In Vivo Stimulation of Low-Density Lipoprotein Degradation by Insulin

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SUMMARY

The effect of insulin on low-density lipoprotein (LDL) metabolism in vivo was evaluated using the euglycemic insulin clamp technique. In seven subjects, mononuclear cells isolated after a 4-h insulin infusion degraded more ^{125}I-labeled LDL than cells isolated after a saline infusion in six control subjects. In addition, insulin caused the accelerated disappearance of ^{125}I-labeled LDL from plasma in subjects previously injected with autologous ^{125}I-LDL. Infusion of saline had no such effect. These data suggest that insulin, in vivo, stimulates LDL catabolism and could thereby influence LDL cholesterol levels. Insulin-induced stimulation of LDL catabolism could account for the reduction of LDL-cholesterol levels observed in intensively treated type I diabetic patients. DIABETES 33:333–338, April 1984.

Accelerated vascular disease is a major cause of morbidity and mortality in insulin-dependent diabetes.[1,2] This may in part be due to abnormalities in lipoprotein metabolism and lipoprotein levels that have been noted in these patients.[3–6] Observed elevations of low-density lipoprotein (LDL) levels in particular may predispose to premature vascular disease in nondiabetic individuals.[7] Increased LDL cholesterol levels have been noted in insulin-dependent diabetic patients,[8] and studies examining the effect of intensive insulin therapy on LDL cholesterol levels have shown a decrease of approximately 20% in these levels.[9–12]

The change in LDL cholesterol level associated with improved diabetic control could be due to decreased LDL cholesterol synthesis or increased degradation. Chait et al. have shown previously that insulin can increase LDL degradation in cultured human fibroblasts by increasing LDL receptor activity.[13] If insulin produced this same effect on cells in vivo, insulin therapy in insulin-deficient diabetic subjects could decrease the level of LDL cholesterol by increasing peripheral LDL degradation. Previous studies from our laboratory have shown that the endogenous hyperinsulinemia that is associated with the use of total parenteral nutrition results in an accelerated catabolism of LDL in man.[14] We therefore sought to directly determine whether insulin can stimulate LDL catabolism in vivo, using the euglycemic insulin clamp technique in healthy lean male volunteers. The effect of exogenous insulin on extrahepatic LDL degradation was studied in circulating mononuclear cells, because these cells can be quickly isolated to reflect the in vivo milieu, and have been shown to possess the classic high-affinity LDL receptor[15] first described in fibroblasts.[16] In addition to studying changes in cellular LDL degradation, the effect of insulin infusion on autologous ^{125}I-LDL disappearance from plasma was examined. This protocol allowed us to study the influence of insulin without changes in other variables such as body weight, diet, activity, or glucose and LDL levels that might complicate more long-term studies using insulin-treated diabetic patients.

METHODS

Subjects. Lean healthy males, aged 20–40 yr, underwent euglycemic insulin clamps or 0.25% normal saline infusions after an overnight fast. Peripheral blood mononuclear cells were isolated before and at the end of the infusions in 13 studies, while 5 subjects had 10 insulin and/or saline infusions several days after the injection of autologous ^{125}I-labeled LDL. All the subjects were normolipidemic, nonsmokers, and were receiving no medications.

Insulin clamp. Insulin (1 mU/kg/min) was infused to obtain steady-state plasma insulin levels of approximately 80 μU/ml. Euglycemia was maintained with a dextrose infusion that

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was started 5 min after the beginning of the insulin infusion. Glucose levels were measured every 5 min during the 4-h study. Blood for lipoprotein analysis and for the separation of mononuclear cells was obtained before and immediately after the infusion. The amount of glucose metabolized during the 4-h study in mg/kg/min (M) was calculated using previously described methods.[17]

Control studies were performed in which 0.25% saline was infused at a rate that delivered equivalent amounts of fluid to the intravascular space (as measured by changes in hematocrit and plasma albumin levels).

Preparation of cells. Venous blood (40 ml) was drawn immediately before and immediately after a 4-h euglycemic insulin clamp or saline infusion. Peripheral blood mononuclear cells were isolated by a modification of the method of Boyum.[18] The blood was collected in heparinized syringes (10 U heparin/ml blood) and divided into 10-ml aliquots. These were diluted with equal volumes of 0.15 M NaCl. Ficoll-Paque (15 ml) was layered under each 20-ml mixture of blood and saline and was centrifuged at 400 × *g* for 40 min at room temperature. The resultant mononuclear cell band was washed twice in Hanks' balanced salt solution and then taken up in RPMI 1640 medium. The cells were then plated at a density of 2.5×10^6 cells/35-mm dish in 10% (v/v) pooled human lipoprotein-deficient serum. Cell identification was done by Wright-Giemsa staining of cytospin preparations or by analysis of Coulter channelyzer volume distributions. Monocytes comprised 20–40% of the mononuclear cells; the rest were lymphocytes.

LDL degradation. Low-density lipoprotein (d = 1.019–1.063 g/ml) from normal volunteers was prepared by preparative ultracentrifugation and was iodinated with ^{125}I using the iodine monochloride method as modified for lipoproteins.[19] The degradation of ^{125}I-labeled LDL at a concentration within the high-affinity binding range was used as an integrated index of LDL receptor activity.[20] In three studies, specific, high-affinity degradation was measured by subtracting the ^{125}I-LDL degradation that occurred in the presence of a 20-fold excess of unlabeled LDL from total ^{125}I-LDL degradation. Freshly isolated mononuclear cells were immediately incubated with ^{125}I-LDL (7.5 μg/ml) (with or without excess unlabeled LDL) in 10% lipoprotein-deficient serum for a 4-h degradation assay. At the end of the incubation, an aliquot of the medium was obtained for determination of the TCA-soluble degradation products that were not free iodide.[13,21] Non-cell-associated degradation, observed under identical conditions in cell-free dishes, was subtracted to give a measure of cell-specific LDL degradation. The time from each blood sampling to the beginning of the LDL degradation assay was approximately 120 min.

Catabolism of ^{125}I-LDL in vivo. Autologous LDL (d = 1.019–1.063 g/ml) was prepared from five normal male volunteers and was iodinated as described above. Supersaturated KI was administered for 3 days before the injection of ^{125}I-LDL, and was continued for the duration of the study. Approximately 50 μCi of autologous ^{125}I-LDL was injected after an overnight fast. A 4-h euglycemic hyperinsulinemic clamp and/or saline infusion was performed on each subject. Blood samples were obtained for measurement of plasma radioactivity every 6–8 h on the first day and on the days preceding and following the infusions. Daily samples were obtained on all other days. More than 98% of plasma radioactivity was TCA-precipitable and has been previously shown to be entirely within LDL.[19] The subjects remained on their usual diets and performed their usual daily activities for the duration of the study. Analysis of plasma radioactivity was performed using Matthews' method[22] and the SAAM computer program[23] on a Vax 11/780 digital computer.

Lipoprotein quantification and insulin levels. Plasma was separated by ultracentrifugation, and lipoprotein cholesterol and triglyceride were determined by standard Lipid Research Clinic methods using a Beckman AutoAnalyzer II (Beckman Instruments, Fullerton, California).[24] Insulin was measured using a double-antibody radioimmunoassay technique.[25]

Statistics. Values before and after the clamp were compared using the paired *t* test. Insulin and saline infusions were compared using the *t* test for two means.[26]

RESULTS

Lipoprotein and insulin levels. The changes produced by the 4-h insulin infusion on the levels of LDL cholesterol, HDL cholesterol, VLDL triglycerides, and VLDL cholesterol are shown in Table 1. Very-low-density lipoprotein triglyceride levels fell substantially after only 4 h of hyperinsulinemia ($P < 0.01$) as did VLDL-cholesterol ($P < 0.05$). Saline infusion produced no decrease in these parameters. Neither LDL cholesterol nor HDL cholesterol levels changed significantly during the insulin or control infusions. Plasma insulin values rose from 7.4 ± 1.2 μU/ml at baseline to 80.1 ± 4.4 μU/ml during the insulin infusion (mean ± SEM). The increase occurred during the first 30 min of the infusion and remained

TABLE 1
Plasma lipoproteins before and after insulin infusion (mg/dl)

	LDL-C		HDL-C		VLDL-TG		VLDL-C	
Subject	Pre	Post	Pre	Post	Pre	Post	Pre	Post
1	128	118	53	48	50	16	16	10
2	97	95	38	37	41	25	9	5
3	96	96	58	58	48	22	13	7
4	89	80	62	63	6	2	4	3
5	63	65	35	36	43	30	13	10
6	139	133	57	56	27	20	7	6
7	128	138	45	45	79	71	9	11

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; VLDL-TG: very-low-density lipoprotein triglyceride; and VLDL-C: very-low-density lipoprotein cholesterol.

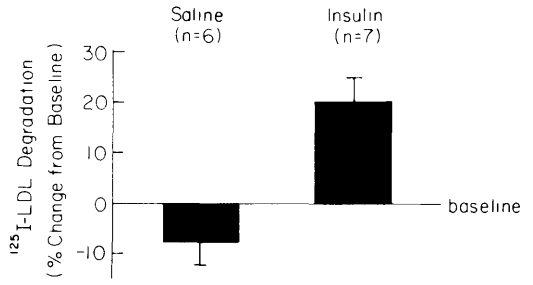

FIGURE 1. The effect of insulin and saline infusions on LDL degradation by freshly isolated mononuclear cells. Values shown are mean ± SEM of the postinfusion values, expressed as percent change from baseline (i.e., preinfusion values). Before the infusion of insulin, freshly isolated mononuclear cells degraded 12.2 ± 2.3 ng LDL protein/2.5 × 10^6 cells/4 h (mean ± SEM). Before the infusion of saline the values for cellular degradation were 8.4 ± 1.1 ng/2.5 × 10^6 cells/4 h.

stable during the balance of the study. Mean fasting blood glucose was 78 ± 2.4 mg/dl (mean ± SEM) and the mean coefficient of variation for glucose during the clamp studies was 15%.

LDL degradation by mononuclear cells. The changes in ^{125}I-LDL degradation by mononuclear cells isolated before and after the insulin or saline infusion are shown in Figure 1. Because monocytes, rather than lymphocytes, account for most of the LDL receptor activity in freshly isolated mononuclear cells,[27,28] the proportion of monocytes in each cell isolate was quantified. There was no difference in the proportion of monocytes in cell preparations isolated before versus after the infusions. Monocyte number, therefore, did not influence the observed changes in LDL degradation.[28] Hyperinsulinemia increased total ^{125}I-LDL degradation by an average of 20 ± 6% (mean ± SEM). When specific high-affinity ^{125}I-LDL degradation by monocytes was measured (as described in METHODS), a similar increase in ^{125}I-LDL degradation of 23 ± 3% was observed (Table 2). Control studies during which 0.25% saline was infused produced no significant change in LDL degradation (−7 ± 5%) (Figure 1). Comparing degradation values expressed as a percent of baseline values, the change in ^{125}I-LDL degradation produced by insulin was found to be significant ($P < 0.02$, paired t test), while that produced by saline was not ($P > 0.2$). The difference between postinfusion values for saline versus insulin was highly significant ($P < 0.005$; Student's t test).

To determine whether the variability in the stimulation of LDL receptor activity was related to insulin effects on glucose metabolism, the relationship between percent increase in LDL degradation and the amount of glucose metabolized (M) was examined. No correlation between M and percent stimulation of LDL receptor activity was observed (data not shown).

Disappearance of autologous ^{125}I-LDL. In addition to the experiments using mononuclear cells, an attempt was made to determine whether the 4-h insulin or saline infusion influenced the disappearance of autologous ^{125}I-LDL. The ^{125}I-LDL plasma disappearance curves for five individuals are shown in Figures 2 and 3. To analyze the data, the Matthews' analysis was used to generate the computer-derived curves, extrapolating through the insulin infusion period to show what would be expected if no infusion were performed. To show how these predicted and the observed values compare, we divided the observed radioactivity by the computer-predicted radioactivity.

Insulin infusions alone were performed in two subjects. An additional two subjects received either an insulin or saline infusion, followed 2 days later by the other infusion. Plasma radioactivity, measured before the infusions, oscillated around an ideal line (observed/predicted = 1). In all subjects, radioactivity measured during the insulin infusion showed a fall that persisted for 24 h. The saline infusions produced little change in the plasma radioactivity curves.

In one subject (subject #5) insulin infusions were performed 2 and 10 days after the injection of autologous ^{125}I-LDL (Figure 3, a and b). This same subject underwent a second study during which only 0.25% saline was infused on days 2 and 10. The infusion of insulin was associated with a reduction in ^{125}I-LDL radioactivity on both days 2 and 10, although the insulin-mediated fall in plasma radioactivity was not as marked as that seen in the other subjects. Observed plasma radioactivity remained below predicted levels for 24 h after the insulin infusion and thereafter returned to baseline.

Finally, a series of computer simulations were performed in which the fractional catabolic rate was increased by as much as 100% for the duration of the clamp and as long as 24 h afterward. The effect on the plasma curve was small and approximated the change produced by the insulin clamp. Thus, the small change in the plasma curve produced by insulin could represent a substantial change in LDL fractional catabolic rate.

DISCUSSION

This study examines the effect of the infusion of insulin on LDL degradation in vivo. The decision to study mononuclear cells was based on prior evidence that these cells, which can be quickly isolated, reflect the in vivo metabolic milieu. Different in vivo metabolic perturbations have been shown to produce changes in insulin binding,[29] lysosomal enzyme activity,[30] and cholesterol synthesis[31] by freshly isolated mononuclear cells. Of particular interest, LDL receptor activity has been shown to be responsive to changes in dietary cholesterol content.[32] We now report that a short-term insulin infusion stimulates LDL degradation in freshly isolated mononuclear cells, presumably reflecting a general stimulation of cellular LDL catabolism in vivo.

We have recently demonstrated that insulin in vitro does not stimulate LDL degradation in cultured human monocyte-macrophages.[33] In those studies, the in vitro exposure to insulin, followed by the measurement of LDL degradation,

TABLE 2
Receptor-mediated ^{125}I-LDL degradation by freshly isolated mononuclear cells. Effects of insulin (mean ± SEM of triplicate values)

Subject	Before insulin (ng/2.5 × 10^6 cells/4 h)	After insulin (ng/2.5 × 10^6 cells/4 h)	% Change
1	8.0 ± 0.7	10.0 ± 0.2	+25%
4	7.2 ± 0.2	8.4 ± 0.5	+17%
5	4.6 ± 0.4	5.8 ± 1.7	+26%

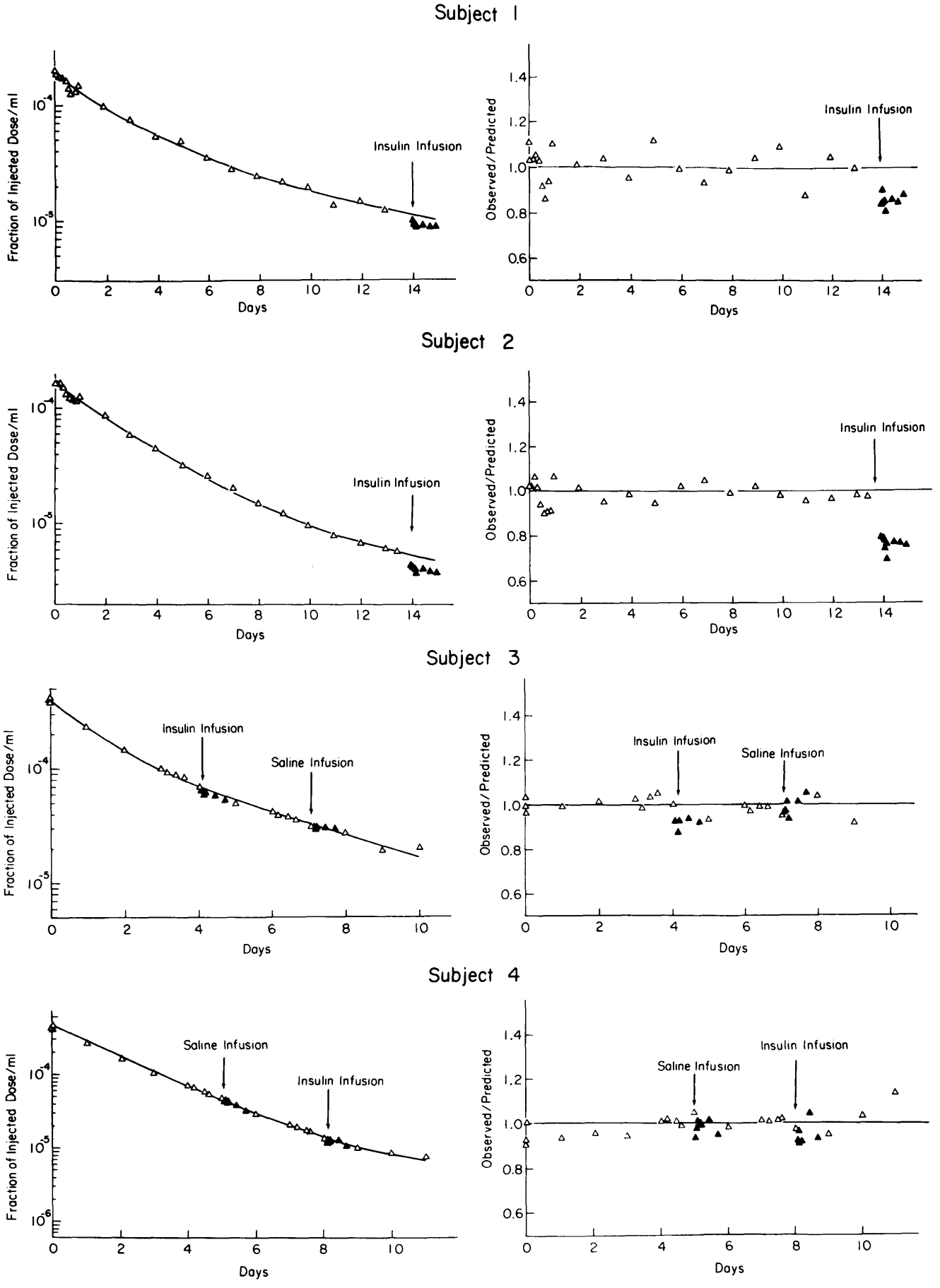

FIGURE 2. The effect of insulin and saline on the disappearance of injected ^{125}I-LDL from plasma in four subjects. Values measured before the infusion (△) and values measured during and 24 h after the infusion (▲). The curves shown on the right represent observed plasma radioactivity divided by predicted radioactivity.

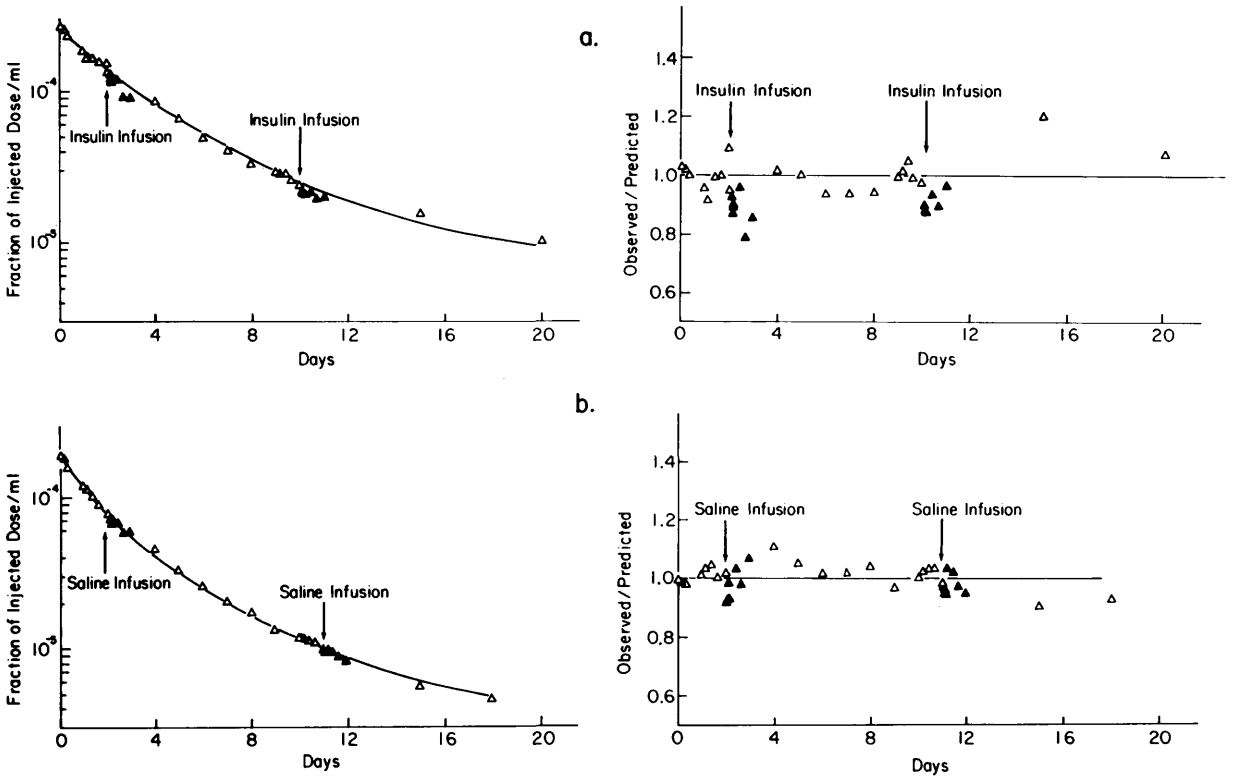

FIGURE 3. The effect of insulin (a) or saline (b) on the disappearance of ^{125}I-LDL from plasma in a fifth subject. The symbols are as described in Figure 2.

resulted in the cells being in culture for a longer time than in the presently described studies. When human monocytes are placed in culture, there are profound, time-dependent changes in cell morphology and metabolism, including an increase in low-density lipoprotein receptor activity as a function of time in culture.[33] The increased time in culture necessary to study the effects of insulin in vitro might, therefore, mask small increments in LDL degradation stimulated by insulin.

In the present study, considerable variation was observed in basal LDL degradation by freshly isolated mononuclear cells. All of the subjects were lean, on no medications, and were normolipidemic according to Lipid Research Clinic criteria. These studies were carried out over the course of several months and different lots of human lipoprotein-deficient serum and LDL were used, perhaps explaining some of the variability. All paired comparisons, however, were made with identical lots of lipoprotein-deficient serum and LDL. It is also of interest that in the 3 subjects in whom specific LDL degradation was measured, the variability is markedly decreased, suggesting that the large differences in basal degradation may be due to non-receptor-mediated uptake and degradation of LDL.

The observed acceleration in the disappearance of autologous ^{125}I-LDL from plasma produced by insulin is consistent with our findings in freshly isolated mononuclear cells. In all cases, the short-term infusion of insulin was associated with observed levels of plasma radioactivity that were less than values predicted on the basis of the preceding radioactive decay curve, while the infusion of saline had no such effect. This observation suggests that insulin resulted in an accelerated catabolism of LDL after only 4-h administration. Since the duration of the insulin infusion was short compared with the relatively long half-life of LDL in plasma, only small changes, of the magnitude observed, would be expected to occur. In view of the long half-life of LDL in relation to the duration of the insulin infusion, it is also not surprising that LDL-cholesterol levels did not change during the 4 h of hyperinsulinemia. The fall of VLDL triglyceride and cholesterol levels is consistent with the shorter half-life of VLDL versus LDL particles, and the previously demonstrated rapid stimulation of adipose tissue lipoprotein lipase activity by the insulin clamp.[34]

When prolonged endogenous hyperinsulinemia was previously evoked by the use of i.v. hyperalimentation, a more marked deviation of the LDL radioactivity decay curve was observed,[14] yet the changes observed were qualitatively similar to those observed in the present study. The finding that a 4-h infusion of insulin stimulates the catabolism of injected ^{125}I-labeled LDL adds credence to the observation that the cellular metabolism of LDL is enhanced by insulin infusion. Taken in conjunction with our previous in vitro findings of insulin-induced stimulation of LDL receptor activity in cultured skin fibroblasts,[13] and of accelerated LDL catabolism in association with the more prolonged hyperinsulinemia that occurs with total parenteral nutrition,[14] the findings from the present study provide strong support for a regulatory role of insulin in LDL catabolism.

Brown and Goldstein have recently suggested that LDL receptor activity may determine plasma levels of LDL.[35] Any stimulus to increase LDL receptor activity would lead to an increase in LDL fractional catabolic rate and, for a stable

synthetic rate, an eventual fall in plasma LDL levels. The results reported here suggest that insulin infusion can stimulate LDL receptor activity in freshly isolated mononuclear cells, which are representative of cells in vivo. The plasma radioactivity curves suggest that insulin's action is transient, as might be expected for a hormonal regulatory effect. The observed fall in LDL cholesterol level in intensively treated insulin-dependent diabetic patients[9-12] could thus result from an insulin-mediated stimulation of LDL degradation or from depression of LDL synthesis. Kissebah et al. have recently reported a study of LDL transport kinetics in a group of non-insulin-dependent diabetic patients.[36] In their group of moderately severe diabetic subjects, LDL fractional catabolic rate was reduced, with normal LDL synthetic rates. Although our data provide no information concerning LDL synthesis, they suggest that insulin can directly stimulate LDL catabolism in vivo. This insulin-induced enhancement of LDL catabolism and subsequent reduction of LDL cholesterol levels may favorably alter the risk for premature vascular disease in diabetic patients.

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