In Vitro Transcriptional Induction of the Human Apolipoprotein A-II Gene by Glucose

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Type 2 diabetic patients present high triglyceride and low HDL levels, significant determinants for the risk of atherosclerosis. Transgenic mice overproducing human apolipoprotein (apo)A-II, one of the two major apos of HDLs, display the same lipid disorders. Here, we investigated the possible regulation of apoA-II gene expression by glucose. In primary rat hepatocytes and in HepG2 cells, the transcription of the human apoA-II gene was upregulated by glucose. This response was mediated by a hormone-responsive element within the enhancer of the apoA-II promoter and was dependent on hepatocyte nuclear factor-4α. Accordingly, in transgenic mice, the human apoA-II gene is stimulated by a high-carbohydrate diet after fasting and at weaning. By contrast, the apoA-II mRNA level is not modified in streptozotocin-induced diabetic rats. In transgenic mice overexpressing the human apoA-II gene, plasma human apoA-II concentration was positively correlated with blood glucose levels. These mice displayed a marked delay in plasma glucose tolerance as compared with control mice. We hypothesize that the following pathogenic pathway might occur in the course of type 2 diabetes: increased apoA-II level causes a rise in plasma triglyceride level and glucose intolerance, resulting in hyperglycemia, which in turn might further increase apoA-II gene transcription. *Diabetes* 53:672–678, 2004

The leading cause of death for individuals with diabetes is coronary artery disease (CAD). The risk of developing premature arterial atherosclerosis is two to three times higher in patients with type 2 diabetes than in the general population. Type 2 diabetic patients have been shown to display dyslipidemia, including hypertriglyceridemia and low levels of HDL (1).

Epidemiological data have demonstrated that the frequency of CAD is inversely related to plasma HDL level (2). The protective effects of HDLs against CAD have been attributed to their roles in reverse cholesterol transport (RCT)—the process by which excess cholesterol is transferred from extrahepatic tissues to the liver (3)—and in the protection of low density lipoprotein (LDL) against oxidative modifications (4).

Apolipoprotein (apo)A-I and apoA-II are the two major HDL apos. As apoA-I stimulates all of the steps of RCT, the role of HDLs in protecting against atherosclerosis has been attributed to apoA-I. The role of apoA-II remains unclear. In humans, it has been suggested that high plasma apoA-II concentrations are proatherogenic (5). Studies in transgenic mice overproducing apoA-II have not come to clear conclusions concerning the atherogenicity of apoA-II. The results obtained depended on the type of the diet (regular or atherogenic diet), the origin of the apoA-II gene expressed in mice (human or murine), and the genetic background of the mice (6,7). In recent studies of transgenic mice overproducing human apoA-II, we demonstrated that apoA-II acts as a physiological modulator of HDL metabolism. The overproduction of human apoA-II, at three times normal concentrations (transgenic line λ), resulted in a large decrease in HDL levels, associated with very high postprandial levels of very low density lipoproteins (VLDLs). Human apoA-II was found to associate with these VLDLs, impairing their catabolism by lipoprotein lipase (8,9). Further evidence for such a role of apoA-II was provided by a recent clinical study reporting that decreasing plasma apoA-II concentration increases clearance of large-sized postprandial VLDLs (10).

Hyperglycemia may be involved in changes in gene expression observed in type 2 diabetes. Glucose regulates the expression not only of the genes involved in glucose metabolism (11), but also that of genes involved in HDL metabolism, including those encoding apoA-I, human phospholipid transfer protein (PLTP), ATP-binding cassette transporter-A1 (ABC-1), scavenger receptor-BI (SRBI), and hepatic lipase (12). These effects on gene expression provide a molecular basis for the role of hyperglycemia in the modifications to HDL metabolism observed in diabetes.

In this study, we investigated whether the apoA-II gene was regulated by glucose. We showed that glucose upregulated human apoA-II gene transcription. This regulation was mediated by a hormone responsive element (HRE) within the apoA-II enhancer and a transcriptionally active form of hepatocyte nuclear factor (HNF)-4α. In transgenic

mice producing various amounts of human apoA-II, we established a strong positive correlation between blood glucose and human apoA-II levels, and we showed a substantial delay in glucose tolerance in mice overproducing human apoA-II.

RESEARCH DESIGN AND METHODS

Transgenic mice. Transgenic mice were producing human apoA-II at physiological plasma concentrations (line β) (9) and at three times higher levels (line λ) (8). Male β-transgenic mice were used for fasting/refeeding studies. The fasted mice ($n = 7$) were deprived of food for 36 h, and the fasted/refed mice ($n = 9$) were fasted for 36 h and then refed for 18 h with the standard carbohydrate-rich diet (51.2% carbohydrate, 23.5% protein, and 5% lipid by weight). For assessment of the correlation between blood glucose and plasma human apoA-II levels, male and female β- ($n = 20$) and λ-transgenic ($n = 24$) mice were fed ad libitum with the diet. Mice were anesthetized by intraperitoneal injection of avertine (0.25 mg/g) and were killed between 9 and 11 A.M. Blood was collected onto EDTA, and plasma was isolated. Blood glucose was determined with the Glucotrend plus test (Roche Diagnostics, Mannheim, Germany). Human apoA-II was assayed in plasma by immunonephelometry, using a specific antibody (Dade Behring), as previously described (13). Livers were removed and immediately frozen in liquid N_2 for subsequent RNA analysis. For the glucose tolerance test, male and female β- and λ-transgenic mice and control C57Bl/6J mice (four animals in each group) were fasted overnight. Animals were anesthetized by intraperitoneal injection of 0.15 mg/g Imalgene (Merial, Lyon, France). A bolus of glucose (2 mg/g) was injected intraperitoneally and blood glucose measured from the tail vein at the indicated times. Animal care and the experimental procedures used in this study conformed to French guidelines for animal studies.
RT real-time PCR. Human apoA-II transgenic mouse liver RNA and streptozotocin-induced diabetic rat liver RNA ($n = 3$), kindly given by I. Guillet-Deniau (14), were analyzed by RT real-time PCR (LightCycler System, Roche). Reverse transcription was performed with 1 μg RNA and Moloney murine leukemia virus reverse transcriptase (Gibco BRL). Human and rat apoA-II mRNA levels were determined with the SYBR Green I dye. We carried out 30 cycles of PCR in a SYBER Green buffer containing 3 mmol/l $MgCl_2$, as follows: 10 s of denaturation at 95°C and 10 s of annealing at 55°C for human apoA-II or 65°C for rat apoA-II and 10 s of extension at 72°C. The mouse coding strand primer was 5′CGCAGCAACTGTGCTACTCC3′, and the mouse noncoding strand primer was 5′CTCTCCACACATGGCTCCTT3′. The rat coding strand primer was 5′TTTAACTGACTATGGCAAGGATTT3′, and the rat noncoding strand primer was 5′CTGGTTTCTCCTCGGGGCTCATTA3′. As an internal control, we determined levels of 18S RNA with the Ribosomal RNA control kit (18S rRNA; Applied Biosystems).
Rat hepatocyte primary culture. Hepatocytes were isolated from fed male Wistar rats as previously described (15). Cells were plated at a density of 8 million cells per 100-mm diameter tissue culture plate for RNA analysis and 1 million cells per 60-mm diameter tissue culture plate for transfection experiments and were allowed to adhere for 4 h. For RNA analysis, the medium was replaced with M199 medium (5.5 mmol/l glucose) or M199 medium supplemented with 20 mmol/l glucose (25.5 mmol/l total glucose). The cells were incubated for a further 24 h. Total RNA was extracted from rat hepatocytes in the appropriate amount of RNAplus solution (Appligene) according to the manufacturer's instructions. Rat hepatocyte RNA samples (15 μg) were analyzed by northern blotting, as previously described (8), and the membrane was hybridized with a rat apoA-II cDNA probe. An 18S probe was used as an internal standard. Autoradiographs were analyzed by densitometry, with densities expressed in arbitrary units. For transfection experiments, the medium was replaced with M199 medium. Then, 30 μg DOTAP liposomal transfection reagent (Boehringer Mannheim) and 5 μg plasmid DNA in 20 mmol/l Hepes, pH 7.4, were added to the medium for overnight transfection, according to the reagent manufacturer's instructions. After transfection, the medium was removed and replaced with M199 medium or M199 medium supplemented with 20 mmol/l glucose. The cells were then incubated for a further 48 h. Experiments were performed in triplicate and repeated three times.
HepG2 cell culture. Human hepatoma HepG2 cells were plated at a density of 0.4 million cells per well in 6-well tissue culture plate. The medium used was Dulbecco's modified Eagle's medium (DMEM)-glutamax, 1 g/l glucose (5.5 mmol/l) (Gibco) supplemented with 10% FCS and 1% penicillin/streptomycin, and the cells were allowed to adhere overnight. HepG2 cells were transfected by incubation for 24 h with 1 μg of plasmid DNA in opti-MEM medium supplemented with 20 μg of lipofectin reagent (Gibco BRL) according to the manufacturer's instructions. After transfection, the medium was replaced with DMEM without sodium pyruvate and glucose, supplemented with glutamine,

1% FCS, 1% penicillin/streptomycin, and the indicated concentration of glucose, and the cells were incubated for a further 24 h. Experiments were performed in triplicate and repeated three to five times.
Chloramphenicol acetyl transferase assay. Transfected rat hepatocytes or HepG2 cells were recovered and chloramphenicol acetyl transferase (CAT) activity (as counts per minute) was measured in a liquid phase assay, as previously described (16). At days 22 and 37, individual liver samples ($n = 8$) from transgenic mice expressing the CAT gene under the control of the ($-911/+29$) human apoA-II gene promoter were homogenized and assayed for CAT activity. The proportion of chloramphenicol converted to acetylated was determined by thin layer chromatography (17).
Vectors and oligonucleotides. The plasmid containing the CAT gene under the control of the $-911/+29$ human apoA-II promoter (-911) and plasmids containing human apoA-II promoter constructs with deletions (-614, -440, -230, -80, and E-AB) have been described elsewhere (16,18,19). The plasmid encoding the dominant-negative form of HNF-4α, CD1b, was kindly provided by Dr. M. Hadzopoulou-Cladaras (Department of Biology, Aristotelian University of Thessaloniki, Thessaloniki, Greece) and has been described elsewhere (20). The mutants were constructed by inverse PCR with the Quick Exchange Mutagenesis kit (Stratagene) and sequenced. The M1 mutant was constructed from the $-911/+29$ plasmid with the coding strand oligonucleotide 5′-GGTG ATCAAATGACCAGTAGCCTTCAACCTTTACCCTGG-3′ and the complementary noncoding strand oligonucleotide. The M2 mutant was constructed from the LM2 plasmid (19) with the coding strand oligonucleotide 5′-GAACTGAT AAGGTGATCAAAATACCAGTAGCCTTCAACCTTTACCC-3′ and the complementary noncoding strand oligonucleotide. The M3 mutant has been described elsewhere (19) as Km1Lm2. The M4 mutant was constructed from the Km1Lm2 plasmid (19), with the coding strand oligonucleotide 5′-CCA GGTGCCTTCAACGCACAGACCTGGTAGAAGCCTCTTATTCACC-3′ and the complementary noncoding strand oligonucleotide.
Statistical analysis. Results were expressed as means ± SEM. The statistical significance of differences was determined by unpaired t tests after ANOVA, performed with Excel software. The statistical significance of the correlation between plasma human apoA-II concentration and blood glucose and that of the glucose tolerance test in transgenic mice was calculated by two-way ANOVA, carried out with Graph-Pad Prism software.

RESULTS

We investigated the response of apoA-II gene expression to changes in diet by performing in vivo analysis on transgenic mice expressing human apoA-II at physiological levels (line β) (9). A refeeding with a high-carbohydrate diet induced an increase in hepatic mRNA level and plasma concentration of human apoA-II, 2.5- and 1.5-fold, respectively (Fig. 1A). Hepatic mRNA levels of the endogenous mouse genes Glut-2 and L-PK, which are known to be induced by carbohydrates, were increased by factors of 8 and 3.6, respectively, following refeeding (data not shown). Previous studies have reported that apoA-II gene expression is induced during weaning (21). Weaning is characterized by a switch from maternal milk to a high-carbohydrate diet. Moreover, in transgenic mice expressing the CAT reporter gene under the control of the human apoA-II ($-911/+29$) promoter, the CAT activity was stimulated three times at weaning (Fig. 1B). However, in streptozotocin-induced diabetic rats with hyperglycemia (14), rat apoA-II mRNA levels were not modified when compared with control rats (Fig. 1C).

Since in vivo studies do not allow the determination of the respective role of hormones and dietary substrates on apoA-II gene expression, we looked for a direct effect of glucose on apoA-II gene expression in primary rat hepatocytes. Rat apoA-II mRNA levels were twice as high in the presence of 25.5 mmol/l glucose than in the presence of 5.5 mmol/l glucose (Fig. 2A). This effect was not affected by insulin, as a similar doubling was observed in the absence of insulin and at all insulin concentrations tested. We transiently transfected rat hepatocytes with the CAT reporter gene under the control of the human ($-911/+29$)
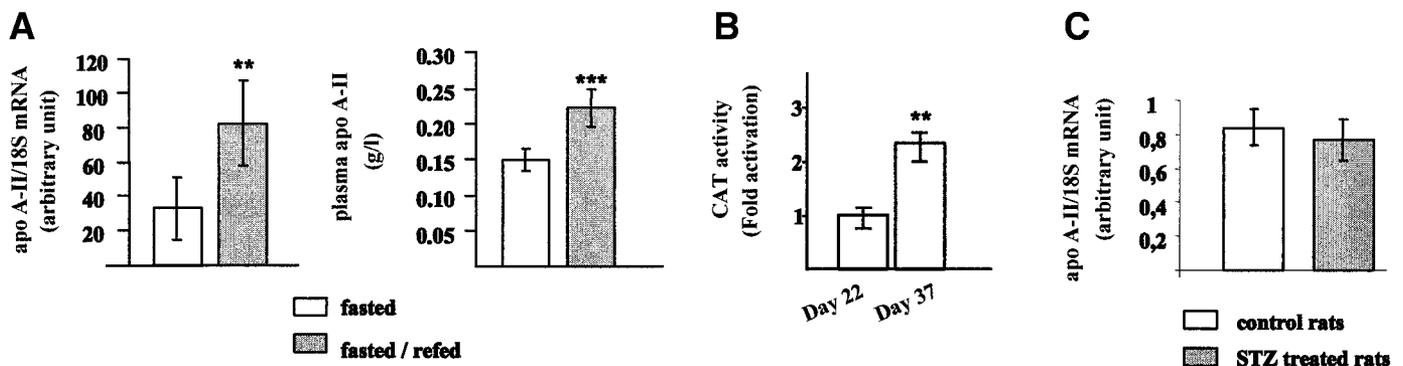
FIG. 1. In vivo regulation of apoA-II gene expression. *A*: Liver mRNA and plasma human apoA-II levels after refeeding with a high-carbohydrate diet in transgenic mice expressing human apoA-II (line β). *B*: Hepatic CAT activities from suckling (22d) and weaned (37d) transgenic mice expressing the CAT reporter gene under the control of the human (−911/+29) apoA-II promoter. CAT activity was expressed as fold activation, with respect to CAT activity arbitrarily set at 1, at day 22. *C*: Hepatic rat apoA-II mRNA from streptozotocin (STZ)-induced diabetic rats. ***P < 0.001 and **P < 0.01 with respect to the fasted group in *A* and to the suckling group (day 22) in *B*.

apoA-II promoter, which is sufficient to restrict CAT expression to the liver in vivo (17). The CAT reporter gene activity was three times higher in the presence of 25.5 mmol/l glucose than in the presence of 5.5 mmol/l glucose (Fig. 2B). In HepG2 cells, the CAT activity displayed 2-, 1.3-, and 3.7-fold induction by 5 mmol/l glucose with respect to 10 mmol/l lactate/1 mmol/l pyruvate, 1 mmol/l glucose, and no glucose (0 mmol/l), respectively (Fig. 2C). Increasing the concentration of glucose up to 20 mmol/l did not increase the level of induction. As observed with rat hepatocytes, induction of the human apoA-II promoter by glucose in HepG2 cells did not depend on the presence of insulin in the medium (data not shown). Thus, glucose itself stimulates transcription of the apoA-II gene, in vitro, independently of insulin action.

To determine the region(s) of the apoA-II promoter responsible for the response to glucose, we transiently transfected HepG2 cells with the CAT reporter gene under the control of various fragments of the human apoA-II promoter. Deletion of the (−911/−614) apoA-II enhancer abolished the response to glucose of the CAT reporter gene. When the (−911/−614) apoA-II enhancer was fused to the proximal promoter −80/+29 (E-AB), the CAT

activity was induced twofold by glucose (Fig. 3B). Thus, the (−911/−614) apoA-II enhancer is required for activation of the CAT reporter gene by glucose.

We then attempted to identify the glucose response element of the apoA-II enhancer. A glucose response element or carbohydrate response element (ChoRE) has been identified in several glucose-regulated genes; the ChoRE consists of two E-boxes (CANNTG) separated by five nucleotides (11). The human apoA-II enhancer contains multiple E-boxes, located between −797 and −721 (Fig. 3A), which bind the transcription factors upstream stimulatory factors (16). To identify a ChoRE, we generated full-length −911/+29 mutants M1 to M3 (Fig. 3A), by inserting specific mutations into the E-boxes. Upstream stimulatory factor binding was impaired (data not shown), but these mutations did not abolish the induction by glucose of CAT reporter activity in transiently transfected HepG2 cells (Fig. 3C). Therefore, these multiple E-boxes, located between −797 and −735, did not behave as a ChoRE.

Optimization of the response of the L-PK gene to glucose stimulation is based on cooperation between the ChoRE and an HRE (22,23). The human apoA-II enhancer pos-
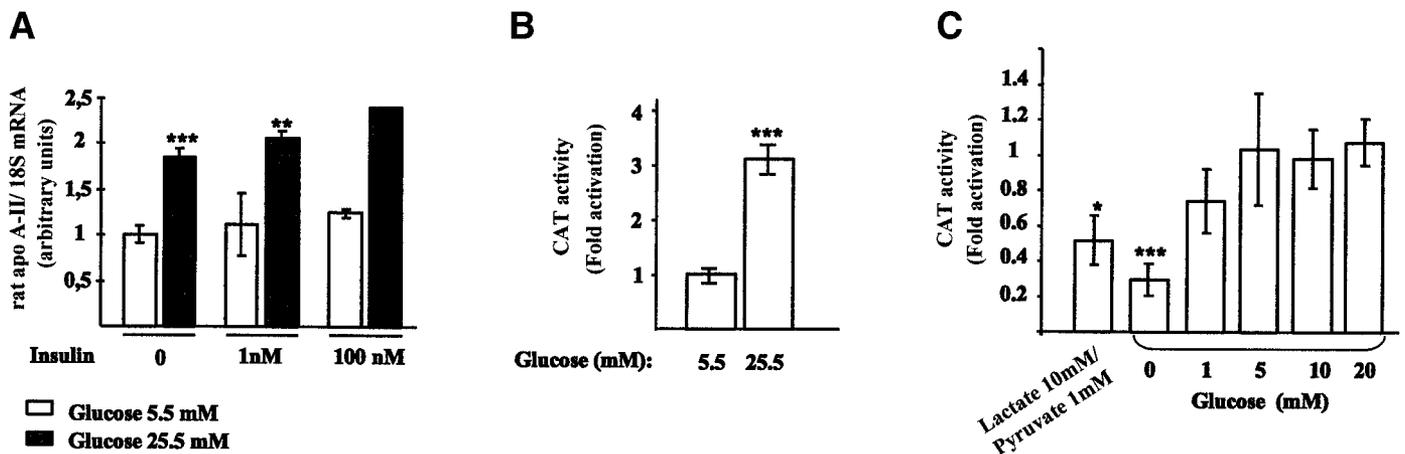

FIG. 2. ApoA-II gene transcription is stimulated by glucose in vitro. *A*: ApoA-II mRNA level is induced by glucose in primary rat hepatocytes. The results are expressed as fold activation with respect to the value obtained in the presence of 5 mmol/l glucose arbitrarily set at 1. *B* and *C*: Transcription of the human apoA-II gene is induced by glucose. Rat hepatocytes (*B*) and HepG2 cells (*C*) were transiently transfected with the CAT reporter gene under the control of the human (−911/+29) apoA-II promoter. CAT activity was expressed as fold activation with respect to CAT activity arbitrarily set at 1 in the presence of 5 mmol/l of glucose. ***P < 0.001, **P < 0.01, and *P < 0.05 with respect to the results obtained with 5 mmol/l glucose .
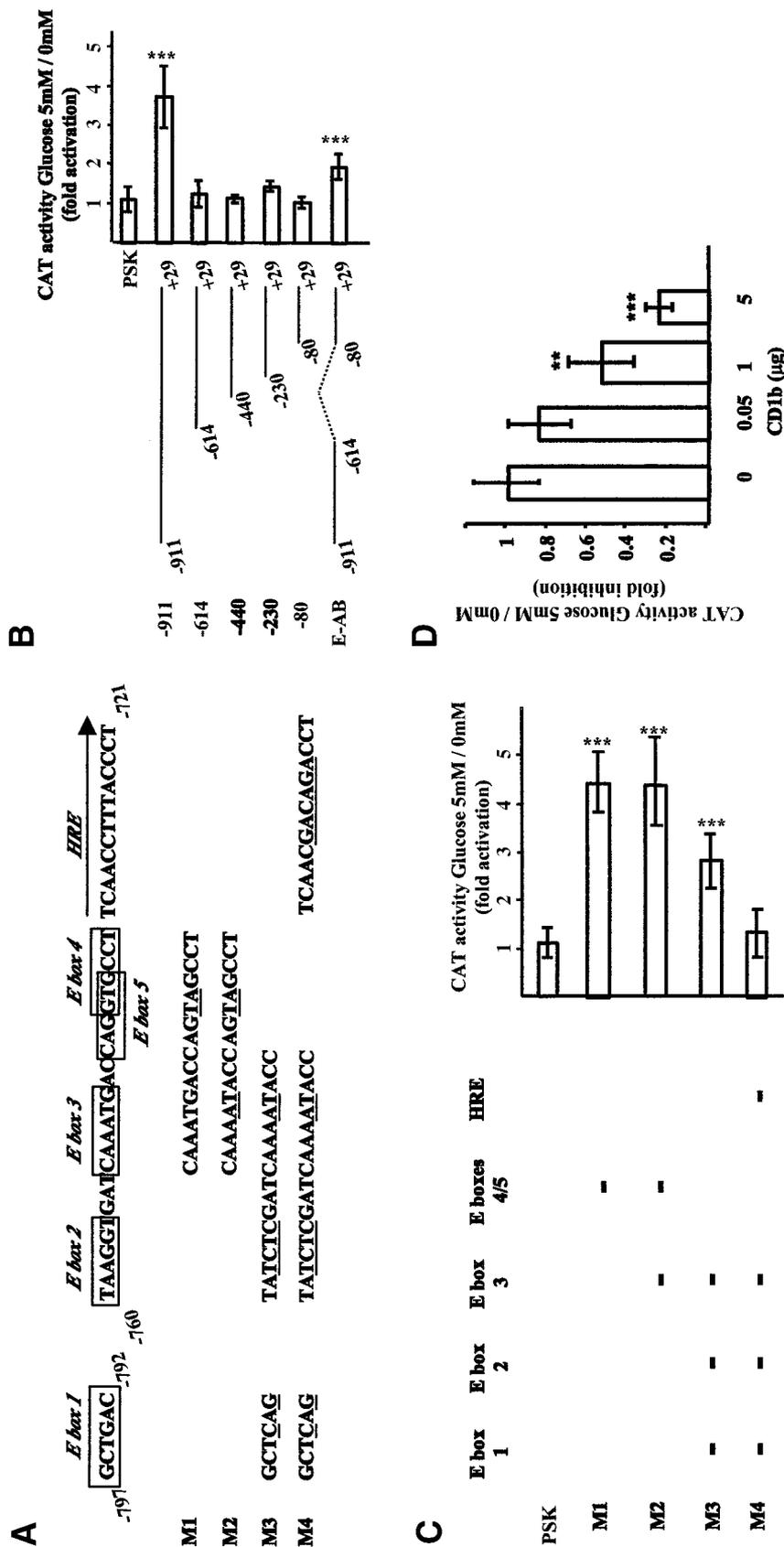
FIG. 3. The HRE in the human apoA-II enhancer is responsible for the stimulation by glucose of apoA-II gene transcription. *A*: Schematic representation of the −797/−721 sequence of the apoA-II promoter. Mutations in the E-boxes and in the HRE are described. HepG2 cells were transiently transfected with the CAT reporter gene under the control of various deletions (*B*) or mutations (*C*) of the human (−911/+29) apoA-II promoter. *B*: The hatched line indicates that the various regions are fused. *C*: The mutated nature of the sequences are indicated by dashes. pBluescript SK (PSK) vector (from Stratagene) is an insert-less vector used as a negative external control, and the CAT activity ratio obtained with PSK transfection was arbitrarily set at 1. *D*: HepG2 cells were transiently transfected with the CAT reporter gene under the control of the human (−911/+29) apoA-II promoter in the presence of various amounts of plasmid encoding a dominant-negative form of HNF-4α, CD1b. Inhibition by CD1b was determined as the ratio of fold stimulation by glucose in the presence of CD1b to fold stimulation in the absence of CD1b. $**P < 0.01$ and $***P < 0.001$, with respect to PSK in *A* and *C* and with respect to the absence of CD1b in *D*.
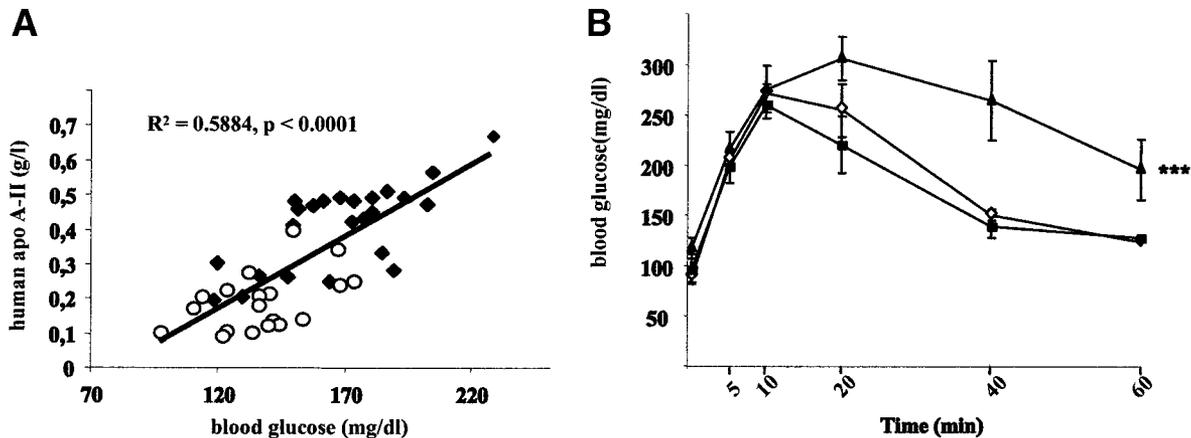
FIG. 4. In vivo effect of human apoA-II overproduction. *A*: Correlation between blood glucose and concentration of human apoA-II in the plasma of transgenic mice. ○, line β; ♦, line λ. *B*: Glucose tolerance test in transgenic mice. ▲, line λ; ■, line β; ◇, wild-type. The wild-type mice were C57Bl/6J. ***P < 0.001, with respect to wild-type and line β.

sesses an HRE located between −734 and −721 that binds members of the nuclear receptor family (24) and is contiguous to the multiple E-boxes (Fig. 3*A*). We therefore inserted an additional mutation into mutant M3 (to generate mutant M4, Fig. 3*A*) to impair HNF-4 binding to the HRE (data not shown). The CAT reporter activity of mutant M4 in transiently transfected HepG2 cells was not induced by glucose (Fig. 3*C*). We therefore investigated a possible role of HNF-4 in the effect of glucose on apoA-II gene transcription using a dominant-negative mutant of HNF-4α (20). We transiently transfected HepG2 cells with increasing amounts of the mutated form of HNF-4α, CD1b, and the CAT reporter gene under the control of the (−911/+29) human apoA-II promoter. The stimulation of CAT activity by glucose, arbitrarily fixed at 1, was inhibited dose-dependently by CD1b (Fig. 3*D*). Thus, either a mutation in the HRE or impairment of the transactivation activity of HNF-4α prevented the induction of human apoA-II promoter activity by glucose.

Transgenic mice overexpressing human apoA-II (line λ) display high plasma triglyceride and low HDL levels (8), a dyslipidemic profile similar to that observed in type 2 diabetes. However, glycemia had not yet been determined. To evaluate the pathophysiological relevance of our results, we therefore investigated the relationship between apoA-II gene expression and glucose in two lines of transgenic mice, line β, which produced human apoA-II at physiological concentration (9), and line λ, which overproduced three times the human apoA-II (8). Blood glucose was found to be strongly correlated (R^2 = 0.588, P < 0.0001) with the level of human apoA-II in transgenic mice of the β- and λ-lines (Fig. 4*A*). We investigated whether apoA-II was involved in blood glucose kinetics by carrying out a glucose tolerance test in these transgenic mice (Fig. 4*B*). Mice overproducing human apoA-II (line λ) displayed a marked delay in blood glucose clearance with respect to control mice (C57Bl/6J) and transgenic mice producing physiological levels of human apoA-II (line β).

DISCUSSION

In this study, we investigated the relationship between apoA-II and glucose levels. We found that glucose stimulated (in vitro) the hepatic transcription of the apoA-II gene. We also found that overexpression of the human apoA-II gene in transgenic mice was accompanied by a marked delay in glucose tolerance. These results are consistent with plasma concentrations of human apoA-II being positively correlated with glycemia in transgenic mice.

It has been shown that plasma apoA-II levels are controlled mainly by its rate of synthesis in the liver rather than its catabolism (25,26). Low plasma concentrations of apoA-II have recently been associated with a common polymorphism in the proximal promoter of the human apoA-II gene that leads to a decrease in apoA-II gene transcription (10). In the present study, we showed that the consumption of a high-carbohydrate diet activates the apoA-II gene transcription in vivo. By contrast, in streptozotocin-induced diabetic rats, the apoA-II mRNA levels remained unaffected despite hyperglycemia. This might be due to the effect of counter-regulatory hormones such as corticosteroids, which are elevated in streptozotocin-induced diabetic animals and are known to decrease apoA-II mRNA levels (27). However, in primary rat hepatocytes and HepG2 cells, glucose stimulated the apoA-II transcription independently of insulin action. The response to glucose required both the enhancer (−911/−614) and the promoter (−80/+29), a region that restricted the human apoA-II gene expression to liver cells (16–19,28). The glucose effect was prevented only when the HRE within the enhancer was mutated and when the active form of HNF-4α is inhibited. HNF-4α has not previously been directly involved in the glucose response but was considered to serve as an accessory factor (22,23). Specific inactivation of HNF-4α gene in the liver abolishes expression of the genes involved in the control of lipid metabolism, such as apoC-III, apoB, and apoA-II, and also inhibits microsomal triglyceride transfer protein (29). Moreover, in humans, mutations in the HNF-4α gene are responsible for type 1 maturity-onset diabetes of the young (MODY1). Significant alterations in triglyceride metabolism have been observed in MODY1 patients and are thought to result from impaired hepatic synthesis of apoA-II, apoB, and apoC-III (30,31). These data indicate that HNF-4α is a major in vivo regulator of genes involved in the control of lipid homeostasis, via the control of apo gene expression.

The modulation of HNF-4α activity by phosphorylation is well documented (32–36). In the fasting state, an increase in serine/threonine phosphorylation of hepatic HNF-4α decreases its DNA binding activity (32). Furthermore, after the consumption of a carbohydrate-rich diet, HNF-4α phosphorylation is decreased and HNF-4α promotes expression of the target genes. The kinases involved in these mechanisms are currently unknown. The AMP-activated protein kinase has been implicated in the inhibition of glucose-dependent gene expression (37) and has been shown to phosphorylate HNF-4 (38,39). Therefore, when glucose is abundant, the increase in binding of HNF-4α to the target gene may be mediated by inactivation of the phosphorylation of HNF-4α.

The overproduction of human apoA-II decreases plasma HDL concentration in a dose-dependent manner (8,40) and induces postprandial hypertriglyceridemia (8,9). This phenotype resembles the dyslipidemia observed in diabetes, suggesting that stimulation of the hepatic synthesis of apoA-II may contribute to the dyslipidemia syndrome that occurs in patients with type 2 diabetes. Several studies in humans have linked the apoA-II gene to type 2 diabetes (41–44). In our transgenic mice, there is a positive correlation between blood glucose and the plasma human apoA-II. A delay in glucose tolerance was impaired in the λ-line overproducing human apoA-II in accordance to the glucose intolerance observed in transgenic mice overproducing murine apoA-II (45). Conversely, apoA-II knockout mice display low plasma levels of free fatty acids, glucose, and insulin, suggesting hypersensitivity to insulin (46). Together with our results, this suggests that the overproduction of apoA-II may contribute to glucose intolerance and that plasma apoA-II levels may be involved in diabetes pathogenesis.

In conclusion, we show that human apoA-II gene transcription is induced by glucose in vitro, and that this regulation requires an HRE that interacts with HNF-4α. Our results allow us to hypothesize a possible pathogenic pathway. An increasing apoA-II level causes hypertriglyceridemia and hypoHDLemia. The dyslipidemia results in glucose intolerance; this promotes a rise in blood glucose, which in turn might increase apoA-II gene transcription, thus establishing a vicious circle that may contribute to type 2 diabetes.

REFERENCES

1. Evans M, Khan N, Rees A: Diabetic dyslipidaemia and coronary heart disease: new perspectives. *Curr Opin Lipidol* 10:387–391, 1999
2. Gordon DJ, Rifkind BM: High-density lipoprotein: the clinical implications of recent studies. *N Engl J Med* 321:1311–1315, 1989
3. Sviridov D, Nestel P: Dynamics of reverse cholesterol transport: protection against atherosclerosis. *Atherosclerosis* 161:245–254, 2002
4. Banka CL: High density lipoprotein and lipoprotein oxidation. *Curr Opin Lipidol* 7:139–142, 1996
5. Parra HJ, Arveiler D, Evans AE Cambou JP, Amouyel P, Bingham A, McMaster D, Schaffer P, Douste-Blazy P, Luc G, et al.: A case-control study of lipoprotein particles in two populations at contrasting risk for coronary heart disease: the ECTIM study. *Arterioscler Thromb* 12:701–707, 1992
6. Blanco-Vaca F, Escola-Gil JC, Martin-Campos JM, Julve J: Role of apoA-II in lipid metabolism and atherosclerosis: advances in the study of an enigmatic protein. *J Lipid Res* 42:1727–1739, 2001
7. Kalopissis AD, Pastier D, Chambaz J: Apolipoprotein A-II: beyond genetic associations with lipid disorders and insulin resistance. *Curr Opin Lipidol* 14:165–172, 2003
8. Boisfer E, Lambert G, Atger V, Tran NQ, Pastier D, Benetollo C, Trottier JF, Beaucamps I, Antonucci M, Laplaud M, Griglio S, Chambaz J, Kalopissis AD: Overexpression of human apolipoprotein A-II in mice induces hypertriglyceridemia due to defective very low density lipoprotein hydrolysis. *J Biol Chem* 274:11564–11572, 1999
9. Pastier D, Dugue S, Boisfer E, Atger V, Tran NQ, van Tol A, Chapman MJ, Chambaz J, Laplaud PM, Kalopissis AD: Apolipoprotein A-II/A-I ratio is a key determinant *in vivo* of HDL concentration and formation of pre-beta HDL containing apolipoprotein A-II. *Biochemistry* 40:12243–12253, 2001
10. Van't Hooft FM, Ruotolo G, Boquist S, deFaire U, Eggertsen G, Hamsten A: Human evidence that the apolipoprotein A-II gene is implicated in visceral fat accumulation and metabolism of triglyceride-rich lipoproteins. *Circulation* 104:1223–1228, 2001
11. Vaulont S, Vasseur-Cognet M, Kahn A: Glucose regulation of gene transcription. *J Biol Chem* 275:31555–31558, 2000
12. Tu AY, Albers JJ: Glucose regulates the transcription of human genes relevant to HDL metabolism: responsive elements for peroxisome proliferator–activated receptor are involved in the regulation of phospholipid transfer protein. *Diabetes* 50:1851–1856, 2001
13. Fournier N, Cogny A, Atger V, Pastier D, Goudouneche D, Nicoletti A, Moatti N, Chambaz J, Paul JL, Kalopissis AD: Opposite effects of plasma from human apolipoprotein A-II transgenic mice on cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells. *Arterioscler Thromb Vasc Biol* 22:638–643, 2002
14. Guillet-Deniau I, Mieulet V, Le Lay S, Achouri Y, Carre D, Girard J, Foufelle F, Ferre P: Sterol regulatory element binding protein-1c expression and action in rat muscles: insulin-like effects on the control of glycolytic and lipogenic enzymes and UCP3 gene expression. *Diabetes* 51:1722–1728, 2002
15. Foretz M, Carling D, Guichard C, Ferre P, Foufelle F: AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. *J Biol Chem* 273:14767–14771, 1998
16. Ribeiro A, Pastier D, Kardassis D, Chambaz J, Cardot P: Cooperative binding of upstream stimulatory factor and hepatic nuclear factor 4 drives the transcription of the human apolipoprotein A-II gene. *J Biol Chem* 274:1216–1225, 1999
17. Le Beyec J, Benetollo C, Chauffeton V, Domiar A, Lafrenière M-J, Chambaz J, Cardot P, Kalopissis AD: The –911/+29 sequnce of human apolipoprotein A-II promoter is sufficient to confer liver-restricted expression, developmental changes and response to a high-fat diet in transgenic mice. *Transgenics* 2:211–220, 1998
18. Chambaz J, Cardot P, Pastier D, Zannis VI, Cladaras C: Promoter elements and factors required for hepatic transcription of the human Apo A-II gene. *J Biol Chem* 266:11676–11685, 1991
19. Cardot P, Chambaz J, Kardassis D, Cladaras C, Zannis VI: Factors participating in the liver-specific expression of the human apolipoprotein A-II gene and their significance for transcription. *Biochemistry* 32:9080–9093, 1993
20. Hadzopoulou-Cladaras M, Kistanova E, Evagelopoulou C, Zeng S, Cladaras C, Ladias JAA: Functional domains of the nuclear receptor hepatocyte nuclear factor 4. *J Biol Chem* 272:539–550, 1997
21. Staels B, Auwerx J, Chan L, van Tol A, Rosseneu M, Verhoeven G: Influence of development, estrogens, and food intake on apolipoprotein A-I, A-II, and E mRNA in rat liver and intestine. *J Lipid Res* 30:1137–1145, 1989
22. Diaz Guerra MJ, Bergot MO, Martinez A, Cuif MH, Kahn A, Raymondjean M: Functional characterization of the L-type pyruvate kinase gene glucose response complex. *Mol Cel Biol* 13:7725–7733, 1993
23. Liu Z, Thompson KS, Towle HC: Carbohydrate regulation of the rat L-type

pyruvate kinase gene requires two nuclear factors: LF-A1 and a member of the c-myc family. *J Biol Chem* 268:12787–12795, 1993

24. Ladias JA, Hadzopoulou-Cladaras M, Kardassis D, Cardot P, Cheng J, Zannis V, Cladaras C: Transcriptional regulation of human apolipoprotein genes ApoB, ApoCIII, and ApoAII by members of the steroid hormone receptor superfamily HNF-4, ARP-1, EAR-2, and EAR-3. *J Biol Chem* 267:15849–15860, 1992

25. Ikewaki K, Zech LA, Kindt M, Brewer HB Jr, Rader DJ: Apolipoprotein A-II production rate is a major factor regulating the distribution of apolipoprotein A-I among HDL subclasses LpA-I and LpA-I:A-II in normolipidemic humans. *Arterioscler Thromb Vasc Biol* 15:306–312, 1995

26. Ikewaki K, Zech LA, Brewer HB Jr, Rader DJ: Apo A-II kinetics in humans using endogenous labeling with stable isotopes: slower turnover of apo A-II compared with the exogenous radiotracer method. *J Lipid Res* 37:399–407, 1996

27. Staels B, van Tol A, Chan L, Verhoeven G, Auwerx J: Variable effects of different corticosteroids on plasma lipids, apolipoproteins, and hepatic apolipoprotein mRNA levels in rats. *Arterioscler Thromb* 11:760–769, 1991

28. Lacorte JM, Fourniat E, Pastier D, Chambaz J, Ribeiro A, Cardot P: The proximal element of the human apolipoprotein A-II promoter increases the enhancer activity of the distal region. *Biochem J* 318:681–688, 1996

29. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ: Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol* 21:1393–1403, 2001

30. Lehto M, Bitzen PO, Isomaa B, Wipemo C, Wessman Y, Forsblom C, Tuomi T, Taskinen MR, Groop L: Mutation in the HNF-4α gene affects insulin secretion and triglyceride metabolism. *Diabetes* 48:423–425, 1999

31. Shih DQ, Dansky HM, Fleisher M, Assmann G, Fajans SS, Stoffel M: Genotype/phenotype relationships in HNF-4α/MODY1: haploinsufficiency is associated with reduced apolipoprotein (AII), apolipoprotein (CIII), lipoprotein(a), and triglyceride levels. *Diabetes* 49:832–837, 2000

32. Viollet B, Kahn A, Raymondjean M: Protein kinase A-dependent phosphorylation modulates DNA-binding activity of hepatocyte nuclear factor 4. *Mol Cell Biol* 17:4208–4219, 1997

33. Ktistaki E, Ktistakis NT, Papadogeorgaki E, Talianidis I: Recruitment of hepatocyte nuclear factor 4 into specific intranuclear compartments depends on tyrosine phosphorylation that affects its DNA-binding and transactivation potential. *Proc Natl Acad Sci U S A* 92:9876–9880, 1995

34. Jiang G, Nepomuceno L, Yang Q, Sladek FM: Serine/threonine phosphorylation of orphan receptor hepatocyte nuclear factor 4. *Arch Biochem Biophys* 340:1–9, 1997

35. Wang B, Cai SR, Gao C, Sladek FM, Ponder KP: Lipopolysaccharide results in a marked decrease in hepatocyte nuclear factor 4 alpha in rat liver. *Hepatology* 34:979–989, 2001

36. Guo H, Wei J, Inoue Y, Gonzalez FJ, Kuo PC: Serine/threonine phosphorylation regulates HNF-4alpha-dependent redox-mediated iNOS expression in hepatocytes. *Am J Physiol Cell Physiol* 284:C1090–C1099, 2003

37. Woods A, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferre P, Foufelle F, Carling D: Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol Cell Biol* 20:6704–6711, 2000

38. Leclerc I, Lenzner C, Gourdon L, Vaulont S, Kahn A, Viollet B: Hepatocyte nuclear factor-4α involved in type 1 maturity-onset diabetes of the young is a novel target of AMP-activated protein kinase. *Diabetes* 50:1515–1521, 2001

39. Hong YH, Varanasi US, Yang W, Leff T: AMP-activated protein kinase regulates HNF4alpha transcriptional activity by inhibiting dimer formation and decreasing protein stability. *J Biol Chem* 278:27495–27501, 2003

40. Escola-Gil JC, Marzal-Casacuberta A, Julve-Gil J, Ishida BY, Ordonez-Llanos J, Chan L, Gonzalez-Sastre F, Blanco-Vaca F: Human apolipoprotein A-II is a pro-atherogenic molecule when it is expressed in transgenic mice at a level similar to that in humans: evidence of a potentially relevant species-specific interaction with diet. *J Lipid Res* 39:457–462, 1998

41. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennett PH, Knowler WC: An autosomal genomic scan for loci linked to type 2 diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 63:1130–1138, 1998

42. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ: A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 48:1175–1182, 1999

43. Vionnet N, Hani El-H, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Lepretre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. *Am J Hum Genet* 67:1470–1480, 2000

44. Elbein SC, Chu W, Ren Q, Wang H, Hemphill C, Hasstedt SJ: Evaluation of apolipoprotein A-II as a positional candidate gene for familial type 2 diabetes, altered lipid concentrations, and insulin resistance. *Diabetologia* 45:1026–1033, 2002

45. Castellani LW, Goto AM, Lusis AJ: Studies with apolipoprotein A-II transgenic mice indicate a role for HDLs in adiposity and insulin resistance. *Diabetes* 50:643–651, 2001

46. Weng W, Breslow JL: Dramatically decreased high density lipoprotein cholesterol, increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. *Proc Natl Acad Sci U S A* 93:14788–14794, 1996