Insulin-secreting \(\beta\)-Cell Dysfunction Induced by Human Lipoproteins*

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Diabetes is associated with significant changes in plasma concentrations of lipoproteins. We tested the hypothesis that lipoproteins modulate the function and survival of insulin-secreting cells. We first detected the presence of several receptors that participate in the binding and processing of plasma lipoproteins and confirmed the internalization of fluorescent low density lipoprotein (LDL) and high density lipoprotein (HDL) particles in insulin-secreting \(\beta\)-cells. Purified human very low density lipoprotein (VLDL) and LDL particles reduced insulin mRNA levels and \(\beta\)-cell proliferation and induced a dose-dependent increase in the rate of apoptosis. In mice lacking the LDL receptor, islets showed a dramatic decrease in LDL uptake and were partially resistant to apoptosis caused by LDL. VLDL-induced apoptosis of \(\beta\)-cells involved caspase-3 cleavage and reduction in the levels of the c-Jun N-terminal kinase-interacting protein-1. In contrast, the proapoptotic signaling of lipoproteins was antagonized by HDL particles or by a small peptide inhibitor of c-Jun N-terminal kinase. The protective effects of HDL were mediated, in part, by inhibition of caspase-3 cleavage and activation of Akt/protein kinase B. In conclusion, human lipoproteins are critical regulators of \(\beta\)-cell survival and may therefore contribute to the \(\beta\)-cell dysfunction observed during the development of type 2 diabetes.

Abnormalities in both glucose and lipid homeostasis are involved in the pathogenesis of type 2 diabetes (1). The lipid phenotype observed in diabetic patients is characterized by low levels of HDL\textsuperscript{1} cholesterol, moderate elevations in triglyceride-rich remnant particles, borderline to high LDL cholesterol, and an increase in plasma free fatty acids (2). Chronically elevated concentrations of free fatty acids have been shown to induce a state of insulin resistance and to alter \(\beta\)-cell function. The susceptibility of these cells to apoptosis is enhanced, and their insulin content and glucose-stimulated insulin release are significantly reduced (3, 4). Overt type 2 diabetes is present when the \(\beta\)-cell mass cannot compensate for insulin resistance. In Zucker diabetic fatty rats, free fatty acids are elevated before the onset of diabetes, leading to triglyceride accumulation in the islets and increased \(\beta\)-cell death (5, 6).

Although the contribution of free fatty acids to the development of diabetes has been studied extensively, the role of lipoproteins in \(\beta\)-cell function is only partially documented. Grupping et al. (7) described the presence of LDL binding sites in rodent and human pancreatic \(\beta\)-cells. More recently, Cnop et al. (8) demonstrated the uptake of LDL and VLDL in lipid-storing vesicles of rat and human \(\beta\)-cells. Given the key role of lipid homeostasis in \(\beta\)-cell function and survival, we hypothesized that lipoproteins modify the susceptibility of \(\beta\)-cells to apoptosis. Here we describe the presence of functional lipoprotein receptors in mouse pancreatic islets and in a transformed insulin-secreting \(\beta\)-cell line. We demonstrate that human purified VLDL and LDL induce a dose-dependent increase in the rate of apoptosis and a decrease in the levels of insulin transcript. In contrast, HDL efficiently antagonizes cell death by mechanisms that include activation of protein kinase B (Akt/ PKB) and inhibition of caspase-3 cleavage. Finally, we establish that the LDL receptor (LDLR) and the c-Jun N-terminal kinase (JNK) signaling pathway play an important role in apoptosis induced by lipoproteins. These results demonstrate how the modifications in lipoproteins observed in type 2 diabetes could contribute to the pathogenesis and progression of \(\beta\)-cell failure.

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\textsuperscript{1} The abbreviations used are: HDL, high density lipoprotein; Akt/ PKB, protein kinase B; apoE2R, apolipoprotein E receptor 2; BrdUrd, bromodeoxyuridine; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl coenzyme A reductase; I-BJFIP-1, islet-brain 1/c-Jun N-terminal kinase-interacting protein-1; JNK, c-Jun N-terminal kinase; LDL, low density lipoprotein; LDLR, low density lipoprotein receptor; LDLR\textsuperscript{Δ/−}, LDL receptor knock-out; LRP, LDL receptor-related protein; PBS, phosphate-buffered saline; SIR, scavenger receptor class B type I; TUNEL, terminal nucleotidyl transferase-mediated UTP nick end labeling; VLDL, very low density lipoprotein; VLDLR, very low density lipoprotein receptor; WT, wild-type.
EXPERIMENTAL PROCEDURES

Lipoprotein Preparation—Blood was collected from healthy donors. Plasma VLDL, LDL, and HDL fractions were isolated by sequential ultracentrifugation (VLDL: LDL density, 1.019-1.006 g/mL; density, 1.21), dialyzed against PBS, and then filter sterilized using a 0.22-μm microfilter. Protein and cholesterol concentrations were measured using the Bio-Rad protein assay and the cholesterol CHOD-PAP method (Roche Diagnostics). Samples were analyzed by SDS-PAGE to assess the integrity of the apolipoproteins and the purity of the different lipoprotein preparations. The fractions contained less than 0.112 units of endotoxin/μg of cholesterol as determined by the kinetic chromogenic technique (Endotell, Allschwil, Switzerland). Cholesterol BODIPY-human HDL3 was reconstituted as described elsewhere (9).

Cell Culture and Pancreatic Islet Isolation—The insulin-secreting cell line βTC3 (cells over 120 passages) was cultured in RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml streptomycin, 100 units/ml penicillin, and 2 mM glutamine (Invitrogen). Both 11.1 mM and 5 mM glucose concentrations were used. Interleukin-1β (Alexis, Carlsbad, CA) was used at a concentration of 0.3 ng/ml, tumor necrosis factor-α (Alexis) at a concentration of 0.3 ng/ml, and interferon-γ (R&D Systems) at a concentration of 15 ng/ml. Lipoproteins were used at the following cholesterol concentrations: VLDL, 1.6 mM, 3.1 mM, 6.2 mM; LDL, 1.6 mM, 3.1 mM, 6.2 mM; HDL, 1.0 mM. Cell-permeable JNK inhibitor 1 (t-serestiosemer; Alexis) was added at a concentration of 1.5 μM 30 min before the addition of lipoproteins and again after 18 and 36 h.

Islets were isolated from C57BL/6 wild-type (WT) mice and mice lacking the LDL receptor (LDLr−/−) (ages 2–3 months; Jackson Laboratory ME) by the method of the Liberase RI Purified Enzyme Blend (Roche Diagnostics) according to the manufacturer’s protocol. Islets were hand-picked to purity and cultured in βTC3 culture medium supplemented with 1% M HEPES, pH 7.4, and 0.1% 50 mM β-mercaptoethanol (Invitrogen).

RNA Extraction, Reverse Transcription PCR, and Quantitative Reverse Transcription PCR—Total RNA was extracted from βTC3 cells using the RNAqueous-4PCR kit (Ambion, Houston, TX) according to the manufacturer’s protocol and treated with DNase to remove residual contaminations with DNA. RNA was reverse transcribed and cDNA amplified by PCR using Superscript II reverse transcriptase, Taq polymerase, and oligo(dT) primers (Invitrogen). The primers used for the VLDL receptor (VLDLR) were 5′-CAG TGG CTG TCC CTC ACT CGG-3′ and 5′-ACT TAC AGT GAC AAG AAA G-3′. For the LDLr, the primer sequences were 5′-CAG ATA CGG CGA ATA GA-3′ and 5′-TGG CTT CTT CAA AC-3′. Insulin primer sequences were 5′-GCC CCT TGG TGG TGA TGA-3′ and 5′-CAT GTT CAA GTT CCA TTT-3′. Scavenger receptor class B type 1 (SR-BI) primer sequences were 5′-GGA GAG TGG TGG TGA TGA-3′ and 5′-TCT GGT TGC TGC TGC TC-3′. 3-Hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoA reductase) primer sequences were 5′-TGG CCA ACC CCT ACC TCA-3′ and 5′-CAT CAA CCA GAG CAC ACC AG-3′. Tubulin primer sequences were 5′-GAA GGA TGC TGG CAA TAA CT-3′ and 5′-GGT GGT GAG GAT GGA ATT GT-3′. Glycereraldehyde-3-phosphate dehydrogenase primer sequences were 5′-TCA AGA ACG TGG TGA AGC AG-3′ and 5′-AAG GTG GAG TGG TAC GT-3′. Insulin primer sequences were 5′-TGG CTT CTT CTT CTA CAC ACC AC-3′ and 5′-TCT AGT TGC AAG TGC ATG TCT CCA-3′. Cycin B1 primer sequences were 5′-AGG CAA GAA GAG CGA AG-3′ and 5′-GAT CTT GAA GAG TGT CAA GAT CAG G-3′. After amplification (activation at 95 °C for 5 min, denaturing at 95 °C for 0.5 min, primer annealing at 51 °C for 0.5 min, and chain extension at 72 °C for 0.5 min) through 32 cycles, the whole volume of the PCR was separated by electrophoresis in a 3% agarose gel.

We performed quantitative real-time PCR using the LightCycler Instrument (Roche Diagnostics) and the QuantiTect SYBR Green PCR kit (Qiagen). For HMGC-oA reductase quantification, cells were starved for 12 h and then incubated in the presence or absence of 3.0 mM LDL cholesterol for 15 h. For insulin and cycin B1 quantification, cells were incubated for 48 h with different lipoprotein fractions. Total RNA was extracted and reverse transcribed as described above. The cDNA was subsequently amplified with the appropriate primers (initial activation at 95 °C for 15 min, denaturing at 95 °C for 15 s, primer annealing at 51 °C for 20 s, and chain extension at 72 °C for 18 s) through 40 cycles. Data were analyzed with the LightCycler Software (Roche Diagnostics). Tubulin and glycereraldehyde-3-phosphate dehydrogenase were used for normalization.

Western Blotting—Cells were washed once in cold PBS and lysed in Promega passive lysis buffer (Promega, Madison, WI). A total of 40 μg of proteins was subjected to SDS-PAGE and transferred onto a nitrocellulose membrane by electroblotting. Membranes were blocked by incubation for 1 h in a buffer containing 0.1% Tween 20 and 5% milk and incubated overnight at 4 °C with the primary antibodies. The immune-reactive bands were visualized using a chemiluminescent substrate (ECL) after incubation of the nitrocellulose filters for 1 h with secondary anti-rabbit or anti-mouse antibodies conjugated to horseradish peroxidase. Densitometric scanning of films was performed using Quantity One software (Bio-Rad).

Antisera directed against SR-BI, LDLR, LRP, VLDR, and apoER2 have been described previously (10–14). The following antibodies were purchased commercially: anti-cleaved caspase (Asp-175; Cell Signaling Technology, Beverly, MA), anti-JIP-1 (BD Biosciences, Basel, Switzerland), and anti-α-tubulin (clone B-5-1-2; Sigma). Antisera were used at dilutions ranging from 1:250 to 1:1,000.

Immunohistochemistry—Murine pancreases were fixed in a solution containing 1% paraformaldehyde and subsequently 30% sucrose. 12 μm sections were prepared and blocked for 1 h in a solution containing 1% bovine serum albumin and 0.3% Triton X-100 (Sigma). Incubation with the primary antisera (dilutions 1:250) was performed at 4 °C for 24 h. After several washes in PBS, sections were incubated for 30 min with secondary Alexa 488-conjugated anti-rabbit IgG (Molecular Probes, Eugene, OR) at a dilution of 1:400. Slices were analyzed with a confocal microscope (Leica Microsystems, Glattbrugg, Switzerland).

Lipoprotein Uptake—βTC3 cells and mouse islets were seeded onto coverslips, starved for 4 h, and incubated with cholesteryl BODIPY-human HDL3, FL LDL (10 μg/ml medium; Molecular Probes) in the presence or absence of excess unlabeled HDL1 (100 μg/ml medium). After incubation for 2 h at 37 °C, cells were fixed and analyzed by light (for βTC3 cells) or confocal (for islets) microscopy. Microscope settings were kept strictly identical between different conditions. Similar experiments were performed with cholesteryl BODIPY-human HDL3 (30 μg/ml medium) in the presence or absence of excess unlabeled HDL3 (600 μg/ml medium).

Hoechst 33342 Staining, Immunocytochemistry, and TUNEL—βTC3 cells were grown on laminin/poly-L-lysine-coated coverslips. After 2 days, the culture medium was changed, and cytokines and/or lipoproteins were added. Incubations were performed for 48 h. Cells were then fixed for 15 min with 1% paraformaldehyde in PBS. To evaluate the number of apoptotic cells, cells were incubated in the presence of Hoechst 33342 (dilution 1:1,500; Molecular Probes) for 3.5 min, and the nuclear morphology was analyzed under a fluorescence microscope. The number of cells displaying a pycnotic (highly condensed) nucleus and/or a fragmented nucleus was evaluated blind by two different investigators. A minimum of 250 cells in four separate experiments was counted for each condition. To evaluate the number of nucleic acid, fixed cells were stained for 7 min with 1 μg SYTOX Green (Molecular Probes). For immunocytochemistry, 10 views of each condition were captured at high magnification with a confocal microscope, and three-dimensional images were generated using the Imaris software (Silicon Graphics, Inc., Mountain View, CA).

For immunocytochemistry, fixed cells were incubated for 1 h in blocking buffer (1% bovine serum albumin, 0.3% Triton X-100) and overnight at 4 °C with the primary antisera (dilutions: anti-SR-BI, anti-LDLR, anti-LRP, anti-apoER2, 1:250; anti-cleaved caspase-3, 1:40). After four washes in PBS, coverslips were incubated for 30 min with secondary Alexa 488-conjugated anti-rabbit IgG at a dilution of 1:400. For cleaved caspase-3, cells were counterstained for 3 min with Hoechst 33342 (dilution 1:1,500), and views were taken under a fluorescence microscope (Abode Systems). For SR-BI, LDLR, VLDLR, and apoER2, views were taken with a confocal microscope. For TUNEL, fixed cells were incubated for 2 min at 4 °C in permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate). Coverslips were then rinsed, and 50 μl of TUNEL reaction mixture (Roche Diagnostics) was added. Coverslips were incubated in a humidified chamber for 60 min at 37 °C and counterstained for 3 min with Hoechst 33342.

Quantification of Apoptosis in Islets—Mouse islets were incubated for 48 h in the presence of lipoproteins, fixed with 1% paraformaldehyde in PBS, and stained for 7 min with 1 μg SYTOX Green. For each islet, 10–15 views spanning the entire islet were captured at high magnification, and 2 μm were taken with a confocal microscope. The numbers of healthy and apoptotic nuclei were then evaluated using the Neurolucida software (MicroBrightField, Inc., Williston, VT).

In Situ Cell Proliferation—βTC3 cells were seeded onto coverslips...
and incubated for 48 h in the absence of serum and/or in the presence of lipoproteins. BrdUrd (Roche Diagnostics) was added to the cells for 1 h at a final concentration of 10 μM. Cells were then fixed in 1% paraformaldehyde in PBS for 15 min and incubated for 1 h with 2 N HCl. Immunostaining with anti-BrdUrd (clone IIB5; Monosan, Uden, The Netherlands) diluted 1:100 and counterstaining with Hoechst 33342 were performed as described above. Counts were made by scoring the number of cells positive for BrdUrd incorporation.

**Akt/PKB Assay**—Akt activity was determined using a kit from Cell Signaling Technology. βTC3 cells were starved for 15 h and incubated with HDL at a concentration of 1.0 mM for 5 min, 20 min, and 60 min. Akt was immunoprecipitated from 200 μg of total protein equivalent of cell lysate with anti-Akt antibody linked to agarose beads overnight at 4 °C. Immunoprecipitates were washed and resuspended in kinase buffer supplemented with 200 μM ATP and 1 μg of glycogen synthase kinase-3 fusion protein. After 30 min at 30 °C the reaction was stopped, and the extent of glycogen synthase kinase-3 phosphorylation was analyzed by Western blotting with a specific anti-phosphoglycogen synthase kinase-3 antibody.

**Statistical Analysis**—Results are presented as means ± S.E. Unpaired two-tailed Student’s t test was used to compare groups.

**RESULTS**

**Lipoprotein Receptor Expression in Insulin-secreting Cells**—We first evaluated whether the genes encoding several receptors implicated in the binding and the internalization of lipoproteins islets and βTC3 cells. Mice pancreatic sections were stained with hematoxylin and eosin (A) and incubated with antisera raised against SR-BI (B), LDLR (C), LRP (D), VLDLR (E), and apoER2 (F). Total cell lysates from βTC3 cells were separated on SDS-PAGE, transferred to nitrocellulose membranes, and incubated with the same antibodies (G). Arrowheads indicate expected apparent molecular masses.

**Lipoprotein-induced β-Cell Dysfunction**

![Fig. 1. Lipoprotein receptors are present in pancreatic islets and βTC3 cells.](image)

![Fig. 2. Assessment of lipoprotein uptake and HMG-CoA reductase expression.](image)
lipoproteins were expressed in the insulin-secreting cell line βTC3. Using reverse transcription PCR, we detected the transcripts for LDLR and three other members of the LDLR family: LRP, VLDLR, and apoER2. We also detected the transcript for SR-BI, which mediates cholesterol uptake from HDL particles (data not shown). Immunohistochemical analysis of mouse pancreatic sections, using specific antibodies for these receptors, revealed a strong labeling in islets (Fig. 1, A–F). Similar data were obtained in βTC3 cells (data not shown). Western blot analysis of protein extracts from βTC3 cells revealed bands of the expected molecular masses for these receptors (Fig. 1G) (10–13). Note that the antiserum directed against LRP recognizes two bands at ~600 and 85 kDa (12).

Uptake of LDL and HDL Particles in βTC3 Cells and Murine Pancreatic Islets and Effects on HMG-CoA Reductase Transcription—We next examined the uptake and storage of LDL and HDL particles in βTC3 cells and in freshly isolated murine pancreatic islets. After incubation for 2 h with fluorescent HDL or LDL, βTC3 cells accumulated high quantities of labeled lipids (Fig. 2, A and C). Fluorescence was reduced markedly when cells were incubated with an excess of nonfluorescent LDL or HDL particles (Fig. 2, B and D), assessing the specificity of the uptake. We performed similar experiments on murine pancreatic islets isolated from WT and LDLR-deficient (LDLR−/−) mice. Islets from WT animals displayed an intense cytoplasmic signal when incubated with fluorescent LDL (Fig. 2E), whereas islets from LDLR−/− mice showed only a faint signal (Fig. 2F). The effect of LDL particles on HMG-CoA reductase expression was also investigated. As shown in Fig. 2G, incubation of βTC3 cells with LDL reduced HMG-CoA reductase mRNA by 83% (p < 0.01). Taken together, these data indicate that insulin-secreting cells express functional LDL receptors.

Assessment of Apoptosis in β-Cells Exposed to Lipoproteins and Effects on Insulin Transcript—Apoptosis is characterized by morphological changes that include chromatin condensation, cellular shrinkage, membrane blebbing, and the formation of apoptotic bodies. Changes in nuclear morphology are recognized as among the most reliable criteria to assess apoptosis. Cells were stained either with Hoechst 33342 or SYTOX Green, and the rate of apoptosis was evaluated by counting the number of cells displaying a pycnotic (highly condensed) and/or a fragmented nucleus (Fig. 3A). Both dyes provide the same information, but SYTOX Green has the advantage of being more stable than Hoechst and easily detected at 488 nm with a standard confocal microscope. We studied in detail the morphological changes that take place in the nuclei of β-cells undergoing apoptosis. Three-dimensional images were generated showing the differing morphologies of a normal nucleus (Fig. 3D), an apoptotic nucleus (Fig. 3E), and a nucleus in early mitosis (Fig. 3F). To assess apoptosis in primary islets accurately, we developed a novel experimental approach that allowed us to scan the three-dimensional islet structure and therefore did not require cell dissociation. Fig. 4 shows the appearance of an islet incubated in normal medium (Fig. 4, A and B) and after 2 days treatment with 3.1 mM VLDL (Fig. 4, C and D), 6.2 mM LDL (Fig. 4, E and F), or 1.0 mM HDL (Fig. 4, G and H). Apoptotic nuclei appear highly fluorescent at low magnification and display characteristic features at higher magnification (arrows, Fig. 4). To confirm the data obtained by morphological studies, we performed a TUNEL assay (Fig. 3C) and immunocytochemistry for cleaved caspase-3 (Fig. 3B). All
cells with an altered nuclear morphology assessed by Hoechst staining were positive for TUNEL. In contrast, cleaved caspase-3 was detected only in apoptotic cells treated with cytokines or VLDL, but not with LDL (data not shown).

As shown in Fig. 5A, a 2-day culture with increasing concentrations of VLDL or LDL in RPMI containing 11.1 mM glucose induced a dose-dependent increase in the apoptotic rate in βTC3 cells. The effects increased steadily over a 48-h time course (data not shown). Serum deprivation was used as a positive control and induced a 4.3-fold increased apoptotic rate. In contrast, HDL particles had a protective effect against β-cell death (Fig. 5B). In the presence of 1.0 mM HDL cholesterol, the basal apoptotic rate was reduced significantly. In addition, HDL particles antagonized the proapoptotic effects of serum deprivation or of cytokine treatment. Cell death induced by high doses of LDL could be partially abolished by HDL particles. To determine whether elevated glucose concentrations played a role in the adverse effects of VLDL and LDL, we performed similar experiments with βTC3 cells incubated with medium containing 5.0 mM glucose. No differences were observed in the apoptotic rate compared with experiments conducted with 11.1 mM glucose concentrations.

We then confirmed in freshly isolated islets the effects observed in βTC3 cells with the exception of HDL, which did not decrease the basal apoptotic rate in control islets (Figs. 4 and Fig. 5C). To evaluate the role of lipoprotein receptors in LDL-induced apoptosis, we incubated islets from LDLR−/− and WT mice with LDL particles. Elevated VLDL or LDL significantly increased the apoptotic rate in WT mice. The increase in apoptosis under LDL treatment was significantly lower in LDLR−/− mice compared with WT mice. We also confirmed the beneficial effects of HDL on islet cell survival (Fig. 5C). In WT mice, cell death induced by LDL particles was decreased by 46% in the presence of HDL. Taken together, these data demonstrate a deleterious effect of VLDL and LDL particles which requires, at least in part, the presence of the LDLR and which can be antagonized by HDL particles.

Finally, we examined the role of human lipoproteins on insulin mRNA (Fig. 5D). As measured by quantitative reverse transcription PCR, insulin mRNA was reduced markedly under VLDL or LDL exposure. HDL particles had no influence and did not alleviate the detrimental effects of elevated LDL on insulin transcript levels. LDL at a lower concentration (3.1 mM) still reduced insulin mRNA but without inducing cell death.

Caspase-3 Activation and Contribution of the JNK Pathway to VLDL-induced Apoptosis—Caspase-3 is one of the key executioners of programmed cell death. Therefore, we quantified the amount of the cleaved active form by Western blotting in βTC3 cells (Fig. 6A). Serum deprivation, cytokines, and 3.1 mM VLDL were all associated with increased levels of the activated cleaved protein. Surprisingly, 6.2 mM LDL did not induce any cleavage. Activation of caspase-3 was partially antagonized by HDL particles, confirming the survival-promoting role of these lipoproteins.

Islet-brain 1/JNK-interacting protein-1 (IB1/JIP-1) is a scaffold protein that interacts with several components of the JNK signaling pathway (15). We examined whether this pathway is implicated in apoptosis induced by lipoproteins. Incubations with 3.1 mM VLDL reduced IB1/JIP-1 levels by 56%, whereas LDL or HDL had no effect (Fig. 6B). A cell-permeable peptide inhibitor of the JNK pathway (16) partially inhibited apoptosis induced by VLDL, but not by LDL particles (Fig. 6C). Taken together, these data suggest that cell death triggered by VLDL depends on caspase-3, IB1/JIP-1, and JNK.

**FIG. 4.** Assessment of apoptosis in mouse islets treated with lipoproteins. Mouse islets were cultured for 48 h in the presence of lipoproteins, stained with SYTOX Green, and analyzed with a confocal microscope at low (×25) and high (×63) magnification. A and B, control; C and D, 3.1 mM VLDL; E and F, 6.2 mM LDL; G and H, 1.0 mM HDL. Arrows indicate apoptotic nuclei.

**Effects of Lipoproteins on Cell Proliferation, Cyclin B1 Transcription, and Akt/PKB Activation—**Cell proliferation in βTC3 cells exposed to lipoproteins was evaluated by quantitating BrdUrd incorporation (Fig. 7A). Elevated concentrations of VLDL (3.1 mM) or LDL (6.2 mM) significantly decreased the number of cells positive for BrdUrd incorporation, whereas HDL caused a slight increase that was not statistically significant (p = 0.064). Cyclin B1 is expressed predominantly in the G2/M phase of cell division and participates in the control of cell proliferation. Cyclin B1 mRNA was reduced by 39% in cells treated with 3.1 mM VLDL and by 29% when treated with 6.2 mM LDL (Fig. 7B), suggesting a mechanism by which these lipoproteins reduce cell proliferation. Finally, HDL particles induced activation of Akt/PKB, known as a key regulator of cellular survival (Fig. 7C).

**DISCUSSION**

This study shows that several members of the LDLR family are expressed in β-cells. Because islets from LDLR−/− mice display markedly decreased LDL uptake compared with islets from WT mice, we propose LDLR to be the main receptor responsible for LDL internalization in β-cells. ApoER2 can also...
bind LDL particles with lower affinity (17), which may explain why low levels of LDL uptake are still observed in islets from LDLR\(^{-/-}\) mice. Down-regulation of HMG-CoA reductase expression in \(\beta\)-cells treated with LDL confirms that these cells express functional receptors for LDL particles. SR-BI is unrelated to LDLR and has been shown to mediate selective uptake of cholesterol from HDL particles (10). Our data demonstrate for the first time the presence of SR-BI in \(\beta\)-cells concomitant with the uptake of HDL.

Exposure of insulin-secreting cells to human VLDL and LDL induces an increase in apoptosis which depends on both the duration of the incubation and the concentration of particles. Cell death triggered in this way appears to occur via two different mechanisms. Caspase-3 is strongly activated in the presence of elevated VLDL concentrations, whereas cellular disassembly and packaging into apoptotic bodies, which take place in the presence of LDL, are independent of caspase-3. Moreover, VLDL, but not LDL, reduces the levels of IB1/JIP-1. This protein is expressed at high levels in pancreatic \(\beta\)-cells and has been shown to play an important role in controlling the activity of the JNK signaling pathway (18). A mutation in the MAPK8IP1 gene encoding IB1 has been associated with a familial form of type 2 diabetes (19). Recently, Bonny et al. (16) engineered cell-permeable peptide inhibitors of JNK corresponding to the minimal JNK binding domain of IB1/JIP-1 which resulted in a marked inhibition of \(\beta\)-cell death triggered by interleukin-1\(\beta\). These peptides could partially antagonize VLDL-induced apoptosis, but they had no effect on incubations performed with LDL. These data indicate that VLDL induces apoptosis concomitant with a reduction in IB1/JIP-1 levels and an activation of the JNK pathway. A study by Bonny et al. (18) implicated this reduction in IB1/JIP-1 levels in increased apoptosis.

Our study establishes that the LDLR plays an important role...
in β-cell death caused by LDL. When exposed to elevated LDL concentrations, islets from LDLR/−/− were more resistant to apoptosis than islets from WT mice. However, apoptotic rates were slightly higher than in control conditions, which may be explained by the presence of other lipoprotein receptors that were shown to be present in LDLR/−/− mice.

We show that in contrast to VLDL and LDL, HDL promotes β-cell survival and protects against apoptosis. HDL efficiently antagonizes the proapoptotic effects of serum deprivation and incubation with cytokines or lipoproteins. In the presence of HDL, caspase-3 cleavage is reduced markedly, and Akt/PKB is activated. Akt inhibits apoptosis by phosphorylating a variety of substrates, including Bad, FKHR, glycolgen synthase kinase-3 (GSK3) and glycogen synthase kinase-3 β (GSK3β).

**FIG. 6.** Caspase-3 activation and contribution of the JNK pathway in VLDL-induced apoptosis. BTC3 cells were incubated for 48 h in the absence of serum (starvation) or in the presence of cytokines (interleukin-1β/tumor necrosis factor-α/interferon-γ), VLDL, LDL, and HDL. Lysates were subjected to immunoblot analysis with anti-cleaved caspase-3 (A) and anti-IB1/JIP-1 antibodies (B). The amount of protein was quantitated by densitometry. Tubulin was used for normalization. Data are the means ± S.E. of a minimum of four separate experiments.

**FIG. 7.** Effects of lipoproteins on βTC3 cell proliferation, cyclin B1 transcription, and Akt/PKB activation. A, βTC3 cells were incubated for 48 h in the presence of lipoproteins and subsequently for 1 h with BrdUrd. The number of nuclei positive for BrdUrd was evaluated in three independent experiments. Data are the means ± S.E. ***, p < 0.01 compared with control conditions. B, cyclin B1 mRNA levels were measured by quantitative PCR. Data are the means ± S.E. of a minimum of four separate experiments. ***, p < 0.01 compared with control conditions. C, βTC3 cells were incubated with HDL, and Akt/PKB activity was determined by measuring the in vitro phosphorylation of glycogen synthase kinase-3 (P-GSK).
nase-3, and caspase-9 (20). In the endocrine pancreas, Akt1 has been shown to regulate β-cell growth and survival (21). In endothelial cells, Akt is a mediator of the antiapoptotic effects of HDL (22).

We demonstrate that lipoproteins have significant effects on cellular proliferation. HDL has been identified as potent mitogens in vascular smooth muscle by stimulating entry into S phase (23). In accordance with this result, HDL increases insulin-secreting cell proliferation in our system. VLDL and LDL, in contrast, induce a decrease in proliferation which is associated with a reduction in cyclin B1 gene expression. This may explain the reduction in proliferation because cyclin B1 protein complexes with p34(cdc2) to form the mitosis-promoting factor.

Finally, we establish that VLDL and LDL, but not HDL, markedly reduce the level of insulin transcript. These effects are not attributable solely to apoptosis triggered by these lipoproteins because they occur even with doses too low to cause apoptosis or when apoptosis is inhibited with HDL. Deleterious effects of free fatty acids on insulin transcription are known (24), but our data are the first to demonstrate a similar role for lipoproteins.

Lipoprotein concentrations used in our study are in the normal or supraphysiological range observed in human plasma. Because pancreatic islets are highly vascularized structures with fenestrated capillaries (25), the settings chosen in our experiments probably reflect the conditions found in vivo. Recently, Cnop et al. (26) studied the role of LDL in rat β-cell death. Surprisingly, LDL toxicity occurred with doses far below physiological concentrations, and the effects on β-cells were described as necrosis. Our study shows that higher concentrations are required to cause death in βTC3 cells and primary mouse islets, with features characteristic of apoptosis. Differences between the species studied might explain these discrepancies.

The present study is in line with genetic studies and pharmacological interventions in humans. It has recently been suggested that a locus on chromosome 9 is linked to both cholesterol levels and diabetes mellitus (27). Blood lipoprotein levels have been confirmed as important predictors for the onset of type 2 diabetes, and preventative administration of the HMG-CoA reductase inhibitor pravastatin was associated with a reduction of 30% in the occurrence of type 2 diabetes in the West of Scotland Coronary Prevention Study (WOSCOPS) (28).

In conclusion, we propose that the changes in plasma lipoproteins observed during type 2 diabetes are not only a consequence of the disease but may contribute to the pathogenesis of the disease itself.

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