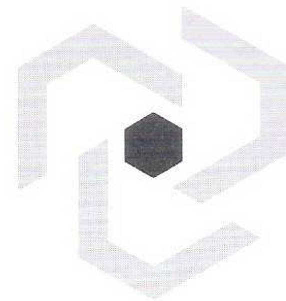

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Plasma Glucose Regulation and Insulin Secretion in Hypertriglyceridemic Mice

Abstract

In this study, we examined glucose homeostasis and insulin secretion in transgenic mice overexpressing the human apolipoprotein CIII gene (apo CIII tg). These mice have elevated plasma levels of triglycerides, FFA and cholesterol compared to control mice. The body weight, plasma glucose, and insulin levels, glucose disappearance rates, areas under the *ip*GTT curve for adult (4–8 mo. old) and aged (20–24 mo. old) apo CIII tg mice and the determination of insulin during the *ip*GTT were not different from those of control mice. However, an additional elevation of plasma FFA by treatment with heparin for 2–4 h impaired the *ip*GTT responses in apo CIII tg mice compared to saline-treated mice. The glucose disappearance rate in heparin-treated transgenic mice was slightly lower than in heparin-treated controls. Glucose (22.2 mmol/l) stimulated insulin secretion in isolated islets to the same extent in saline-treated control and apo CIII tg mice. In islets from heparin-treated apo CIII tg mice, the insulin secretion at 2.8 and 22.2 mmol glucose/l was lower than in heparin-treated control mice. In conclusion, hypertriglyceridemia *per se* or a mild elevation in FFA did not affect insulin secretion or insulin resistance in adult or aged apo CIII tg mice. Nonetheless, an additional elevation of FFA induced by heparin in hypertriglyceridemic mice impaired the *ip*GTT by reducing insulin secretion.

Key words

Glucose Homeostasis · Insulin Secretion · Apolipoprotein CIII · Transgenic Mice

Abbreviations

NIDDM, non-insulin-dependent diabetes mellitus; IDDM, insulin-dependent diabetes mellitus; LpL, lipoprotein lipase; ITT, insulin tolerance test; GTT, glucose tolerance test; FFA, free fatty acid; tg, transgenic; apo CIII, apolipoprotein CIII; VLDL, very low density lipoprotein.

Introduction

Insulin resistance in type 2 diabetic subjects can be provoked by acquired and/or genetic factors. One of the most important of these factors is hyperlipidemia together with elevated plasma FFA levels [1]. An increase in FFA oxidation leads to a reduction in glucose uptake, oxidation and storage, and an increase in glucose production [2–4]. However, the precise mechanism by which elevation of FFA impairs glucose use is still controversial. According to the glucose-fatty acid cycle proposed by Handle 37 years ago, a rise in FFA oxidation increases intramitochondrial acetyl-CoA/CoA and $NADH/NAD^+$ ratios with a consequent inactivation of pyruvate dehydrogenase. These alterations raise the citrate concentration, thereby abolishing phosphofructokinase activity with an accumulation of glucose-6-phosphate, which ultimately inhibits hexokinase activity and leads to a decrease in glucose uptake [5–6]. Alternatively, the increased insulin resistance provoked by the elevation of FFA has been ascribed to the inhibition of glucose transporters and/or glucose phosphoryla-

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tion with a subsequent reduction in the rate of glucose oxidation and muscle glycogen synthesis [7,8]. Normal glucose homeostasis is dependent on the interaction between tissue sensitivity to insulin and insulin secretion. Thus, an increase in the insulin resistance requires progressive increases in the insulin secretion rate [9]. If the compensatory hyperinsulinemia is not sufficient to maintain euglycemia, hyperglycemia may occur [1]. On the other hand, an increase in FFA oxidation by pancreatic B-cells stimulates insulin secretion by raising the $[Ca^{2+}]_i$ associated with PKC activation [10]. A rise in FFA oxidation in β-cells also impairs glucose-induced insulin secretion [11 – 14].

Transgenic mice expressing the human apolipoprotein CIII (apo CIII tg) exhibit hypertriglyceridemia and elevated plasma cholesterol and FFA levels [15 – 17], and are a suitable model for studying the association between hypertriglyceridemia on the one hand and insulin resistance and secretion on the other [18,19]. However, hypertriglyceridemic transgenic mice are neither insulin-resistant nor hyperinsulinemic [20].

In the present work, we have confirmed and extended the observation that hypertriglyceridemia *per se* does not affect insulin resistance in adult (4 – 8 mo) or aged (20 – 24 mo) apo CIII tg mice. However, additional FFA increases induced by heparin impaired the *ip*GTT in transgenic, but not in control mice. This effect is the result mainly of a reduction in insulin secretion.

Materials and Methods

Materials

Collagenase was purchased from Boehringer. Antiserum against insulin was kindly provided by Dr Leclercq-Meyer (Free University of Brussels, Brussels, Belgium). Rat standard insulin was obtained from Novo-Nordisk (Copenhagen) and human recombinant insulin (Humulin R) from Eli Lilly Co. (Indianapolis, IN). Bovine serum albumin (fraction V) and chemicals were from Sigma Chemical Co. (St. Louis, MO). The anesthetics Rompun and Vetanarcol were from Bayer S.A. (Germany) and König (Brazil), respectively. Liquemine was obtained from Roche (Brazil).

Animals

Human apo CIII transgenic mice (line 3707) kindly donated by Dr. Alan R. Tall (Columbia University, NY) were bred in the animal facilities of the Department of Physiology and Biophysics, UNICAMP. The experiments involving animals were approved by the university's Ethics Committee and were in accordance with the *Guidelines on the Handling and Training of Laboratory Animals* published by the Universities Federation for Animal Welfare (1992). The mice had access to standard laboratory chow and water *ad libitum* and were housed at 22 ± 2 °C on a 12 h light/dark cycle. Male and female homozygous apo CIII transgenic and non-transgenic littermates aged 4 – 24 months were used in this study. Blood for measuring plasma glucose [21], triglyceride [22], cholesterol [23] and insulin [24] was taken from either the retro-orbital plexus or the tail of anesthetized mice. FFA measurements were performed using an enzymatic colorimetric method according to the manufacturer's instructions (Wako Chem. USA, Inc., VA).

Methods

Glucose-tolerance test

Intraperitoneal glucose tolerance tests (*ip*GTT) were performed at 4 – 8 months or 20 – 24 months of age. After 12 – 15 h of fasting, the mice were injected with glucose (2 g/kg) of glucose intraperitoneally [25]. Blood samples for glucose determination were obtained from the cut tip of the tail at 0, 15, 30, 60 and 120 min. The insulin was determined during the *ip*GTT at 0, 30 and 120 min after glucose injection. Glucose response during the glucose tolerance test was evaluated by estimating the total area under the glycemia vs. time curves using the trapezoidal method [26].

Insulin-tolerance test

Intraperitoneal (*ip*) or intravenous (*iv*) insulin-tolerance tests (ITT) were performed in fed mice aged 4 – 8 months. The *ip*ITT consisted of a bolus intraperitoneal injection of regular insulin (0.75 U/kg). Blood samples were obtained from the cut tip of the tail at 0, 15, 30 and 60 min for glucose determination [25]. The *iv*ITT consisted of a bolus injection of regular insulin (0.1 U/kg) into the inferior cava vein of the anesthetized mice, which had previously been laparotomized. Blood samples were obtained from the cut tipp of the tail at 0, 4, 8, 12 and 16 min. The rate constant for serum glucose disappearance (Kitt) was calculated from the formula $0.693/t_{1/2}$. The serum glucose $t_{1/2}$ was calculated from the slope of the least square analysis of the serum glucose concentrations from 0 – 16 min after intravenous insulin injection [27].

Insulin secretion from isolated pancreatic islets

Fasted mice were killed by decapitation, and the pancreas was removed. Islets were isolated by handpicking after collagenase digestion of the pancreas. Groups of five islets were first incubated for 30 min at 37 °C in Krebs-bicarbonate buffer containing 2.8 mmol glucose/l and equilibrated with 95 % O_2/5 % CO_2 at pH 7.4. The solution was then replaced with fresh Krebs-bicarbonate buffer and the islets were further incubated for 1 h with medium containing 2.8 or 22.2 mmol glucose/l. The incubation medium contained (in mmol/l): NaCl 115, KCl 5, $NaHCO_3$ 24, $CaCl_2$ 2.56, $MgCl_2$ 1, and BSA 0.3 % (w/v) [28]. The insulin released after 15 min and 1 h was quantified as previously described [24] using rat insulin as the standard.

Statistics

The results are presented as the mean ± SEM for the number of mice (*n*) indicated. When working with islets, *n* refers to the number of experiments performed (120 islets per group per experimental condition, distributed in groups of five islets each). The data were analyzed by one-way analysis of variance (ANOVA) followed by the Tukey-Kramer test for individual differences between groups. Non-paired *t*-tests were used whenever appropriate, and $p < 0.05$ indicated a significant difference.

Results

The body weight, plasma glucose and insulin levels (Table **1**), glucose disappearance rate (Fig. **1 A**) and area under the *ip*GTT curve (Fig. **1 B**) of adult apo CIII tg mice (4 – 8 mo old) were not different from those of control mice. The mean plasma insulin levels during *ip*GTT at 30 and 120 min were 0.41 ng/ml ± 0.06 and 0.201 ng/ml ± 0.03 for controls, and 0.31 ng/ml ± 0.05 and

Table 1 Body weight and plasma glucose, insulin, cholesterol, triglyceride and FFA levels in fasted control and apo CIII tg mice

		Control		*Apo CIII tg*	
Body weight (g)	M	29.4 ± 0.5	(15)	30.4 ± 1.0	(15)
	F	23.8 ± 1.0	(15)	24.5 ± 1.0	(15)
Glucose (mg/dl)	Fasted	89 ± 4.6	(15)	81 ± 2.8	(15)
	Fed	135 ± 4.4	(8)	142 ± 2.4	(8)
Insulin (ng/ml)	Fasted	0.17 ± 0.08	(6)	0.15 ± 0.03	(6)
	Fed	0.96 ± 0.3	(16)	0.98 ± 0.2	(13)
Cholesterol (mg/dl)		97 ± 4.2	(10)	117 ± 5.4*	(10)
Triglycerides (mg/dl)		86 ± 5.5	(10)	573 ± 61*	(10)
FFA (mmol/l)		1.3 ± 0.17	(10)	3.0 ± 0.14*	(10)

Mean ± SEM of *n* (in parenthesis); *$p < 0.01$ vs. control.

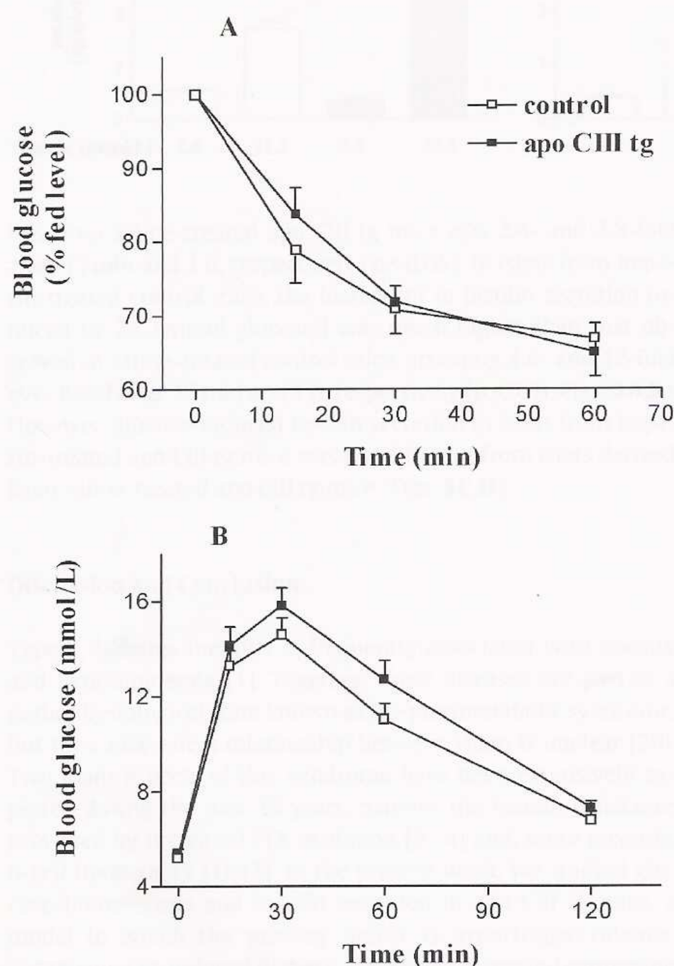

Fig. 1 Insulin and glucose tolerance tests in apo CIII tg and control mice. In **A** eight fed adult mice received a bolus intraperitoneal injection of regular insulin (0.75 U/kg). Blood samples were obtained from the cut tipp of the tail at 0, 15, 30 and 60 min for glucose measurements. In **B** fasted adult mice received glucose (2 g/kg) intraperitoneally. Blood samples were obtained from the cut tip of the tail at 0, 15, 30, 60 and 120 min for glucose measurements. The points are means ± SE of 8 – 12 mice.

0.2 ng/ml ± 0.04 for apo CIII tg mice, respectively. These values were not different between the two groups. However, the plasma cholesterol, triglyceride and FFA levels of apo CIII tg were higher than in control mice (Table **1**). The differences in triglyceride, FFA and cholesterol levels between apo CIII tg and control mice persisted in aged animals (20 – 24 mo old), whereas the plasma glucose levels as well as *ip*GTT values were no different between the two groups of aged mice.

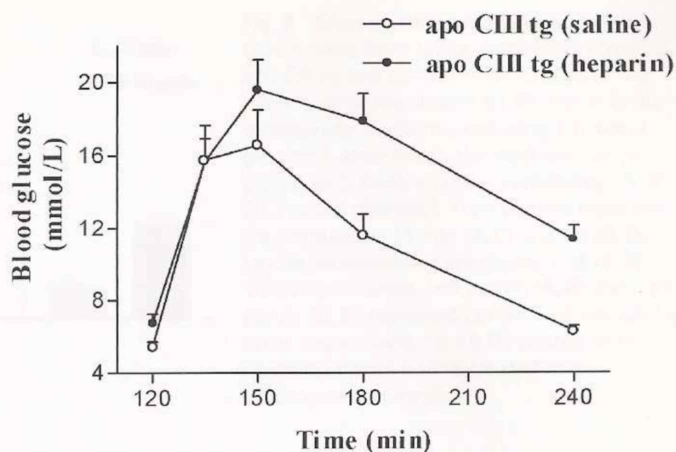

Fig. 2 Glucose tolerance tests in saline- and heparin-treated apo CIII tg mice. The mice were treated with three subcutaneous injections of saline or heparin at 0, 90 and 180 min and received glucose (2 g/kg) intraperitoneally at 120 min. Blood samples were obtained from the cut tip of the tail at 120, 135, 150, 180 and 240 min for glucose measurements. The points are means ± SE of 10 – 11 mice per group.

Heparin significantly increased the plasma FFA levels of control and apo CIII tg mice. The mean plasma FFA levels 90 to 240 min after heparin administration were 4.3 ± 0.2 and 2.8 ± 0.14 mmol/l for apo CIII tg and control mice, respectively (n = 10 for both groups; $p < 0.01$). These values were significantly higher than those obtained prior to heparin administration. The administration of saline did not interfere with the plasma FFA levels in apo CIII tg mice, but significantly reduced the levels in control mice at the end of the period of infusion (130 – 240 min, not shown). The glucose disappearance rates were 3.51 ± 0.34 %/min (n = 9) and 3.47 ± 0.27 %/min (n = 8) for saline-treated apo CIII tg and control mice, respectively. The corresponding rates for heparin-treated apo CIII tg and heparin-treated control mice were 2.88 ± 0.36 %/min (n = 11) and 4.12 ± 0.45 %/min (n = 10), respectively. Although these values were significantly different ($p < 0.05$), they were not different from control or apo CIII tg mice treated with saline. In addition, the *ip*GTT showed an increased area under the glycemic curve comparing heparin-treated apo CIII tg mice to saline-treated apo CIII tg mice (18.7 ± 1.0 mmol/l × 120 min vs. 13.6 ± 1.2 mmol/l × 120 min; n = 10 for both groups; $p < 0.01$; Fig. **2**). Since heparin strongly interferes with the radioimmunoassay, we unable to measured plasma insulin during *ip*GTT of the heparin-treated mice.

In another series of experiments, we examined the insulin secretion from isolated islets from both control and transgenic mice previously treated with saline or heparin. Basal insulin secretion in the presence of 2.8 mmol glucose/l after a 15 min (Figs. **3A,C**) or 1 h incubation (Figs. **3B,D**) was similar in islets from control and transgenic saline-treated mice. In the presence of 22.2 mmol glucose/l, the insulin secretion in control islets increased to 1.6- and 3.0-fold over basal after 15 min and 1 h incubation, respectively ($p < 0.05$ in both groups), whereas the increase in insulin secretion provoked by 22.2 mmol glucose/l in is-

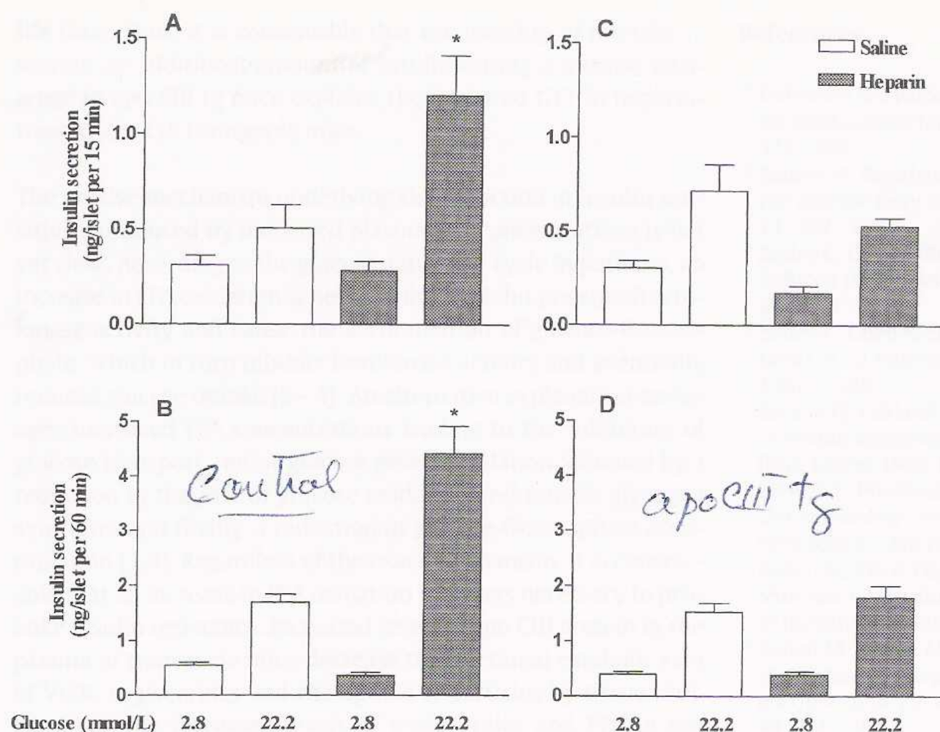

Fig. 3 Glucose stimulation of insulin secretion in islets from saline-and heparin-treated apo CIII tg and control mice. Groups of five islets were preincubated for 45 min in Krebs-bicarbonate medium containing 5.6 mmol glucose/l, after which the medium was replaced with Krebs solution containing 2.8 or 22.2 mmol glucose/l. The columns represent the cumulative 15 min (**A, C**) and 1 h (**B, D**) insulin secretion and are means ± SE of 20–35 groups of islets. Left panels (**A, B**) and right panels (**C, D**) represent control and apo CIII tg mice, respectively. *p < 0.05 related to respective control (saline-treated mice 22.2 mmol glucose/l).

lets from saline-treated apo CIII tg mice was 2.4- and 3.8-fold after 15 min and 1 h, respectively ($p < 0.05$). In islets from heparin-treated control mice, the increment in insulin secretion induced by 22.2 mmol glucose/l was much higher than that observed in saline-treated control mice, attaining 4.6- and 12-fold over basal after 15 min and 1 h, respectively ($p < 0.01$, Figs. **3A,B**). However, glucose-induced insulin secretion in islets from heparin-treated apo CIII tg mice was not different from islets derived from saline-treated apo CIII tg mice (Figs. **3C, D**).

Discussion and Conclusion

Type 2 diabetes mellitus is frequently associated with obesity and hyperlipidemia [1]. Together, these diseases are part of a pathophysiological state known as the plurimetabolic syndrome, but the cause-effect relationship between them is unclear [29]. Two main aspects of this syndrome have been extensively explored during the past 15 years, namely, the insulin resistance provoked by increased FFA oxidation [2–4] and, more recently, B-cell lipotoxicity [11,13]. In the present work, we studied glucose homeostasis and insulin secretion in apo CIII tg mice, a model in which the primary defect is hypertriglyceridemia. Streptozotocin-induced diabetic mice show increased expression of apo CIII mRNA. This upregulation of apo CIII is abolished by insulin administration [30]. These findings suggest that overexpression of the apo CIII gene could be responsible for the hypertriglyceridemia observed in IDDM. Similarly, apo CIII tg mice have significantly higher plasma triglycerides, cholesterol and FFA levels than non-transgenic littermate control mice [15–17].

In agreement with previous results [20], glycemia and insulinemia in adult (4–8 mo old) apo CIII tg mice were similar to those of the controls. In aged apo CIII tg mice, glycemic control may be hampered, although the data for 20–24 months-old transgenic mice were not significantly different from aged non-transgenic mice. Thus, hypertriglyceridemia with increased plasma FFA, at least at the levels observed in these apo CIII tg mice, did not affect the glucose homeostasis of adult or aged mice. Insulin resistance has been reported in humans with increased plasma FFA concentrations produced by the concomitant administration of triglycerides and heparin [7,31]. Plasma FFA levels in these subjects were significantly lower than those found in apo CIII tg mice. However, the increase observed after treatment with heparin was much greater in humans – four-fold in contrast to less than two-fold in transgenic mice. We have no explanation for this difference between human and hypertriglyceridemic transgenic mice, except that the effect of increased plasma FFA concentrations on insulin resistance may be species-specific [20]. A further possibility is that elevated levels of plasma triglycerides, and in specially FFA, are necessary but not sufficient to provoke alterations in insulin action in target tissues. We addressed the latter possibility by provoking an additional increase in plasma FFA levels through the administration of heparin to control and transgenic mice. Both groups showed significant increases in plasma FFA levels compared to those before heparin administration. A deterioration of glucose homeostasis in transgenic heparin-treated mice was observed, based on the increased area under the *ipGTT* curve and the lower Kitt values in these mice compared to the corresponding controls.

Parallel experiments showed that the glucose-induced insulin secretion by isolated islets incubated for 15 min or 1 h was similar in control- and transgenic saline-treated mice. However, in heparin-treated mice, the glucose-induced insulin secretion after a 15 min or 1 h incubation period was significantly higher than that observed in islets from saline-treated control mice. Heparin treatment failed to further increase insulin secretion in apo C III tg mice. Compelling evidence suggests that acute exposure to high concentrations of FFA renders the B-cells more sensitive to glucose [32], whereas a chronic exposure to high levels of FFA reduces the glucose-stimulation of insulin secretion [33,34]. The latter situation seems to be the case for apo CIII tg mice that present chronic high levels of plasma FFA during their

life time. Thus, it is conceivable that the inability of the islet to secrete an additional amount of insulin during a glucose challenge in apo CIII tg mice explains the impaired GTT in heparin-treated apo CIII transgenic mice.

The precise mechanism underlying the reduction in insulin sensitivity produced by increased plasma FFA concentrations is not yet clear. According to the glucose-fatty acid cycle hypothesis, an increase in FFA oxidation is necessarily to inhibit phosphofructokinase activity and cause the accumulation of glucose-6-phosphate, which in turn inhibits hexokinase activity and eventually reduces glucose uptake [2–4]. An alternative explanation envisages increased FFA concentrations leading to the inhibition of glucose transport and/or glucose phosphorylation followed by a reduction in the rate of glucose oxidation and muscle glycogen synthesis, and finally, a reduction in glucose-6-phosphate accumulation [7,8]. Regardless of the exact mechanism, it is conceivable that an increase in FFA oxidation is always necessary to provoke insulin resistance. Increased levels of apo CIII protein in the plasma of transgenic mice decrease the fractional catabolic rate of VLDL-triglycerides and the uptake of particles by tissue [20]. However, the increased levels of triglycerides and FFA in the plasma of transgenic mice may not necessarily indicate an increase in FFA oxidation. Furthermore, if FFA oxidation is indeed increased, it is apparently not sufficiently increased to alter cellular metabolism and provoke insulin resistance in apo CIII transgenic mice. Recently, the presence of LpL was demonstrated in islets cells. In addition, LpL-deficient mice islets secrete more insulin than islets from animals with normal LpL activity. These LpL-deficient mice have higher fasting insulin and lower blood glucose levels than wild-type mice [35,36].

In conclusion, the additional increase in plasma FFA levels resulting from enhanced lipoprotein lipase activity produced by heparin in apo CIII tg mice may be responsible for the reduced insulin secretion by islets and the consequently impaired response to the *ip*GTT in these transgenic mice.

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