Effects of Insulin Treatment on Lipoprotein Composition and Function in Patients With IDDM

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Normolipidemic patients of both sexes with insulin-dependent diabetes mellitus have the same pervasive changes in lipoprotein surface and core lipid composition. The disproportionate increase observed in their lipoprotein free (unesterified) cholesterol relative to the predominant surface phospholipid lecithin (phosphatidycholine) is reflected by elevation of the FC-L ratio in their whole plasma, VLDL, HDL_2, and HDL_3. As a possible consequence of this qualitative disturbance, cholesteryl ester transfer is pathologically increased and the mass of cholesteryl ester transferred from HDL to VLDL+LDL is significantly greater in IDDM patients than in control subjects at 1, 2, and 4 hr ($P < 0.001$). Consistent with accelerated CET in vivo, the TG-CE core lipid ratio was decreased in VLDL from six subjects (IDDM 9.5 ± 0.8 vs. control 12.9 ± 3.4; $P < 0.01$) and increased in their HDL (diabetic 0.55 ± 0.11 vs. control 0.42 ± 0.04: $P < 0.025$). These abnormalities in lipoprotein composition and CET do not correlate with glycemic control and persist after intensive management with s.c. insulin. They may be related to the peripheral hyperinsulinemia that is an unavoidable consequence of conventional s.c. insulin administration because preliminary studies indicate that these disturbances in lipoprotein composition and function are reversed when systemic insulin levels are lowered and insulin is delivered into the portal circulation from an i.p. catheter connected to an implanted programmable s.c. insulin pump. *Diabetes* 41 (Suppl. 2):107–10, 1992

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IDDM, insulin-dependent diabetes; FC, free cholesterol; L, lecithin; VLDL, very-low-density lipoprotein; HDL, high-density lipoprotein; CET, cholesteryl ester transfer; CE, cholesteryl ester; LDL, low-density lipoprotein; TG, triglyceride; CSII, chronic s.c. insulin-infusion therapy; CIT, conventional s.c. insulin therapy; apoB, apolipoprotein B; CETP, cholesterol ester transfer protein; LpL, lipoprotein lipase.

It has been generally believed that lipoproteins do not pose a cardiovascular risk in insulin-treated IDDM patients because the routinely performed measurements of plasma triglyceride, cholesterol, and HDL-C are normal (1). Particularly puzzling has been that in many IDDM patients, HDL-C levels may actually be increased, reflecting higher concentrations of HDL_2, which in nondiabetic populations is believed to be the more antiatherogenic of the two HDL subfractions (2). It has become apparent recently that changes in lipoprotein composition can affect their functional properties and potential atherogenicity. Therefore, we were intrigued by the possibility that normolipidemic IDDM patients may have qualitative alterations in the plasma diabetic lipoproteins that might adversely affect their transport and metabolism.

To address this question, we undertook lipoprotein composition studies initially in a group of otherwise healthy older IDDM women with long histories of insulin treatment and a wide spectrum of glycemic control. As observed repeatedly earlier, their plasma lipids were remarkably normal and virtually identical to control values (3). Their lipoproteins, however, demonstrated an overall enrichment in free (unesterified) cholesterol relative to the surface phospholipid lecithin, resulting in a significant increase in the plasma FC-L ratio. This finding was of interest because Kuksis et al. showed in a Lipid Research Clinic study performed in Toronto that a disturbance of this type in lipoprotein FC was a potent new cardiovascular risk factor with a predictive power that exceeded that of total plasma and LDL cholesterol (4). Our finding this same alteration in normolipidemic IDDM patients indicated that they shared an abnormality in lipoprotein composition with nondiabetic patients with hypercholesterolemia. The obvious question arose whether it contributed to the accelerated atherogenesis that each group experienced. Later, this survey of lipo-

protein composition was extended to men with IDDM with a broad range of glycemic control, and an identical abnormality in lipoprotein FC was observed, with evidence that the VLDL+LDL fraction and HDL subfractions were all involved (5). In neither of these studies was there a correlation between the extent of this disturbance and glycemic control. In the latter one, the IDDM HDL subfractions (which did not differ in their total cholesterol content) were enriched in TG relative to cholesteryl ester and as a result had increases in their TG-CE ratios.

To further examine the question of whether this compositional abnormality could be reversed by intensive treatment with chronic s.c. insulin-infusion therapy and at the same time begin to assess the role of insulin in the genesis of this disturbance, we isolated and studied lipoproteins in a group of IDDM patients in a collaborative effort with colleagues at the University of Helsinki in Finland (6). Here, similar studies were performed in very well controlled IDDM subjects after 6-mo periods of intensive insulin treatment with CSII and conventional (s.c.) insulin therapy. Despite excellent glycemic control during both treatment periods (HbA_1; CIT 9.6 ± 0.7%; CSII 9.4 ± 0.5%; reference group 6–9%), the previously described abnormal increases in plasma and lipoprotein FC-L ratios and in the HDL TG-CE ratios persisted. Although results of this study could not exclude that these abnormalities were closely linked to the diabetes, they raised the possibility that they also might in some way be related to insulin treatment itself. While there is in vitro evidence from tissue culture studies suggesting that certain cellular actions of insulin may be atherogenic in people with diabetes (7), there is little experimental data indicating that insulin-induced alterations in lipoprotein composition may play a role in accelerating the development of macrovascular complications in diabetes.

What is the clinical relevance of these alterations in lipoprotein composition? It is now recognized that free cholesterol gradients among lipoproteins and between lipoproteins and cells are a critical determinant of the directional flux of cholesterol (8). Such gradients normally affect the efflux of FC from cells to an acceptor lipoprotein such as HDL, the first step in reverse cholesterol transport; conversely, they can affect the delivery of cholesterol to cells independent of the LDL receptor (9). Consequently, abnormalities in the FC content of lipoproteins can have pervasive effects on both the delivery of cholesterol and its removal from cells. Another step in reverse cholesterol transport, the transfer of cholesteryl ester from HDL to the apoB-containing lipoproteins, was shown by Morton (10) to be positively regulated by the FC content of VLDL.

To determine whether the enrichment we found in FC in VLDL+LDL in IDDM patients increased the rate of CET, we studied 4 IDDM patients receiving CIT. On this occasion, we measured their CET by both isotopic and mass-transfer assays and examined in further detail the composition of individual lipoproteins to determine whether they revealed evidence of enhanced in vivo neutral lipid transfer. Consistent with the in vitro studies of Morton (10), we observed that CET was accelerated in these IDDM patients studied with both assays (Figs. 1,2;

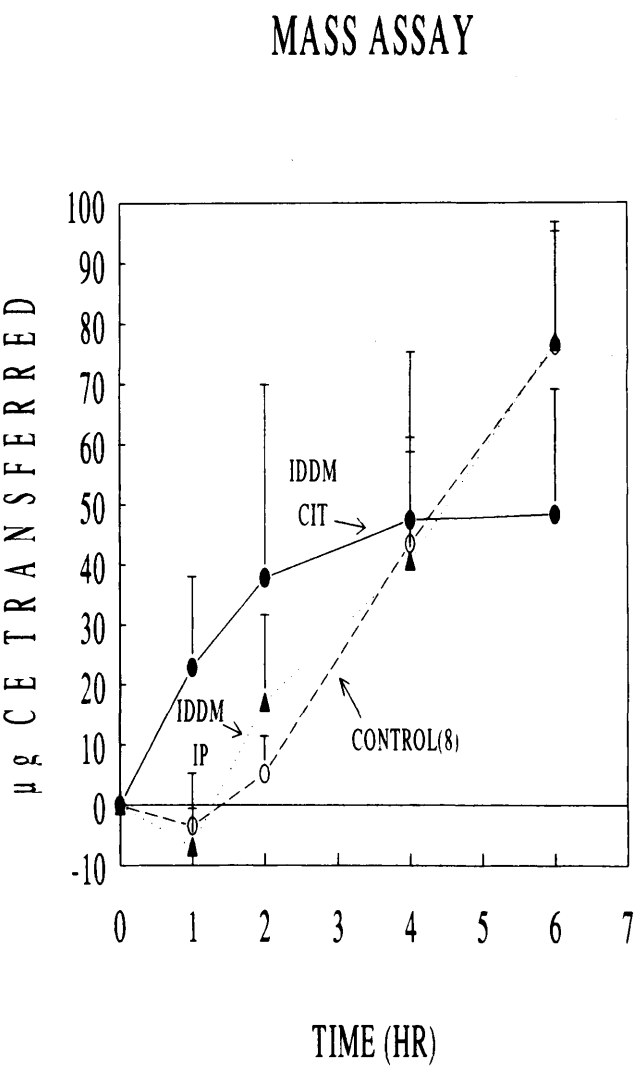

FIG. 1. Mass of cholesteryl ester transferred from HDL to apoB-containing lipoproteins in 4 IDDM patients after 3 mo periods of conventional (*closed circles*) and i.p. insulin (*closed triangles*) treatment and control subjects (*open circles*).

11). No correlation was apparent between glycemic control and the changes in CET. To obtain more information about the underlying mechanism and discern the potential roles of the acceptor (VLDL, LDL) and donor (HDL) lipoproteins and cholesterol ester transfer protein, we performed a series of recombination experiments with lipoprotein fractions isolated by preparative ultracentrifugation from IDDM and control subjects. In these, we first found that CET was accelerated only in a system that contained IDDM d < 1.063 fractions containing VLDL+LDL; the IDDM d > 1.063 fraction containing HDL, CETP, and VHDL did not increase the rate of transfer when combined with control d < 1.063 fractions, indicating that the defect we had observed in CE mass transfer was attributable to one (or more) of the acceptor lipoproteins. Next, we combined VLDL and LDL alone from IDDM patients at plasma concentration with controls' d < 1.063 fractions containing the CE donor HDL and CETP and found that IDDM VLDL and not LDL was dysfunctional. In

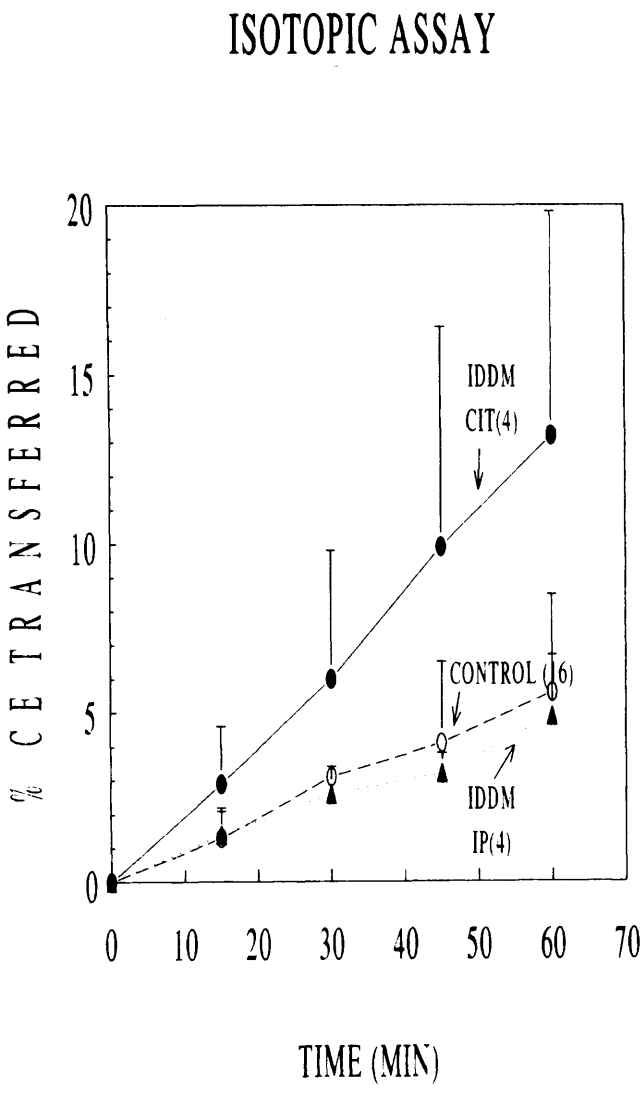

FIG. 2. Transfer of labeled cholesteryl ester from HDL to apoB-containing lipoproteins in IDDM patients after 3 mo periods of conventional (*closed circles*) and i.p. insulin (*closed triangles*) treatment and control (*open circles*) subjects.

further studies of a similar design we found that the largest and most TG-enriched subfraction, $VLDL_1$, was the only one of the three VLDL fractions that behaved abnormally (11).

Analysis of surface and core lipids revealed that $VLDL_1$ was enriched in CE relative to TG and, as observed earlier, had an increased FC-L ratio. HDL, on the other hand, had a relative increase in TG, so that its TG-CE ratio was significantly higher than that of control HDL (11). These directional changes in core lipid composition were precisely the type one would expect to find if CET were accelerated in vivo. Because animal species that are susceptible to dietary-induced atherosclerosis, such as the rabbit and humans, have high levels of CET (12), our finding this acceleration in normolipidemic IDDM patients suggests that this abnormality may be a newly recognized factor that contributes to their well-recognized predisposition to macrovascular complications. Dullaart et al. (13) reported a similar increase in CET with a somewhat different assay method.

DISCUSSION

Although the precise effects on the metabolism and clearance of lipoproteins modified by CET in IDDM is still unclear, Klein et al. (14) showed that $VLDL_1$ from IDDM patients similar to those we studied causes increased CE formation when incubated with human monocyte-macrophages in tissue culture. When radiolabeled LDL from nondiabetic subjects is incubated with CETP and then exposed to arterial smooth muscle cells and fibroblasts, Chait and co-workers demonstrated that its receptor-mediated binding and degradation are reduced (15). It is therefore possible that a subpopulation of VLDL in IDDM patients becomes atherogenic as a consequence of their being modified by CET. Particles generated in this way could also be more susceptible to other modifications that further enhance their atherogenicity. Normally, CET is a postprandial phenomenon whose peak activity appears to parallel that of lipoprotein lipase (16). We therefore considered whether CET and LpL might function coordinately, because Sammet and Tall (17) showed that CET was enhanced when VLDL was first treated with LpL. Nikkila et al. reported earlier that IDDM patients who were in better clinical control and therefore more fully "insulinized" had evidence of higher lipase activities (18). This series of related observations suggested to us the possibility that peripheral hyperinsulinism resulting from conventional s.c. insulin therapy may continuously activate LpL during the non-fed state. As a result of LpL-induced changes in VLDL composition, the affinity of VLDL for CETP would be enhanced and CET stimulated. In this hypothetical model, elevated insulin levels achieved through CIT are a major pathogenetic factor. The opportunity to perform a preliminary study in a few well-controlled IDDM patients who were treated with insulin delivered i.p. into the portal circulation by a permanently implanted programmable pump provided a unique human model to test this hypothesis. In collaboration with Dr. Fred Dunn's group at Duke University Medical Center, where this particular insulin-delivery device (Infusaid) is being tested, we examined CET serially in four IDDM patients during CIT and after 3 mo. of i.p. treatment. Excellent glycemic control was achieved with both modes of insulin delivery (HbA_{1c}, CIT 6.8 ± 1.2%; i.p. 6.4 ± .5; reference group <6.4%); i.p. treatment was well tolerated, and hypoglycemia, rare (19).

Although plasma lipids (TG, cholesterol, HDL-C, HDL_2, HDL_3) were not significantly changed during i.p. therapy when insulin levels decline substantially (19), CET returned to normal (Figs. 1,2). This finding suggests that hyperinsulinism associated with CIT may in fact activate LpL and stimulate CET. Studies are in progress to determine the effect of i.p. treatment on lipoprotein composition and to document the apparent reduction in peripheral circulating insulin levels. These preliminary results suggest a new pathogenetic role for hyperinsulinism in IDDM, as an activator of CET.

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