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Surface Binding, Internalization and Degradation by Cultured Human Fibroblasts of Low Density Lipoproteins Isolated from Type 1 (Insulin-Dependent) Diabetic Patients: Changes with Metabolic Control

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Summary. A previous study of low density lipoprotein metabolism by cultured cells focused on the metabolism of normal lipoproteins in vitro by fibroblasts isolated from diabetic patients. No abnormalities were found. We have followed the opposite approach. Using normal human fibroblasts as test cells we compared the metabolism in vitro of low density lipoproteins isolated from diabetic patients before and after metabolic control. We found a significant decrease ($p < 0.02$) in internalization and degradation of low density lipoproteins isolated from diabetic patients before metabolic control when compared with those isolated from normal control subjects or from the same patients after metabolic control. The observed changes were mainly apparent in intracellular degradation. To evaluate whether the observed differences in low density lipoprotein behaviour were correlated with lipid or apolipoprotein composition, we measured cholesterol, triglyceride, apolipoprotein B and total protein levels in the low density lipoproteins tested. A significant decrease ($p < 0.05$) of the triglyceride/protein ratio was found in post-control low density lipoproteins suggesting that a high triglyceride content may interfere with low density lipoprotein metabolism. The present study represents the first observation that metabolic control in diabetes mellitus can alter low density lipoprotein-cell interaction and suggests a possible mechanism for the enhanced incidence of atherosclerosis in diabetic patients.

Key words: Type 1 (insulin-dependent) diabetic patients, low density lipoprotein, pre-control LDL, post-control LDL; human fibroblasts, LDL uptake, LDL degradation, cell culture, LDL lipids, LDL apolipoprotein B, LDL-cell interaction.

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There is an increased incidence of atherosclerosis in diabetes mellitus [1]. One feature common to both atherosclerosis and diabetes is the existence of hyperlipaemia. A postulated mechanism for the development of atherosclerosis in patients with hyperlipaemia was proposed by Goldstein and Brown [2]. These authors stated that the low density lipoprotein (LDL) pathway functions to protect against atherosclerosis by allowing cells to take up LDL to satisfy cholesterol needs and to maintain simultaneously plasma LDL concentration below threshold levels for atherosclerosis. If this mechanism would, indeed, be of biological significance, a decrease in the amount of LDL taken up and catabolized by the cells, either due to possible defects in the LDL pathway or to constitutional changes of the lipoprotein that could prevent its adequate uptake and/or intracellular metabolism, would eventually lead to the development of atherosclerosis.

The LDL pathway has been studied previously in fibroblasts isolated from diabetic patients by Chait et al. [3] and no abnormalities were found. We therefore decided to study the interaction between normal human fibroblasts and LDL isolated from insulin-dependent diabetic patients before and after strict metabolic control with insulin.

Patients and Methods

Patients

Sixteen Type 1 (insulin-dependent) diabetic patients (ten females and six males), ranging in age from 10 to 31 years were studied. Their weight varied within 10% of ideal body weight. Thirteen patients were hospitalized from 4 to 20 days (mean 11 days). They were all placed on a normocaloric diet, consisting of 20% calories as protein, 35% fat and 45% carbohydrate. An attempt was made to maintain these patients with a normal exercise level during the hos-

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pitalization period. Four patients were treated with a closed-loop insulin-delivery system (Biostator glucose controller, Miles Laboratories, Elkhart, Indiana) for 2 days followed by continuous SC insulin infusion administered by portable infusion pump (Model AS2C Autosyringe, Hookset, New Hampshire, USA). Nine patients were treated with short-acting insulin given SC four times daily. Twenty-four hour urinary glucose excretion was determined daily in all patients. Mean plasma glucose levels were obtained four times daily (fasting, before lunch and dinner and at midnight) during the first 48 h and in the 4 days preceding discharge in some patients and daily in others. Haemoglobin A_{1c} (HbA_{1c}) was measured in all patients on admission and at discharge. In all patients fasting blood samples were collected for lipoprotein studies within 24 h of admission and within 48 h of discharge.

The remaining three patients were only studied during a period of poor metabolic control. Fasting blood samples were collected in these three patients for lipoprotein studies and for glucose and HbA_{1c} determinations. Nine of the 16 patients were studied simultaneously with an age- and sex-matched control subject.

As control subjects we used 11 healthy volunteers (six females and five males) ranging in age from 16 to 30 years. In four of the normal control subjects blood was collected twice within 6–9 day intervals between bleeds. Informed consent, as approved by the Institutional Review Board for Human Research of the Medical University of South Carolina was obtained from all adult subjects involved in the study. Parental consent was obtained for minors.

Cells

Fibroblasts were obtained from pooled neonatal foreskins by enzymatic dissociation and sieving through nylon mesh as described previously [4]. Cultures were maintained on a mixture of medium F12K (85%) prepared as described [4] and fetal calf serum (15%; Sterile Systems, Logan, Utah, USA) buffered with N-2-hydroxyethyl piperazine N′-2 ethanesulphonic acid (HEPES) (25 mmol/l, pH 7.6), in an atmosphere of 5% CO_2/95% air. Primary cultures were treated with penicillin (50 U/ml) and gentamicin (50 µg/ml), but antibiotics were withdrawn after the first passage and they were only added again at the beginning of each experiment.

Second-passage cells were stored by freezing in liquid nitrogen, and thereafter were thawed as needed. All experiments were performed in the third to eighth passage of the cells, using the same pool of fibroblast cells.

Lipoprotein and Lipoprotein Deficient Serum

Low density lipoprotein ($d > 1.019$, < 1.063 g/ml) were isolated from plasma by sequential ultracentrifugation in a preparative ultracentrifuge (Beckman L5–50) after appropriate adjustment of density with solid KBr [5]. LDL preparations were washed by ultracentrifugation, stored under nitrogen, in the dark. Before labelling, the LDL were dialysed against a 0.15 mol/l NaCl solution containing EDTA 1 mmol/l (pH 7.4). Each isolated LDL migrated as a homogenous peak on polyacrylamide gel lipoprotein electrophoresis [6]. LDL, passed through Acrodisc filter (0.2 µm pore size) in order to remove aggregates, were iodinated with ^{125}I by the McFarlane procedure as modified by Bratzler et al. [7]. LDL isolated from each patient before and after metabolic control were iodinated simultaneously and studied in the same experiment.

The integrity of ^{125}I-labelled LDL was assessed by incubating, in parallel, increasing concentrations of ^{125}I-labelled LDL (5–80 µg/ml of medium) and ^{125}I-LDL (5 µg/ml) progressively diluted with unlabelled LDL to equal concentrations (5–80 µg/ml medium). The same LDL preparation was used in its native form (unlabelled) and after ^{125}I-labelling to perform these experiments. Binding, internalization and degradation of ^{125}I-labelled LDL and ^{125}I-labelled LDL diluted with unlabelled LDL were similar at all concentrations studied after an appropriate correction for the changes in specific activity due to dilution. Radioactivity localized in the lipid moiety of LDL was determined after a Folch lipid extraction [8] and ranged from 1.6% to 8% of the total radioactivity. No significant differences in the radioactivity of the lipid moieties of LDL were found between LDL isolated from diabetic subjects before metabolic control (4.8%); after metabolic control (3.8%) and from normal subjects (3.4%).

Lipoprotein deficient plasma was separated by ultracentrifugation after adjustment of density to 1.25 g/ml with solid KBr [5]. After removal of the lipoproteins contained in the top fraction, the bottom fraction was recentrifuged, under the same conditions to ensure complete removal of lipoproteins. The plasma was clotted with thrombin using the method of Brown et al. [9] modified by the addition of calcium (2.5 mg/ml). It was then dialyzed against a 0.15 mol/l NaCl solution (pH 7.4) and sterilized by filtration (Acrodisc filter, 0.45 µm pore size) before use. Its total cholesterol content was below 60 ng/mg of protein.

Binding, Internalization and Degradation of ^{125}I-LDL

Five X 10^5 cells were inoculated into 60 mm tissue culture dishes (Falcon Products, Cockeysville, Maryland, USA) in F12K medium containing 15% fetal calf serum, penicillin (50 U/ml) and gentamicin (50 µg/ml). After incubation for 3–4 days, the medium was removed, the cells were washed with Hank's balanced salt solution (HBSS) and fresh medium containing antibiotics and lipoprotein deficient serum (2.5 mg protein/ml medium) was added (2 ml/dish). Twenty-four hours later, the medium was removed again and fresh medium with the same composition and containing ^{125}I-LDL in three different concentrations (10,20 and 40 µg/ml of medium) was added. In each experiment LDL isolated from diabetic patients before and after metabolic control were studied. LDL from age- and sex matched normal subjects were tested simultaneously in seven of the experiments but only two concentrations (10 and 40 µg LDL/ml medium) were used for the normal LDL in some of the experiments. Cultures were incubated for 18 h, at 37° C, in a humidified incubator equilibrated with 5% CO_2 in air. After incubation, the medium was removed, the cell layer was washed five times, two times with cold HBSS containing 2 mg/ml of bovine serum albumin, and three times with the same solution containing no bovine serum albumin. Afterwards the cell layer was treated for 5 min with a solution of 0.05% trypsin and 0.01% EDTA in Ca^{++} and Mg^{++} free HBSS. Cell pellets were obtained by centrifugation at 4 °C, 2,500 rev/min, for 45 min. The amount of surface-bound and internalized LDL was calculated by dividing, respectively, the radioactivity released by trypsin (i.e. in the supernatant) and the radioactivity in the cell pellet by the specific activity of LDL. Duplicate determinations of ^{125}I-LDL binding and internalization varied by less than 10% of the mean values. The medium removed before trypsin treatment was used to study degradation by the method of Bierman et al. [10]. Net degradation was calculated as the difference between values obtained from identical incubations in the presence and absence of cells. Duplicate determinations of ^{125}I-LDL degradation varied by less than 5% of the mean values. All results were expressed as ng LDL/mg of cell protein. Protein was determined in the cell pellets by the method of Lowry et al. [11]. The cell pellets were solubilized overnight, at 4° C, with 300 µl of 1 mol/l KOH and the final volume was adjusted to 1 ml with 0.9% saline. The lipids contained in the cell pellets were extracted by the method of Folch et al. [8] and the radioactivity associated with it determined. As a rule the radioactivity contained in the lipid portion of the cell pellets was 2%–5% higher than that associated with the lipoprotein. The radioactivity associated with the lipid portion was subtracted from the surface binding and internalization values.

The above protocol was designed to study LDL behaviour under culture conditions that led to up-regulation of LDL receptors.

Table 1. Surface binding, internalization and degradation by normal human fibroblasts of LDL isolated from 13 diabetic patients before and after metabolic control

	Diabetic patients before control	Diabetic patients after control	changes	p
Binding (ng LDL/mg cell protein)				
10 μg LDL/ml medium	181 ± 17	203 ± 19	21.3 ± 11.4	NS
20 μg LDL/ml medium	300 ± 31	332 ± 34	32.2 ± 22.3	NS
40 μg LDL/ml medium	440 ± 46	503 ± 48	62.3 ± 32.5	NS
Internalization (ng LDL/mg cell protein)				
10 μg LDL/ml medium	772 ± 51	906 ± 50	134 ± 45	< 0.02
20 μg LDL/ml medium	1127 ± 72	1359 ± 91	232 ± 68	< 0.01
40 μg LDL/ml medium	1749 ± 128	2064 ± 132	315 ± 111	< 0.02
Degradation (ng LDL/mg cell protein)				
10 μg LDL/ml medium	4096 ± 505	5461 ± 497	1364 ± 437	< 0.01
20 μg LDL/ml medium	6178 ± 788	7752 ± 696	1574 ± 529	< 0.02
40 μg LDL/ml medium	8162 ± 842	10527 ± 921	2366 ± 781	< 0.02

Results expressed as mean ± SEM; NS = not significant

In order to evaluate the LDL metabolism when LDL receptors were down-regulated, a modified protocol was used. After the initial cell growth of 3–4 days in F12K medium containing 15% fetal calf serum and antibiotics, the cells were washed and fresh medium containing antibiotics, lipoprotein deficient serum (2.5 mg protein/ml medium) and the unlabelled LDL to be studied (100 μg/ml of medium) was added (2 ml/dish).

Twenty-four hours later, the medium was removed, the cells washed and fresh medium containing ^{125}I-LDL in two concentrations (10 and 40 μg/ml of medium) was added and the cultures were incubated for 18 h, as described. The next steps of the protocol were similar to the ones already described for the study of LDL behaviour under conditions of up-regulation of LDL receptors.

LDL isolated from two diabetic patients in poor metabolic control and two sex- and age-matched normal subjects were studied simultaneously under culture conditions set for the study of LDL behaviour under up- and down-regulation of LDL receptors. In all of the experiments described above, each value represents the mean of duplicate incubations and measurements.

Other Methods

Plasma and 24 h urinary glucose was assayed using the glucose oxidase method as adapted for use in the Beckman glucose analyzer [12]. HbA_{1c} was measured by isoelectric focussing of erythrocyte haemolysates over a pH gradient of 6–8, according to the method of Spicer et al. [13] with slight modifications.

Cholesterol and triglyceride levels were measured using the semi-automated method standardized by the Lipid Research Clinics Program [14]. Free and total cholesterol were assayed on a Sigma 3 gas-chromatograph equipped with a hydrogen flame ionization detector, using a glass column packed with 3% OV-17 on 100–120 mesh Supelcoport and N_2 as the gas carrier. The column temperature was maintained at 280° C and the flow of the carrier gas was 40 ml/min. LDL were extracted with chloroform: methanol (2:1) and washed according to the method of Folch et al. [8]. For the total cholesterol assay one aliquot of the washed chloroform phase was hydrolyzed by the method of Ishikawa et al. [15]. As internal standard, 5-α-cholestane was used.

Apolipoprotein B levels were measured using a slightly modified version of Laurell's electro-immunodiffusion technique [16] similar to the one described by us for apolipoprotein AI [17]. Anti-apo B antisera (1.5 ml/l of the Agarose/Dextran 10 solution) was used. LDL isolated from a normal donor by ultracentrifugation (d > 1.030 and < 1.050) was used to calibrate the assay. The dilutions of the LDL to be assayed ranged from 1/40 to 1/240. All of them were run in duplicate, at least in two different dilutions.

Statistical analysis was performed using the mean paired Student's t-test and the Wilcoxon signed rank Test to compare differences between paired data. For evaluation of differences between means, in unpaired data, Wilcoxon rank sum test and Student's t-test were used. Correlation coefficients (r) were determined by linear regression analysis. Statistical significance of the correlation coefficients was determined by the method of Fisher and Yates.

Results

Results for surface binding, internalization and degradation of LDL isolated from 13 diabetic patients before and after metabolic control are summarized in Table 1. There was no significant change in surface binding. A significantly lower internalization and intracellular degradation ($p < 0.02$) for the LDL isolated from diabetic patients before metabolic control was observed at all concentrations studied.

Since LDL isolated from patients before metabolic control were stored longer than those obtained after metabolic control, we examined the effect of LDL storage on LDL binding, internalization and degradation by fibroblasts. LDL of four normal volunteers were isolated from two separate blood collections 6–9 days apart. LDL prepared from the first collections were stored under the same conditions as the patient pre-control LDL samples. No differences between the metabolism of stored and fresh LDL isolated from normal subjects were found. To examine further the effect of LDL storage on LDL metabolism, we compared the differences observed in degradation between pre- and post-control LDL with the length of hospitalization time. No significant correlation was found ($r = 0.14$).

In Figure 1 the mean changes in LDL surface binding, internalization and degradation between pre- and post-control LDL, isolated from diabetic subjects, and between stored and fresh LDL isolated from normal subjects are represented for 10 µg LDL/ml medium. A statistically significant increase in internalization and degradation was found in the post-control LDL from diabetic patients.

The difference in the handling of pre- and post-control LDL was observed in nine of the 13 diabetic patients studied. Two of the four patients who failed to show a difference were admitted in fair control (HbA_{1c} 11.5% and 11%; plasma glucose 10.38 and 13.04 mmol/l). The other two patients, admitted in poor control, showed a slightly increased degradation but failed to show increased internalization. The mean concentrations of total LDL and VLDL plasma cholesterol, total plasma triglycerides, plasma glucose and HbA_{1c} are represented in Table 2, for all patients and normal subjects studied.

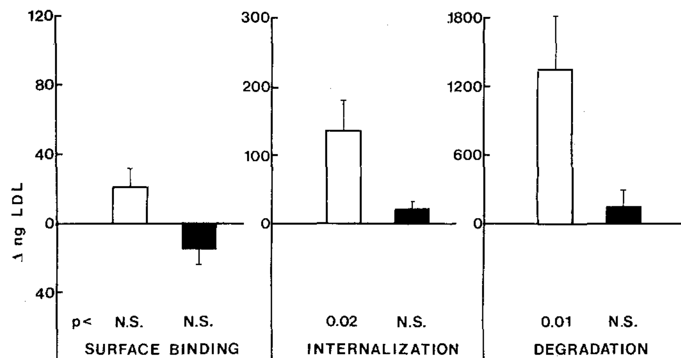

Fig. 1. Differences of surface binding, internalization and degradation of LDL isolated from 13 diabetic patients before and after metabolic control and of LDL isolated from two different plasma samples collected from four normal subjects with 6–9 day intervals between bleeds. The differences are expressed as mean ± SEM for a concentration of 10 µg LDL/ml of medium. The absolute levels of the three parameters studied were adjusted to ng LDL/mg cell protein, as described in Methods. □ = diabetic patients (post-control LDL minus pre-control LDL); ■ = normal subjects (fresh LDL minus stored LDL); NS = not significant

To evaluate the effect of control in the handling of pre- and post-control LDL by fibroblasts, we compared the differences in HbA_{1c} and glucose observed in each patient with the differences observed in the degradation of pre- and post-control LDL. A significant correlation was obtained ($r = 0.558, p < 0.05$ for HbA_{1c} and $r = 0.559, p < 0.05$ for glucose). Furthermore, we compared the differences in the plasma LDL cholesterol obtained in each patient before and after metabolic control with the differences observed in the degradation of pre- and post-control LDL isolated from the same patient and we found a significant correlation ($r = 0.586, p < 0.05$).

To compare the binding, internalization and degradation of patient-derived LDL with that obtained from normal subjects, LDL from seven of the 16 diabetic patients were studied simultaneously with LDL from seven sex- and age-matched control subjects. The results are summarized in Table 3. LDL isolated from normal subjects were degraded by fibroblasts to a greater extent ($p < 0.05$) than those obtained from diabetic patients before metabolic control. The degradation of the LDL isolated from the normal subjects was similar to that of LDL obtained from diabetic patients after metabolic control. In order to determine the influence of culture conditions, leading to

Table 2. Plasma lipid an lipoproteins, glucose and HbA_{1c} levels in normal and diabetic subjects before and after metabolic control

	Total cholesterol (mmol/l)	LDL-cholesterol (mmol/l)	VLDL-cholesterol (mmol/l)	Triglycerides (mmol/l)	Plasma glucose (mmol/l)	HbA_{1c} (% of total HbA)
1) Diabetic patients before control ($n = 16$)	5.98 ± 1.86 (231 ± 72)[a]	3.36 ± 0.93 (130 ± 36)[a]	1.68 ± 1.42 (65 ± 55)[a]	3.29 ± 2.11 (291 ± 187)[a]	20.6 ± 7.94 (372 ± 143)[a]	16.0 ± 3.5
2) Diabetic patients after control ($n = 13$)	4.27 ± 0.83 (165 ± 32)	2.77 ± 0.65 (107 ± 25)	0.47 ± 0.36 (18 ± 14)	0.89 ± 0.47 (79 ± 42)	6.11 ± 2.66 (110 ± 48)	11.4 ± 2.7
3) Normal subjects ($n = 11$)	4.04 ± 0.72 (156 ± 28)	2.35 ± 0.67 (91 ± 26)	0.41 ± 0.28 (16 ± 11)	0.87 ± 0.38 (77 ± 34)	4.27 ± 0.67 (77 ± 12)	5.0 ± 0.3
p value (1 versus 2)	< 0.01	NS[b]	< 0.01	< 0.01	< 0.001	< 0.01
p value (1 versus 3)	< 0.001	< 0.01	< 0.01	< 0.01	< 0.001	< 0.001

Results expressed as mean ± SD; NS = not significant

[a] Levels in parentheses expressed in mg/dl. [b] $p < 0.05$ when comparing the levels obtained in diabetic patients before and after metabolic control ($n = 13$)

Table 3. Binding, internalization and degradation by normal fibroblasts of LDL isolated from seven diabetic patients and from seven age- and sex-matched control subjects

	Diabetic patients before control	Diabetic patients after control[a]	Normal subjects
Binding (ng LDL/mg cell protein)			
10 μg LDL/ml medium	142 ± 45	171 ± 71	151 ± 38
40 μg LDL/ml medium	315 ± 100	418 ± 161	367 ± 143
Internalization (ng LDL/mg cell protein)			
10 μg LDL/ml medium	747 ± 214	891 ± 188	869 ± 132
40 μg LDL/ml medium	1706 ± 461	2122 ± 510	2011 ± 225
Degradation (ng LDL/mg cell protein)			
10 μg LDL/ml medium	3488 ± 1429[b]	4988 ± 919	5402 ± 1379
40 μg LDL/ml medium	7575 ± 2784[b]	10,172 ± 1540	10,883 ± 2399

Results expressed as mean ± SD
[a] Six patients only. [b] $p < 0.05$ compared with normal control subjects

Table 4. Relative cholesterol (free and total), triglyceride and protein composition of LDL isolated from diabetic subjects before and after metabolic control and from normal subjects

	Cholesterol/ protein	Triglyceride/ protein	Cholesterol/ triglyceride	Free/total cholesterol[a]
Diabetic patients before metabolic control (n = 11)	1.56 ± 0.57	0.52 ± 0.23[b]	4.04 ± 3.60	0.23 ± 0.03
Diabetic patients after metabolic control (n = 11)	1.61 ± 0.33	0.34 ± 0.13	5.34 ± 1.65	0.26 ± 0.05
Normal subjects (n = 8)	1.64 ± 0.23	0.29 ± 0.06	6.16 ± 2.31	0.24 ± 0.08

Results expressed as mean ± SD
[a] From five to eight subjects in each group. [b] $p < 0.05$ compared with diabetic patients after control and normal subjects

up- or down-regulation of LDL receptors, we studied, under both sets of conditions, two diabetic patients in poor metabolic control and two sex- and age-matched normal subjects. The studies were performed using 10 and 40 μg of LDL/ml of medium. In both sets of culture conditions, at the two concentrations studied, we found that the LDL isolated from the poorly controlled diabetic patient was less degraded by fibroblasts than that isolated from the matched normal control.

In order to determine whether changes in LDL lipid composition contributed to the results observed, we measured cholesterol, triglycerides and total protein in the pre- and post-control LDL isolated from 11 of the 16 hospitalized patients studied and in eight of the 11 normal control subjects from whom enough LDL was available (Table 4). The ratio between free and total cholesterol was determined in the LDL isolated from eight patients in poor metabolic control; five patients in good metabolic control and six normal subjects (Table 4). A significant decrease of the LDL triglyceride/protein ratio ($p < 0.05$) was observed in the post-control LDL. To evaluate further possible alterations of the LDL composition, we compared total LDL-protein and LDL-apolipoprotein B concentrations in LDL isolated from six patients in poor metabolic control, five in good metabolic control, and five normal subjects. The apolipoprotein B content was 97.8 ± 3.4%, 98. ± 3.3% and 98.1 ± 4.6% of the total LDL protein levels (mean ± SD), respectively in the normal subjects and in the patients in good and poor metabolic control.

Discussion

Our results indicate that normal fibroblasts showed decreased uptake and intracellular degradation of LDL isolated from diabetic patients in poor metabolic control. The differences observed were probably not related to LDL aging since storage of LDL from normal control subjects did not affect uptake or

degradation. There was also no relationship between degradation and the length of patient hospitalization.

These differences were consistent in 9 of 13 patients studied. The other four did not follow the general trend. Two of these latter patients were hospitalized in fair control which may have explained the negative results. The other two patients showed some increase in degradation but not internalization. To support further the role of metabolic control in our findings, a significant correlation was found between the differences in HbA_{1c} and glucose and the differences in the degradation of pre- and post-control LDL.

Our results also show that the degradation of LDL isolated from age- and sex-matched normal subjects is similar to that isolated from diabetic patients in good metabolic control. In contrast, degradation of LDL from normal subjects is significantly higher than that obtained with LDL isolated from diabetics in poor control. A similar finding was observed when two patients in poor metabolic control and two sex- and age-matched normal subjects were studied under culture conditions that led to down-regulation of LDL receptors. The latter experiment was performed since it was more likely to reproduce the conditions encountered by the peripheral cells in vivo.

These results suggest a possible mechanism for enhanced atherosclerosis in diabetic patients. As postulated by Goldstein and Brown [2], the LDL pathway protects against atherosclerosis, allowing cells to take up LDL to satisfy cholesterol needs and to maintain plasma LDL concentrations below threshold levels for atherosclerosis. In diabetics, no abnormalities in the LDL pathway have been shown to date [3] but in this study, we demonstrate that there is a significant decrease of internalization and catabolism of the LDL isolated from diabetic patients in poor control. The decrease in the internalization and catabolism of pre-control LDL is not due to alterations in the number of cell receptors since we used the same pool of normal human fibroblasts in all our experiments. It is most probably due to alterations in LDL composition.

Whatever the mechanism might be, this decrease could eventually lead to plasma LDL concentrations that exceed the threshold levels for atherosclerosis. In support of this concept, we found that the degree of impairment of degradation of the LDL isolated from diabetic subjects in poor control was related to a rise in plasma LDL-cholesterol levels.

As we mentioned before, the differences observed in LDL behaviour are likely to be due to alterations in LDL composition. These alterations might be in LDL lipid composition, LDL apoprotein composition and/or in the carbohydrate moiety of apolipoprotein LDL. The lipid composition of LDL isolated from diabetic patients has been studied previously by Schonfeld et al. [18] who reported an increased triglyceride content of LDL. In our present studies we found similar alterations in the composition of LDL isolated from diabetic patients before metabolic control. Since the pre-control LDL were less internalized and degraded by fibroblasts than those isolated from the same patients after metabolic control, a logical conclusion would be that the significant increase of the triglyceride content of LDL that we observed can interfere with its metabolic behaviour. It has been postulated that the triglyceride content of LDL may greatly influence the fluidity of the lipid core of the particle [19]. However, it is not clear that this would affect LDL metabolism by fibroblasts. Whether the alteration in the triglyceride/protein ratio is the main or only factor determining the metabolic properties of diabetic LDL, cannot be concluded from our experiments. Further studies of LDL lipid composition are needed. In particular, phospholipids must be quantitated, since they are major lipid constituents of the LDL surface. According to Steinberg [20], the apoprotein-phospholipid interaction confers the specificity for LDL to be recognized by the cell.

Alterations in LDL apoprotein composition could also explain these changes in LDL behaviour. The LDL protein consists mainly of apolipoprotein B, but also contains small amounts of apolipoprotein C [21]. Havel [22] has shown that when the content of the C apoproteins is increased in VLDL and chylomicrons, their uptake by perfused livers is inhibited. In order to explore indirectly the possibility that a similar phenomenon may occur in LDL, we determined the apolipoprotein B content of LDL and related that with the LDL total protein content. No differences existed between patients in poor and good metabolic control and normal subjects.

Finally, differences in the carbohydrate moiety of LDL could also explain the alterations in LDL behaviour. Morell et al. [23] postulated that one of the roles of the carbohydrate portion of glycoproteins is the regulation of protein catabolism. Studies on the function of the carbohydrate moiety of apolipoprotein LDL have been carried out. Shireman and Fisher [24] found no differences in the uptake of LDL when its carbohydrate moiety was removed completely. In this study, no reference to changes in LDL charge was made and catabolism was not studied. Other authors found significant differences in LDL internalization and clearance after removal of sialic acid radicals from native LDL [25]. The removal of sialic acid led to alterations in LDL charge.

Non-enzymatic glycosylation of Apo B, secondary to hyperglycaemia, could also lead to alterations of LDL behaviour. Non-enzymatic glycosylation of

several proteins have been described in diabetes [26] and some differences in behaviour of glycosylated proteins have been found including decreased solubility and greater aggregability [27]. Furthermore, Witztum et al. [28] recently found that LDL glycosylated in vitro was less degraded by fibroblasts than normal LDL.

The precise mechanisms of altered LDL-cell kinetics in diabetes mellitus are unknown and await further studies. However, the present study represents the first observation that metabolic control in diabetes mellitus can alter LDL-cell interaction and may have relevance to the problems of atherosclerosis in diabetic patients.

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