Low Density Lipoprotein Receptor Activity in Fibroblasts Cultured from Diabetic Donors

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SUMMARY

Low density lipoprotein (LDL) receptor activity was evaluated in cultured skin fibroblasts from diabetics and nondiabetic controls to evaluate whether intrinsic abnormalities of the LDL pathway exist, which might account for the premature atherosclerosis associated with diabetes mellitus. LDL receptors did not differ between cells grown from 16 diabetics (7 insulin-dependent, 9 non-insulin-dependent) or from 16 nondiabetic controls. An inverse relationship between LDL receptor activity and cell density was observed ($y = 1.35x^{-1.22}$, $r = 0.90$, $P < 0.001$), which appeared the same for diabetic and nondiabetic cells. Normalized values for LDL degradation by diabetic and nondiabetic cell strains were 1.52 ± 0.42% of added LDL/10^6 cells and 1.34 ± 0.28, respectively (P = NS). The kinetics of the LDL receptor also appeared to be the same in cells derived from a diabetic and a nondiabetic donor. LDL receptor activity in diabetic cells increased appropriately in response to physiologic concentrations of insulin in the incubation medium.

Thus, LDL receptor activity appears to be normal in diabetic cell strains. Therefore, these results do not support the possibility that alterations in the LDL pathway contribute to the accelerated atherosclerosis associated with diabetes. DIABETES *28*:914–918, October 1979.

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The premature development of atherosclerosis is the major cause of morbidity and mortality among diabetics.[1–3] Hyperlipidemia is well established as a risk factor for coronary artery disease in the general population,[4] although the interrelationship among diabetes, hyperlipidemia (to which diabetics appear particularly prone[3]), and atherosclerosis is less clear.

Primary cellular abnormalities intrinsic to genetic diabetes may also play a role in the pathogenesis of atherosclerosis, particularly as they might affect arterial wall cells. Cellular abnormalities that have been demonstrated in skin fibroblasts cultured from diabetic subjects include altered life span,[5,6] slow growth,[7] reduced cloning efficiency,[8] increased lipogenesis,[9] and altered protein synthesis.[7] In another disorder associated with premature atherosclerosis, familial hypercholesterolemia, abnormalities of the cell surface low density lipoprotein (LDL) receptor have been demonstrated.[10] LDL levels also appear to be higher in uncontrolled diabetics than in appropriate control populations.[11–13] Therefore, the present study was undertaken to evaluate whether any intrinsic abnormality of the LDL receptor exists in cells obtained from diabetic donors, which might contribute to the hyperlipidemia and premature atherosclerosis associated with diabetes mellitus.

METHODS

Cell culture. Cultured fibroblasts were derived from punch skin biopsies from the arms of diabetic subjects and from nondiabetic controls (Table 1). The diabetic subjects were seven insulin-dependent diabetics aged 19–29 at the time of biopsy and nine non-insulin-dependent diabetics aged 29–69. Of the latter nonketosis prone group, four received insulin to lower their blood glucose levels and hence to reduce symptoms related to hyperglycemia. The nondiabetic control group consisted of 16 subjects aged 20–70 with fasting plasma glucose levels <115 mg/dl.[14] Cells were grown in monolayer as previously described[15,16] and used

TABLE 1
Subject data

No.	Age	Sex	ID/NID*	Insulin-treated
		Diabetic		
1	19	F	ID	+
2	22	M	ID	+
3	22	M	ID	+
4	23	F	ID	+
5	24	M	ID	+
6	27	F	ID	+
7	28	M	NID	−
8	29	F	ID	+
9	29	M	NID	+
10	31	F	NID	−
11	41	M	NID	−
12	49	F	NID	+
13	52	F	NID	+
14	53	F	NID	+
15	56	M	NID	+
16	69	M	NID	−
		Nondiabetic		
1	20	M		
2	22	F		
3	24	M		
4	25	M		
5	26	M		
6	26	F		
7	27	F		
8	27	M		
9	28	F		
10	29	M		
11	36	M		
12	41	M		
13	60	M		
14	60	M		
15	66	M		
16	70	M		

* ID = insulin-dependent; NID = non-insulin-dependent.

between the 3rd and 15th passages. Stock cultures were maintained at 37°C in humidified 95% air, 5% CO_2 in 250-ml tissue culture flasks in modified Dulbecco Vogt medium containing 10% pooled human serum. For determination of LDL binding, uptake, and degradation, confluent monolayers from stock flasks were incubated with 0.05% trypsin for 10 min at 37°C to dissociate the cells. Approximately 1×10^5 cells were then seeded into 60-mm-diam plastic Petri dishes in 4 ml modified Dulbecco Vogt medium containing 10% pooled human serum. The cells were then grown for 7–10 days with three to four replacements of fresh growth medium. Twenty-four hours before the addition of ^{125}I-LDL, the medium was changed to one containing 10% lipoprotein-deficient serum. ^{125}I-labeled LDL (7.5 μg/ml) was then added for the determination of LDL binding, uptake, and degradation, as previously described.[15–17]

Lipoproteins. Human LDL (d 1.019–1.063) and lipoprotein-deficient serum (d > 1.25) were prepared from plasma of healthy subjects by sequential preparative ultracentrifugation as previously described.[15,16] The LDL was iodinated by the iodine monochloride method as modified for lipoproteins,[18] as previously described,[15–17] and sterilized by Millipore filtration. Thirty micrograms of ^{125}I-LDL specific activity (120–240 cpm/ng protein) was added to each dish for the determination of its binding and degradation.

Assays. After 4 h incubation of ^{125}I-LDL with cells at 37°C, the bound LDL was released from cell surfaces by trypsinization (0.05%) for 10 min at 37°C.[17] Remaining cell-associated radioactivity after trypsinization was used as a measure of LDL uptake. LDL degradation was measured as the trichloroacetic acid (TCA)-soluble nonfree iodide counts released into the incubation medium during 24 h incubation of cells with ^{125}I-LDL. Free iodide in the TCA-soluble phase of the medium was extracted by chloroform after its conversion to I_2 by hydrogen peroxide.[17] Dishes containing ^{125}I-LDL but no cells were incubated in parallel, and degradation by cells was determined by subtracting LDL degradation in these dishes from total degradation.

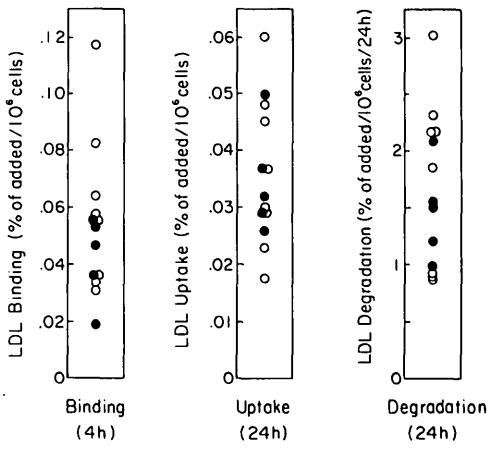
Effect of insulin on LDL degradation. Confluent cells were incubated with medium containing 10% lipoprotein-deficient serum with or without added insulin (100 μU/ml final concentration; purified single component obtained from Eli Lilly and Co.) for 48 h before the addition of labeled lipoprotein. ^{125}I-LDL was then added for measuring its degradation over the ensuing 24 h.

RESULTS

In the first experiment, LDL binding and degradation were determined in skin fibroblasts from eight diabetics and five nondiabetic controls. A wide range of values was obtained for LDL binding, uptake, and degradation, with complete overlap between diabetic and nondiabetic cell strains (Figure 1). The variation between cell strains was in part related to the number of cells in the dish at the time of the experiment. An inverse relationship was observed between cell number/dish and both LDL binding (Figure 2A) and degradation (Figure 2B), dishes with sparse numbers of cells having higher LDL receptor activity than dishes with a more confluent cell layer. No difference in the relationship between cell number and LDL binding or degradation was observed between diabetic and nondiabetic cell strains (Figure 2).

When LDL degradation was evaluated as a function of number of cells per dish for all 32 cell strains tested, an inverse relationship was observed which was nonlinear at low cell densities. The best linear fit for the data was a double log plot ($y = 1.35x^{-1.22}$; $r = 0.90$; $P < 0.001$) (Figure 3A) from which the relationship between cell number and LDL

Figure 1. Binding, uptake, and degradation of ^{125}I-labeled low density lipoprotein by cultured skin fibroblasts of eight diabetic (○) and five normal (●) subjects (7.5 μg/ml ^{125}I-LDL was added per dish).



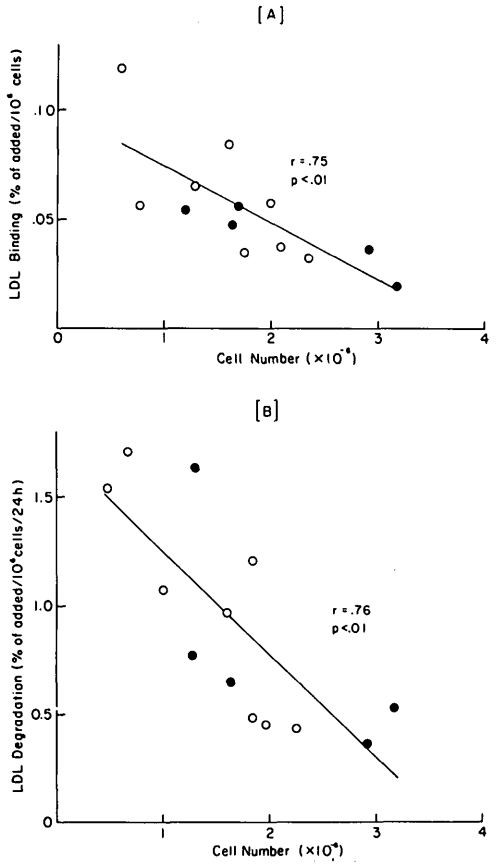


Figure 2. Relationship between cell density and LDL binding (A) and degradation (B). Subjects are the eight diabetics (○) and five nondiabetics (●) from Figure 1.

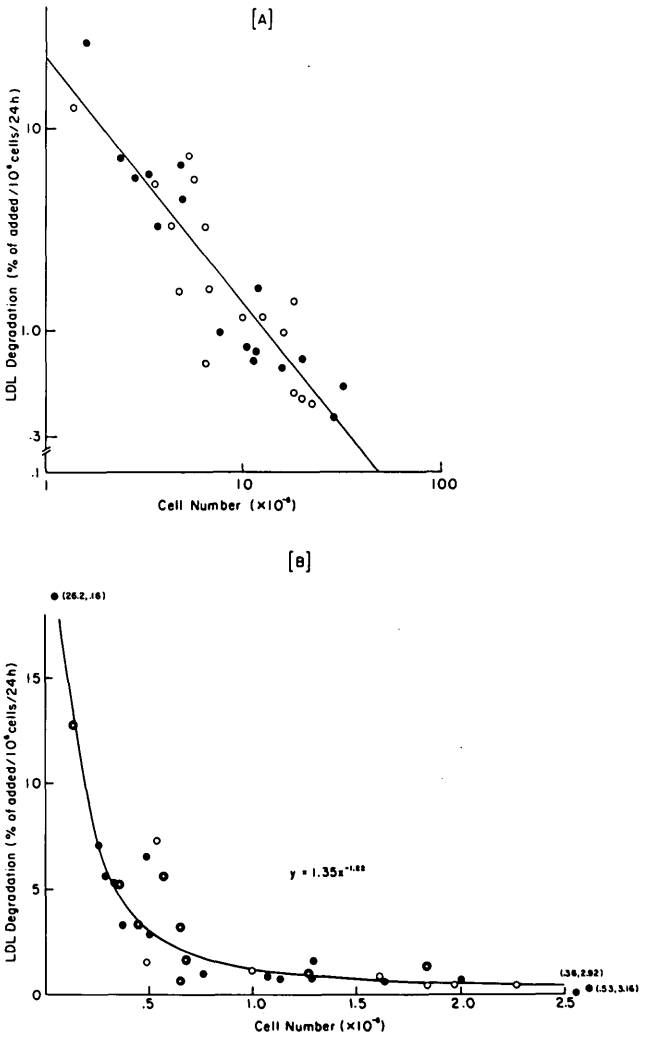


Figure 3. Relationship between cell density and LDL degradation. (A) Log–log plot from 16 diabetic (○) and 16 nondiabetic (●) controls. $y = 1.35x^{-1.22}$, r = 0.90, P < 0.001. (B) Curvilinear relationship derived from A. O, Insulin-dependent diabetic; ○, non-insulin-dependent diabetic; ●, normal. LDL (7.5 μg/ml) was added per dish. A degradation rate of 1% of added LDL/10⁶ cells/24 h translated into 300 ng/10⁶ cells/24 h.

degradation shown in Figure 3B was derived. Again no difference in the relationship of these two variables is apparent between diabetic and nondiabetic cells or between cells from insulin-dependent or non-insulin-dependent diabetics (Figure 3B). To compare LDL degradation directly between diabetic and nondiabetic strains, it was necessary to eliminate cell density as a variable. Therefore, the data for each group were normalized[19–21] according to the relationship observed in Figure 3 to that which would be expected at a cell density of 10^6 cells per dish. When normalized in this manner, LDL degradation by diabetic cells was 1.52 ± 42% of added LDL/10^6 cells/24 h (mean ± SEM, N = 16), while LDL degradation by nondiabetic cells was 1.34 ± 0.28% (N = 15; P = NS). The nondiabetic strain with the lowest cell number was eliminated from analysis because the normalized value was more than 10 standard deviations from the mean and hence satisfied the criteria for an outlyer.

When kinetics of LDL receptor activity in a single diabetic cell strain (#11, Table 1) were compared to that in a nondiabetic strain (#11, Table 1), saturability was demonstrated in both and very similar values for "apparent Km" were obtained (Figure 4).

To test whether LDL metabolism by "diabetic cells" was responsive to insulin in a manner similar to that previously demonstrated in nondiabetic cell strains,[15,16] four diabetic and four nondiabetic cell strains were incubated with or without insulin (100 μU/ml) for 48 h before the addition of ^{125}I-LDL. LDL degradation was stimulated in all eight cell strains; furthermore, no differences were observed in the degree of stimulation between cells from diabetic and nondiabetic donors (Table 2).

DISCUSSION

The inverse relationship between LDL receptor activity and cell density observed in this study is consistent with our findings[21] and those of others[22,23] in cultured arterial smooth muscle cells and endothelial cells.[23] It is also consistent with the observation that LDL binding and degradation decrease when cultured fibroblasts reach confluence.[24] An explanation is not readily apparent. This relationship may result from the exposure of fewer LDL receptors when cells are in close contact. Also, it could represent a physiologic phenomenon in which nonconfluent cells have increased numbers of receptors to facilitate the transport of exogenous cholesterol for synthesis of new membranes required for proliferation. Whichever, diabetic cells demonstrate no difference in the relationship between cell density and LDL receptor activity when compared with nondiabetic cells.

LDL receptor activity, assayed either as binding or degra-

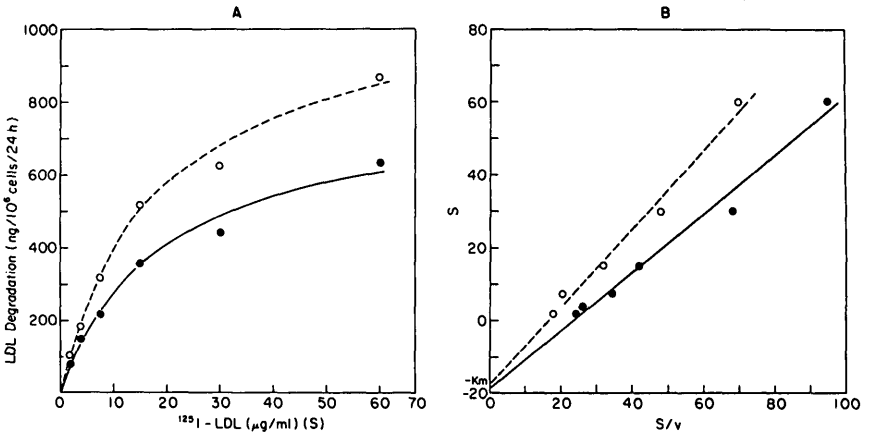


Figure 4. Saturation kinetics of LDL degradation by skin fibroblasts from a diabetic (○——○) and a normal (•——•) subject. (A) ^{125}I-LDL concentration curve. (B) Linearization plot[16,26] of data from A. The slope of the line = "Bmax," while the point of intersection with the y axis = "−Km".

dation of ^{125}I-labeled LDL, did not differ between cells grown from diabetic or nondiabetic donors. Furthermore, no differences between strains from subjects with insulin-dependent and non-insulin-dependent diabetes were apparent, nor did diabetic strains differ in this respect from normals. Donor age did not correlate with LDL degradation in either diabetics or nondiabetics (data not shown). Therefore, no evidence for an intrinsic abnormality of the LDL receptor was demonstrated. LDL saturation kinetics were similar in a diabetic and nondiabetic cell strain, and cell strains from diabetic donors responded normally[15,16] to stimulation by physiologic concentrations of insulin. These findings suggest that the regulation of LDL receptor activity by insulin also is normal in diabetes.

Atherosclerosis is characterized by an accumulation of cholesterol esters in arterial smooth muscle cells, possibly as a result of disturbed cellular lipoprotein metabolism. Since the characteristics of the LDL receptor in cultured human arterial smooth muscle cells appear to be similar to those demonstrated on cultured skin fibroblasts,[25] the latter cell type would appear to be an appropriate model for LDL binding and degradation by extrahepatic cells including arterial smooth muscle cells. Assuming that LDL receptors in cultured fibroblasts are a valid model for LDL receptor activity in arterial smooth muscle cells in vivo, it is then unlikely that the cholesterol accumulation in arteries of diabetic patients is related to defective LDL receptors.

TABLE 2
Effect of insulin (100 μU/ml) on LDL binding and degradation by diabetic and nondiabetic skin fibroblasts

Binding (% of added/10⁶ cells)			Degradation (% of added/10⁶ cells/24 h)		
Basal	Insulin	% ↑	Basal	Insulin	% ↑
Nondiabetic					
0.21	0.28	33	3.60	4.57	27
0.18	0.33	83	3.24	4.74	46
0.37	0.51	38	5.64	8.38	49
0.17	0.25	47	0.76	1.03	36
		$\bar{x}$ = 50 ± 23			40 ± 10
Diabetic					
0.23	0.35	52	2.77	5.62	103
0.12	0.20	67	1.35	2.62	94
0.23	0.28	22	3.57	4.03	13
1.06	1.35	27	1.55	2.07	34
		$\bar{x}$ = 42 ± 21			61 ± 44

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