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Insulin decreases plasma cholesteryl ester transfer but not cholesterol esterification in healthy subjects as well as in normotriglyceridaemic patients with type 2 diabetes

R. P. F. Dullaart*, S. C. Riemens*, L. M. Scheek† and A. van Tol†

*University Hospital, Groningen, and †Erasmus University, Rotterdam, the Netherlands

Abstract

Background Plasma cholesterol esterification (EST) and subsequent cholesteryl ester transfer (CET) from high-density lipoproteins (HDLs) towards apolipoprotein (apo) B-containing lipoproteins are key steps in HDL metabolism.

Materials and methods The effects of exogenous hyperinsulinaemia on plasma CET and EST, measured with isotope methods, were evaluated in 10 male normotriglyceridaemic (plasma triglycerides $<2{\cdot}0\,\mathrm{mmol\,L^{-1}}$) patients with type 2 diabetes and 10 individually matched healthy subjects during a two-step hyperinsulinaemic euglycaemic clamp over 6–7 h.

Results No between-group differences in baseline plasma lipid parameters were observed, but the HDL cholesteryl ester content was lower ($P<0{\cdot}02$) and the HDL triglyceride content was higher ($P<0{\cdot}05$) in diabetic patients. Baseline CET and EST were similar in the groups. In both groups, hyperinsulinaemia decreased plasma triglycerides ($P<0{\cdot}01$) and the HDL triglyceride content ($P<0{\cdot}01$) compared with saline infusion in healthy subjects, whereas the HDL cholesteryl ester content increased ($P<0{\cdot}05$ vs. saline infusion) in diabetic patients. CET was similarly decreased by hyperinsulinaemia in both groups ($P<0{\cdot}01$ vs. saline infusion). In contrast, the change in EST in either group was not different from that during saline administration. In the combined group, baseline CET was positively correlated with plasma triglycerides ($R_s=0{\cdot}68$, $P<0{\cdot}01$). The HDL cholesteryl ester content was negatively ($R_s=-0{\cdot}48$, $P<0{\cdot}05$) and the HDL triglyceride content was positively ($R_s=0{\cdot}64$, $P<0{\cdot}01$) correlated with CET.

Conclusion Insulin infusion decreases plasma CET in conjunction with a fall in triglycerides but does not decrease cholesterol esterification in healthy and type 2 diabetic subjects, indicating that acute hyperinsulinaemia has a different effect on these processes involved in HDL metabolism. Despite unaltered fasting plasma CET, HDL core lipid composition was abnormal in diabetic patients, suggesting that additional mechanisms may contribute to changes in HDL metabolism in diabetes mellitus.

Keywords Cholesteryl ester transfer, cholesterol esterification, free fatty acids, high-density lipoproteins, insulin, triglycerides, type 2 diabetes mellitus.
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Introduction

The process of reverse cholesterol transport, whereby cholesterol is transported from peripheral tissues back to the liver for excretion and conversion to bile acids, is regarded as representing an important defence mechanism against atherosclerosis development [1–4]. This pathway consists of several metabolic steps, including uptake of unesterified cholesterol from cell surfaces by extracellular acceptors such as pre-β high-density lipoproteins (HDLs) and subsequent cholesterol esterification by lecithin cholesterol acyltransferase (LCAT) [2–7]. HDL cholesteryl

Department of Endocrinology, University Hospital, Groningen, (R. P. F. Dullaart, S. C. Riemens); Department of Biochemistry, Cardiovascular Research Institute (COEUR), Erasmus University, Rotterdam, the Netherlands (L. M. Scheek, A. van Tol).

Correspondence to: Dr R. P. F. Dullaart, Department of Endocrinology, State University Hospital Groningen, PO Box 30.001, 9700 RB Groningen, the Netherlands. E-mail: R.P.F.Dullaart@int.AZG.NL

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esters can then be taken up by the liver by various mechanisms [3,4,8,9]. In man, HDL cholesteryl esters are also transferred to very low- and low-density lipoproteins (VLDLs and LDLs) by the cholesteryl ester transfer protein (CETP), and uptake of these apolipoprotein (apo) B-containing lipoproteins by the liver provides an additional route for hepatic delivery of cholesterol originating from HDL [4,10,11].

Despite continued controversy [12–15], there is recent epidemiological evidence supporting the hypothesis that hyperinsulinaemia is a cardiovascular risk factor [16]. The effects of insulin on reverse cholesterol transport are incompletely understood. Insulin may inhibit cellular cholesterol efflux *in vitro* [17,18], as well as cholesterol uptake in hepatic cells, either directly [18] or by lowering hepatic lipase activity [19]. Little is known about the effects of insulin on plasma cholesterol esterification and (CET). These processes are not only governed by the plasma levels of LCAT and lipid transfer proteins, but are to an important extent also positively related to the plasma triglyceride concentration [10,20–22]. Because insulin acutely lowers plasma triglycerides [23–25], it is plausible to postulate that these processes could be influenced by exogenous hyperinsulinaemia. In type 2 diabetic patients with adequate metabolic control, plasma cholesterol esterification has been reported to be unaltered [26,27] or increased [22]. Plasma CET has been found to be decreased [28], unchanged [27] or increased [22,26,29,30]. These equivocal results may, at least in part, be attributed to selection of subjects, with hypertriglyceridaemic type 2 diabetic patients showing increased plasma cholesterol esterification [22] and CET [22,30].

The purpose of the present report was to compare the effects of exogenous hyperinsulinaemia on plasma CET and cholesterol esterification in type 2 diabetic patients and healthy subjects. The type 2 diabetic and healthy subjects were individually matched for fasting plasma triglycerides, and only normotriglyceridaemic individuals participated to minimize confounding effects of dyslipidaemia on these processes.

Methods

The present study was approved by the medical ethics committee of the University Hospital Groningen, and all participants provided written informed consent. In healthy subjects, diabetes mellitus was excluded by a glucose tolerance test with fasting venous blood glucose $\geq 6{\cdot}7\,\text{mmol}\,\text{L}^{-1}$ and a 2-h post-load glucose $\geq 10{\cdot}0\,\text{mmol}\,\text{L}^{-1}$ as cut-off levels [31]. Type 2 diabetes mellitus was diagnosed according to National Diabetes Data group criteria [31]. Age at onset of diabetes was >40 years, and none of the patients was treated with insulin. Only non-smoking men participated to avoid the effects of smoking [32,33] and the menstrual cycle [34] on lipid levels. Fasting plasma triglycerides $\geq 2{\cdot}0\,\text{mmol}\,\text{L}^{-1}$, total cholesterol $\geq 6{\cdot}5\,\text{mmol}\,\text{L}^{-1}$, severe obesity, defined as a body mass index (BMI, calculated as weight divided by height squared) $>30\,\text{kg}\,\text{m}^{-2}$, clinically manifest cardiovascular disease, hypertension (systolic blood pressure >160 mmHg and diastolic blood pressure >90 mmHg), thyroid, kidney and liver dysfunction, alcohol use >3 drinks per day and the use of medication other than oral glucose-lowering agents were exclusion criteria. The type 2 diabetic patients were individually matched with healthy subjects for age (within 5 years), BMI (within $1\,\text{kg}\,\text{m}^{-2}$) and fasting plasma triglycerides (within $0{\cdot}5\,\text{mmol}\,\text{L}^{-1}$).

The study subjects continued their habitual diet and the type 2 diabetic patients took their glucose-lowering drugs until the study day. Alcohol consumption was avoided on the day before the study, and the participants fasted from 20.00 h onwards. They were in the supine position after 08.00 h. A hand vein was cannulated and the catheter was kept patent with a saline drip. (NaCl, $154\,\text{mmol}\,\text{L}^{-1}$, $30\,\text{mL}\,\text{h}^{-1}$). This hand was placed in a thermoregulated box with an ambient temperature of 55°C in order to obtain arterialized venous blood. A contralateral antecubital vein was used for infusion of glucose (20% by volume, to which potassium chloride 20 mmol per litre of glucose was added to prevent hypokalaemia) and insulin. Baseline blood samples were taken after 1 h of supine rest. Thereafter, a two-step hyperinsulinaemic, euglycaemic clamp was started. Insulin was infused at a rate of $30\,\text{mU}\,\text{kg}^{-1}\,\text{h}^{-1}$ and of $150\,\text{mU}\,\text{kg}^{-1}\,\text{h}^{-1}$, with the first step lasting 3 h (4 h in diabetic patients) and the second step lasting 3 h. Each step was primed by an insulin bolus of $5\,\text{mU}\,\text{kg}^{-1}$. Blood glucose was maintained at $4{\cdot}6\,\text{mmol}\,\text{L}^{-1}$ by varying the glucose infusion. To be able to compare possible changes in parameters due to a dilutional effect of glucose infusion or to a time effect with the effects of insulin, the healthy subjects were restudied within 4 weeks, receiving 1200 mL of saline (NaCl, $154\,\text{mmol}\,\text{L}^{-1}$) over 6 h. This volume was similar to the mean volume infused in healthy subjects during the hyperinsulinaemic clamp.

Laboratory measurements

Blood was collected into EDTA-containing tubes ($1{\cdot}5\,\text{mg}\,\text{mL}^{-1}$) and was directly placed on ice. Plasma was obtained within 30 min by low-speed centrifugation for 15 min at 4°C. Samples were kept frozen at −70°C and analysed within 2 months.

Plasma CET was measured by a radioisotope method essentially as described previously [35]. In brief, a tracer amount of [^{3}H]-cholesterol, complexed to albumin, was incubated overnight at 4°C with 0·3 mL of the individual plasma samples to be analysed. The tracer contained 150 000 dpm and 3 nmol of unesterified cholesterol. After this incubation, an equilibrium is reached between the labelled unesterified cholesterol and the unesterified cholesterol present in the plasma sample, i.e. the specific radioactivity of unesterified cholesterol is the same in all lipoprotein classes (VLDL, LDL and HDL). This specific radioactivity is determined by measuring plasma unesterified cholesterol mass. Subsequently, the plasma samples

were incubated at 37°C for 3 h. During this incubation radioactive cholesteryl esters are formed on HDL by the LCAT reaction, and these newly synthesized cholesteryl esters are distributed by CETP among the various acceptor lipoprotein particles (VLDL, LDL, HDL). To measure CET, i.e. the transfer of cholesteryl esters to VLDL and LDL, these lipoproteins are precipitated from the incubation mixture after the 3 h incubation at 37°C by addition of phosphotungstate/$MgCl_2$. The precipitate is then collected by low-speed centrifugation, washed once with phosphotungstate/$MgCl_2$ solution, collected again and suspended in 0·2 mL of saline. The cholesteryl esters are extracted with 0·3 mL of methanol + 0·4 mL of hexane by vortexing the mixture. Separation of the hexane phase from the methanol–saline phase is achieved by low-speed centrifugation. Subsequently, the hexane is applied to a silica column. Another 0·4 mL of hexane is added to the methanol–saline phase and the procedure is repeated. The second hexane fraction is applied to the same silica column. Radioactive unesterified cholesterol, which is also present to some extent in the hexane phase, is separated from the labelled cholesteryl esters by elution with 3 mL of hexane–diethylether (6:1, v/v). Unesterified cholesterol remains bound to the silica, whereas the cholesteryl esters are eluted [36]. The accumulation of labelled cholesteryl esters in LDL and VLDL is linear with time during the whole incubation period. CET is calculated using the specific radioactivity of cholesteryl esters present in the incubation mixtures as described [35]. The chemical composition of the substrate lipoproteins in this assay reflects the actual situation *in vivo* at the time of blood sampling. The rate of distribution of newly formed cholesteryl esters to VLDL and LDL is closely correlated with the net cholesteryl ester mass transfer from HDL to VLDL and LDL under conditions of LCAT inhibition, so that the CET assay can be regarded as an accurate estimate of cholesteryl ester mass transfer in plasma [30]. The influence of the minor cholesterol esterification in LDL (< 4% of total) can be ignored for the CET assay [35].

Cholesterol esterification in total plasma (EST) was assayed as described previously [35], using the same incubation system as for the CET assay. After incubation for 1 h at 37°C, cholesteryl esters are extracted with hexane from the complete incubation mixture and radioactive unesterified cholesterol is separated from the labelled cholesteryl esters on the silica columns system. The cholesterol esterification rate is linear with time for 5 h, indicating an excess of unesterified cholesterol in the assay system [35]. CET and EST assays were performed using the same batches of [^{3}H]-cholesterol–albumin complex, all assays were performed in duplicate, and the CET and EST measurements are expressed in nmol mL^{-1} h^{-1}. The within-assay coefficients of variation of plasma CET and EST are 7·1% and 5·4% respectively.

Lipids were measured in plasma and in the HDL fraction after precipitation of apolipoprotein (apo) B-containing lipoproteins with polyethylene glycol-6000 [37]. VLDL + LDL were calculated as the difference between plasma and the supernatant fraction. Total cholesterol and free cholesterol were measured by gas chromatography. Cholesteryl ester was calculated as the difference between total and free cholesterol. Triglycerides and phospholipids were measured as described [38]. ApoAI and ApoB were measured by immunoturbidimetry (Boehringer Mannheim, Germany, cat. nos 726478 and 726494, respectively). Plasma free fatty acids (FFA) were measured

Table 1 Clinical characteristics, blood glucose and plasma insulin at baseline and during the hyperinsulinaemic clamp in type 2 diabetic patients and healthy subjects

	Type 2 diabetic patients ($n = 10$)	Healthy subjects ($n = 10$)
Age (years)	53 ± 8	54 ± 9
Diabetes duration (years)	9 ± 8	–
Body mass index (kg m^{-2})	25·8 ± 2·6	25·7 ± 2·9
Waist–hip ratio	0·97 ± 0·04	0·94 ± 0·09
Blood pressure		
systolic/diastolic (mmHg)	131 ± 14/83 ± 7	133 ± 8/85 ± 4
Alcohol (U day^{-1})	0·6 ± 0·9	0·6 ± 1·0
HbA_{1c} (%)	7·5 ± 1·5*	5·5 ± 0·7
Blood glucose (mmol L^{-1})		
Baseline	8·3 ± 1·1*	5·3 ± 0·4
First insulin step	4·4 ± 0·2‡	4·7 ± 0·3†
Second insulin step	4·5 ± 0·2‡	4·8 ± 0·3†
Plasma insulin (mU L^{-1})		
Baseline	10 ± 5	12 ± 3
First insulin step	31 ± 6‡	31 ± 8‡
Second insulin step	170 ± 26‡	163 ± 46‡

Data are means ± SD. Blood glucose levels at the last hour of each insulin step and plasma insulin concentrations at the end of each insulin step are given.

*$P < 0·01$ compared with healthy subjects. †$P < 0·05$, ‡$P < 0·001$ campared with baseline.

using a kit from Wako (Germany, cat. no. 994-754-75409). Blood glucose was analysed on an APEC Glucose Analyser (Apec, Danvers, MA, USA). Glycated haemoglobin (HbA_{1c}) was measured by high-performance liquid chromatography (Bio-Rad, Veenendaal, The Netherlands; normal range 4·6–6·1%).

Statistical analysis

Data are expressed as means ± SD, unless stated otherwise. Baseline parameters in healthy subjects and type 2 diabetic patients were compared by unpaired Wilcoxon tests. Between-group differences in changes in parameters after insulin and saline administration were evaluated by Kruskal–Wallis analysis of variance. Within-group changes in parameters after insulin and saline infusion were analysed by Friedman's two-way analysis of variance. Duncan's method was applied to correct for multiple comparisons. Bivariate correlations were evaluated by Spearman's rank correlation analysis (R_s). A two-sided P-value $< 0{\cdot}05$ was considered significant.

Results

As shown in Table 1, there were no differences in age, BMI, waist–hip ratio, blood pressure and alcohol intake between the diabetic patients and healthy subjects. One of the diabetic patients was treated with diet alone, four were treated with diet plus sulphonylurea, and five were treated with diet in combination with sulphonylurea and metformin. HbA_{1c} and baseline blood glucose were higher in diabetic patients. Plasma insulin levels were not different between the groups. During the clamp blood glucose reached target levels in each group and plasma insulin rose similarly in diabetic and healthy subjects.

Table 2 demonstrates that there were no differences in baseline plasma total cholesterol, triglycerides, VLDL + LDL cholesterol, apoAI, apoB and FFAs between the groups. HDL-cholesterol was not significantly different between the groups, but the HDL cholesteryl ester content was lower and the HDL triglyceride content was higher in diabetic patients than in the healthy subjects. Plasma CET and EST were not different between the groups.

During the hyperinsulinaemic clamp, plasma triglycerides decreased similarly in diabetic and healthy subjects. In both groups, the fall was significantly larger than the change during the saline infusion experiment that was carried out in healthy subjects (Fig. 1a). HDL-cholesterol decreased during hyperinsulinaemia in diabetic and healthy subjects, as well as during saline administration (Fig. 1b). In diabetic patients, the decrease in HDL-cholesterol during hyperinsulinaemia was smaller than that during saline infusion, whereas in healthy subjects the difference in change in HDL-cholesterol compared with saline infusion did not reach significance ($P < 0{\cdot}10$). Compared with saline infusion, hyperinsulinaemia resulted in an increase in the HDL cholesteryl ester content (expressed in mol% of total lipids) in diabetic patients. In healthy subjects, the change in the HDL cholesteryl ester content after insulin was not significantly different ($P = 0{\cdot}10$) from the change during saline infusion (Fig. 1c). The HDL triglyceride content decreased in both groups compared with saline infusion (Fig. 1d). Plasma FFAs decreased during hyperinsulinaemia in both groups, whereas prolonged fasting during the saline experiment was accompanied by an increase in plasma FFAs (Fig. 1e). During hyperinsulinaemia plasma apoB decreased in diabetic and healthy subjects compared with saline infusion (Fig. 1f). A decrease in plasma apoAI during hyperinsulinaemia was observed in both groups, but its change was not different from the change during saline infusion ($P > 0{\cdot}10$ for diabetic and healthy subjects, data not shown).

Table 2 Baseline plasma (apo) lipoproteins, high-density lipoprotein (HDL) lipid composition, free fatty acids, cholesteryl ester transfer (CET) and cholesterol esterification rate (EST) in type 2 diabetic patients and healthy subjects

	Type 2 diabetic patients ($n = 10$)	Healthy subjects ($n = 10$)
Plasma total cholesterol ($mmol\,L^{-1}$)	4·89 ± 0·67	5·06 ± 0·71
Plasma triglycerides ($mmol\,L^{-1}$)	1·26 ± 0·41	1·06 ± 0·28
VLDL + LDL-cholesterol ($mmol\,L^{-1}$)	3·79 ± 0·83	3·85 ± 0·90
HDL cholesterol ($mmol\,L^{-1}$)	1·09 ± 0·30	1·22 ± 0·28
Apolipoprotein AI ($g\,L^{-1}$)	1·24 ± 0·24	1·27 ± 0·15
Apolipoprotein B ($g\,L^{-1}$)	0·94 ± 0·20	0·86 ± 0·22
HDL cholesteryl ester (mol%)	37·9 ± 3·3†	42·5 ± 4·8
HDL triglycerides (mol%)	8·4 ± 2·6*	6·1 ± 1·7
HDL free cholesterol (mol%)	7·4 ± 1·4	6·4 ± 2·0
HDL phospholipids (mol%)	46·3 ± 4·4	44·9 ± 3·5
Plasma free fatty acids ($\mu mol\,L^{-1}$)	587 ± 137	490 ± 83
Plasma CET ($nmol\,mL^{-1}\,h^{-1}$)	22·8 ± 10·4	22·1 ± 9·7
Plasma EST ($nmol\,mL^{-1}\,h^{-1}$)	72·4 ± 23·1	65·2 ± 18·0

Data are means ± SD.
*$P < 0{\cdot}05$, †$P < 0{\cdot}02$ from healthy subjects.

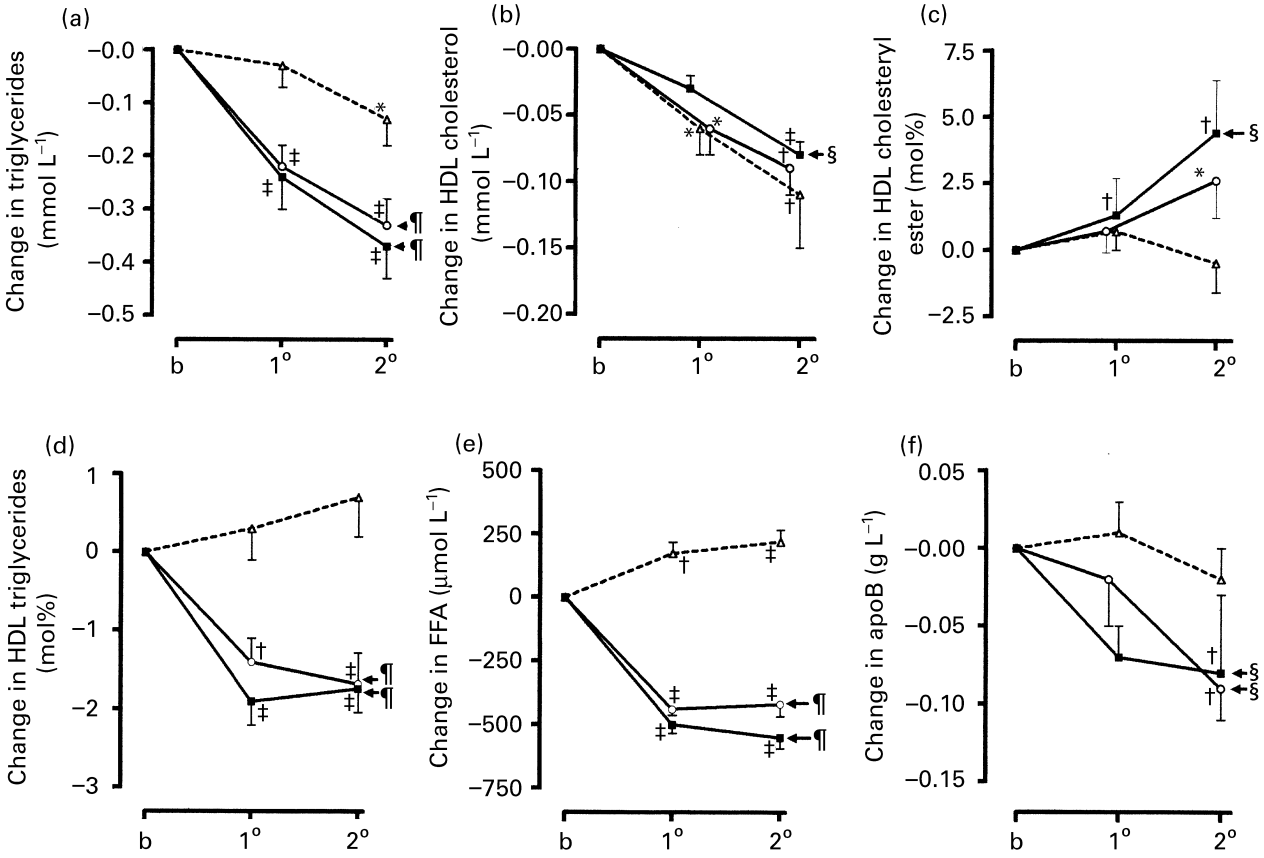

Figure 1 Changes in plasma triglycerides (a), high-density lipoprotein (HDL)-cholesterol (b), HDL cholesteryl ester content (c), HDL triglyceride content (d), plasma free fatty acids (e) and plasma apo B (f) in response to hyperinsulinaemia in type 2 diabetic patients (■), in healthy subjects (○) and during saline infusion in healthy subjects (Δ). b Indicates baseline, 1° and 2° indicate measurements after 3 (4) h of insulin infused at 30 mU kg^{-1} h^{-1}, respectively, and after 3 h of insulin infused at 150 mU kg^{-1} h^{-1}. Data are means ± SEM. *$P<0{\cdot}05$, †$P<0{\cdot}01$, ‡$P<0{\cdot}001$ compared with baseline. §$P<0{\cdot}05$, ¶$P<0{\cdot}01$ compared with overall change during saline infusion in healthy subjects.

Plasma CET was similarly decreased by hyperinsulinaemia in both groups. Its decrement in diabetic and healthy subjects was larger than that during saline infusion (Fig. 2a). In contrast, the decrease in plasma EST that occurred in diabetic and healthy subjects was not different from its decrement during saline infusion (Fig. 2b).

Baseline plasma CET was positively correlated with plasma triglycerides ($R_s=0{\cdot}68$, $P<0{\cdot}01$, in combined subjects; $R_s=0{\cdot}82$, $P<0{\cdot}01$, in healthy subjects, $R_s=0{\cdot}51$, NS in diabetic patients). Baseline plasma EST was also related to plasma triglycerides ($R_s=0{\cdot}52$, $P<0{\cdot}05$, in combined subjects), but its correlation did not reach significance in the separate groups. No relationships of baseline plasma CET and EST with plasma FFAs were observed ($R_s=0{\cdot}08$, NS, and $R_s=-0{\cdot}15$, NS, in combined subjects respectively). The HDL cholesteryl ester content at baseline was negatively correlated with plasma CET ($R_s=-0{\cdot}48$, $P<0{\cdot}05$, in combined subjects; $R_s=-0{\cdot}71$, $P<0{\cdot}05$, in healthy subjects; $R_s=-0{\cdot}35$, NS, in diabetic patients). The baseline HDL triglyceride content was positively correlated with plasma CET ($R_s=0{\cdot}64$, $P<0{\cdot}01$, in combined subjects; $R_s=0{\cdot}78$, $P<0{\cdot}02$, in healthy subjects; $R_s=0{\cdot}52$, NS, in diabetic patients).

Discussion

The present study has shown that plasma CET and EST are unaltered in normotriglyceridaemic type 2 diabetic patients compared with normolipidaemic healthy subjects. Despite exclusion of hypertriglyceridaemic subjects, plasma CET and EST were positively correlated with plasma triglycerides in the combined subjects. This finding supports the notion that even a normal plasma triglyceride-rich lipoprotein level is a determinant of plasma CET as well as of EST. It was anticipated that the hyperinsulinaemic euglycaemic clamp induced haemodilution, necessitating the comparison of changes in lipoprotein parameters after insulin with those observed after volume expansion. Indeed, significant decreases in plasma triglycerides, HDL-cholesterol, CET and EST were observed during saline infusion in healthy subjects. A potentially important

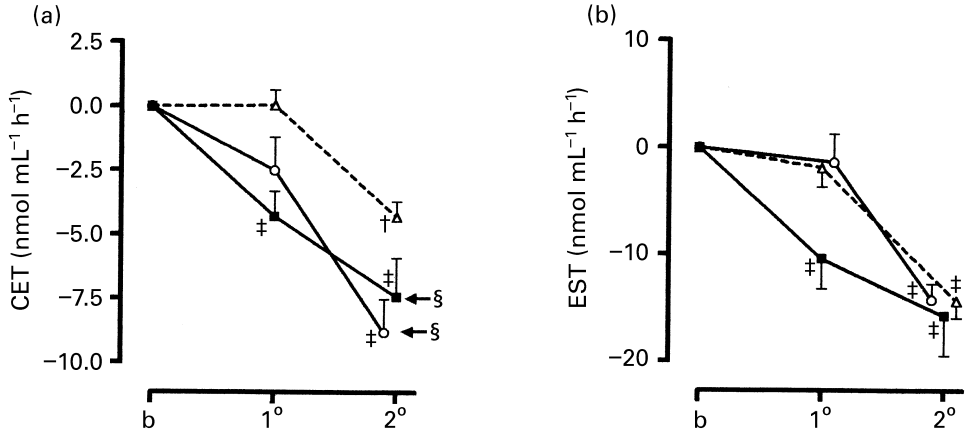

Figure 2 Changes in plasma cholesteryl ester transfer (CET, a) and cholesterol esterification rate (EST, b) in response to hyperinsulinaemia in type 2 diabetic patients (■), in healthy subjects (○) and during saline infusion in healthy subjects (Δ). b Indicates baseline, 1° and 2° indicate measurements after 3 (4) h of insulin infused at 30 mU kg^{-1} h^{-1}, respectively, and after 3 h of insulin infused at 150 mU kg^{-1} h^{-1}. Data are means ± SEM. *$P < 0{\cdot}05$, †$P < 0{\cdot}01$, ‡$P < 0{\cdot}001$ compared with baseline. §$P < 0{\cdot}01$ compared with overall change during saline infusion in healthy subjects.

novel finding of our study was that exogenous hyperinsulinaemia compared with the saline infusion experiment decreased plasma CET similarly in normotriglyceridaemic type 2 diabetic patients and in healthy subjects. Compared with saline infusion, insulin administration did not affect plasma EST in either group. Hence, the present data suggest that acute hyperinsulinaemia has a different effect on plasma CET in comparison with cholesterol esterification.

By the process of CET, cholesteryl esters are transferred from HDL towards lipoproteins of lower density classes, whereas triglycerides are transferred in the opposite direction [10,11]. The rate of CET is governed not only by the composition and concentration of cholesteryl ester donor and acceptor lipoproteins [10,20,28], but also by the amount of active CETP [22,39]. Another lipid transfer protein, phospholipid transfer protein (PLTP), may enhance the CETP-mediated CET [22,40]. Furthermore, *in vitro* experiments have shown that CET from HDL towards VLDL is stimulated during lipolysis as a consequence of accumulation of fatty acids in VLDL remnants [41,42]. It is likely that the decrease in plasma CET during hyperinsulinaemia can be attributed mainly to a decrease in plasma triglycerides, reflecting a lowered cholesteryl ester acceptor lipoprotein concentration. In addition, a decrease in plasma PLTP activity [25,43] could contribute to the lower plasma CET after insulin. Although a small decrease in plasma CETP activity, as a measure of CETP mass, has been reported in response to insulin infusion in non-diabetic subjects [44], no such effect was found during endogenous [25] and exogenous [43] hyperinsulinaemia in our earlier studies. Thus, it seems unlikely that changes in the plasma CETP level *per se* are important in this lowering of plasma CET. Of note, the current experiments challenge the possible effect of the ambient plasma FFA level on plasma CET and its fall during hyperinsulinaemia. Firstly, baseline plasma CET was not correlated with plasma FFA. This finding is in accord with other studies [25,43] but differs from the positive correlation between plasma FFA and CET from HDL towards VLDL and LDL, observed in a group of normolipidaemic individuals, which included subjects with very high fasting plasma FFA levels [42]. Secondly, changes in plasma FFA and CET were dissociated during saline infusion, as plasma FFA increased, whereas plasma CET decreased, probably because of plasma dilution.

The process of CET provides a plausible metabolic intermediate to explain the negative relationship of HDL cholesterol and HDL cholesteryl ester content to plasma triglycerides [10,45]. Indeed, the HDL cholesteryl ester content was negatively correlated with plasma CET, whereas the HDL triglyceride content was positively correlated with plasma CET. These relationships should be regarded as effects of CET and reciprocal triglyceride transfer on HDL core lipids [46], rather than as support of the possibility that cholesteryl ester depletion and triglyceride enrichment of HDL is a primary abnormality that causes a reduction in CET out of HDL [26,28,47]. In keeping with this view, we propose that the decrease in plasma CET coinciding with the fall in triglycerides provides a mechanism for the lack of decrease in HDL-cholesterol [23] and in the HDL cholesteryl ester content, as well as for the decrease in the HDL triglyceride content in response to acute hyperinsulinaemia. Obviously, we cannot exclude contributions of other mechanisms to these changes in HDL core lipids during hyperinsulinaemia, but it seems unlikely that the reported decrease in hepatic lipase activity [19] is primarily responsible because this would result in an increase rather than a decrease in the HDL triglyceride content.

Our study confirms that plasma cholesterol esterification in total plasma is positively correlated with plasma

triglycerides [21,22]. Consequent to the process of CET and the actions of lipases, HDL particle size and cholesteryl ester content are inversely related to plasma triglycerides [10,45]. Because LCAT preferentially interacts with small, cholesteryl ester-poor HDL [21,45], the positive relationship between plasma triglycerides and EST is probably explained by an effect of triglycerides on HDL composition. The fall in plasma EST after insulin was not different from that during saline infusion. This lack of effect of acute hyperinsulinaemia on plasma EST as opposed to the decrease in plasma CET is of interest, because plasma cholesterol esterification and CET are thought to be integrated processes [48]. The mechanisms responsible for the absence of a change in plasma EST after insulin are unknown.

The unaltered plasma CET [22,27,30] and cholesterol esterification [22,27,49] in type 2 diabetic patients compared with triglyceride-matched healthy subjects is in agreement with other studies. However, a decrease in plasma CET was originally reported in severely hyperglycaemic type 2 diabetic patients [50]. Some studies, in contrast, showed an increase in CET out of HDL in diabetic plasmas, in association with abnormalities in VLDL composition, apart from its triglyceride content [26,29]. Despite unchanged plasma CET and EST, HDL was found to be cholesteryl ester poor and triglyceride rich [51] in normotriglyceridaemic type 2 diabetic patients. This finding indicates that, besides CET and cholesterol esterification, measured in the fasting state, additional mechanisms contribute to an abnormal HDL core lipid composition in the diabetic state. An elevated post-prandial triglyceride-rich lipoprotein response has been observed in type 2 diabetic patients compared with healthy subjects matched for fasting plasma triglycerides [52]. This could result in sustained alterations in HDL composition in fasting plasma from diabetic patients as observed in the present study.

Although the process of CET in plasma is part of the reverse cholesterol transport pathway in man, this does not necessarily imply that inhibition of plasma CET represents a proatherogenic effect of insulin. In fact, accelerated plasma CET has been documented in dyslipidaemic conditions associated with an increased cardiovascular risk [22,26,39,53]. On the other hand, epidemiological studies suggest that subjects with partial genetic CETP deficiency have an increased cardiovascular risk [54]. It could be possible that the consequence for atherogenesis of a lowering in plasma CET by insulin is dependent on the balance between hepatic catabolism of apoB-containing lipoproteins and the efficacy of non-CET routes for cholesterol delivery to the liver [55,56].

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