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No change in apolipoprotein AI metabolism when subcutaneous insulin infusion is replaced by intraperitoneal insulin infusion in type 1 diabetic patients

Laurence Duvillard[a,b,*], Emmanuel Florentin[a,b], Sabine Baillot-Rudoni[c], Marie-Laure Lalanne-Mistrich[c], Agnès Brun-Pacaud[c], Jean-Michel Petit[a,b,c], Jean-Marcel Brun[c], Philippe Gambert[a,b], Bruno Vergès[a,b,c]

[a] *INSERM U498, Dijon F-21000, France*
[b] *University of Burgundy, IFR 100, Dijon F-21000, France*
[c] *Department of Endocrinology and Metabolic Diseases, CHU Dijon Bocage Hospital, Dijon F-21000, France*

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Abstract

In type 1 diabetic patients, the replacement of subcutaneous insulin infusion by intraperitoneal insulin infusion restores the normal physiological gradient between the portal vein and the peripheral circulation, which is likely to modify HDL metabolism. This stable isotope kinetic study was designed to compare HDL apolipoprotein (apo) AI metabolism in seven type 1 diabetic patients first treated by continuous subcutaneous insulin infusion by an external pump and then 3 months after the beginning of intraperitoneal insulin infusion by an implantable pump. Glycaemic control was comparable under subcutaneous and intraperitoneal insulin infusion (HbA1c = 7.34 ± 0.94% versus 7.24 ± 1.00%, NS). HDL composition was similar under both insulin regimens (esterified cholesterol = 20.1 ± 2.5% versus 24.0 ± 3.0% (NS), free cholesterol = 3.4 ± 1.1% versus 3.3 ± 0.9% (NS), triglycerides = 2.4 ± 0.9% versus 2.1 ± 0.9% (NS), phospholipids = 22.7 ± 5.3% versus 25.2 ± 6.5% (NS) and proteins = 51.2 ± 6.3% versus 45.5 ± 4.7% (NS)). The replacement of subcutaneous insulin infusion by intraperitoneal insulin infusion induced significant changes neither in apoAI fractional catabolic rate, nor in apoAI production rate, nor in apoAI pool size (respectively, 0.199 ± 0.051 pool d^{-1} versus 0.211 ± 0.017 pool d^{-1}, 12.0 ± 3.2 mg kg^{-1} d^{-1} versus 12.1 ± 1.8 mg kg^{-1} d^{-1}, 60.4 ± 5.0 mg kg^{-1} versus 57.5 ± 7.5 mg kg^{-1}). In conclusion, HDL metabolism is not modified by the replacement of subcutaneous insulin infusion by intraperitoneal insulin infusion when glycaemia is well controlled under both insulin regimens. As far as HDL metabolism is concerned there is no advantage in favour of one way of insulin administration or another.

Keywords: External pump; HDL; Implantable pump; Insulin; Kinetic study; Apolipoprotein AI; Type 1 diabetes mellitus

In type 1 diabetic patients, insulin is most commonly administered subcutaneously, either by multiple daily injections or continuously by an external pump. Alternatively, in case of frequent episodes of severe hypoglycaemia or excessive weight gain, an implantable pump is proposed to the patients [1]. Implantable pumps deliver insulin intraperitoneally with a resorption mainly in the portal vein, allowing the restoration of the negative physiological gradient between the portal and peripheral systems.

We recently demonstrated that the replacement of continuous subcutaneous insulin infusion (CSII) by continuous intraperitoneal insulin infusion (CIPII) in type 1 diabetic patients did not modify very low density lipoprotein (VLDL)

* Corresponding author at: Unité INSERM 498, Hôpital du Bocage, BP 77908, 21079 Dijon Cédex, France. Tel.: +33 3 80 29 36 46; fax: +33 3 80 29 36 61.
E-mail address: laurence.duvillard@chu-dijon.fr (L. Duvillard).

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and intermediate density lipoprotein production rate and fractional catabolic rate and induced a trend to a decrease in low density lipoprotein FCR [2].

As far as HDL are concerned, CIPII has been demonstrated to induce a decrease in HDL2 cholesterol and an increase in HDL3 cholesterol, compared to CSII [3]. Concomittantly, hepatic lipase (HL) activity was enhanced. Changes in cholesteryl ester transfer protein (CETP) activity have also been reported, although they are variable and contradictory. In a first paper CETP activity was increased while CSII was replaced by CIPII [4], whereas, it was decreased in a second paper [5].

CETP and HL play a major role in reverse cholesterol transport, in which cholesterol in excess is mobilized from peripheral cells and transported back to the liver by HDL particles [6–10]. CETP exchanges cholesteryl esters from HDL for triglycerides (TG) from TG-rich lipoproteins. The TG molecules transferred to HDL are secondary hydrolyzed by HL. During the remodelling of HDL by the combined role of CETP and HL, apolipoprotein (apo) AI is released and catabolized. Apo AI is also released after the interaction of HDL with scavenger receptor BI (SRBI) which promotes the selective uptake of cholesteryl ester molecules. HL facilitates the transfer of these molecules from the HDL core to SRBI by hydrolyzing HDL phospholipids at the surface of HDL [9].

While changes in the activity of CETP and HL have been shown, nothing is known about apoAI turnover rate when CSII is replaced with CIPII in type 1 diabetic patients. Apo AI kinetic studies allow to appreciate the dynamic of reverse cholesterol transport process, taking into account the different factors likely to influence this process. In order to get further insight into changes in HDL metabolism that could occur while the transition of CSII to CIPII, we performed two apolipoprotein AI stable isotope kinetic studies in type 1 diabetic patients: first under CSII by an external pump and then 3 months later under CIPII by an implantable pump.

1. Subjects and methods

1.1. Subjects

Seven type 1 diabetic patients (6 men and 1 woman, mean age = 48 ± 6.5 years) participated in that study. They had no diabetic complications (microalbuminuria < 20 $\mu g\,min^{-1}$) and were not taking any medication known to affect lipid metabolism. Two kinetic studies were performed in each subject: first one, while patients were under CSII, then second one, 3 months after the beginning of CIPII by an implantable pump.

CSII was accomplished using a 506 or 507 Minimed pump (Northridge, CA, USA). Insulin infusion was continuous with additional boluses administered at each mealtime. CIPII was accomplished using a 2007C or 2007A Minimed pump (Northridge, CA, USA) implanted subcutaneously in the abdomen with the catheter inserted into the peritoneal cavity. Insulin infusion was continuous with additional boluses administered at each mealtime.

The protocol was approved by the Dijon University Hospital Ethics Committee and written informed consent was obtained from each subject before the study.

1.2. Experimental protocol

The kinetic study was performed in the fed state. Food intake, with a leucine poor diet (1700 $kcal\,d^{-1}$, 55% carbohydrates, 39% fats and 7% proteins), was fractionated into small portions which were provided every 2 h starting 6 h prior to the tracer infusion up to the end of the study, in order to avoid important variations in apolipoprotein secretion, as previously performed by our group [11–13]. The endogenous labelling of apolipoproteins was carried out by administration of L-[1-^{13}C] leucine (99 atom%; Eurisotop, Saint Aubin, France), dissolved in 0.9% NaCl solution. At 08.00 h, each subject received intravenously a primed infusion of 0.7 $mg\,kg^{-1}$ of tracer immediately followed by a 16 h constant infusion of 0.7 $mg\,kg^{-1}\,h^{-1}$. Blood samples were drawn in tubes without anticoagulant but with a gel separator (Becton Dickinson, Meylan, France) at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15 and 16 h after the primed infusion. Serum was separated by centrifugation of $3000 \times g$ for 10 min at 4 °C.

The usual basal insulin infusion was not modified during the kinetic study. Four boluses of insulin (4–6 units) were administered at 08.00, 12.00, 18.00 and 22.00 h, before meals that contained more than 10 g carbohydrates. The same total amount of insulin was administered in the boluses as usual except that it was fractionated into four instead of three boluses. During the kinetic study capillary glycaemia was measured every 2 h.

1.3. Determination of apolipoprotein leucine ^{13}C enrichment

VLDL and HDL were isolated from plasma by a gradient ultracentrifugation, using a SW41 rotor in a L90 apparatus (Beckman Instruments, Palo Alto, CA, US). Then they were dialysed against a 10 $mmol\,l^{-1}$ ammonium bicarbonate buffer pH 8.2 containing 0.01% EDTA and 0.013% sodium azide and were delipidated 1 h at −20 °C using 10 volumes of diethylether-ethanol 3:1. VLDL apoB and HDL apoAI were isolated by preparative discontinuous SDS-PAGE on a 3 and 15% gel, respectively. The delipidated VLDL fractions were solubilised in 0.05 M Tris buffer pH 6.8, containing 3% SDS, 3% mercaptoethanol and 10% glycerol. The delipidated HDL fractions were solubilised in the same buffer without any mercaptoethanol. After staining with Coomassie blue R-250, apoB-100 and apoAI were cut from the gel, before being hydrolyzed in 6 N HCl for 16 h at 110 °C. The samples were then centrifuged to remove polyacrylamide and the supernatants were lyophilised in a Speed Vac (Savant