Plasma Phospholipid Transfer Protein Activity Is Lowered by 24-h Insulin and Acipimox Administration

Blunted Response to Insulin in Type 2 Diabetic Patients

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Cholesteryl ester transfer protein (CETP) transfers cholesteryl esters from HDL to VLDL and LDL. Phospholipid transfer protein (PLTP) transfers phospholipids between lipoproteins, converts HDL_3 into larger and smaller particles, and is involved in pre–β-HDL generation. We examined the effects of 24-h hyperinsulinemia (30 mU · kg^{-1} · h^{-1}) and 24-h Acipimox (250 mg/4 h) on plasma lipids as well as CETP and PLTP activities (measured with exogenous substrate assays) in eight healthy and eight type 2 diabetic subjects. After 24 h of insulin, plasma free fatty acids (FFAs), HDL cholesterol, and plasma apolipoprotein AI decreased in healthy subjects and type 2 diabetic patients ($P < 0.05$). Plasma triglycerides did not significantly change in either group. After 24 h of Acipimox, all parameters, including plasma triglycerides, decreased in both groups ($P < 0.05$). Insulin decreased plasma PLTP activity by 17.6% after 24 h in healthy subjects ($P < 0.05$) and 10.2% in diabetic patients ($P < 0.05$ vs. baseline; $P < 0.05$ vs. healthy subjects). Acipimox lowered PLTP activity by 10.3% in healthy subjects ($P < 0.05$) and 11.3% in diabetic patients ($P < 0.05$). When insulin was infused for 3 h after Acipimox, a further decrease was found only in healthy subjects. Plasma CETP activity decreased by 9.5% after 24 h of insulin in healthy subjects ($P < 0.05$), but not in diabetic patients. Acipimox did not decrease plasma CETP activity in either group. In healthy subjects, the PLTP responses with insulin and Acipimox were larger than the changes in CETP activity ($P < 0.05$). These findings suggest that there is a metabolic link between the regulation of plasma FFA and PLTP, but not CETP. The PLTP response to insulin is blunted in type 2 diabetes. *Diabetes* 48:1631–1637, 1999

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Received for publication 30 September 1998 and accepted in revised form 15 April 1999.

AU, arbitrary units; CETP, cholesteryl ester transfer protein; CV, coefficient of variation; FFA, free fatty acid; LCAT, lecithin:cholesterol acyltransferase; PLTP, phospholipid transfer protein.

Cholesteryl ester transfer protein (CETP) and phospholipid transfer protein (PLTP) are key factors in HDL metabolism (1–6). CETP mediates the heteroexchange of cholesteryl esters and triglycerides between HDL and VLDL and LDL (1,2). PLTP specifically mediates lipoprotein phospholipid transfer and may enhance CETP-catalyzed cholesteryl ester transfer out of HDL (7–10). Furthermore, PLTP converts small-sized HDL (HDL_3) into larger and smaller HDL particles (3,4,11). On incubation of HDL_3, PLTP increases and CETP decreases HDL size distribution (12). The PLTP-induced HDL conversion is enhanced when HDL is enriched with triglycerides (13). During the PLTP-mediated HDL remodeling, lipid-poor pre-β-HDL particles are produced that act as initial acceptors of free cholesterol from cell surfaces (14,15). Thus, among other factors, CETP and PLTP are currently considered to be important determinants of the process of reverse cholesterol transport in which HDL shuttles cholesterol from peripheral cells to the liver (5,6,14,16).

Abnormalities in the regulation of plasma CETP and PLTP may accompany high plasma triglyceride levels and be involved in altered HDL metabolism in type 2 diabetes. Acute exogenous hyperinsulinemia was reported to decrease plasma CETP activity in type 2 diabetic, but not healthy, subjects (17). In contrast, a blunted CETP lowering was found in type 2 diabetes in another study (18). Using endogenous hyperinsulinemia, we documented a lowering in plasma PLTP but not in CETP activity in healthy subjects (19). In lean healthy subjects, only PLTP activity decreased after 6 h of exogenous hyperinsulinemia in conjunction with a decline in plasma free fatty acids (FFAs) and triglycerides, while obese type 2 diabetic patients had a diminished PLTP response (20). This raises the possibility that insulin affects plasma PLTP via an effect on FFA and triglyceride metabolism. At present, it is uncertain whether the decrease in plasma PLTP is sustained and different from the change in plasma CETP during prolonged hyperinsulinemia. Moreover, it is unknown whether plasma PLTP activity is also lowered when FFA availability is decreased by other interventions. In the present experiments, we documented the plasma PLTP and CETP activity responses to 24-h moderate hyperinsulinemia and to the nicotinic acid derivative Acipimox. Furthermore, it was evaluated whether the lipid transfer protein responses

were different in type 2 diabetic patients compared with healthy subjects.

RESEARCH DESIGN AND METHODS

Subjects. Eight male patients with type 2 diabetes and eight healthy males participated in the studies. Only nonsmoking men participated to exclude effects of smoking (21,22) and the menstrual cycle (23) on lipid levels. Type 2 diabetes was diagnosed according to National Diabetes Data Group criteria (24). In all patients, diabetes was diagnosed after the age of 40 years, and none of the patients had suffered from ketoacidotic periods or was treated with insulin. In the nondiabetic subjects, diabetes was excluded by a 75-g oral glucose tolerance test using fasting venous blood glucose level <6.7 mmol/l and 2-h blood glucose level <7.8 mmol/l as cut-off values. Fasting plasma total cholesterol >8.0 mmol/l or triglycerides >4.5 mmol/l was an exclusion criterion. None of the participants had familial hyperlipidemia or suffered from clinically manifest cardiovascular disease. Thyreotropin levels, liver function tests, and serum creatinine concentrations were within normal ranges, and blood pressure was <160/95 mmHg in all subjects. No medication other than sulfonylurea and metformin was used. Maximum alcohol intake was three beverages per day. BMI was calculated as weight divided by height squared. Waist-to-hip ratio was measured as the ratio of the smallest circumference between rib cage and iliac crest and the largest circumference between waist and thigh (25). The study protocol was approved by the ethics committee of the University Hospital Groningen, and all participants gave written informed consent.

Study design. The participants maintained their usual diets and the diabetic patients continued their blood glucose–lowering drugs except on the study days. They did not use alcohol on the days before the studies and fasted from 2000 overnight. Thereafter, the subjects were studied on two separate days in random order, with a washout period of 1 week in between. The two study days consisted of *1*) a 24-h euglycemic-hyperinsulinemic clamp in the healthy subjects or a 24-h isoglycemic-hyperinsulinemic clamp in diabetic subjects and *2*) 24 h of Acipimox administration, with an additional 3-h euglycemic-hyperinsulinemic clamp (isoglycemic for diabetic patients). In the diabetic patients, an extra 4-h euglycemic-hyperinsulinemic clamp was performed at the end of each study day. On the 24-h study days, participants consumed a 1,000-kcal diet consisting of 37% carbohydrate, 38% fat, and 24% protein divided over breakfast, lunch, and dinner. They remained in the supine position after 0800 on the study days.

The subjects were admitted at 0700 for the insulin and Acipimox studies. One hand vein was cannulated, and the cannula was kept open with a saline drip (154 mmol/l NaCl, 30 ml/h). This hand was placed in a thermoregulated box with an ambient temperature of 55°C to obtain arterialized venous blood (26). An antecubital vein of the contralateral arm was used for administration of dextrose and insulin. After 1 h of supine rest (0800), baseline blood samples were obtained for measurement of blood glucose, insulin, lipids, (apo)lipoproteins, FFAs, and CETP and PLTP activity levels. Further blood samples were taken after 8 and 24 h and thereafter at 27 h (control subjects) and 31 h (diabetic subjects). During the insulin day, insulin was given at a rate of 30 mU · kg^{-1} · h^{-1}, preceded by an insulin bolus of 5 mU/kg. Blood glucose was measured at 10-min intervals. A variable dextrose infusion (20% wt/wt) with potassium chloride (20 mmol per liter of dextrose) to prevent hypokalemia was given to maintain the blood glucose level. In the healthy subjects, target blood glucose was kept ~0.4 mmol/l below the fasting level to inhibit endogenous insulin secretion. In the diabetic patients, target blood glucose level was the fasting baseline value during the isoglycemic-hyperinsulinemic clamp, while target blood glucose was 4.2 mmol/l during the euglycemic period.

On the Acipimox day, the participants received 250 mg Acipimox (5-methylpyrazine-carboxylic acid 4-oxide) every 4 h, starting at 1000, until 1000 on the next day. Blood glucose was measured 8 and 24 h after the start of the study and at 10-min intervals during the subsequent glucose clamps (with insulin again infused at a rate of 30 mU · kg^{-1} · h^{-1}).

Laboratory measurements. Blood was collected into tubes containing EDTA (1.5 mg/ml) and was placed on ice immediately. Plasma was separated from blood cells within 30 min by centrifugation at 3,000 rpm for 15 min at 4°C. Samples were frozen at –80°C before assay.

Blood glucose was measured with a glucose analyzer (APEC, Danvers, MA). Plasma free insulin was assayed by radioimmunoassay (Novo Nordisk Immunochemical Department, Copenhagen). Plasma FFAs were measured enzymatically using a kit from Wako Chemicals (Osaka, Japan; catalog no. 994-75409). Lipids were measured in plasma and in the HDL-containing supernatant fraction after removal of apolipoprotein B (apoB)-containing lipoproteins with polyethylene glycol-6000 (27). VLDL and LDL were calculated as the difference between plasma and the HDL fraction. Total cholesterol was measured by gas chromatography. In the HDL fraction, free cholesterol was measured by a modification of a previously published method (28) in which the hydrolysis step was omitted. HDL cholesteryl ester was calculated as the difference between total and free cholesterol. Triglycerides were measured enzymatically. Apos AI and B were assayed by immunoturbidimetry (Boehringer Mannheim, Almere, the Netherlands; catalog no. 726478 and 726494, respectively).

CETP activity was determined after removal of VLDL and LDL from each sample by precipitation with Mg^{2+}/phosphotungstate (9,29). The isotope assay measures the transfer of cholesteryl ester between [1-^{14}C]oleate—cholesteryl ester—labeled LDL and an excess of unlabeled pooled normal HDL. LCAT was inhibited with dithiobis-2-nitrobenzoic acid. CETP activity was calculated as the bidirectional transfer between labeled LDL and HDL. The plasma CETP activity level obtained by this method is strongly correlated with CETP mass (30) and is not affected by the endogenous plasma lipoproteins. Plasma PLTP activity was assayed with a phospholipid vesicles-HDL system (9,29). Plasma samples were incubated with [^{3}H]phosphatidylcholine-labeled liposome vesicles and an excess of pooled normal HDL. Subsequently, the vesicles were precipitated with a mixture of NaCl, $MgCl_2$, and heparin (final concentrations of 230 mmol/l, 92 mmol/l, and 200 U/ml, respectively). Plasma PLTP activity levels are linearly related to the amount of plasma added to the incubation system. The method is specific for PLTP and is not influenced by the phospholipid transfer promoting properties of CETP (9). Plasma activity levels of CETP and PLTP were assayed in duplicate; the measurements were performed in one run using the same batches of substrates. The within-assay coefficients of variation (CVs) were 3.7 and 4.1% for CETP and PLTP, respectively. The activity levels of CETP and PLTP were related to their activities in human pool plasma, which was included in each run, and are expressed in arbitrary units (AU) corresponding to the percentage of the activities in the pool plasma.

Statistical analysis. Data are expressed as means ± SD unless stated otherwise. Within-group changes in parameters were evaluated by Friedman's nonparametric two-way analysis of variance. Changes in parameters after addition of insulin on the Acipimox day and between isoglycemia and euglycemia in the diabetic group were evaluated by the paired Wilcoxon test. Differences in parameters between the healthy and type 2 diabetic subjects were analyzed with the unpaired Wilcoxon test. Duncan's method was used to correct for multiple comparisons. A two-sided P value <0.05 was taken as significant.

RESULTS

Shortly after the first intake of Acipimox, one of the healthy subjects had a rash that lasted 2 hours. He could continue the study. No further side effects were seen.

BMI, waist-to-hip ratio, and blood pressure were higher in type 2 diabetic patients than in healthy subjects (Table 1). Metabolic control was acceptable in the diabetic patients. In addition to diet, three diabetic patients were treated with sulfonylurea alone and five received sulfonylurea in combination with metformin. Alcohol intake was similar in the groups.

On both study days, baseline blood glucose was higher in diabetic patients, whereas plasma insulin was not different between groups (Table 1). Blood glucose decreased by ~0.4 mmol/l in healthy subjects and remained unaltered in diabetic patients during the 24-h insulin clamp. Plasma insulin rose similarly in both groups. The overall CV of blood glucose during the 24-h insulin clamp was 12.7 ± 2.9% in healthy subjects and 9.5 ± 3.4% in diabetic patients. No changes in plasma insulin were observed in healthy subjects after 8 and 24 h of Acipimox administration, whereas in diabetic patients plasma insulin rose slightly after 8 h. Blood glucose decreased significantly only in the diabetic patients. When insulin was added to Acipimox, plasma insulin rose similarly in both groups (Table 1).

No differences in any of the baseline plasma lipid and (apo)lipoprotein parameters were observed between the study days in each group. The values of the Acipimox day are shown in Table 2. At baseline, plasma cholesterol, VLDL and LDL cholesterol, and apoB levels were higher in the diabetic group. HDL cholesterol and plasma apoAI levels were not significantly different between the groups. On both study days, baseline plasma triglycerides were higher in diabetic than in healthy subjects (Table 3). The changes in plasma triglycerides after 8 and 24 h of insulin infusion did not reach significance in either group. With Acipimox, plasma triglycerides decreased in both groups after 24 h, and a further

TABLE 1
Clinical characteristics, blood glucose, and plasma insulin in healthy subjects and type 2 diabetic patients

	Healthy subjects	Type 2 diabetic patients
n	8	8
Age (years)	51 (41–64)	59 (51–73)
BMI (kg/m^2)	24.0 ± 2.4 (20.8–27.2)	27.8 ± 1.5* (25.4–30.3)
Waist-to-hip ratio	0.92 ± 0.06	1.00 ± 0.04*
Diabetes duration (years)	—	4 (2–8)
Blood pressure, systolic/diastolic (mmHg)	127 ± 14/83 ± 6	140 ± 8†/89 ± 3†
HbA_{1c} (%)	5.1 ± 0.3	7.1 ± 0.8*
Alcohol (U/day)	0.6 (0–2)	0.4 (0–2)
24-h insulin infusion		
Blood glucose (mmol/l)		
Baseline	4.7 ± 0.4	8.3 ± 1.4*
8 h	4.2 ± 0.4‡	8.2 ± 1.7*
24 h	4.3 ± 0.4‡	7.9 ± 2.2*
Plasma insulin (mU/l)		
Baseline	10.7 ± 4.3	22.8 ± 14.1
8 h	40.6 ± 15.6§	60.9 ± 30.7§
24 h	32.1 ± 7.4§	44.4 ± 17.4‡
24-h Acipimox administration		
Blood glucose (mmol/l)		
Baseline	4.3 ± 0.7	7.8 ± 1.6*
8 h	4.8 ± 0.5	6.0 ± 1.4‡
24 h	4.1 ± 0.4	6.2 ± 0.8§
27 h	4.0 ± 0.3	7.9 ± 1.8
Plasma insulin (mU/l)		
Baseline	10.9 ± 3.6	14.7 ± 7.9
8 h	12.2 ± 7.8	19.3 ± 10.9‡
24 h	10.7 ± 7.1	14.1 ± 14.0
27 h	33.3 ± 7.5§	40.8 ± 16.3§

Data are means ± SD or medians (range). *P < 0.01, †P < 0.05 vs. healthy subjects; ‡P < 0.05, §P < 0.02 vs. baseline.

decrease was noted when insulin was added (Table 3). Baseline plasma FFAs were also higher in diabetic than healthy subjects on both study days (Table 3). Plasma FFAs decreased in both groups after 8 and 24 h of insulin infusion to levels that were not different in diabetic and healthy subjects. With Acipimox, plasma FFAs also decreased after 8 and 24 h in each group. The decrement was most pronounced after 8 h. A further fall in plasma FFAs was observed in both groups when insulin was given after 24 h of Acipimox. The plasma FFA levels attained during this experiment were again not different between the groups (Table 3).

During insulin administration, HDL cholesterol fell by 8.9 ± 2.2% (P < 0.01) and 12.0 ± 2.7% (P < 0.001) at 8 and 24 h of insulin in healthy subjects and similarly by 5.4 ± 2.5% (P = 0.05) and 9.4 ± 2.0% (P < 0.001) in type 2 diabetic patients. With Acipimox, HDL cholesterol decreased by 5.5 ± 1.3% (P < 0.05) and 6.8 ± 1.9% (P < 0.01) at 8 and 24 h in healthy subjects and by 6.0 ± 2.0% (NS) and 6.5 ± 3.0% (P < 0.01) in type 2 diabetic patients. These decreases in HDL cholesterol were due to a slight fall in HDL free cholesterol (data not shown) as well as cholesteryl ester and were accompanied by decreasing HDL triglycerides (Table 4). Plasma apoAI fell by 7.5 ± 5.7%

TABLE 2
Baseline plasma (apo)lipoproteins and PLTP and CETP activity in healthy subjects and type 2 diabetic patients

	Healthy subjects	Type 2 diabetic patients
n	8	8
Plasma total cholesterol (mmol/l)	4.72 ± 0.76	5.40 ± 0.56
VLDL and LDL cholesterol (mmol/l)	3.58 ± 0.79	4.40 ± 0.56*
HDL cholesterol (mmol/l)	1.15 ± 0.41	1.01 ± 0.18
Plasma apolipoprotein AI (g/l)	1.21 ± 0.25	1.15 ± 0.09
Plasma apolipoprotein B (g/l)	0.79 ± 0.15	0.98 ± 0.14*
Plasma PLTP activity (AU)	94 ± 15	92 ± 10
Plasma CETP activity (AU)	90 ± 14	70 ± 13†
Plasma PLTP/CETP activity ratio	1.06 ± 0.22	1.33 ± 0.20†

Data are means ± SD. *P < 0.05, †P < 0.02 vs. healthy subjects.

TABLE 3
Plasma triglycerides and FFAs in healthy subjects and type 2 diabetic patients during insulin and Acipimox administration

	Healthy subjects	Type 2 diabetic patients
n	8	8
Plasma triglycerides (mmol/l)		
Insulin infusion		
Baseline	0.93 ± 0.36	1.78 ± 0.71*
8 h	1.03 ± 0.47	2.00 ± 1.00†
24 h	0.79 ± 0.44	1.52 ± 0.67
Acipimox administration		
Baseline	1.08 ± 0.57	1.86 ± 0.47*
8 h	1.20 ± 0.46	1.81 ± 0.50†
24 h	0.78 ± 0.41‡	1.34 ± 0.34*‡
27 h	0.56 ± 0.31‡§	1.00 ± 0.31*‡§
Plasma FFAs (μmol/l)		
Insulin infusion		
Baseline	644 ± 301	1,293 ± 434‖
8 h	215 ± 140‡	254 ± 104‡
24 h	242 ± 215‡	170 ± 124‡
Acipimox administration		
Baseline	665 ± 269	1,135 ± 232‖
8 h	277 ± 177‡	210 ± 72‡
24 h	517 ± 262¶	314 ± 76‡
27 h	51 ± 50‡§	73 ± 44‡§

Data are means ± SD. 27-h Acipimox administration is after 3 h of concomitant insulin infusion. *$P < 0.02$, †$P < 0.05$, ‖$P < 0.01$ vs. healthy subjects; ‡$P < 0.01$, ¶$P < 0.02$ vs. baseline; §$P < 0.05$ vs. before insulin.

TABLE 4
HDL lipids in healthy subjects and type 2 diabetic patients during insulin and Acipimox administration

	Healthy subjects	Type 2 diabetic patients
n	8	8
HDL cholesteryl ester (mmol/l)		
Insulin infusion		
Baseline	0.92 ± 0.24	0.83 ± 0.12
8 h	0.84 ± 0.25*	0.79 ± 0.14
24 h	0.81 ± 0.28*	0.75 ± 0.12*
Acipimox administration		
Baseline	0.94 ± 0.32	0.84 ± 0.15
8 h	0.88 ± 0.28†	0.80 ± 0.13
24 h	0.86 ± 0.26*	0.79 ± 0.11†
27 h	0.86 ± 0.24*	0.81 ± 0.12
HDL triglycerides (mmol/l)		
Insulin infusion		
Baseline	0.13 ± 0.02	0.17 ± 0.04
8 h	0.13 ± 0.02	0.15 ± 0.03
24 h	0.11 ± 0.03*	0.12 ± 0.03†
Acipimox administration		
Baseline	0.15 ± 0.04	0.19 ± 0.04
8 h	0.13 ± 0.03	0.14 ± 0.03*
24 h	0.12 ± 0.04*	0.11 ± 0.02*
27 h	0.09 ± 0.02*	0.09 ± 0.02*

Data are means ± SD. 27-h Acipimox administration is after 3 h of concomitant insulin infusion. *$P < 0.01$, †$P < 0.05$ vs. baseline.

($P < 0.01$) and 9.9 ± 6.9% ($P < 0.001$) in healthy and diabetic subjects after 24 h of insulin and by 8.3 ± 6.1% ($P < 0.01$) and 5.5 ± 3.3% ($P < 0.05$) after 24 h of Acipimox.

Baseline plasma PLTP activity was not different between the groups; CETP activity was lower in diabetic patients (Table 2). As a result, the PLTP/CETP activity ratio was 25% higher in diabetic patients. During insulin infusion, plasma PLTP activity gradually decreased in both groups, but its percent decrease at 24 h was larger in healthy than in diabetic subjects (17.6 ± 6.0 vs. 10.2 ± 6.6%, $P < 0.05$ for the difference in change between the groups) (Fig. 1*A*). Acipimox also lowered plasma PLTP activity, but there was no between-group difference in response (10.3 ± 9.4 and 11.3 ± 5.8% in healthy and diabetic patients, respectively, at 24 h, NS) (Fig. 1*B*). When insulin was infused after Acipimox, a significant further lowering in PLTP was observed only in healthy subjects. Plasma CETP activity diminished slightly during insulin infusion in healthy subjects and only temporarily in diabetic patients (Fig. 1*C*). Plasma CETP activity was not significantly decreased by Acipimox in either group. Its level was slightly lower compared with baseline when insulin was added in healthy subjects (Fig. 1*D*). After 24 h, the percentage decrements in plasma PLTP activity were larger than those in plasma CETP activity with insulin ($P < 0.01$) and Acipimox ($P < 0.05$) in healthy subjects and with Acipimox ($P < 0.02$) in diabetic patients. In the diabetic group, no effect of euglycemia compared with isoglycemia was seen on PLTP or CETP activity after insulin or Acipimox plus insulin (data not shown).

DISCUSSION

This study demonstrates for the first time that plasma PLTP activity is decreased by 24-h insulin infusion as well as by Acipimox administration in healthy subjects. Thus, the acute lowering of plasma PLTP activity is sustained with prolonged insulin administration and also occurs when plasma FFA availability is decreased by another intervention that inhibits adipose tissue lipolysis. In type 2 diabetic patients, the PLTP-lowering effect of insulin was blunted both when evaluated during 24 h of infusion and when insulin was added for 3 h after Acipimox, in accordance with the diminished plasma PLTP response after acute hyperinsulinemia in obese type 2 diabetic patients (20). Our study also shows that the effect of insulin on plasma PLTP activity was clearly larger than that on CETP activity, and that Acipimox did not decrease plasma CETP activity. This extends recent findings demonstrating that the plasma CETP activity level is not significantly decreased by acute endogenous (19) and exogenous (20) hyperinsulinemia. It appears therefore that interventions affecting FFA and triglyceride metabolism have different effects on plasma PLTP compared with CETP activity levels.

Before intervention, plasma PLTP activity was similar and CETP activity was lower in type 2 diabetic patients compared with healthy subjects. The unaltered PLTP activity in type 2 diabetes is in keeping with another report (16) but differs from the notion that plasma PLTP activity is higher in obese type 2 diabetic patients than in lean healthy subjects (20). Because plasma PLTP activity is correlated with BMI and plasma triglycerides (20,29), this apparent discrepancy is probably explained by less pronounced differences in BMI and plasma triglycerides in the diabetic and healthy subjects in this study compared with those in our previous study (20).

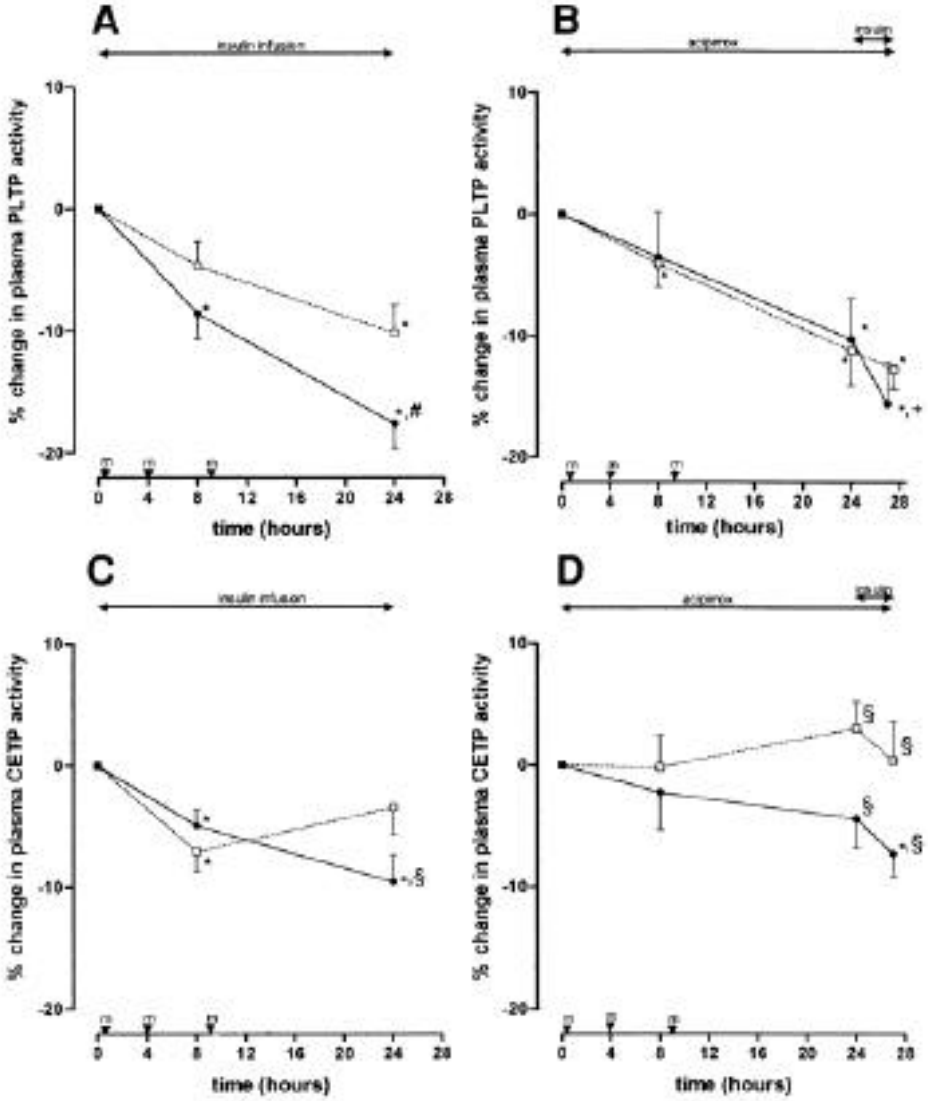

FIG. 1. Percent change in plasma PLTP and CETP activity during 24-h insulin infusion (*A*, *C*) and Acipimox administration with an additional 3-h insulin infusion (*B*, *D*) in healthy subjects (●) and type 2 diabetic patients (□). Data are mean values and SE. **P* < 0.05 vs. baseline; #*P* < 0.05 vs. change in type 2 diabetic patients; +*P* < 0.05 vs. before insulin; §*P* < 0.05 vs. change in PLTP. m, meal intake.

The mean between-group difference in BMI was 3.8 kg/m^2 in the current study and 9.3 kg/m^2 in our previous report (20). Plasma triglycerides were only 72% higher in the diabetic patients compared with healthy subjects; this difference was 275% in the earlier study (20). Plasma CETP activity, as an estimate of its mass, has been reported unchanged (10,20,31) or slightly lower (32) in type 2 diabetes. The higher plasma PLTP/CETP ratio in type 2 diabetes (10,20, and the present study) suggests that the diabetic state is associated with an abnormal regulation of PLTP relative to CETP, apart from the absolute level of each lipid transfer protein.

Insulin inhibits adipose tissue lipolysis, thereby lowering plasma FFAs (33). Both the decrease in FFA availability and direct suppression of hepatic triglyceride and apoB secretion are thought to contribute to the decrease in plasma triglycerides following acute hyperinsulinemia (34–36). Acipimox also inhibits hormone-sensitive lipase in adipose tissue, but its mechanism of action may be different from that of insulin (37). Unlike insulin, Acipimox does not directly inhibit hepatic lipoprotein secretion (38). In the present study, FFA availability, as judged by plasma level after 24 h of insulin infusion, was not different between type 2 diabetic patients and healthy subjects. During acute hyperinsulinemia, similar plasma FFA levels in type 2 diabetic and healthy subjects have also been shown previously, provided that the individuals under study were not severely obese (36,39,40). In other reports, plasma FFA levels during hyperinsulinemia were higher even in type 2 diabetic patients without severe obesity (41,42). In our study, the Acipimox-induced decrease in plasma FFAs was most pronounced initially but was still present after 24 h. Moreover, the lowering in plasma triglycerides was sustained, suggesting continued inhibition of adipose tissue lipolysis. Taken together, the present observations are in keeping with the hypothesis that there is a metabolic link between the regulation of plasma FFAs and PLTP activity.

The exact mechanisms responsible for such an association are unknown. In mice, circulating PLTP is likely to be derived from a variety of tissues, with large contributions of liver, adipose tissue, and lung (43). In humans as well, PLTP mRNA is present in many tissues (44). Among other possibilities, insulin and Acipimox may lower PLTP secretion by liver, adipose tissue, or both, in conjunction with inhibition of FFAs or VLDL release. It is unlikely that the diminished plasma PLTP lowering by insulin in type 2 diabetes, as observed in the current study, is related to abnormal plasma FFA regulation. Since the direct effect of insulin to inhibit hepatic lipoprotein

secretion was found to be impaired in type 2 diabetes (39), it is possible that abnormal regulation of PLTP in liver could provide a clue to this blunted PLTP response. Obviously, it cannot be excluded at present that insulin and Acipimox affect PLTP secretion by other tissues or affect PLTP catabolism.

A novel observation of our study is that HDL total cholesterol, cholesteryl ester, and triglycerides, as well as plasma apoAI decrease after 24 h of insulin and Acipimox administration in both diabetic patients and healthy subjects. In comparison, HDL cholesterol is not decreased after 2–5 h of hyperinsulinemia (17,45), except in one study (18). High-dose Acipimox does not decrease HDL cholesterol after 3 days (46), whereas HDL cholesterol is unaltered (47,48) or increased (49) with long-term administration of this nicotinic acid derivative. Thus, the duration of insulin or Acipimox administration seems to be crucial for the effects on HDL cholesterol.

HDL metabolism is affected by many factors including lecithin:cholesterol acyltransferase (LCAT), lipid transfer proteins, and lipases (1,2,50). During intravascular lipoprotein remodeling, phospholipids and free cholesterol are transferred from triglyceride-rich lipoproteins to HDL particles, where cholesteryl ester is produced by the LCAT reaction (50). The observed decreases in HDL total cholesterol and cholesteryl ester are unlikely explained by effects on the process of cholesteryl ester transfer between lipoproteins or by changes in lipase activities. In fact, a decrease in plasma triglycerides is expected to lower the transfer of cholesteryl ester out of HDL (10,51), thereby increasing HDL cholesterol. Stimulation of lipoprotein lipase (33) or a possible lowering in hepatic lipase activity (52) by insulin would also result in a raised HDL cholesterol.

PLTP facilitates the transfer of phospholipids (7) and free cholesterol to HDL in vitro (53). It may therefore be speculated that the decrease in HDL cholesterol induced by insulin and Acipimox could be due in part to a lowering of plasma PLTP activity in conjunction with a trend toward lower plasma triglycerides. PLTP is able to generate pre-β-HDL particles (5,15,54), which are considered to be anti-atherogenic (15,16). This PLTP-mediated HDL conversion is enhanced when HDL is enriched with triglycerides (13). Whether a decrease in plasma PLTP activity with insulin and Acipimox has adverse consequences for reverse cholesterol transport remains to be elucidated.

ACKNOWLEDGMENTS

The present study was supported by grant 94-130 from the Dutch Diabetes Foundation.

The expert technical assistance of P. van den Berg, T. van Gent, and L.M. Scheek is much appreciated.

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