After this report, several *BDNF* SNP analyses appeared, as mentioned in the Introduction. These SNPs were generally considered risk factors for both diseases, but recent reports have not mentioned this possibility. According to Shimizu et al [43], the rates of *BDNF* SNP depend on ethnic differences. Our incidence of Val66Met was almost the same as that found by these authors which was also estimated from Japanese data. In addition, Desai et al. [44] recently described a difference in Val66Met and C270T distribution patterns of Caucasian Americans and African-Americans [44]. In case of C270T polymorphism, our results were similar to those reported by another Japanese group [13] as well as to the findings observed in African-Americans [44].

As for the risk of sporadic DLB, we reported that ApoE is a predisposing factor for this disease as it is for AD [32]. Moreover, paraoxonase-1 [45] is associated with the Lewy body stage [45]. However, harboring a *BDNF* polymorphism has not conclusively been shown to raise the PD risk, while no data are available on DLB.

The aim of our current study was to determine whether the Val66Met and C270T polymorphisms represent risk factors for developing AD or DLB, and for this task we used autopsy samples, with which the diagnosis had been confirmed. BDNF has been found to promote survival of all major neuronal tissue types affected in AD and PD/DLB, such as hippocampal and neocortical neurons, cholinergic septal and basal forebrain neurons, and nigral dopaminergic neurons [2]. Taken together, these findings indicate that BDNF plays a pivotal role in protecting hippocampal and nigral neurons, and evidence of its dysfunction could be suggestive of AD or DLB pathogenesis.

Among our neuropathologically diagnosed samples, no subgroup showed an association with *BDNF* SNPs (table 2a). *BDNF* SNPs might raise the risk of AD, but our data could not confirm this. As for DLB, none of the DLB subgroups showed any relationship to *BDNF* polymorphisms C270T and Val66Met. Our DLB samples were taken from a total of 35 patients, and the size of this group might have been too small to establish statistical significance.

It is known that ApoE polymorphism is a strong risk factor for AD and DLB [32]. With this in mind, we filtered our data according to the ApoE ε4+/– status (table 3), but we failed to detect any correlation.

In conclusion, variation in the C270T rate was too great to provide a useful measure of the genetic risk for a particular individual, and as for theVal66Met SNP, no correlations were observed with our Japanese AD and DLB patients. In assessing the biological function of BDNF in AD, DLB, and PD, we could not detect any association with *BDNF* SNPs. To reach at a final conclusion, the next step must involve the study of the genetic background of individual patients with a sufficient number of samples, for which the neuropathological diagnosis has been confirmed.

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Deficiency of the Very Low-Density Lipoprotein (VLDL) Receptors in Streptozotocin-Induced Diabetic Rats: Insulin Dependency of the VLDL Receptor

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Hyperlipidemia is a common feature of diabetes and is related to cardiovascular disease. The very low-density lipoprotein receptor (VLDL-R) is a member of the low-density lipoprotein receptor (LDL-R) family. It binds and internalizes triglyceride-rich lipoproteins with high specificity. We examined the etiology of hyperlipidemia in the insulin-deficient state. VLDL-R expression in heart and skeletal muscle were measured in rats with streptozotocin (STZ)-induced diabetes. STZ rats showed severe hyperlipidemia on d 21 and 28, with a dramatic decline in VLDL-R protein in skeletal muscle (>90%), heart (~50%) and a loss of adipose tissues itself on d 28. The reduction of VLDL-R protein in skeletal muscle could not be explained simply by a decrease at the transcriptional level, because a dissociation between VLDL-R protein and mRNA expression was observed. The expression of LDL-R and LDL-R-related protein in liver showed no consistent changes. Furthermore, no effect on VLDL-triglyceride production in liver was observed in STZ rats. A decrease in postheparin plasma lipoprotein lipase activity started on d 7 and continued to d 28 at the 50% level even though severe hyperlipidemia was detected only on d 21 and 28. In rat myoblast cells, serum deprivation for 24 h induced a reduction in VLDL-R proteins. Insulin (10^{-6} M), but not IGF-I (10 ng/ml), restored the decreased VLDL-R proteins by serum deprivation. These results suggest that the combination of VLDL-R deficiency and reduced plasma lipoprotein lipase activity may be responsible for severe hyperlipidemia in insulin-deficient diabetes. (*Endocrinology* 146: 3286–3294, 2005)

IN DIABETES MELLITUS, hyperlipidemia is often observed as a result of impaired insulin action (1, 2), and their causal relations to macrovascular disease and diabetic macroangiopathy have been discussed (3). For the insulin-deficient diabetic model, streptozotocin (STZ)-induced diabetic rats (STZ rats) have been used for the study of diabetic hyperlipidemia (4–14). Several mechanisms of diabetic hyperlipidemia in STZ rats have been proposed, including increased intestinal absorption of dietary cholesterol (10–14), increased very low-density lipoprotein (VLDL) production in liver at an early period after STZ treatment (4), and decreased removal of VLDL-triglyceride (TG) from the circulation (4, 5). Lipoprotein lipase (LPL) hydrolyzes circulating TG, leading to the release of free fatty acids (FFAs), which are stored as TG in adipose tissue and serve as energy sources in skeletal muscle and heart (15). Several studies have measured LPL activity in skeletal muscle and heart in insulin-deficient diabetic animal models. Decreased activity (7, 9, 16), no change in activity (5, 17), and increased activity (6, 8) all have been observed. Variations in LPL activity probably contribute to the abnormalities in lipoprotein metabolism and the duration of the diabetic state. Recently, it has been reported that intestinal acyl-coenzyme A:cholesterol acyltransferase (ACAT-2), microsomal triglyceride transfer protein (MTP), and ATP-binding cassette transporter (ABCG5/G8) are also related to hyperlipidemia in STZ rats (12–14). However, the precise mechanisms of insulin-deficient diabetic hyperlipidemia are unclear.

The VLDL receptor (VLDL-R) is a member of the low-

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Abbreviations: ABC, ATP-binding cassette transporter; ACAT, acyl-coenzyme A:cholesterol acyltransferase; apo, apolipoprotein; FA, fatty acid; FFA, free fatty acid; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; LPL, lipoprotein lipase; LRP, low-density lipoprotein receptor-related protein; MTP, microsomal triglyceride transfer protein; RAP, receptor-associated protein; SD, Sprague Dawley; STZ, streptozotocin; TC, total cholesterol; TG, triglyceride; TGPR, triglyceride production rate; VLDL, very low-density lipoprotein; β-VLDL, β-migrating very low-density lipoproteins.

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density lipoprotein (LDL) receptor (LDL-R) family and is most abundant in extrahepatic tissues such as brain, heart, skeletal muscle, and adipose tissue (18). Because heart and skeletal muscle use fatty acids (FAs) as an energy source, and adipose tissue use FAs for energy storage, the VLDL-R is thought to play a role in the delivery of FAs as TG-rich lipoproteins to peripheral tissues (19). The VLDL-R binds with high affinity apolipoprotein E (apoE)-containing particles, such as VLDL and intermediate density lipoprotein from Watanabe heritable hyperlipidemic rabbits, as well as β-VLDL obtained from cholesterol-fed rabbits, but does not bind LDL. In contrast, VLDL from fasted normal human subjects binds with lower affinity than VLDL prepared from Watanabe heritable hyperlipidemic rabbits or β-VLDL from cholesterol-fed rabbits. The low-affinity binding of fasted human VLDL to the VLDL receptor can be overcome by enriching VLDL with either apoE or LPL (18–21). There are three mechanisms between LPL and the VLDL-R: 1) direct binding to the receptor, 2) mediation of the binding lipoprotein particles to heparan sulfate proteoglycans before interaction with the receptor, and 3) its lipolytic activity, converting VLDL particles to smaller remnants (apoE-rich particles) before these can become endocytosed by receptors. After our findings of unique ligand-binding specificity of the VLDL-R for VLDL particles, Niemeier and colleagues (22) showed that the same mechanism was operating for chylomicron particles. The VLDL-R also interacts with numerous other ligands, including LPL (21, 23), urokinase plasminogen activator/plasminogen activator inhibitor-1 complex (23), receptor-associated protein (RAP) (24), and the atherogenic lipoprotein(a) (25). VLDL-R expression, mostly in macrophages, has been demonstrated in human and rabbit atherosclerotic lesions (25–28), and we suggested that the VLDL-R contributes to macrophage foam cell formation in the early phase of atherosclerosis via uptake of remnant lipoproteins (29). Furthermore, we showed a novel VLDL-R pathway for FA metabolism in the heart (30). Taken together, these findings suggest that the VLDL-R plays an important role in lipoprotein metabolism of VLDL and other TG-rich lipoprotein particles in concert with LPL as a peripheral lipoprotein receptor (31).

It has recently been reported that VLDL-R mRNA in skeletal muscle is reduced in experimental hypothyroidism and is increased in hyperthyroidism (32). Reductions of VLDL-R expression have been described in rats with chronic renal insufficiency and nephrotic syndrome and in Imai rats with spontaneous focal glomerulosclerosis (33–35). Rats with these conditions showed marked hypertriglyceridemia, elevated plasma VLDL concentration, and impaired VLDL clearance.

To investigate the role of the VLDL-R in the disorder of lipoprotein metabolism in insulin-deficient diabetes, we studied VLDL-R expression in STZ rats and rat myoblasts (L6 cells).

Materials and Methods

Experimental animals and cultured myoblasts

Adult 8-wk-old male Sprague Dawley (SD) rats (280–310 g) were obtained from Japan SLC (Shizuoka, Japan). Rats were randomly divided into nondiabetes (control) and diabetes (STZ) groups. The animals were made diabetic under halothane anesthesia, followed by injection of STZ (60 mg/kg body weight, iv; Sigma-Aldrich Corp., St. Louis, MO) into the tail vein. An equivalent volume (1 ml/kg) of saline was administered to the nondiabetic controls. Hyperglycemia was tested 24 h after STZ administration by a blood glucose meter. All STZ-treated rats were kept for 1–4 wk after STZ injection, at which time they were killed after 5 h of fasting, and their tissues and plasma samples were collected. All animals were maintained under a 12-h light (0700–1900 h), 12-h dark cycle and given a standard laboratory diet (Oriental Yeast, Tokyo, Japan) and water. All experiments were conducted in accordance with the National Institutes of Health and Welfare Guide for the Care and Use of Laboratory Animals. Rat myoblasts (L6 cells, JCRB9081) were purchased from Health Science Research Resources Bank (Osaka, Japan).

Antibodies against VLDL-R, LDL-R, LDL-R-related protein-1 (LRP1), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Rabbit polyclonal antibody (VR2) to the carboxyl terminus of the VLDL-R was made using a synthetic peptide, CASVGHTYPAISVVSTDDDLA, which is encoded in several tissues and species (29). The specificity of rabbit antibody VR2 was confirmed by immunoblotting against a membrane fraction from IdlA-7 cells (LDL-R-deficient Chinese hamster ovary cells) expressing human type 1 VLDL-R, human LDL-R, and human apoE receptor 2 (data not shown). Hybridoma cells producing a monoclonal antibody against rat LDL-R (IgG 4A4, CRL-1898) and rat LRP1 (IgG 11H4, CRL-1936) were purchased from American Type Culture Collection (Manassas, VA). Anti-GAPDH monoclonal antibody was purchased from Chemicon International (Temecula, CA).

Isolation of membrane fraction from tissues and Western blot analysis

Membrane fractions were prepared according to a standard method (36). Cellular protein was measured using the bicinchoninic acid protein assay kit (Pierce Chemical Co., Rockford, IL). SDS-PAGE was performed on the fractions with 5–20% slab gels containing 0.1% sodium dodecyl sulfate. Total membrane and cell protein (30 μg/lane) for heart, liver, and skeletal muscle were applied and transferred to a polyvinylidene difluoride membrane (Millipore Corp., Bedford, MA) using a Trans-blot (Atto, Tokyo, Japan). Detection of antibodies was performed using a second antibody and was visualized by enhanced chemiluminescence (ECL, Pharmacia Biotech, Uppsala, Sweden).

RNA extraction and Northern blot analysis

Total RNA from heart, liver, and skeletal muscle (soleus muscle) was extracted using the guanidinium thiocyanate method with phenol-chloroform extraction (37). Total RNA (15 μg) was loaded onto a 1% agarose-gel with 9% formaldehyde, which was separated in MOPS [3-(N-morpholino)-propanesulfonic acid] buffer. The RNAs were transferred on to the nylon membrane (GeneScreen Plus, NEN Life Science Products, Boston, MA) by capillary transfer. After UV cross-linking, the membrane was prehybridized and hybridized with cDNA fragments labeled [γ-^{32}P]deoxy-CTP by the random primer method, using Random Primer DNA Labeling kits (Takara Shuzo Co., Ltd., Shiga, Japan). The VLDL-R probe was prepared from digested rat VLDL-R cDNA.

RT-PCR

To analyze isoforms of VLDL-R mRNA, RT-PCR was carried out as previously described (20). cDNA was synthesized from 10 μg total RNA from heart and skeletal muscle, using oligo(deoxythymidine)$_{18}$ primer and SuperScript. One tenth of the cDNA was subjected to PCR with a sense primer (5′-CTAGTCAACAACCTGAATGATG-3′) and an antisense primer (5′-AAGAATGGCCCATGCAGAA-3′). The cDNA was amplified with 250 nM of each primer and 0.75 U *Taq* DNA polymerase in a 50-μl volume of buffer, as recommended by the supplier. The reaction mixture was heated to 94 C for 3 min, followed by 30 cycles of reannealing at 62 C for 1 min, elongation at 72 C for 1 min, and denaturation at 94 C for 1 min. The PCR products were separated by agarose gel electrophoresis and stained with ethidium bromide.

Hepatic VLDL production with Triton WR1339 (TG secretion rate)

On d 28 after saline or STZ treatment, SD (control) and STZ rats were food-deprived overnight. Each rat was injected in the tail vein at 250 mg/kg body weight with a 150 g/liter solution of Triton WR1339 (Sigma-Aldrich Corp.) in 9 g/liter NaCl. Blood samples of 100 μl were drawn before the Triton WR1339 injection and 45, 90, 135, 180, and 360 min later. The plasma TG concentration was measured in each sample as described below. The TG secretion rate was calculated from the increments in the plasma TG concentration per minute, multiplied by plasma volume (estimated as 4% of the body weight). The result was expressed as millimoles per hour per 100 g body weight (38).

Serum measurements

Blood samples were collected from the heart into plane glass tubes after 5 h of food deprivation. After clotting, samples were centrifuged, and serum was collected and stored at −80 C until assayed. Serum glucose, TG, and cholesterol levels were measured with kits (Daiichi Pure Chemicals, Tokyo, Japan). The high-density lipoprotein (HDL) cholesterol concentration was measured after precipitation of apoB-containing lipoprotein with dextran sulfate, phosphotungustate, and magnesium chloride. The VLDL/LDL cholesterol concentration was calculated by subtraction of HDL cholesterol from total cholesterol (TC). Serum insulin was measured with using a commercial kit (SCETI Co., Ltd., Tokyo, Japan). Postheparin plasma was collected 5 min after iv injection of 500 U heparin/kg. Plasma LPL activity was measured using a commercial kit (Progen Biotechnik, Heidelberg, Germany).

Scanning and statistical analysis

The results were scanned and analyzed using the Intelligent Quantifier System (Genomic Solutions, Ann Arbor, MI). Values are presented as the mean ± SD. Statistical analysis was performed using the Mann-Whitney *U* test, and the level of statistical significance was set at $P < 0.05$.

Results

General characteristics and lipid profile

The STZ injection caused a reduction in serum insulin levels that was accompanied by hyperglycemia (Fig. 1, A and B, and Table 1). Body weight gain over 4 wk was reduced in the diabetic rats (STZ rats) compared with the controls (SD rats; 392.5 ± 36.84 *vs.* 253.75 ± 29.73 g; $P < 0.05$). In STZ rats, serum TC and TG concentrations increased gradually, and a progressive rise with time was detected. The TC concentration was 2.5- and 4.2-fold higher than the control values on d 21 and 28, respectively ($P < 0.05$; Fig. 1C). Serum TG levels were also 6.7- and 12.7-fold higher than the controls on d 21 and 28 ($P < 0.05$), respectively (Fig. 1D). The serum VLDL/LDL cholesterol concentration was significantly increased by 5.2-fold on d 28 ($P < 0.05$; Table 1). Agarose-gel electrophoresis showed high concentrations of broad β-migrating lipoproteins in STZ rats on d 28, indicating that high TC and TG concentrations were due to the accumulation of remnant lipoproteins (VLDL remnant and chylomicron remnant). Lane C in Fig 1 shows a human plasma lipoprotein pattern with LDL-sized particles and HDL-sized particles. SD rats contained mainly HDL-sized particles on d 0 and 28, but STZ rats on d 28 contained mainly the remnant lipoproteins (broad β-migrating lipoproteins) that were usually detected in human type III dyslipidemia (Fig. 1E). Serum levels of T_3 in STZ rats showed decreases of 26% on d 28, but these were not statistically significant, and there was no elevation of TSH in STZ rats (Table 1). It was unlikely that severe hyperlipidemia on d 28 was due to hypothyroidism caused by a long-term diabetic state.

Expression of VLDL-R protein in heart and skeletal muscle

The expression of VLDL-R protein in heart tissue showed no change in the control rats, whereas in STZ rats it had decreased to 70% on d 14 and 21 and to 50% on d 28 (Fig. 2, A and C). The expression of VLDL-R protein in skeletal muscle in STZ rats had decreased to 60% on d 3, to 30% on d 14, and to 10% on d 21. On d 28, VLDL-R in skeletal muscle was less than 10% of that in the controls (Fig. 2, B and D). LDL-R protein levels in skeletal muscle showed no consistent changes in control or STZ rats (data not shown).

mRNA levels of VLDL-R in heart and skeletal muscle

In heart tissue, there was little change in the mRNA level of VLDL-R in either STZ or control rats during these periods (Fig. 3A). Also in skeletal muscle, the mRNA level of VLDL-R did not change during the study periods, but it increased on d 7 in control rats and decreased slightly on d 28 in STZ rats (Fig. 3B). It was intriguing that there was a dissociation between VLDL-R protein and mRNA expression in STZ rats. To obtain exact results for VLDL-R mRNA levels in heart and skeletal muscle, we also checked VLDL-R/GAPDH mRNA levels on d 28 by scanning the VLDL-R and GAPDH density. Figure 3C indicates that VLDL-R/GAPDH mRNA levels were decreased by 40% and 24% in heart and skeletal muscle, respectively, but we could not find a statistical significance in the difference between the two groups. Even though we could not clearly indicate the discrepancy between VLDL-R protein and mRNA in heart, it was obvious that the decreased VLDL-R protein level was not related to the VLDL-R mRNA level in skeletal muscle. In contrast, the splice isoform of the VLDL-R mRNA (20) examined by RT-PCR showed no significant difference in the expression of type 1 and type 2 VLDL-R mRNA in heart and skeletal muscle during the study periods (data not shown).

Hepatic TG production rate (TGPR) and postheparin plasma LPL activity

TGPRs were determined after Triton WR1339 injection, which prevents VLDL catabolism and thereby allows TGPR to be calculated. The TGPR during 3 h on d 28 showed no statistically significant difference between control and STZ rats (0.12 ± 0.03 *vs.* 0.10 ± 0.03 mmol/h/100 g body weight). TGPR over 6 h was greater in the controls than in STZ rats (0.13 ± 0.01 *vs.* 0.10 ± 0.02 mmol/h/100 g body weight), although this was not statistically significant (Fig. 4A). There was no difference in VLDL production by liver between the two groups, indicating that the hyperlipidemia in STZ rats on d 28 was not due to VLDL overproduction in liver. In contrast, heparin-releasable plasma LPL activity in STZ rats was reduced early on d 7 compared with the control level, even though there was no significant lipoprotein abnormality between SD and STZ rats (Fig. 1, C and D). On d 21 and 28, severe hyperlipidemia was observed in STZ rats, but the plasma LPL activity level was not changed during the study period (~50% of that in control rats; Fig. 4B). These data

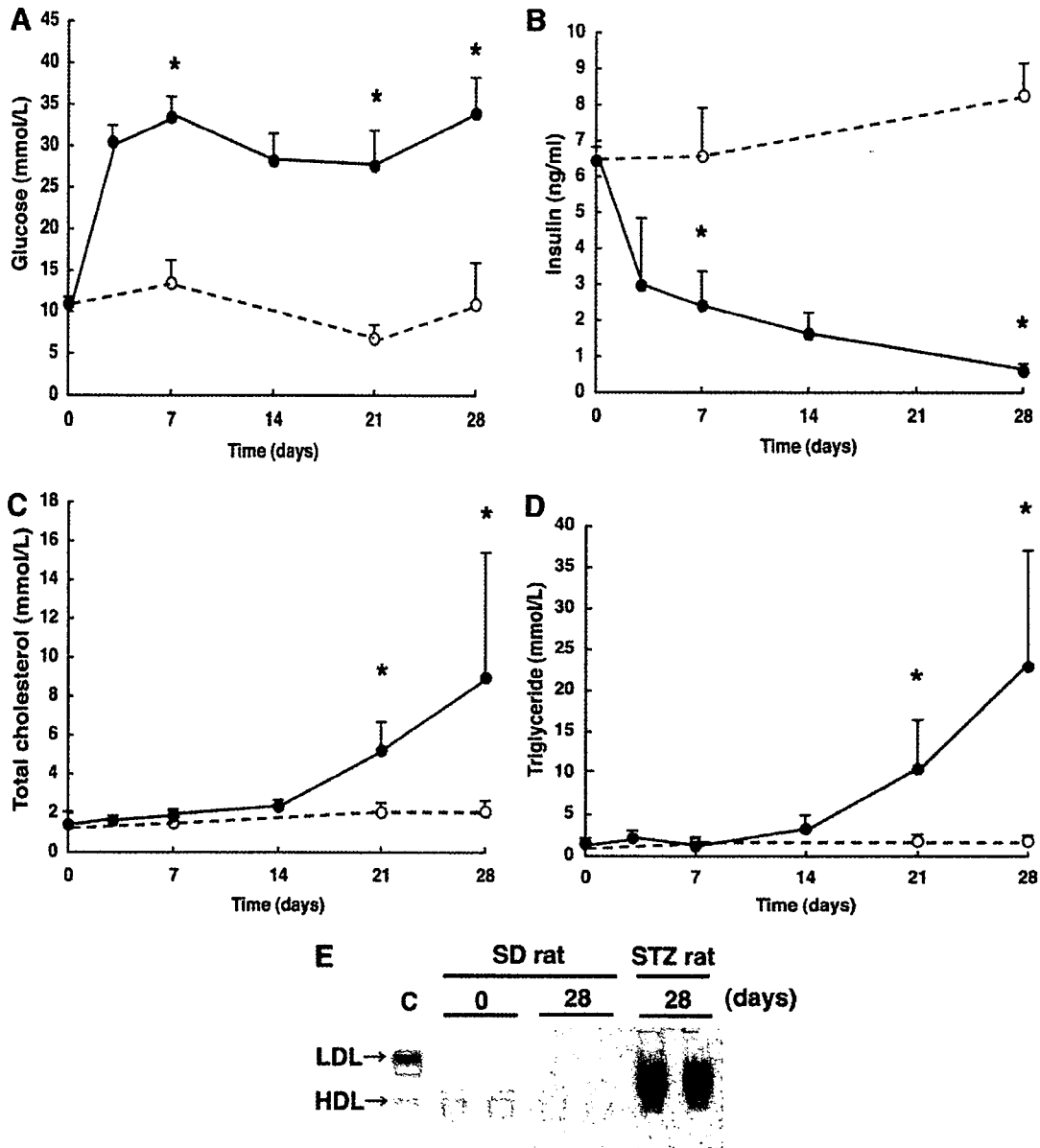

FIG. 1. Serum glucose (A), insulin (B), TC (C), and TG (D) levels in control (SD rats) and STZ rats. Results are presented as the mean ± SD of five SD (○) and eight STZ (●) rats at each time point. The lipoprotein pattern (E) was analyzed by agarose-gel electrophoresis. C indicated a human plasma lipoprotein pattern showing LDL-sized particles and HDL-sized particles. *, $P < 0.05$ compared with controls.

indicated that a modest insulin deficiency quickly induced low plasma LPL activity, and a severe insulin deficiency was necessary for a reduction of VLDL-R expression in heart and skeletal muscle in STZ rats.

VLDL-R expression in adipose tissue

VLDL-R is also expressed in adipose tissue. In STZ rats on d 28, the total mass of adipose tissue almost disappeared in heart, intestine, kidney, and testis (data not shown), indicating an absolute deficiency of VLDL-R protein in adipose tissue of STZ rats produced by a long-term diabetic state. Thus, we were not able to measure VLDL-R expression in adipose tissue.

LDL-R and LRP1 expression in liver

We also examined the expression of other lipoprotein receptors in liver, namely, LDL-R and LRP1, because LDL-R and LRP1 were hepatic remnant lipoprotein receptors. Neither showed any change in SD or STZ rats, and scanning analysis also indicated that insulin-deficient diabetes did not change hepatic LDL-R and LRP1 protein expressions (Fig. 5A).

Effect of insulin and IGF-I on expression of VLDL-R in rat myoblasts (L6 cells)

VLDL-R protein was decreased after deprivation of fetal calf serum for 24 h; treatment with insulin (10^{-6} M), but not

TABLE 1. Characteristics of rats with or without diabetes mellitus

Variables	SD rats	STZ rats
Body weight (g)	392.5 ± 36.84	253.75 ± 29.73[a]
Serum insulin (ng/ml)	8.28 ± 0.88	0.61 ± 0.19[a]
Serum glucose (mmol/liter)	10.96 ± 4.95	33.93 ± 4.30[a]
TC (mmol/liter)	2.12 ± 0.52	8.97 ± 6.45[a]
TG (mmol/liter)	1.79 ± 0.63	22.91 ± 14.13[a]
VLDL/LDL cholesterol (mmol/liter)	0.97 ± 0.17	5.01 ± 3.27[a]
HDL cholesterol (mmol/liter)	0.70 ± 0.07	1.16 ± 0.08[a]
FFA (mmol/liter)	0.81 ± 0.18	1.36 ± 0.39
T_3 (ng/dl)	78.31 ± 7.74	57.85 ± 17.58
TSH (ng/ml)	7.23 ± 2.60	6.35 ± 1.61

After a 5-h fast, blood was taken from SD (control; n = 5) and STZ rats (n = 8) on d 28. Values are presented as the mean ± SD. Statistical significance was analyzed by the Mann-Whitney *U* test.

[a] Significantly different from the controls, $P < 0.05$.

IGF-I (10 ng/ml), restored the VLDL-R proteins (Fig. 5B). Thus, VLDL-R expression was dependent on insulin even in a rat muscle cell line as well as in skeletal muscle in STZ rats.

Discussion

The results of this study raise the possibility that severe hyperlipidemia on d 21 and 28 in STZ rats may be caused in part by VLDL-R deficiency in skeletal muscle, heart, and adipose tissues in addition to decreased plasma LPL activity. We found that VLDL-R protein was greatly reduced in skeletal muscle (to <10%) and was reduced in heart (50%) on d 28. STZ rats also lost adipose tissue itself due to a long-term diabetic state; there was almost total disappearance of VLDL-R expression in adipose tissue on d 28. Reduction of VLDL-R protein was not associated with a reduction of VLDL-R mRNA, especially in skeletal muscle. In contrast, postheparin plasma LPL activity had been reduced at an early stage (on d 7) after STZ injection.

It has been reported that there are several mechanisms for insulin-deficient diabetic hyperlipidemia. Insulin deficiency stimulates lipolysis in adipose tissues, increasing the delivery of FFA from adipose tissues to liver and consequently also the production of TG in liver. Insulin deficiency also reduces plasma LPL activity. Rats injected with STZ showed a marked reduction of serum insulin, hyperglycemia, and hyperlipidemia. The phenotype in our STZ rats was consistent with the results previously described (4–14). The marked increase in serum cholesterol and TG levels in STZ rats was mainly due to elevation of remnant lipoproteins (broad β-migrating lipoproteins by agarose-gel electrophoresis). Accumulated remnant lipoproteins might be made up of chylomicron remnant and VLDL remnant lipoproteins that were specific ligands for the VLDL-R (21, 31). Because there was no difference in the hepatic TGPR between the two groups of rats in our experiments, the remnant lipoproteins in STZ rats were not due to overproduction of VLDL by liver. In contrast, LDL-R and LRP1 expression in liver did not change in STZ rats compared with that in controls. Swami and colleagues (39) reported that hepatic LDL-R levels were unaffected by diabetes using STZ rats. These data indicated

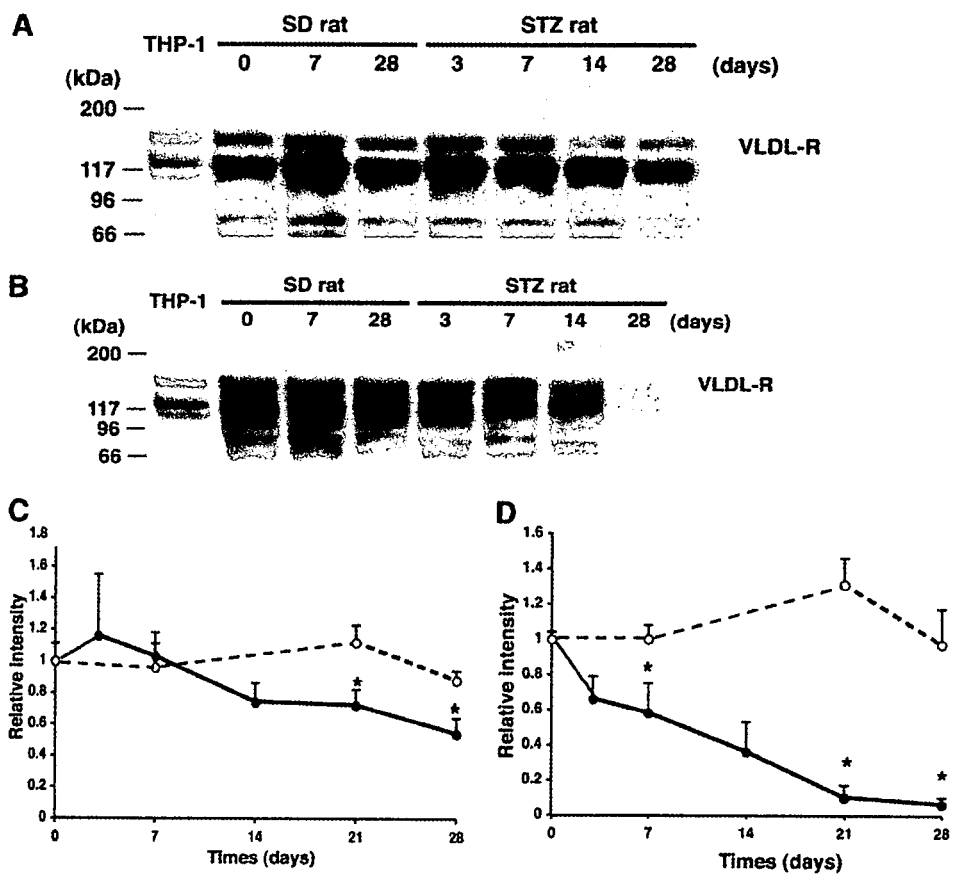

FIG. 2. Western blots analysis of VLDL-R protein of heart (A) and skeletal muscle (B) in representative SD (control) and STZ rats. Total membrane protein (30 μg/lane) from tissues was prepared as described in *Materials and Methods* and subjected to SDS-PAGE. Protein was transferred to a nylon membrane, and VLDL-R protein was detected using polyclonal antibody VR2. Similar results were observed in each of six pairs of rats. The intensities of signals in heart (C) and skeletal muscle (D) were quantified by densitometric scanning (○, SD rats; ●, STZ rats). *, $P < 0.05$ compared with controls.

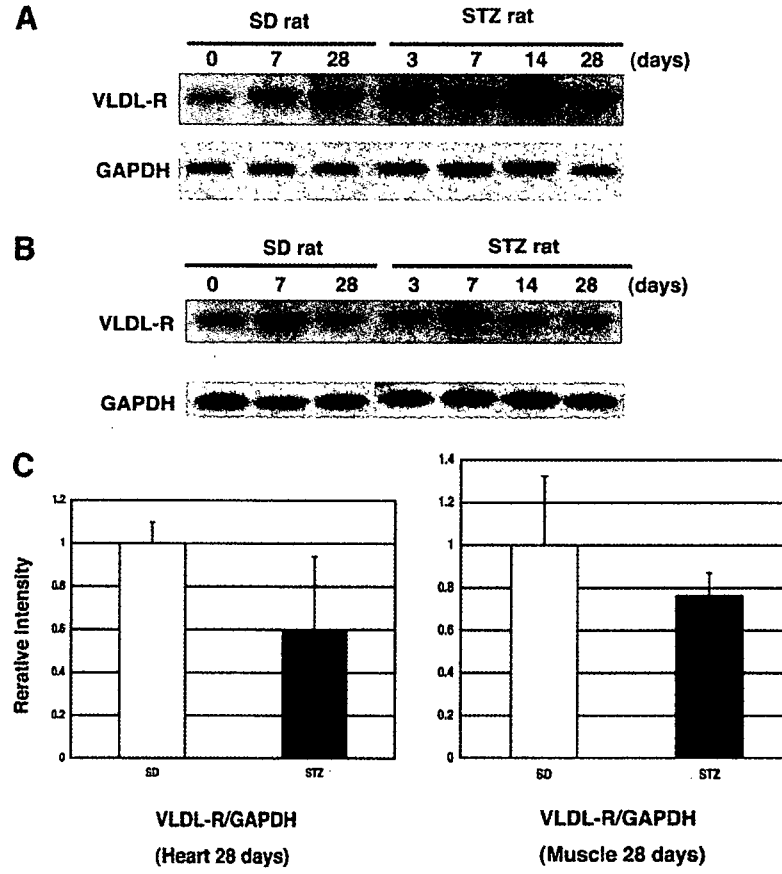

FIG. 3. Northern blot analysis of VLDL-R mRNA in heart (A) and skeletal muscle (B) in representative SD (control) and STZ rats. Total RNA (15 μg/lane) was isolated from tissues as described in *Materials and Methods* and was separated by electrophoresis on 1.0% agarose/9% formaldehyde gel. RNA was transferred to a nylon membrane and hybridized with ^{32}P-labeled 0.5 kb cDNA to rat VLDL-R mRNA and 1.3 kb DNA to rat GAPDH mRNA. The intensities of signals in heart and skeletal muscle on d 28 were quantified by densitometric scanning as VLDL-R/GAPDH mRNA (C). □, SD rats; ■, STZ rats.

that LDL-R and LRP1 (hepatic lipoprotein receptors for remnant lipoproteins) were not responsible for the accumulated remnant lipoproteins in STZ rats.

Our data indicated that severe hyperlipidemia in STZ rats might be due to profoundly decreased VLDL-R protein in skeletal muscle, almost complete loss in adipose tissues, and a decrease to a lesser extent in heart tissue. VLDL-R is normally expressed abundantly in heart, skeletal muscle, and adipose tissue and has a role in the binding and uptake of remnant lipoproteins, such as the intermediate density lipoprotein and chylomicron remnant, in concert with LPL (31). Although VLDL-R knockout mice showed no obvious lipoprotein abnormality, they were resistant to the development of obesity after a high-fat diet or on an *ob/ob* background (40, 41). Furthermore, it has been reported that the metabolism of VLDL-TG was impaired in VLDL-R/LDL-R double-knockout mice, and a long-term fasting state (16 h) in VLDL-R knockout mice produced high plasma TG levels compared with those in wild-mice (42). Goudriaan *et al.* (43) also demonstrated a major role of the VLDL-R in postprandial lipoproteins by enhancing LPL-mediated TG hydrolysis, rather than by mediating FFA uptake. These results indicate that the VLDL-R plays an important role in VLDL-TG metabolism in heart, skeletal muscle, and adipose tissue under conditions of severe stress (fasting or a high-fat diet) or on an LDL-R deficient or *ob/ob* background. It is reasonable that an insulin-deficient state also occurs under conditions of stress, and the experiment in STZ rats gave us a chance to determine the function of the VLDL-R in rats. For the mechanism of insulin-deficient diabetic hyperlipidemia, Chen and colleagues (5) pointed out that hypertriglyceridemia in STZ rats was not due to VLDL overproduction in liver, and a VLDL-TG removal defect associated with insulin deficiency may not be explained simply by the decrease in muscle and adipose tissue LPL activities. In our study, TC and TG concentrations on d 7 were not elevated, even though there was a significant reduction of postheparin plasma LPL activity (~50%) on d 7 in STZ rats. We suggest that the deficiency of VLDL-R protein in heart, skeletal muscle, and adipose tissue might be a crucial role of severe hyperlipidemia in STZ rats in concert with the reduced plasma LPL activity, because LPL accelerated the binding of TG-rich lipoproteins to the VLDL-R. VLDL binding to the VLDL-R is weak, but LPL-treated VLDL is recognized by the VLDL-R with high affinity (21). Reduced plasma LPL activity was also one of the necessary conditions for severe hyperlipidemia in STZ rats on d 21 and 28 in our study. Yagyu *et al.* (44) indicated that the disruption of VLDL-R resulted in hypertriglyceridemia associated with decreased LPL activity in mice. It is likely that remnant lipoprotein particles that could not be taken up by the VLDL-R in skeletal muscle, adipose tissue, and heart accumulated in plasma of STZ rats. Furthermore, in rat myoblasts, VLDL-R expression was insulin dependent. We think that both plasma LPL activity and VLDL-R expression are insulin dependent, but the former is more sensitive to insulin deficiency. Because postheparin plasma LPL activities did not fully reflect specific tissue LPL activity, such as adipose tissue, which probably declined progressively between 7 and

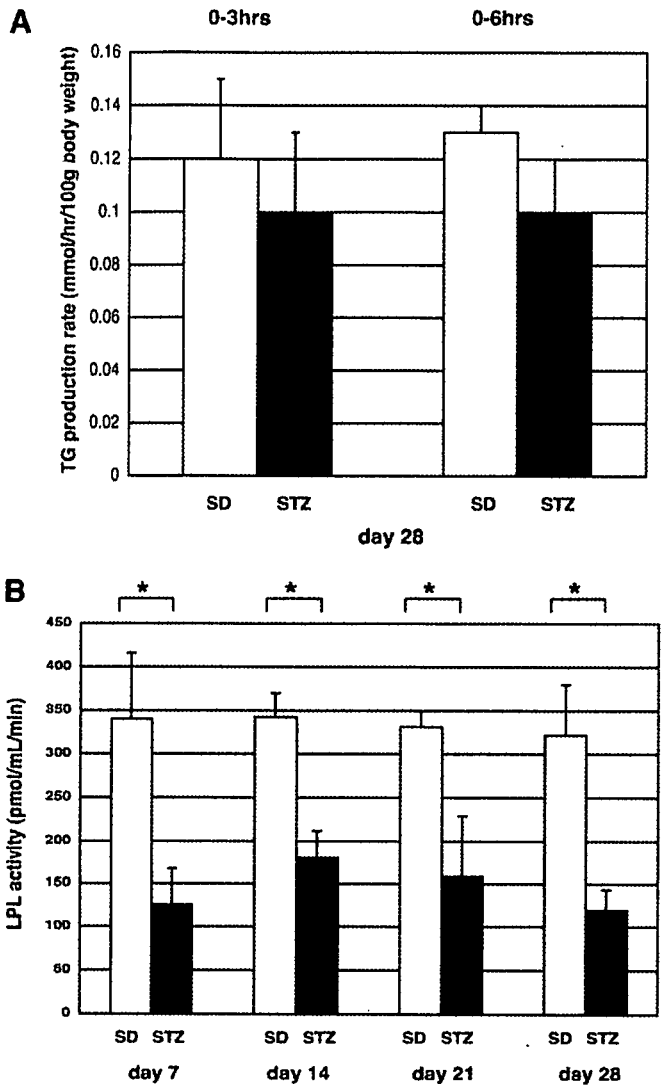

FIG. 4. A, Plasma TGPR after Triton WR1339 injection. SD (control; n = 4) and STZ (n = 3) rats were injected on d 28 with Triton WR1339 (250 mg/kg body weight) and followed for 6 h. The TGPR was calculated as millimoles per hour per 100 g body weight. □, SD rats; ■, STZ rats. B, Postheparin plasma LPL activity in SD (control) and STZ rats. Postheparin plasma was collected 5 min after iv injection of 500 U heparin/kg. Plasma LPL activity was determined by a commercial kit as described in *Materials and Methods*. □, SD rats; ■, STZ rats. *, Significantly different from the controls, $P < 0.05$.

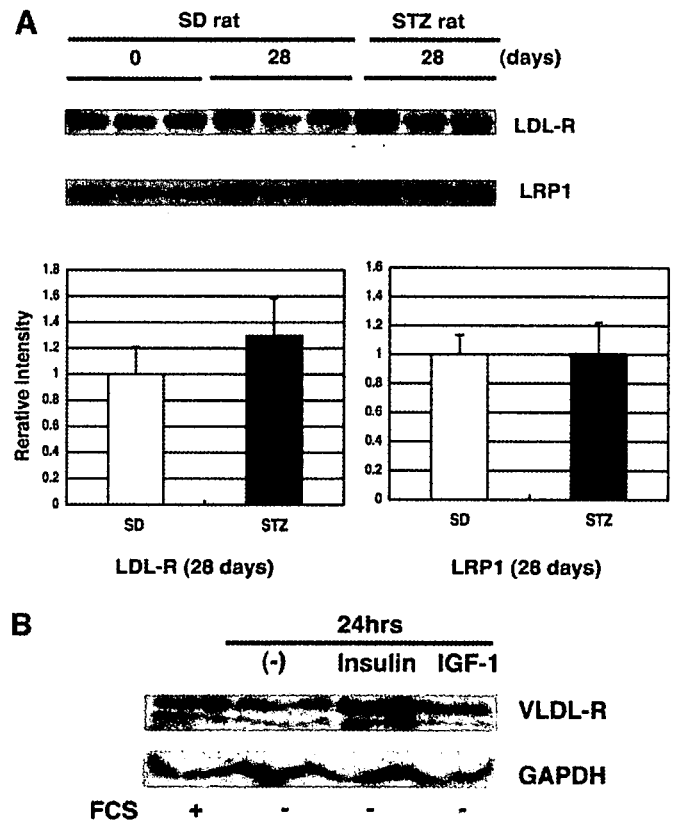

FIG. 5. A, LDL-R and LRP1 protein expression in liver from representative SD (control) and STZ rats by Western blot analysis. LDL-R and LRP1 proteins (30 μg/lane) were detected using specific antibodies IgG 4A4 and IgG 11H4, respectively. The intensities of signals in LDL-R and LRP1 on d 28 were quantified by densitometric scanning. □, SD rats; ■, STZ rats. B, Effects of insulin and IGF-I on the VLDL-R proteins in rat myoblasts (L6 cells). The effects of insulin (10^{-6} M) and IGF-I (10 ng/ml) without fetal calf serum were examined for 24 h. VLDL receptor and GAPDH proteins (30 μg/lane) were detected using specific antibody VR2 and anti-GAPDH antibody, respectively.

28 d in STZ rats, we were not able to neglect the specific tissue LPL activity for hyperlipidemia in insulin-deficient diabetes. We believe that the tissue LPL activity, more so than plasma LPL activity, may be an important factor for the VLDL-R binding of TG-rich lipoproteins in extrahepatic tissues; more detailed experiments will be needed. FA transporters were also candidates for hyperlipidemia in STZ rats, because decreased LPL activity might influence FA transporters or simple diffusion-mediated FFA uptake. However, the decreased plasma LPL-mediated hyperlipidemia by FA transporters or diffusion did not explain the accumulation of remnant lipoproteins in STZ rats. The TGPR by the liver was not increased in STZ rats. Reaven and colleagues (4, 5) also indicated no change or decreased VLDL secretion from liver on d 7 in STZ rats. FFAs may be metabolized into lipoprotein as VLDL, and consequently, VLDL production from liver might be increased in STZ rats if FFAs are responsible for hyperlipidemia.

In addition to plasma LPL activity, VLDL-R expression, tissue-specific LPL and FA transporters, intestinal ACAT-2 (10–12) and MTP (13), also might be proteins responsible for insulin-deficient diabetic hyperlipidemia. However, it is unlikely that those two proteins are involved in the severe hyperlipidemia seen on d 21 and 28 in our study, because ACAT-2 and MTP are increased within 14 d, like the reduced plasma LPL activity. For ABCG5/ABCG8, researchers measured the reduced intestinal and hepatic ABCG5/ABCG8 expression and increased plasma plant cholesterols only 28 d after STZ injection (14). At this time we are not able to exclude ABCG5/ABCG8 as a cause of the severe hyperlipidemia on d 21 and 28, but we do not believe that the accumulated remnant lipoproteins in STZ rats are composed of plant cholesterols.

VLDL-R proteins in skeletal muscle and heart tissue were reduced in rats with chronic renal insufficiency and puro-

mycin-induced nephrotic syndrome due to decreased VLDL-R mRNA levels (33, 34). Both LPL activity and VLDL-R expression in skeletal muscle and adipose tissue were decreased in Imai rats with spontaneous focal glomerulosclerosis (35). In the present study, light and electron microscopic examinations did not show the pathological findings of diabetic nephropathy in the kidneys of STZ rats on d 28 (data not shown), indicating the reduced VLDL-R expression in STZ rats might be due to an insulin-deficient state, not to renal insufficiency. It has also been reported that VLDL-R protein expression in skeletal muscle, but not in heart and adipose tissue, was reduced by thyroidectomy in rats, which was reversed by the administration of excess thyroid hormone (32). This result suggests that VLDL-R expression in muscle is strongly influenced by the thyroid status of the animal. In the present study, hypothyroidism in STZ rats was not observed, thereby excluding it as a major cause of STZ-induced reduction of VLDL-R protein. The reduction of VLDL-R protein in STZ rats was not accompanied by a reduction of VLDL-R mRNA, especially in skeletal muscle. This suggests that the reduction of VLDL-R protein cannot simply be explained by the decreased VLDL-R transcription. This phenomenon may be due to a decreased VLDL-R translation or an increased VLDL-R protein degradation in addition to the modestly decreased VLDL-R transcription.

It has been recently reported that the ligand-binding activity of the VLDL-R is inhibited by protein kinase C-dependent phosphorylation with phorbol 12-myristate 13-acetate in human monocyte-derived THP-1 cells, human endothelial cells, and human vascular smooth muscle cells (45). Hyperglycemic conditions such as diabetes mellitus, which induce protein kinase C activation, resulting in VLDL-R phosphorylation and loss of ligand-binding activity, may impair the uptake of TG-rich lipoproteins in cells. We also examined the VLDL-R-binding activity in a ligand blotting study using RAP, and we observed no changes in RAP-binding activity in heart or skeletal muscle membranes from STZ rats (data not shown).

In conclusion, the severe elevation of serum cholesterol and TG concentrations (remnant lipoproteins) in STZ-induced diabetic rats on d 21 and 28 was accompanied by a deficiency of VLDL-R protein in heart, skeletal muscle, and adipose tissues in cooperation with reduced postheparin plasma LPL activity. This suggests that VLDL-R deficiency may be one of the factors producing the impaired VLDL catabolism in insulin-deficient diabetes. The precise mechanism leading to the dissociation between VLDL-R protein and mRNA in STZ rats is uncertain, and it needs to be determined whether insulin therapy could recover the reduced VLDL-R protein and hyperlipidemia *in vivo*. Additional investigation is required in the future.

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