Triglyceride-Induced Diabetes Associated With Familial Lipoprotein Lipase Deficiency

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Raised plasma triglycerides (TGs) and nonesterified fatty acid (NEFA) concentrations are thought to play a role in the pathogenesis of insulin-resistant diabetes. We report on two sisters with extreme hypertriglyceridemia and overt diabetes, in whom surgical normalization of TGs cured the diabetes. In all of the family members (parents, two affected sisters, ages 18 and 15 years, and an 11-year-old unaffected sister), we measured oral glucose tolerance, insulin sensitivity (by the euglycemic-hyperinsulinemic clamp technique), substrate oxidation (indirect calorimetry), endogenous glucose production (by the [6,6-$^2H_2$]glucose technique), and postheparin plasma lipoprotein lipase (LPL) activity. In addition, GC-clamped polymerase chain reaction–amplified DNA from the promoter region and the 10 coding LPL gene exons were screened for nucleotide substitution. Two silent mutations were found in the father's exon 4 (Glu^{118} Glu) and in the mother's exon 8 (Thr^{361} Thr), while a nonsense mutation (Ser^{447} Ter) was detected in the mother's exon 9. Mutations in exons 4 and 8 were inherited by the two affected girls. At 1–2 years after the appearance of hyperchylomicronemia, both sisters developed hyperglycemia with severe insulin resistance. Because medical therapy (including high-dose insulin) failed to reduce plasma TGs or control glycemia, lipid malabsorption was surgically induced by a modified biliopancreatic diversion. Within 3 weeks of surgery, plasma TGs and NEFA and cholesterol levels were drastically lowered. Concurrently, fasting plasma glucose levels fell from 17 to 5 mmol/l (with no therapy), while insulin-stimulated glucose uptake, oxidation, and storage were all markedly improved. Throughout the observation period, plasma TG levels were closely correlated with both plasma glucose and insulin concentrations, as measured during the oral glucose tolerance test. These cases provide evidence that insulin-resistant diabetes can be caused by extremely high levels of TGs. *Diabetes* 48:1258–1263, 1999

Type 2 diabetes is often associated with increased triglyceride (TG) and nonesterified fatty acid (NEFA) levels (1). Within a certain concentration range, uptake of NEFAs by skeletal muscle is proportional to their plasma level (2). In turn, augmented cellular NEFA uptake leads to increased fat oxidation (3). Furthermore, there is evidence that enhanced hepatic TG synthesis may induce TG accumulation in the muscle fibers of type 2 diabetic patients (4). The lipoprotein lipase (LPL) attached to heparin-sulfate proteoglycans in striated muscle and adipose tissue is the rate-determining enzyme for TG clearance from the blood (5).

Through inhibition of both glucose oxidation (6) and nonoxidative glucose metabolism (mostly glycogen synthesis), increased NEFA uptake is thought to be responsible, at least in part, for the skeletal muscle insulin resistance of type 2 diabetic patients (7). In the liver, increased NEFA oxidation may stimulate glyconeogenesis, thereby contributing to the inappropriate glucose production found in type 2 diabetic patients (8). It has recently been shown in vitro that growth of islets cultured in the presence of elevated NEFA levels results in insulin hypersecretion at low glucose concentrations (9). However, NEFAs seem to have a dual action on β-cells: in the short term they promote insulin release, whereas in the long term they may inhibit insulin secretion, probably by modulating β-cell gene expression (10). Finally, in families with multiple cases of hypertriglyceridemia, raised serum TG levels serve as a risk marker for subsequent development of type 2 diabetes (11).

It has been shown that the natural history of ZDF rats, which provide a useful replica of the human phenotype of adipogenetic type 2 diabetes (12), leads to a praecox 5- to 10-fold increase of the islet TG content, with a rising of TG levels up to more than 50 times in the most advanced stages of the disease. The surfeit of fat in islets is associated with a dose-related biphasic effect, initially enhancing insulin output by stimulating hyperplasia (13,14), but subsequently reversing these compensatory changes when fat content rises to extremely high levels (13,14).

Collectively, these data suggest that a primary increase in plasma NEFA and TG levels can lead to hyperglycemia by multiple converging mechanisms. However, proof of a

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apo, apolipoprotein; BPD, biliopancreatic diversion; CV, coefficient of variation; DGGE, denaturing gradient gel electrophoresis; EGP, endogenous glucose production; HL, hepatic lipase; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; PHP, postheparin plasma; TG, triglyceride.

causative role for lipids in the pathogenesis of type 2 diabetes in humans is not available.

In this study, we investigated insulin sensitivity, LPL activity, and LPL gene sequence in a family with LPL deficiency, in whom two young sisters presented a severe form of insulin-resistant type 2 diabetes associated with severe hypertriglyceridemia. After biliopancreatic diversion (BPD), a surgical operation through which a massive lipid malabsorption is obtained, diabetes was well controlled without any therapy, even on an unrestricted diet.

RESEARCH DESIGN AND METHODS

Subjects. The family, whose anthropometric characteristics are given in Table 1, consisted of five ethnically white members. The mother had a history of recurrent pancreatitis but no evidence of hypertriglyceridemia or type 2 diabetes. The father had moderate hypertriglyceridemia, but was otherwise healthy. The family history of diabetes was negative. The clinical history of the oldest sister (Sib1) has been reported previously (15). Briefly, she first noted an eruption of scattered tubercles with shining colorless papulae at age 12. At the same time, she started to complain of recurrent abdominal pain, and serum amylase levels were found to be elevated. Abdominal ultrasonography showed enlargement of both the liver and the spleen with diffuse brightness of the liver, indicating the presence of a massive steatosis. Both serum TG and cholesterol levels were very high (55.1 and 11.7 mmol/l, respectively), and prolonged treatment with ω-3 fatty acids and gemfibrozil had erratic effects on plasma TG levels. At age 16, Sib1 developed insulin-resistant diabetes, with fasting plasma glucose of 17 mmol/l and postprandial glucose levels of 28 mmol/l in the face of raised fasting plasma insulin concentrations (416 pmol/l, normal range 15–90).

The middle sister (Sib2) developed severe hypertriglyceridemia at age 15, initially without cutaneous lesions, and, a few months later, type 2 diabetes. Around her 16th birthday, scattered skin lesions, very much like those of Sib1, quickly spread all over her body, while TGs reached 29.7 mmol/l and serum cholesterol reached 9.2 mmol/l under dietary restriction. ω-3 fatty acids and gemfibrozil therapy failed also in Sib2.

A low-calorie low-fat diet (1,200 kcal/day, with 15% fat, 40% complex carbohydrates, and 55% protein) combined with oral hypoglycemic therapy (2,550 mg/day metformin) had little benefit in either Sib1 or Sib2, because of their low compliance to the diet. Eventually, both short- and intermediate-acting human insulins were introduced at doses of up to 150 U/day, without achieving glycemic control. At the time of the study, Sib3, the youngest of the three sisters, did not show any clinical alteration.

Surgery. The surgical procedure was a modified (according to Scopinaro [16]) BPD consisting of a gastric antrectomy with stapled closure of the duodenal stump. The small bowel was divided in half, and its proximal end anastomosed to the remaining stomach. The distal end of the ileum, comprising the remaining small bowel carrying the biliopancreatic juice and excluded from food transit, was anastomosed in an end-to-side fashion to the bowel 50 cm proximal to the ileocecal valve (Fig. 1).

Clinical studies. Body composition was estimated based on total body water, as measured by isotopic dilution. Fat-free mass (in kilograms) was obtained by dividing total body water by 0.73 (17). Insulin sensitivity was determined in all family members by the euglycemic-hyperinsulinemic technique (18). In Sib1 and Sib2, the clamp was performed 3 months before and again 8 and 18 months after surgery. Whole-body glucose uptake (*M* value, in micromoles per minute per kilogram) was determined during a primed-constant infusion of insulin (at the rate of 7 pmol · min^{-1} · kg^{-1}) after an overnight fast. In the two diabetic sisters, preclamp glycemia was maintained at 5.5 mmol/l using small boluses of short-acting insulin (Actrapid HM; Novo Nordisk, Bagsvaerd, Denmark). At the time of the clamp studies, subjects were on a weight-maintaining diet consisting of at least 250 g of carbohydrate a day for at least 1 week before each study.

Endogenous glucose production (EGP) was measured in the fasting state as well as during the clamp by a primed-continuous infusion of [6,6-$^2H_2$]glucose, as described previously (19). Respiratory gas exchange was measured in the resting state and during the clamp by open-circuit indirect calorimetry with a canopy (MBM-100, Deltratrac; Datex, Helsinki, Finland). Energy expenditure, the nonprotein respiratory quotient, and substrate oxidation were calculated as the difference between total insulin–mediated glucose uptake and whole-body net glucose oxidation.

Every 3-month interval after surgery, Sib1 and Sib2 underwent a 75-g oral glucose tolerance test (OGTT), with measurements of plasma glucose and insulin concentrations at 2 h postglucose.

The study was conducted according to the Declaration of Helsinki and was approved by the institutional review board. Written informed consent was obtained from the parents in legal representation of their children.

Analytical methods. Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA). Plasma insulin was assayed by microparticle enzyme immunoassay (Abbott, Pasadena, CA). NEFAs and TGs were determined by enzymatic colorimetric methods (Boehringer Mannheim, Mannheim, Germany). HDL cholesterol and HDL_3 cholesterol were quantified by a polyethylene glycol precipitation kit (Quantolip, Immuno Baxter Labs, Morton Grove, IL) combined with a cholesterol oxidase peroxidase-antiperoxidase method (Boehringer Mannheim) for cholesterol measurement that was also used for measuring total cholesterol; HDL_2 cholesterol was calculated as the difference (21). Apolipoprotein (apo) C-II was assayed by the single radial immunodiffusion technique using a kit from Daiichi Chemicals (Tokyo, Japan); the average of values obtained in a sample of 39 healthy adults of either sex was 0.038 ± 0.011 g/l.

Urinary nitrogen was determined by a Beckman BUN Analyzer II. Stool fat, starch, and nitrogen were measured by near-infrared reflectance analysis (22) in 24-h samples for 3 consecutive days.

LPL catalytic activity and immunoreactive mass. An intravenous sampling catheter was inserted into the forearm, heparin (100 IU/kg) was injected into the catheter, and 15 min later, postheparin blood with lipolytic activity was obtained. The catalytic LPL assay measured LPL activity in postheparin plasma (PHP) by

TABLE 1
Anthropometric and biochemical characteristics of the study family

	Father	Mother	Sib1 preoperative	Sib2 preoperative	Sib3
Sex	M	F	F	F	F
Age (years)	46	43	18	15	11
Height (cm)	165	157	156	164	153
Weight (kg)	55.0	48.0	52	54	50.8
BMI (kg/m^2)	20.2	19.5	21.0	20.1	21.7
FFM (kg)	49.8	37.8	43.0	38.8	36.4
FM (kg)	5.2	10.2	9.0	15.2	14.4
TG (mmol/l)	3.2	2.0	55.1	29.7	1.7
Cholesterol (mmol/l)	5.2	5.4	11.7	9.2	4.6
HDL cholesterol (mmol/l)	0.58	1.24	0.51	0.63	1.12
HDL_2 cholesterol (mmol/l)	0.44	0.86	0.34	0.44	0.80
HDL_3 cholesterol (mmol/l)	0.14	0.38	0.17	0.19	0.32
NEFA (μmol/l)	458	521	990	870	462
LPL activity (mU/ml)	145	102	76	117	206
LPL protein (ng/ml)	133	210	116	192	420
Apo C-II (g/l)	0.034	0.031	0.027	0.029	0.035

FFM, fat-free mass; FM, fat mass.

quantitation of the [^{3}H]oleic produced from the LPL-catalyzed hydrolysis of an intralipid emulsion carrying a [^{3}H]triolein label (23). To obtain zero-order kinetics, TGs and the cofactor apo C-II were applied in excess. The specificity of the LPL assay relied on specific inhibition of hepatic lipase (HL) activity by anti-HL antiserum. The liberated fatty acids were isolated from the TGs by a selective extraction procedure of liquid-liquid partitioning (24) and determined by reference to the ^{3}H-labeled tracer, which was quantified by liquid scintillation counting. LPL activity was calculated in nanomoles of fatty acids released per minute per milliliter of PHP (23). An intra-assay coefficient of variation (CV) <5.0% and an interassay CV of <5.3% were considered satisfactory. All PHP LPL activities were measured in triplicate. LPL activity and mass variability in 39 healthy adults of either sex were 152–429 mU/ml, with a median of 294 mU/ml, and 226–1,164 ng/ml, with a median of 462 ng/ml, respectively. Because at present no reference method or certified reference material for LPL activity measurement has been agreed upon, we checked accuracy by exchanging a frozen skim-milk standard with the University of Umeå Department of Physiological Chemistry and Biophysics in Sweden. The LPL protein assay is a solid-phase time-resolved fluoroimmunoassay (25) based on the reaction between the LPL dimer, the binding antibodies, and the europium-labeled detecting antibodies. The established monoclonal antibody 5D2 (26), raised against bovine LPL, was used to both capture and detect the dimeric active form of LPL (27). The Delfia (EG&G Wallac, Turku, Finland) LPL assay was calibrated against a skim-milk LPL standard of 0.6 mU/ng.

Molecular analyses. The promoter and all exons of the LPL gene were amplified by a GC-clamped polymerase chain reaction. Both the promoter and the exons were screened for nucleotide substitution by denaturing gradient gel electrophoresis (DGGE) (28–30). Exons with an abnormal DGGE pattern (3- or 4-band pattern) were sequenced by an automated laser fluorescence DNA sequencer (31,32).

Statistical analysis. Correlation between fasting TG concentrations and the 2-h plasma glucose and insulin levels was assessed by the Spearman rank technique.

RESULTS

A mutation, in which the base replacement does not lead to a change in the amino acid sequence but only to a different codon for the same amino acid Glu^{118}, was detected in exon 4 (GAG to GAA) in one of the father's alleles. A different mutation in exon 8 (ACC to ACA), coding for the amino acid Thr^{361}, and a nonsense mutation (in which the base change generates one of the termination codons) in exon 9 (TCA to TGA), coding for the change of Ser^{447} to Ter, was found in the mother. The two sisters who developed hyperchylomicronemia had the same mutation pattern in exons 4 and 8, whereas the youngest sister inherited the mutations in exons 4 and 9.

PHP LPL activity and LPL protein were, respectively, 145 and 133 in the father, 102 and 210 in the mother, 76 and 116 in Sib1, 117 and 192 in Sib2, and 206 mU/ml and 420 ng/ml in Sib3 (Table 1). Plasma concentrations of apo C-II were normal in all of the family members, which ruled out the possibility that the chylomicronemia of Sib1 and Sib2 might be secondary to an apo C-II deficiency (Table 1). Fasting levels of TGs, NEFAs, and cholesterol declined dramatically after surgery: the lowest levels observed were, respectively, 7.4 mmol/l, 543 µmol/l, and 3.0 mmol/l in Sib1 and 3.2 mmol/l, 482 µmol/l, and 2.8 mmol/l in Sib2. At this time, 83–92% of ingested fat was recovered in the feces.

Preoperative fasting plasma insulin concentrations were six- to eightfold higher in the two diabetic sisters than in the other family members, but concentrations returned to normal after surgery; fasting EGP showed a similar pattern (Table 2). EGP suppression by insulin during the clamp was virtually complete in all studies.

After surgery, glycemic control was normalized, with HbA_{1c} values consistently <6%. Insulin sensitivity (as the *M* value) and its components (net carbohydrate oxidation and nonoxidative glucose disposal) were within the normal ranges (33) in the parents, although a tendency toward low values was observed in the father and in Sib3, whereas values in Sib1 and Sib2 were markedly reduced; both sisters' *M* values increased remarkably after surgery. During the follow-up period, both sisters showed a moderate disposition toward gaining weight (Sib1 went from 52 to 54 kg at 18 months postoperatively, and Sib2 went from 54 to 55.5 kg at 8 months postoperatively). A highly significant correlation between fasting plasma TG concentrations and 2-h plasma glucose levels on repeat OGTTs was found in both Sib1 and Sib2 ($r = 0.99$, $P < 0.0001$ in Sib1; $r = 0.96$, $P < 0.0001$ in Sib2). A direct correlation was also found between fasting plasma TGs and the 2-h plasma insulin values in both patients ($r = 0.99$, $P < 0.0001$ in Sib1; $r = 0.97$, $P < 0.001$ in Sib2) (Figs. 2 and 3).

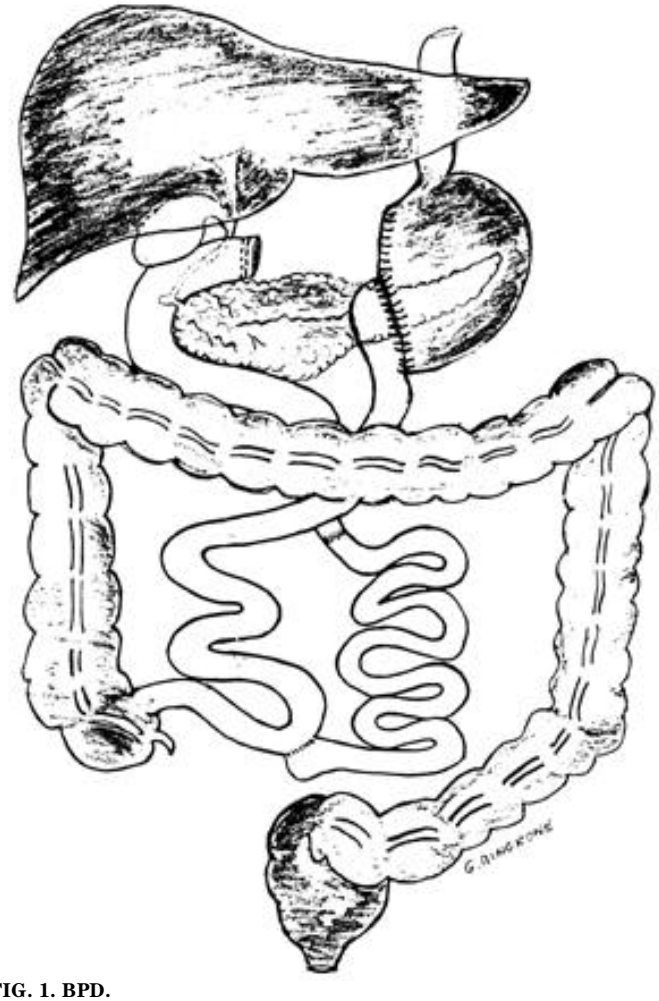

FIG. 1. BPD.

DISCUSSION

Diabetes developed in both Sib1 and Sib2 after the appearance of dyslipidemia. The combination of a negative family history of maturity-onset diabetes of the young, age at onset, and the presence of severe insulin resistance suggests early-onset type 2 diabetes. Complete reversal of the overt diabetes with pronounced amelioration of the insulin resistance occurred when lipid malabsorption was induced by a BPD. TGs and NEFAs were dramatically lowered. Furthermore, over the course of 8 months, the oxidative pathway of glucose disposal was normalized, whereas glycogen synthesis, although definitely improved, was subnormal. Hepatic glucose production

TABLE 2
Fasting plasma insulin concentrations, fasting plasma glucose concentrations, resting energy expenditure (REE), insulin-mediated whole-body glucose uptake (*M*), fasting endogenous glucose production (EGP), insulin-mediated net glucose oxidation (G_{ox}), and nonoxidative glucose disposal (G_{nonox}) in the study family

	Fasting plasma insulin (pmol/l)	Fasting plasma glucose (mmol/l)	REE (kJ/day)	*M* (μmol · min^{-1} · kg^{-1} FFM)	Fasting EGP (μmol · min^{-1} · kg^{-1} FFM)	G_{ox} (μmol · min^{-1} · kg^{-1} FFM)	G_{nonox} (μmol · min^{-1} · kg^{-1} FFM)
Father	14	4.5	6,564	30.5	11.0	14.8	15.7
Mother	11	4.2	7,185	48.5	13.3	33.2	15.3
Sib3	55	4.4	8,159	42.5	13.5	19.1	23.4
Sib1							
Preoperative	416	16.7	7,132	6.7	28.1	6.1	0.6
3 months postoperative	51	4.7	7,368	21.3	19.2	19.3	2.0
18 months postoperative	45	4.6	7,698	23.2	17.1	15.1	8.1
Sib2							
Preoperative	330	15.1	8,068	17.5	23.4	12.9	4.6
3 months postoperative	29	4.7	8,433	28.5	18.4	26.4	2.1
8 months postoperative	40	4.4	8,651	30.5	16.0	20.7	9.8

FFM, fat-free mass.

was also markedly reduced, although not completely normalized. In both sisters we observed a highly significant correlation between fasting TG levels and the values of both plasma glucose and insulin, as repeatedly measured 2 h after a standard oral glucose challenge. Fluctuations of plasma TGs were dependent on the amount of lipids present in the diet, which was unrestricted. The results suggest that the influence of the TG level seems to be strictly on glucose disposal and insulin secretion in the two sisters.

The two sisters who developed hyperlipidemia had the same silent mutations in exons 4 and 8 of the LPL gene (compound heterozygotes), the first inherited from the father and the second from the mother. This kind of mutation does not result in a change in the amino acid sequence of the enzyme and, therefore, no structural alteration of the gene product, but it may explain the reduced amount of LPL protein.

The youngest sister inherited the polymorphism in exon 4 from the father and the nonsense mutation in exon 9 from the mother. At the time of the study, Sib3 had modestly reduced LPL activity with normal values of TGs, total cholesterol, and NEFAs, although HDL cholesterol tended to be low. Although we do not know whether the hypertriglyceridemia of Sib1 and Sib2 is in any way linked to the nucleotide substitution in exon 4 or in exon 8, it will be interesting to follow up with Sib3 for later manifestations of the disease.

At present, the effects of the silent mutations in exons 4 and 8 upon LPL activity and mass have been never published (34–36), but new data from our laboratory reveal that both the mutations alone and in combination are neutral. Recent published data have shown that the termination mutation in exon 9 seems to increase LPL activity (37) and HDL cholesterol (38,39) while decreasing TG concentration in the bloodstream, thus acting as a kind of favorable mutation. In the light of these data, the low LPL activity in Sib1, Sib2, and the mother might be caused by other rare factors hard to detect as intron mutations, e.g., the existence of other genes in linkage disequilibrium, with LPL gene producing a significant functional defect possibly through the control of transcription and mRNA synthesis rates in accordance with previous works (40), or high levels of the LPL inhibitor apo C_{III}.

Maybe high TGs have induced the recurrent pancreatitis in the mother many years ago, and the subsequent malabsorption is the reason why the TGs are normal in the mother in spite of low LPL activity. The mother may be cured by a self-induced malabsorption functioning in the same way as the surgical BPD in the two sisters.

In other cases of LPL deficiency, the incidence of diabetes is not increased in the absence of chronic pancreatitis (41), so the modestly reduced insulin sensitivity (as the *M* value) in the father may provide some evidence for increased genetic susceptibility to diabetes in the sisters.

Genetic susceptibility to insulin resistance and decreased LPL activity are combined in the two sisters as a kind of experiment by Nature. The reduced LPL activity predisposes the individuals to higher levels of serum TGs, and insulin resistance is commonly associated with a higher level of NEFAs, the major determinant of the hepatic VLDL TG secretion. Whether the high level of NEFAs is liberated by LPL in the endothelium or by the hormone-sensitive lipase in the adipocytes is a matter of recent investigation (42). Otherwise, both sisters were cured from their serious illness when the levels of TGs and NEFAs were reduced and their whole-body glucose uptake (*M* value) got close to the subnormal value of their father.

It, therefore, seems to be conceivable that a progressive accumulation of TGs and NEFAs in the two diabetic sisters might have reproduced the experimental picture observed in animals. The effect of plasma TG lowering by fibrates on the parameters of glucose metabolism in humans is still controversial (43,44). One reason could be that fibrates do not reduce fat metabolism but only TG levels in plasma through an activation of the peroxisome proliferator-activated receptor-μ in the liver and fat cells. Correction of extreme hypertriglyceridemia, as it happens in experimental animals and the two diabetic sisters, appears to be associated with marked improvement of insulin resistance and diabetes. Most interestingly, the drastic lowering of TGs, brought about by post-surgery massive lipid malabsorption, resulted in the reversibility of the diabetes. Progression from the insulin-resistant state, in which normoglycemia is maintained by

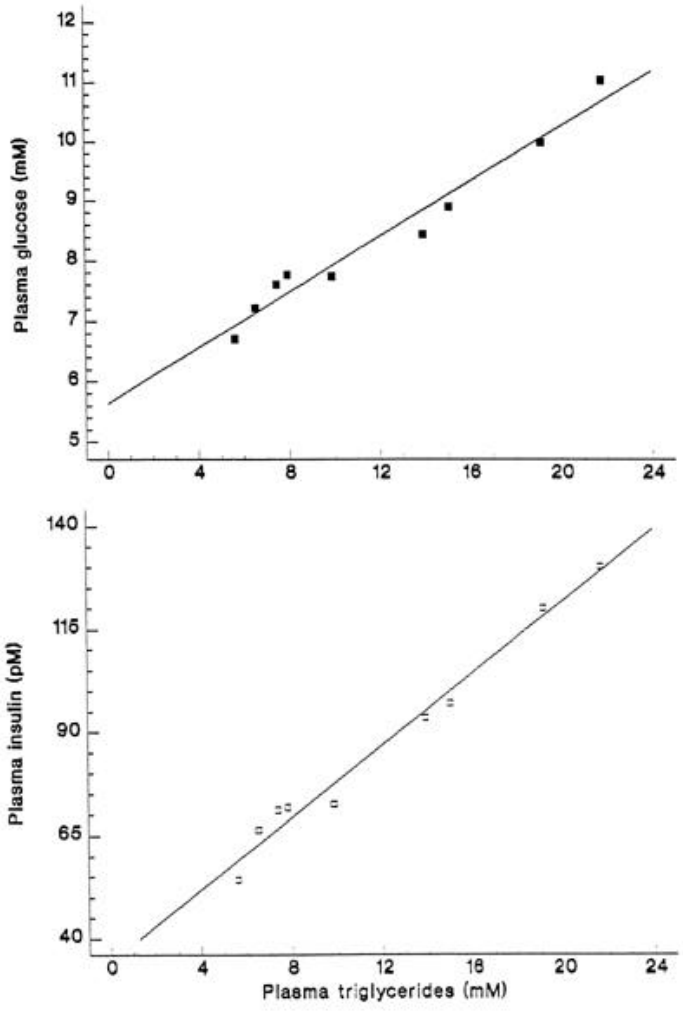

FIG. 2. Fasting plasma TG levels plotted against plasma glucose or plasma insulin concentrations, as measured 2 h after oral glucose loading on repeated occasions in Sib1.

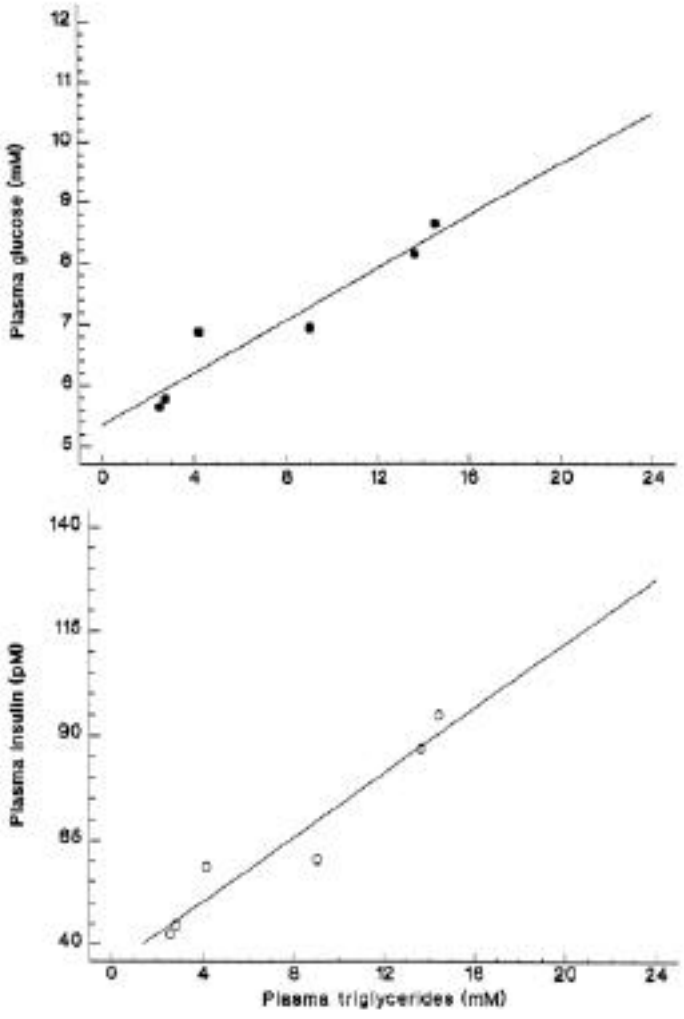

FIG. 3. Fasting plasma TG levels plotted against plasma glucose or plasma insulin concentrations, as measured 2 h after oral glucose loading on repeated occasions in Sib2.

compensatory hyperinsulinemia to overt diabetes (developing rapidly, within 14 weeks of age, in ZDF rats), is likely to be much lower in humans, despite the similarities of the rodent and human syndromes.

In summary, the current investigation seems to substantiate that lipotoxicity may play a pivotal role in the development of diabetes in humans.

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