Cardiovascular diseases are the most common complications observed in type 2 diabetes. The multiple abnormalities in lipoprotein metabolism frequently associated with this disorder play an important role in the premature development of atherosclerosis in type 2 diabetic patients (1–4).

This dyslipidemia is characterized both by quantitative abnormalities consisting in hypertriglyceridemia and decreased plasma HDL cholesterol level and by qualitative abnormalities, mainly small LDL size and triglyceride enrichment of every lipoprotein subclass. LDL cholesterol concentration is similar to that observed in nondiabetic subjects or slightly more elevated. Despite a quite normal concentration, the metabolism of LDL particles is modified in poorly controlled type 2 diabetic patients. Indeed, in several kinetic studies, LDL fractional catabolic rate (FCR) has been found to be significantly slowed down, regardless of whether patients are on oral antidiabetic therapy (5–7). In parallel, LDL synthesis rate is normal or tends to be decreased, resulting in a normal or only moderately increased plasma LDL concentration.

Recently, we demonstrated that replacing oral antidiabetic treatment with insulin therapy corrected the abnormalities of LDL metabolism in type 2 diabetic patients, both by normalizing FCR and increasing synthetic rate (8). Due to these two simultaneous changes, the concentration of circulating LDL particles remains constant.

The aim of this work was to get further insight into the mechanisms responsible for the changes in LDL FCR reported by kinetic studies in type 2 diabetic patients. It has been previously demonstrated that insulin upregulates the mRNA expression of LDL receptor and its expression at the surface of cultured cells (9–11). Because type 2 diabetes is characterized by a defect in insulin action, we decided to test the hypothesis that changes of LDL receptor expression underlie the variations of LDL FCR observed in type 2 diabetic patients both before and after the introduction of insulin treatment.

**OBJECTIVE** — In type 2 diabetic patients with poor metabolic control, kinetic studies have demonstrated that LDL fractional catabolic rate (FCR) is slowed down, whereas it is normalized on insulin therapy. This study was designed to analyze whether variations in the expression of LDL receptors at the cell surface could explain the results observed in kinetic studies.

**RESEARCH DESIGN AND METHODS** — LDL receptors were quantified at the surface of mononuclear cells in fresh fasting blood samples by a flow cytometry method in 21 control subjects and 21 type 2 diabetic patients before and 3 months after the introduction of insulin therapy and concomitant removal of oral antidiabetic drugs.

**RESULTS** — Before insulin treatment, monocyte LDL receptor expression was reduced by 41% (6,439 ± 2,310 vs. 10,846 ± 2,764 receptors per monocyte, *P < 0.001*) in type 2 diabetic patients compared with control subjects. It increased by 57% after 3 months of insulin therapy (10,96 ± 5,657 vs. 6,439 ± 2,310, *P < 0.01*) and was similar to that observed in control subjects.

**CONCLUSIONS** — Our results suggest that insulin plays an important role in the in vivo expression of LDL receptors. Moreover, modulations in the expression of LDL receptors in type 2 diabetic patients either with poor metabolic control or on insulin therapy are likely to contribute to the variations of LDL FCR demonstrated by kinetic studies under those circumstances.
patients performed capillary glucose monitoring three times per day. They were educated in order to adapt insulin doses according to capillary blood glucose level. The goal was to obtain a fasting blood glucose level between 4.5 and 8.25 mmol/l. The patients were asked to increase their insulin dose by 2 units when fasting glycermia was >8.25 mmol/l and to reduce it by 2 units when fasting glycermia was <4.5 mmol/l.

**Experimental protocol**

LDL receptors were quantified at the surface of mononuclear cells in fresh fasting blood samples taken at 8:00 A.M. This analysis was performed twice in type 2 diabetic patients, first before the introduction of insulin therapy and then 3 months later. Blood samples were drawn in tubes with EDTA (Becton Dickinson, Meylan, France). At the same time, blood was taken for the measurement of lipid parameters, glycemia, insulinemia, and glycated HbA1c.

**LDL receptor measurement**

LDL receptor expression was quantified on peripheral mononuclear cells isolated from blood samples immediately after blood drawing. Erythrocytes were lysed by mixing 1 ml blood and 4 ml of an hypotonic solution containing 0.83% NH4Cl, 0.004% EDTA, and 0.1% KHCO3. After a 30-min incubation at 4°C, samples were centrifugated at 300g for 5 min and the pellet was washed twice with the lysis solution. The pellet was then suspended in ice-cold PBS with 1% BSA and 0.1% NaN3. A volume containing 10^6 cells was then centrifuged, supernatant was removed, and cells incubated at 4°C for 30 min with 50 μl of a pure mouse monoclonal anti-LDL receptor IgG2b (clone 15C8) (Oncogene Research Products, Boston, MA). Subsequently, the samples were washed twice with PBS/1% BSA/0.1% NaN3 and resuspended in 1 ml of the same solution. Blanks were processed in parallel with the samples and in the same manner, except that anti-LDL receptor antibody was replaced with PBS/1% BSA/0.1% NaN3. A calibration curve linking the intensity of fluorescence and the number of antigenic sites was established using QIFIKIT. SetUp and Calibration beads (50 μl each) were washed as recommended by the manufacturer and incubated at 4°C for 30 min in the dark with 50 μl goat FITC-labeled anti-mouse IgG diluted 1:50 (QIFIKIT, Dako, Glostrup, Denmark).

**Statistical analysis**

Results are expressed as means ± SD. Statistical calculations were performed using the SPSS software package. Means in control and type 2 diabetic groups were compared using the Student’s t test. Means in type 2 diabetic patients before and after insulin therapy were compared using the paired Student’s t test. Correlation coefficients were calculated by the Spearman test. For type 2 diabetic patient subgroup analysis, the nonparametric Mann-Whitney U test was used. P values <0.05 were considered statistically significant.

**RESULTS**

**Metabolic parameters**

Clinical and glucose metabolism characteristics are presented in Table 1. The type 2 diabetic patients were significantly overweight compared with control subjects (BMI 28.4 ± 3.5 vs. 21.2 ± 2.3 kg/m², P < 0.001) and did not significantly change their weight on insulin therapy (BMI 29.2 ± 3.9 vs. 28.4 ± 3.5 kg/m²). Insulin therapy significantly improved glycemic control in type 2 diabetic patients, as assessed by fasting blood glucose concentration (10.1 ± 2.5 vs. 13.0 ± 3.6 mmol/l, P < 0.001) and HbA1c (8.7 ± 1.5 vs. 10.1 ± 1.9%, P < 0.01). Fasting
plasma insulin level was comparable in type 2 diabetic patients at their entry in the study and in control subjects (8.0 ± 6.0 vs. 5.3 ± 1.7 mU/l) and was significantly higher in insulin-treated patients than in control subjects (15.6 ± 10.9 vs. 5.3 ± 1.7 mU/l, *P* < 0.01).

**Lipid parameters**

Before the introduction of insulin therapy, type 2 diabetic patients presented a 2.5-fold increase in fasting plasma triglycerides compared with control subjects (2.38 ± 1.48 vs. 0.92 ± 0.51 mmol/l, *P* < 0.001) (Table 1). On insulin therapy, plasma triglycerides fell by 25% (1.79 ± 0.82 vs. 2.38 ± 1.48 mmol/l, *P* < 0.05). Nevertheless, plasma triglycerides remained 1.9-fold higher in type 2 diabetic subjects than in control subjects (*P* < 0.001). Total and LDL plasma cholesterol concentrations were initially comparable between patients and control subjects and were not modified by insulin treatment in type 2 diabetic patients. HDL cholesterol concentration was reduced by 26% in type 2 diabetic patients before insulin therapy (1.19 ± 0.24 vs. 1.63 ± 0.42 mmol/l, *P* < 0.001). It increased on insulin therapy (1.38 ± 0.37 vs. 1.19 ± 0.24 mmol/l, *P* < 0.05) but remained 14% lower than that in control subjects (*P* < 0.05).

**Monocyte LDL receptors**

The mean number of LDL receptors at the surface of blood mononuclear cells was reduced on average by 41% (6.439 ± 2,310 vs. 10,846 ± 2,764, *P* < 0.001) in type 2 diabetic patients with poor metabolic control before insulin treatment compared with control subjects (Fig. 1). After 3 months of insulin therapy, LDL receptor expression in type 2 diabetic patients increased by 57% (10,096 ± 5,657 vs. 6,439 ± 2,310, *P* < 0.01) and was similar to that observed in control subjects. Considering type 2 diabetic patients before insulin and control subjects together, LDL receptor expression was negatively correlated with age, BMI, glycemia, and triglycerides (data not shown) and positively correlated with HDL cholesterol. However, none of these correlations remained significant when we analyzed each group separately. LDL receptor expression was correlated with neither total or LDL cholesterol nor insulinemia when considering the two groups together or individually. Moreover, in type 2 diabetic patients, LDL receptor expression was not correlated with glycated HbA1c. A step-by-step multiple regression analysis performed on the data of control subjects and type 2 diabetic patients before insulin treatment, including the presence of diabetes, age, sex, BMI, fasting blood glucose, triglyceridemia, and LDL and HDL cholesterol levels, demonstrated that the number of LDL receptors per monocyte was only dependent on the presence or absence of type 2 diabetes (*P* < 0.001). This factor explained 57% of the variations of LDL receptor expression. The difference of LDL receptor expression in type 2 diabetic patients before insulin treatment with that after was not significantly correlated with any of the differences for BMI, fasting blood glucose, HbA1c, insulinemia, triglycerides, or total, HDL, or LDL cholesterol. Moreover, the increase in LDL receptors in insulin-treated patients was not correlated with the amount of injected insulin, as expressed in units per kilograms of body weight.

We wondered whether LDL receptor expression was parallel to glycemia in type 2 diabetic patients. To try to answer this question, we divided the type 2 diabetic group into two subgroups: patients with fasting glycemia before insulin treatment <12 mmol/l (*n* = 10) and patients with fasting glycemia >12 mmol/l (*n* = 11). Mean glycemia was 10.1 ± 1.4 and 16.1 ± 2.4 mmol/l (*P* < 0.001) in each subgroup, respectively. Before the introduction of insulin therapy, LDL receptor expression was still comparable between these two subgroups (9,612 ± 2,932 vs. 6,283 ± 1,697). After 3 months of insulin therapy, LDL receptor expression was still comparable between these two subgroups (9,696 ± 4,079 vs. 10,459 ± 6,481). The difference of LDL receptor expression after insulin treatment with that before was similar in the two subgroups (3,084 ± 4,313 vs. 4,176 ± 7,219), whereas the difference of fasting glycemia after insulin treatment with that before was significantly lesser in patients with an initial fasting glycemia <12 mmol/l than in patients with an initial fasting glycemia >12 mmol/l (*−0.83 ± 4.05 vs. −5.13 ± 3.60 mmol/l, *P* < 0.01).

**CONCLUSIONS** — This study was designed to test the hypothesis that changes in the expression of LDL receptors in type 2 diabetic patients before and after the introduction of insulin therapy could explain both the decrease of LDL FCR observed in poorly controlled type 2 diabetic subjects and its normalization in insulin-treated patients. By quantifying LDL receptor expression on blood mononuclear cells with a flow cytometry method, we demonstrated for the first time that LDL receptor expression is decreased in type 2 diabetic patients with a poor metabolic control on oral antidiabetic drugs compared with nondiabetic subjects and, moreover, that it is normal-

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**Figure 1**—Expression of monocyte LDL receptors in 21 type 2 diabetic patients before and after insulin therapy and in 21 control subjects. LDL receptors were quantified at the surface of mononuclear cells in fresh fasting blood samples by a flow cytometry method, using a mouse monoclonal anti-LDL receptor antibody and a FITC-labeled goat antimouse antibody. A calibration curve linking the intensity of fluorescence and the number of antigenic sites was established using QIFIKIT (Dako). Values represent the mean number (±SD) of LDL receptors per cell. Means are significantly different between type 2 diabetic patients before insulin and control subjects (*P* < 0.001, Student’s *t* test), as well as between type 2 diabetic patients before and after the introduction of insulin treatment (*P* < 0.01, paired *t* test).
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Liver but not mononuclear cell LDL receptors play a key role in LDL catabolism in vivo. For ethical reasons, receptor quantification on liver biopsies is not currently conceivable. Several studies have shown that LDL receptor measurement on mononuclear cells is physiologically relevant for the determination of LDL receptor status. Indeed, in human mononuclear cells, LDL receptor gene expression has been demonstrated to parallel and be coordinately regulated to gene expression in human liver (13,14). Moreover, the number of LDL receptors on mononuclear cells has been shown to be susceptible to the effect of well-recognized modulators of LDL receptor number such as saturated fatty acids and dietary cholesterol (14,15).

It is noteworthy that we performed LDL receptor measurements on freshly isolated mononuclear cells; therefore, our results are likely to reflect the in vivo situation.

Insulin upregulates both the mRNA expression of LDL receptor and its expression at the surface of cultured cells (9,11). Thus, the variations of the LDL receptor expression we observed both before and after the introduction of insulin therapy in poorly controlled type 2 diabetic patients are likely to be related to insulin and insulin action. We found that plasma insulin level was in a normal range in type 2 diabetic patients before insulin therapy. Nevertheless, type 2 diabetes is characterized by a resistance of peripheral tissues to the action of insulin, and a normal insulinemia in regard to elevated plasma glucose levels indicates a relative insulin deficiency (16). Thus, the decreased expression of LDL receptors we observed in type 2 diabetic patients before the introduction of insulin therapy is likely to be explained by a relative insulin deficiency. This hypothesis is highly strengthened by the second part of our study.

The other major finding of our work is the normalization of the expression of LDL receptors at the mononuclear cell surface in type 2 diabetic patients treated by insulin for 3 months. To our knowledge, only one previous study (17) reported a positive effect of insulin therapy on LDL receptor expression in diabetic patients, but this work differed from ours on several points: only seven patients were recruited, the type of diabetes (type 1 or 2 diabetes) was not specified, and the effect of insulin was studied only in the short term (1 week after the introduction of insulin therapy). In the present study, the normalization of LDL receptor expression is probably directly linked to the administration of insulin, which tended to correct the relative insulin deficiency. At first glance, the lack of correlation between the amount of injected insulin and the difference of the expression of LDL receptors between the two quantifications might be surprising. In fact, insulin action depends on several factors and is not correlated with plasma insulin level, which explains the lack of correlation between either the dose of administrated insulin or plasma insulin level and their effect on the expression of LDL receptors (18,19).

Based on plasma concentration or urinary excretion of mevalonate, cholesterol synthesis has been demonstrated to be both increased in type 2 diabetic patients and normalized by intensive insulin treatment (20,21). Therefore, the decreased expression of LDL receptors in these patients might also be regarded as the consequence of an increased intracellular concentration of cholesterol, related to an enhanced cholesterol synthesis (22). However, in cultured cells, insulin upregulates cholesterol synthesis (23). In light of this observation, the elevated cholesterol synthesis demonstrated in type 2 diabetes, which is characterized by a relative insulin deficiency, cannot be interpreted as a primary event likely to induce a negative feedback on LDL receptor expression. Rather, the increased cholesterol synthesis seems to be the consequence of reduced LDL receptor expression in poorly controlled type 2 diabetic patients. The decrease of cholesterol synthesis in insulin-treated type 2 diabetic patients also seems to be the consequence of increased LDL receptor expression.

The increase in LDL receptor expression we observed in type 2 diabetic patients is, in the strict sense, the result of both removal of oral antidiabetic drugs and addition of insulin. It is highly unlikely that the 41% increase in LDL receptor expression 3 months after the introduction of insulin therapy in type 2 diabetic patients was due to removal of oral antidiabetic drugs. Indeed, sulfonylureas stimulate insulin secretion and metformin stimulates insulin sensitivity. If these two drugs had an effect on LDL receptor expression, they would increase LDL receptor expression. Moreover, in the present study, we think that the influence of antidiabetic drugs on LDL receptor expression is weak. Indeed, in our patients, the glycemic control was poor, indicating that antidiabetic drugs do not effectively improve metabolic disturbances in type 2 diabetes.

A very interesting finding of this work is that the variations of LDL receptors at the cell surface in type 2 diabetic patients parallel the fluctuations of LDL FCR we and others observed in in vivo kinetic studies. We recently demonstrated that LDL catabolism was slowed down by 25% on average in type 2 diabetic patients with poor metabolic control, whereas it was normalized after 3 months of insulin treatment (5,8). In this work, LDL receptor expression was diminished by 41% in poorly controlled patients, as well as normalized in insulin treatment. We wondered which responsibility could be assigned to the decrease of LDL receptors for the decrease of LDL catabolism in patients before insulin treatment. In heterozygous familial hypercholesterolemia, characterized by only 50% functional and efficient LDL receptors, LDL FCR has been demonstrated to be significantly slowed down (24,25). So, the hypothesis that a 41% decrease of the expression of LDL receptors in type 2 diabetic patients is highly implied in the decrease of LDL FCR seems to be relevant. In the same manner, the normalization of the expression of LDL receptors after the introduction of insulin therapy probably plays an important role in the normalization of LDL FCR in these patients.

In type 2 diabetic patients, LDLs are triglyceride rich and glycosylated, which is likely to decrease their affinity to the LDL receptor (26). The correction of these abnormalities by insulin therapy may also contribute to the normalization of LDL FCR in insulin-treated type 2 diabetic patients, parallel to the increase of LDL receptor expression. However, the correction of LDL qualitative abnormalities by insulin therapy is far from being constant in these patients (27,28). It is noteworthy that in our group of type 2 diabetic patients, we observed no significant change of the triglyceride-to-apoB, cholesteryl ester-to-apoB, and triglyceride-to-cholesterol ester ratios and of LDL glycation 3 months after the introduction of insulin therapy (data not shown).
Another apparently intriguing result is the lack of decrease in plasma LDL cholesterol concentration in insulin-treated patients, whereas LDL receptor expression is increased, suggesting that LDL clearance rate is probably also increased. In fact, the in vivo kinetic study we recently performed showed that 3 months after the introduction of insulin therapy in patients with poor metabolic control, the increase in LDL FCR was accompanied by an increase in LDL synthetic rate, explaining why LDL concentration can be unchanged after the introduction of insulin therapy despite an increase in FCR.

In conclusion, we demonstrate that despite oral antidiabetic treatment, LDL receptor expression is decreased in type 2 diabetic patients with a poor metabolic control compared with nondiabetic subjects and that it is normalized after 3 months of insulin therapy. This study therefore favors an important in vivo role of insulin in the expression of LDL receptors. Moreover, the number of LDL receptors at the cell surface evolves in the same way as LDL FCR in type 2 diabetes, suggesting that a low LDL receptor expression may play an important role in the decrease of LDL FCR observed in type 2 diabetic patients with poor metabolic control, whereas the normalization of this expression may contribute to the normalization of LDL FCR in insulin-treated patients.

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References