasma TG concentration would lead to the secondary appearance of insulin resistance and hyperinsulinemia. To test this possibility, various facets of glucose and insulin metabolism were studied in mice made hypertriglyceridemic by insertion of the human apoC-III gene. The results presented show that plasma glucose and insulin

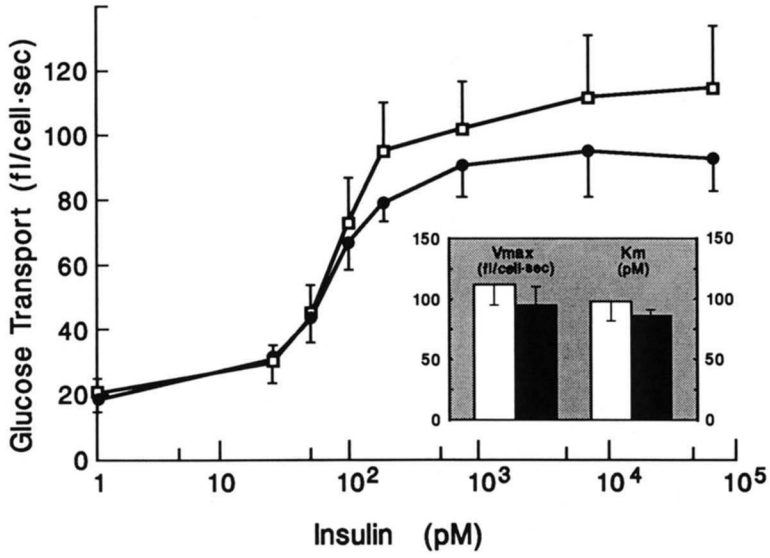

Fig. 1. Insulin-stimulated glucose transport by adipocytes isolated from control (□) and apoC-III transgenic (●) mice. The results represent the mean ± SEM of three experiments, with adipose tissue from three control and three transgenic mice used for each experiment. The results in the insert depict mean ± SEM values for maximal insulin-stimulated glucose transport (V_{max}) and the concentration of insulin resulting in half-maximal transport rate (ED_{50}).

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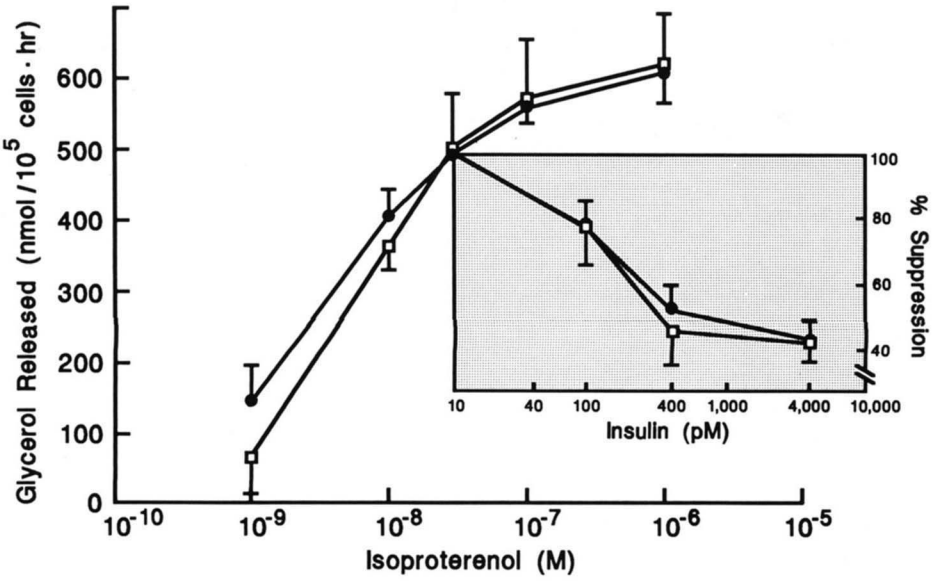

Fig. 2. Isoproterenol-stimulated glycerol released in adipocytes by adipocytes isolated from control (□) and apoC-III transgenic mice (●). The results represent the mean ± SEM of three experiments, with adipose tissue from three control and three transgenic mice used for each experiment. The results in the insert illustrate the ability of insulin to inhibit isoproterenol-stimulated (3×10^{7} M) glycerol release.

concentrations did not increase, and in vivo insulin-mediated glucose uptake was unaffected, despite an approximate tenfold increase in plasma TG concentration. In addition, the ability of insulin to stimulate glucose uptake and inhibit lipolysis in isolated adipocytes in vitro was also similar in the control and hypertriglyceridemic mice. The results of all the experiments were consistent, and demonstrated that a primary elevation in plasma TG concentrations in mice, secondary to the introduction of the human apoC-III gene, had no effect on insulin action.

Although the hypothesis that hypertriglyceridemia, per se, would produce insulin resistance cannot be supported by the results presented, certain caveats should be expressed. For example, it is possible that the effects of hypertriglyceridemia on insulin action may be model-specific. The mechanism of the hypertriglyceridemia has been studied in the human apoC-III transgenic mice (12, 13). These animals accumulate slightly larger than normal VLDL. The VLDL composition is appropriately TG-rich, but there is altered apolipoprotein content with increased apoC-III and diminished apoE. The transgenic mice also have increased free fatty acid levels. Metabolic studies indicate the primary abnormality to be decreased VLDL fractional catabolic rate with a small increase in VLDL-TG but not apoB production. In vitro the transgenic VLDL showed decreased LDL receptor-mediated uptake by tissue culture cells but normal lipolysis by purified lipoprotein lipase. Thus, the hypertriglyceridemia appears due to a prolonged VLDL residence time but not with the accumulation of remnant particles. This implies decreased in vivo lipolysis and tissue uptake, presumably secondary to altered surface apolipoprotein composition and/or elevated free fatty acid levels. Parenthetically, when studied in human beings, resistance to insulin-mediated glucose uptake and compensatory hyperinsulinemia have been strongly associated with increases in hepatic VLDL-TG secretion rates (3–5). Thus, it could be speculated that insulin resistance will only be seen in situations in which the hypertriglyceridemia is primarily due to increased VLDL-TG secretion, not a prolonged VLDL residence time.

In conclusion, the data presented have shown that the presence of hypertriglyceridemia in a transgenic mouse does not necessarily lead to a defect in insulin-stimulated glucose uptake or hyperinsulinemia. There is always uncertainty in extrapolating data from one species to another, and a definitive answer to the question posed at the outset requires evaluation of the relationship between hypertriglyceridemia and insulin resistance in human beings. In this context, comparison of insulin metabolism between normal individuals and hypertriglyceridemic patients with a genetic absence of lipoprotein lipase would be of considerable interest. We are currently planning such experiments.

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