PASI I. NEVALAINEN

Intraperitoneal Versus Subcutaneous Insulin Treatment in Diabetic Patients on Continuous Ambulatory Peritoneal Dialysis Therapy

University of Tampere
Tampere 2001

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ACADEMIC DISSERTATION

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Supervised by
Professor Amos Pasternack
University of Tampere

Reviewed by
Docent Agneta Ekstrand
University of Helsinki
Professor Jorma Viikari
University of Turku

Distribution

University of Tampere
Sales Office
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Tel. +358 3 215 6055
Fax +358 3 215 7685
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I CONTENTS

II LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following six original studies, which are cited in the text by roman numerals **I-VI**.

I Nevalainen PI, Lahtela JT, Mustonen J, Pasternack A (1996): Subcutaneous and intraperitoneal insulin therapy in diabetic patients on CAPD. Perit Dial Int 16: Suppl 1: S288-91.

II Nevalainen PI, Lahtela JT, Mustonen J, Pasternack A (1997): The influence of peritoneal dialysis and the use of subcutaneous and intraperitoneal insulin on glucose metabolism and serum lipids in type 1 diabetic patients. Nephrol Dial Transplant 12: 145-50.

III Nevalainen PI, Lahtela JT, Mustonen J, Taskinen M-R, Pasternack A (1999): The effect of insulin delivery route on lipoproteins in type 1 diabetic patients on CAPD. Perit Dial Int 19: 148-53.

IV Nevalainen PI, Lahtela JT, Mustonen J, Pasternack A (2000): Intraperitoneal insulin reduces plasma leptin concentrations in diabetic patients on CAPD. Perit Dial Int 20: 27-32.

V Nevalainen PI, Kallio T, Lahtela JT, Mustonen J, Pasternack A (2000): High peritoneal permeability predisposes to hepatic steatosis in diabetic continuous ambulatory peritoneal dialysis patients receiving intraperitoneal insulin. Perit Dial Int 20: 637-642.

VI Kallio T, Nevalainen PI, Lahtela JT, Mustonen J, Pasternack A (2001): Hepatic subcapsular steatosis in diabetic CAPD patients treated with intraperitoneal insulin. Description of a typical pattern. Acta Radiol (in press).

III ABBREVIATIONS

ABC1	adenosine triphosphate binding cassette transporter 1
AGE	advanced glycosylation end-product
Apo	apoprotein
BMI	body mass index
CAPD	continuous ambulatory peritoneal dialysis
CE	cholesteryl ester
CETP	cholesteryl ester transfer protein
CV	coefficient of variation
D/D0gluc	the ratio of dialysate to initial dialysate glucose concentration at a given time in a PET
D/Pcrea	the ratio of dialysate to plasma creatinine concentration at a given time in a PET
ESRD	end-stage renal disease
FC	free cholesterol
g	gravity
HbA_{1c}	glycated haemoglobin A fraction 1c
HDL	high density lipoprotein
HL	hepatic lipase
IDDM	insulin dependent diabetes mellitus
IDL	intermediate density lipoprotein
IP	intraperitoneal
IRI	immunoreactive insulin concentration
U	international unit
KT/V_{urea}	urea clearance in litre/total body water in litres
LCAT	lecithin cholesterol acyltransferase
LDL	low density lipoprotein
LPL	lipoprotein lipase
M	glucose disposal rate
M/ΔIRI	insulin sensitivity
NIDDM	non-insulin dependent diabetes mellitus
NPH	neutral protamine Hagedorn
NS	not significant
OB	obesity gene
PCR	protein catabolic rate
PD	peritoneal dialysis
PET	peritoneal equilibration test
Rad	Ras gene associated with diabetes
rpm	rotations per minute
SC	subcutaneous
TG	triglyceride
VLDL	very low density lipoprotein

IV INTRODUCTION

Up to 40% of diabetic patients are faced with nephropathy and renal failure after decades of either insulin dependent diabetes mellitus (IDDM) or non-insulin dependent diabetes mellitus (NIDDM) (Andersen et al. 1983). Diabetic patients with end-stage renal disease (ESRD) are the largest and fastest growing patient group on dialysis therapy. Continuous ambulatory peritoneal dialysis (CAPD) is a commonly used renal replacement therapy option for diabetic patients (Koch et al. 1997, Finnish Registry for Kidney Diseases 1998). Dialysis treatment is an intermediate solution for some patients awaiting renal transplantation. Dialysis therapy should be tailored individually with an aim of not causing any further burden on patient health. The acceleration of atherosclerosis, for example, should be avoided. Atherosclerosis is of major concern, as it causes significant morbidity and mortality. Atherosclerosis is accelerated in diabetes and the uraemic state, but patients with concurrent diabetes and ESRD are a special risk group (de Lemos and Hillis 1996, Muller 1998).

In non-uraemic diabetic patients an implantable pump has been developed to administer insulin intraperitoneally (IP) (Selam et al. 1989, Saudek et al. 1996). CAPD treatment provides a convenient medium for the administration of insulin IP with freedom from subcutaneous (SC) injections in the patient with concurrent diabetes and ESRD. The IP route is theoretically considered to be the most physiologic available way of replacing the comromised endogenous insulin production. IP insulin can cause diurnal plasma insulin levels that mimic the physiological state (Schade et al. 1980a). Compared to SC insulin a similar or better glycaemic control is observed with IP insulin administration in non-uraemic IDDM patients (Selam et al. 1992, Saudek et al. 1996). In addition, severe hypoglycaemic

episodes with IP insulin have been fewer, and blood glucose variation has been less marked, than with SC insulin in both non-uraemic IDDM subjects (Hanaire-Broutin et al. 1995, Dunn et al. 1997) and IDDM patients on CAPD (Lahtela et al. 1995).

IP versus SC insulin treatment has been studied in many aspects in non-uraemic patients with implantable IP or SC insulin pumps. In the present study, the metabolic effects of two different routes of insulin administration have been compared in diabetic patients on CAPD. Special attention was given to glycaemic control, insulin sensitivity, lipoprotein metabolism, plasma leptin and hepatic steatosis.

V REVIEW OF THE LITERATURE

1. Diabetic end-stage renal disease

1.1. Continuous ambulatory peritoneal dialysis in diabetic patients

1.1.1. History of peritoneal dialysis treatment

Peritoneal dialysis (PD) treatment was first used successfully for the treatment of acute renal failure in 1945. The first long-term intermittent PD was initiated in 1956 (McBride 1987). The first report of intermittent PD treatment in diabetic patients was in 1971 (Crossley and Kjellstrand 1971). CAPD treatment was initiated in non-diabetic uraemic patients in 1975 (Popovich et al. 1976, McBride 1987) and a few years later in diabetic uraemic patients (Flynn and Nanson 1979).

1.1.2. Epidemiology of chronic uraemia and peritoneal dialysis treatment in diabetic patients

The incidence of new patients with chronic uraemia in Finland beginning active treatment was 90 patients/million people/year, which translated to 462 patients/year in 1998. The incidence of new dialysis patients in 1998 was 22% higher than in 1997, and 45% higher than in 1994. The fastest growing group was the NIDDM patients, with a 77% increase in patient years between 1994 and 1998. Diabetic ESRD

accounted for 32% of all new patients treated by dialysis (Finnish Registry for Kidney Diseases 1998). The median age of dialysed IDDM patients has increased annually from 42.9 years at the end of 1994 to 45.7 years at the end of 1998. The mean patient age for dialysed NIDDM patients remained more constant during the same time interval (64.4 and 64.7 years, respectively) (personal communication, Finne P, Finnish Registry for Kidney Diseases 1998).

At the end of the year 1998, 26% (n=270) of all dialysis patients in Finland were treated with PD. The corresponding figure for patients with diabetic ESRD was 35% (n=107). Among all PD patients the incidence of episodes of peritonitis was 0.24/patient/year. In diabetic PD patients the corresponding figure was 0.22 episodes/patient/year (personal communication, Finne P, Finnish Registry for Kidney Diseases 1998).

1.1.3. The peritoneum and the physiology of peritoneal dialysis

The peritoneal cavity is lined by a continuous serous membrane with a simple squamous mesothelium underlaid by connective tissue. Physiologically the peritoneum functions as a secretory organ producing fluid that contains mainly phosphatidylcholine acting as a lubricant for moving organs (Dobbie 1994). The function of the peritoneum as a dialysis membrane, however, is still partly unknown.

The lymphatic system of the peritoneal cavity drains primarily through lymphatic vessels located in the subdiaphragmatic peritoneum, further through the retrosternal lymphatics and the right lymphatic duct to the venous circulation (Rippe and Krediet 1994). In the parietal and visceral peritoneal interstitium, a rich network of lymphatic vessels is present (Dobbie 1994). Terminal lymphatic lacunae are

separated from the peritoneal cavity only by thin mesothelial cells (Rippe and Krediet 1994). The lymphatics draining the peritoneal cavity are the main pathways for the absorption of biologically inert particles, colloids, cells, and isosmotic-isoncotic fluid from the peritoneal cavity (Khanna and Nolph 1989).

Solute transport across the peritoneum occurs by way of convection or diffusion. Small solutes mainly gain entry into the peritoneal microcirculation by diffusion. The greater the concentration difference, the greater the net diffusion. Lipid soluble substances diffuse through the lipid portion of the cell membrane (Khanna and Nolph 1989). Transcellular and intercellular transport of water and water-soluble substances are believed to occur mainly via small, protein-restrictive pores (diameter 8-11 nm). A solute restrictive “water-only” pathway (molecular diameter 0.5-3 nm) accounts for the remainder of ultrafiltration. Proteins are transported via large pores (diameter 50 nm), which are extremely few in number (Rippe 1993). Protein loss can be in the nephrotic range of 5-12 g/day (Blumenkrantz et al. 1981). The kinetics of lipoprotein peritoneal clearance are similar to those of other macromolecules and proteins. This supports the concept of passive leakage of lipoproteins from plasma to peritoneal dialysate as the mechanism of loss (Kagan et al. 1990b).

Over time, solute transfer and ultrafiltration may decline with PD treatment. The process can be worsened and accelerated by episodes of peritonitis (Davies et al. 1996). Age, sex, and mean blood pressure do not significantly affect long-term peritoneal transport kinetics (Shin et al. 1999). The most important factor influencing peritoneal clearance kinetics of solutes and proteins is molecular mass (Kagan et al. 1990a). The loss of a macromolecule, however, may perceivably be influenced by the type of dialysis, a local production of the macromolecule, its rate of biosynthesis, its serum concentration, and by the rate of re-uptake by the lymphatics.

During CAPD the continuous exposure to glucose containing dialysates causes diabetiform changes in the peritoneal membrane and vasculature in both diabetic and non-diabetic patients. Microscopic thickening of the basal membrane is seen. These changes include protein glycation and formation of advanced glycation end-products (AGE). Serum AGE levels closely correlate with creatinine clearance. AGE levels are highest in diabetic ESRD patients (Bucala and Vlassara 1995).

1.1.4. Continuous ambulatory peritoneal dialysis treatment in practise

CAPD is usually performed as four exchanges of 1.5-2.5 litres dextrose (glucose anhydrate) containing dialysate. The dwell time of each exchange is 4-8 hours. This schedule is convenient for both solute transfer and ultrafiltration. In patients with high peritoneal transfer rate, automated PD treatment is preferable. In the dialysate solutions used in Finland, the concentration of glucose can be 1.36%, 2.27% or 3.86% (24.5-69.5 mmol/l). When using four 1.5-2.5-litre dialysate bags, the daily amount of instilled glucose is approximately 80-400 grams, depending on the glucose concentration of the solution. Two thirds of this amount is absorbed (Schmitz 1985), equaling 50-270 grams/day. The mean amount of energy derived from the absorbed glucose represents 200-1100 calories per day. Recent PD treatment, in addition to glucose, utilises combination therapies with dialysates containing glucose polymers, amino acids, and bicarbonate-buffered dialysates.

Table 1. Advantages and disadvantages of continuous ambulatory peritoneal dialysis

Advantages	Disadvantages
Steady state hemodynamics and biochemistry	High glucose absorption
Reduced cardiovascular stress	Hypertriglyceridemia
Better preservation of residual renal function	Hypervolemic state
Allows administration of IP insulin	Protein loss
Freedom from machine	Inconvenient schedule for daily activities
Freedom to travel	Distorted body image
Avoidance of the need for vascular access	Risk of peritonitis
	Dialysis solution leak, hernias, fistulae, pleural effusion, abdominal and back pain

1.1.5. Advantages and disadvantages of continuous ambulatory peritoneal dialysis

The main advantages and disadvantages of CAPD treatment are listed in Table 1. In an active patient CAPD provides freedom from the dialysis machine and travelling is less restrective than with hemodialysis. Compared to the fast volume and biochemical changes caused by each treatment session of hemodialysis, CAPD provides relatively steady state hemodynamics and gradual electrolyte shifts. This is thought to be advantageous for diabetic patients with autonomic neuropathy, advanced age, and cardiovascular problems. Vascular access in hemodialysis treatment causes more problems in diabetic patients than peritoneal catheterisation, which is easier to establish and less troublesome. CAPD does not require heparinisation, which may cause hemostasis-related problems (Rippe and Krediet

1994). The peritoneal catheter provides an easy administration route for IP insulin, removing the need for self-injection. During CAPD effective control of blood sugar is achieved through IP insulin administration (Flynn et al. 1979, Lahtela et al. 1995).

In non-diabetic CAPD patients using dialysates with low glucose concentrations, the plasma glucose levels remain stable. With higher concentrations of dialysate glucose, plasma glucose and insulin concentrations are increased in both non-diabetic (Armstrong et al. 1985) and diabetic patients (Wideröe et al. 1983). It is assumed that diabetic patients require higher doses of insulin after commencement of CAPD therapy due to increased glucose load. A presumably compensatory reduction in their intake of oral carbohydrates is observed (von Baeyer et al. 1983). Although the initiation of dialysis decreases insulin resistance (Schmitz 1985), a high dialysate glucose concentration can increase plasma glucose levels (Armstrong et al. 1985).

During CAPD treatment diabetic ESRD progresses, causing decreased residual renal function (Balaskas et al. 1994). With CAPD both non-diabetic (Moist et al. 2000) and diabetic (Rottembourg et al. 1983) patients have a tendency to retain greater residual renal function than hemodialysis patients. Factors contributing to this may include the hypervolemic status (Takeda et al. 1998) and steady state hemodynamics (Khanna 1994) during CAPD therapy.

A major disadvantage of PD is the substantial loss of protein. Peritoneal dialysate contains possibly all the proteins present in serum and intestinal lymph. The lower the molecular weight of a protein, the greater the loss (Kagan et al. 1990a). High peritoneal permeability of macromolecules is a risk factor for malnutrition (Kang et al. 1999).

Protein-energy malnutrition is common (41%) among CAPD patients (Young et al. 1991). One study has suggested that diabetic dialysis patients may have a lower

mean body weight than non-diabetic dialysis patients (Miller et al. 1983). In diabetic patients a higher incidence of mild to moderate malnutrition has been observed than in non-diabetic patients on CAPD, but the incidence of severe malnutrition is similar (Young et al. 1991). Low weekly KT/V_{urea} (urea clearance in litres/total body water in litres) correlates with poor survival in non-diabetic patients regardless of the dialysis method. Loss of residual renal function decreases nutritional indices, weekly KT/V_{urea}, the protein catabolic rate (PCR) and creatinine clearance in patients on CAPD. In this patient group loss of residual renal function is associated with a lower survival rate than in patients with preserved renal function (Maiorca et al. 1995). Nutritional status in dialysis patients is best evaluated by using a variety of markers (Young et al. 1991).

1.2. Atherosclerosis and survival in uraemic diabetes mellitus patients

IDDM patients present with a 3 to 6-fold higher mortality rate than the non-diabetic population. The most frequent causes of death are cardiovascular diseases and ESRD. In NIDDM patients a 1.4 to 3.7-fold higher mortality rate is observed when compared to the non-diabetic population. Cardiovascular events and strokes are the major causes of death in NIDDM patients (Muller 1998).

Cardiovascular disease accounts for almost half of the total mortality in the ESRD population. Diabetic patients with ESRD have a cardiovascular mortality rate twice that of age-matched non-diabetic ESRD patients (de Lemos and Hillis 1996). During CAPD the presence of diabetes is the most important risk factor for mortality (Cueto-Manzano and Correa-Rotter 2000). Whether diabetic patients are treated by hemodialysis or CAPD seems to have no effect on survival rates. This holds true even

after adjusting for the fact that diabetic patients in better clinical condition are prone to be selected for CAPD treatment (Marcelli et al. 1996). In an angiographic study where uraemic IDDM patients were evaluated for the presence of coronary artery disease, it was shown that no patient over age 45 had normal coronary arteries. One or more significant coronary stenoses were found in 81% of the patients. Diabetic renal transplant recipients with coronary artery disease have poor survival rates (Manske et al. 1992).

In a prospective study of 196 diabetic patients on hemodialysis, elevated serum total cholesterol, low density lipoprotein to high density lipoprotein (LDL/HDL) - cholesterol ratio, LDL-cholesterol, and apoprotein (Apo) B levels on admission to dialysis were associated with an increased risk of sudden death and death due to myocardial infarction (Tschöpe et al. 1993). Another prospective study evaluated 412 IDDM and NIDDM patients at the time of initiation of dialysis therapy (CAPD or hemodialysis). Serum Apo A-I, fibrinogen, age and stroke were all found to be independent predictors of both cardiac and non-cardiac death (Koch et al. 1997).

IDDM patients on active renal replacement treatment (dialysis or renal transplantation) in Finland in the year 1998 had a five-year survival rate of 51%. In NIDDM patients the corresponding figure was 11%. In CAPD-treated patients the five-year survival rates were 12% and 7% for IDDM and NIDDM patients, respectively (Finnish Registry for Kidney Diseases 1998).

2. Insulin metabolism in non-uraemic and uraemic diabetic patients

Most of the insulin secreted by the pancreas is transported to the liver via the portal vein. The liver metabolises about 50% of this insulin on the first pass, i.e. before it reaches the systemic circulation. Post-hepatically the kidney plays an important role in insulin degradation by sequestering some 30-80% of the insulin entering the systemic circulation. The role of the kidney increases if exogenous insulin is administered peripherally. Other tissues, mainly skeletal muscle, remove the remainder of circulating insulin (Ferrannini et al. 1983). In target tissues insulin interacts with insulin receptors. The insulin molecule is internalised and degraded together with the receptor (Juul et al. 1986).

Neither subcutaneous, intramuscular nor intravenous routes of insulin administration restore normal insulin levels in IDDM patients. Patients undergoing these treatment modalities are characterised by peripheral hyperinsulinaemia and relative portal hypoinsulinaemia (Schade et al. 1980b). Sustained iatrogenic peripheral hyperinsulinaemia results in insulin resistance (Rizza et al. 1985).

When the glomerular filtration falls below 40 ml/min in chronic renal failure, renal insulin degradation decreases (Rabkin et al. 1970). Clinically this is associated with decreased insulin requirement in IDDM patients. When uraemia emerges and dialysis therapy begins, the insulin requirement is virtually unpredictable in both IDDM and NIDDM patients (Avram et al. 1984). Uraemia results in delayed insulin degradation in extrarenal tissues in laboratory test animals (Mondon et al. 1978). Accumulation of a dialysable uraemic toxin may explain the inhibition of insulin degradation systems. In uraemia, hepatic insulin metabolism is intact (DeFronzo and

Alvestrand 1980). Insulin is not significantly dialysed by either PD (Wideröe et al. 1983) or hemodialysis (Wizemann et al. 1985).

3. Lipoproteins in non-uraemic and uraemic diabetic patients

In contrast with chylomicrons, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL) and LDL particles, HDL is considered to be an antiatherogenic lipoprotein because it is responsible for the reverse cholesterol transport. Low plasma HDL-cholesterol levels predict increased cardiovascular mortality in population studies (Pekkanen et al. 1990, Buring et al. 1992). HDL mediated reverse cholesterol transport is presented in Figure 1. The transfer of free cholesterol (FC) from peripheral cells to HDL is mediated by adenosine triphosphate binding cassette transporter protein 1 (ABC1). Partly the cholesteryl esters in a mature HDL particle are taken up by the liver through the interaction of HDL with either the HDL receptor (scavenger receptor B1 or SR-B1), the LDL (ApoB-100/ApoE) receptor, or hepatic lipase (Kwiterovich 2000). Some of the cholesteryl esters in a mature HDL particle are transferred to Apo B –containing lipoproteins VLDL, IDL and LDL. The liver takes up cholesteryl esters of IDL and LDL through the action of LDL (ApoB-100/ApoE) receptor.

The decompensated IDDM state is commonly accompanied by elevated levels of triglyceride-rich VLDL and LDL particles, cholesterol and Apo B, and decreased HDL-cholesterol. Intensive insulin treatment restores fasting lipoprotein concentrations to non-diabetic levels. With SC insulin treatment, a good or moderate control of plasma glucose levels results in normal or near normal levels of plasma lipids and lipoproteins. Good glycaemic control correlates inversely with plasma

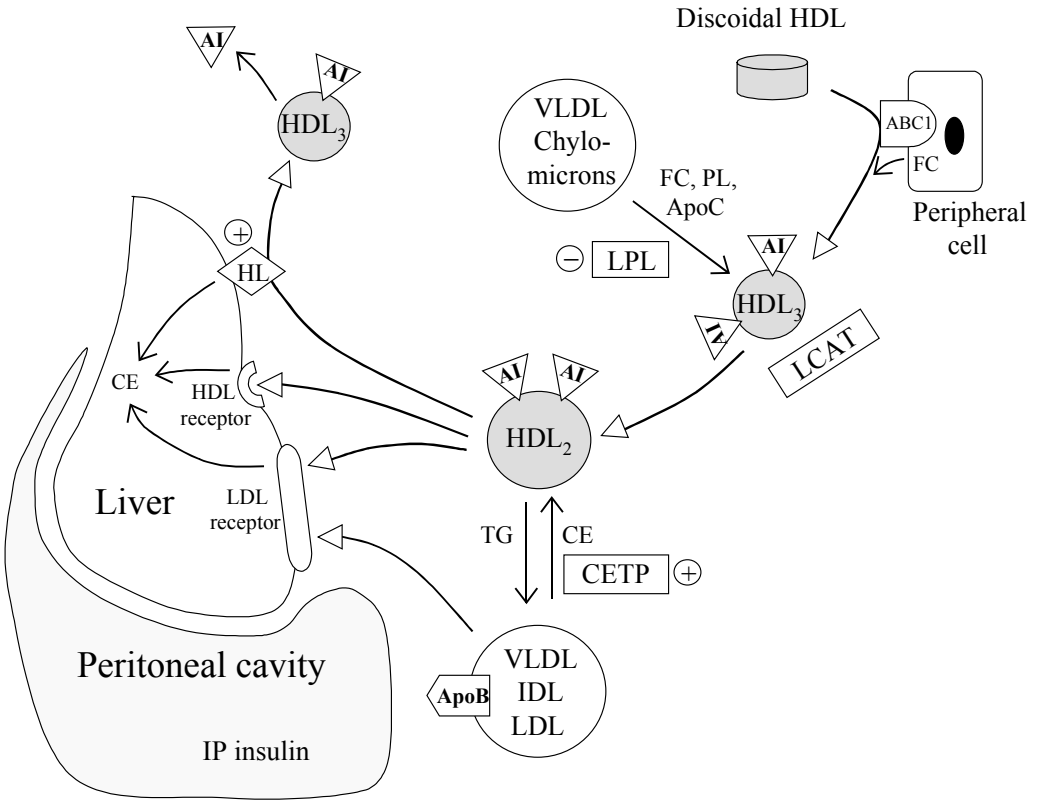


Figure 1. HDL-particles receive free cholesterol (FC), phospholipids (PL), apoprotein (Apo) C from VLDL and chylomicrons through the action of lipoprotein lipase (LPL). HDL particles also accept FC from peripheral cells through the action of ABC1 (adenosine-triphosphate-binding cassette transporter protein 1). HDL_3 particles accumulate cholesteryl esters (CE) via the action of lecithin cholesterol acyltransferase (LCAT) and transform into larger, less dense HDL_2 particles. The latter undergoes cholesteryl ester transfer protein (CETP) -mediated exchange of CE for triglycerides (TG) from Apo B-containing lipoproteins (VLDL, IDL and LDL). Hepatic lipase (HL) hydrolyses TG and CE moieties from HDL_2, or entire HDL_2 particles are taken up by the liver. At the same time HDL_2 is converted to HDL_3 which is subject to Apo A-I loss. The liver can also take up HDL through HDL receptor and LDL receptor (ApoB/ApoE receptor) action. Decrease (–) or increase (+) in enzyme activity by converting insulin administration route from SC to IP in nonuraemic IDDM patients is shown. AI = Apo A-I, IP = intraperitoneal, SC = subcutaneous, LDL = low density lipoprotein, IDL = intermediate density lipoprotein, VLDL = very low density lipoprotein.

concentrations of VLDL-triglycerides, LDL-cholesterol and Apo B, and directly with plasma HDL-cholesterol concentrations. Plasma HDL-cholesterol is elevated in IDDM patients. Primarily HDL_2-cholesterol is increased, but a rise in HDL_3-cholesterol has also been noted. Plasma Apo A-I is commonly elevated. Lipoprotein changes are mainly qualitative and functional in IDDM patients with at least moderate glycaemic control (Taskinen 1990).

Hyperinsulinaemia associated with insulin resistance can result in increased plasma triglycerides and decreased plasma HDL-cholesterol in both healthy and NIDDM subjects. In non-uraemic IDDM patients on SC insulin, plasma HDL-cholesterol correlates with lipoprotein lipase activity, which probably reflects peripheral hyperinsulinaemia and its effect on lipoprotein lipase (Nikkilä et al. 1977, Taskinen 1990). When the peripheral insulin concentration is lowered with IP insulin treatment, the lipoprotein lipase activity decreases (Kazumi et al. 1986, Selam et al. 1994).

Hepatic lipase is involved in the turnover of HDL and Apo B containing lipoproteins. By the action of hepatic lipase, hydrolysed triglycerides and cholesteryl esters are taken up by the liver (Figure 1). The activity of hepatic lipase is normal or increased in diabetes, and could be one explanation for the normal or higher than normal plasma HDL-cholesterol observed in IDDM (Nikkilä et al. 1977, Taskinen 1990). IP insulin has been shown to stimulate hepatic lipase activity more than SC insulin in IDDM patients without renal disease. In one study plasma HDL-cholesterol decreased and plasma HDL_3-cholesterol increased during IP insulin treatment, and the lipoprotein composition was normalised (Ruotolo et al. 1994). The activity of both hepatic and lipoprotein lipase is lowered in uraemia. The activities of these enzymes are not affected by the initiation of hemodialysis treatment (Huttunen et al. 1978).

Non-enzymatic glycosylation and oxidation can modify lipoproteins. The catabolism of HDL is accelerated by glucosylation in laboratory test animals. This could partly explain the decreased concentration of HDL observed in non-uraemic diabetic patients with poor glycaemic control (Witztum et al. 1982). Glycosylation and oxidation of LDL are considered proatherogenic changes because cholesterol-rich LDL becomes more prone to uptake by foam cells (Lyons 1991).

Diabetic nephropathy is associated with increased plasma cholesterol, LDL-cholesterol, VLDL triglycerides and Apo B, and decreased plasma HDL-cholesterol and Apo A-I. These changes are already present at the microalbuminuric stage (Taskinen 1990). High serum triglycerides and low serum HDL-cholesterol are characteristic findings in chronic renal failure of any origin (Avram et al. 1992). Uraemic IDDM and NIDDM patients tend to have lower plasma HDL-cholesterol levels than non-diabetic ESRD patients (Avram et al. 1992).

Weight gain after initiation of CAPD is associated with a further worsening of abnormal lipid levels: increased serum triglycerides and total cholesterol/HDL-cholesterol ratio, decreased HDL-cholesterol (Little et al. 1998). In a study where non-diabetic CAPD patients with negligible or preserved residual renal function were compared, plasma total cholesterol, LDL-cholesterol, Apo B and Apo A-I were found to be lower in the group with negligible renal function (Kagan et al. 1997). In an *in vitro* study the addition of serum from non-diabetic CAPD patients to hepatic cell culture decreased apo A-I and increased apo B synthesis (Shah et al. 1996). The dialysate used in CAPD has a high concentration of glucose, which can stimulate non-enzymatic protein glycosylation. This could increase the catabolism of HDL and to some degree explain decreased HDL-cholesterol after initiation of CAPD (Witztum et al. 1982).

In the normal population high plasma triglyceride concentration and low HDL-cholesterol are associated with small LDL particle size. This lipoprotein pattern is associated with increased atherosclerotic changes (Coresh et al. 1993). An increased level of small LDL particles is more frequent in non-diabetic CAPD patients than those on hemodialysis or healthy controls (O'Neal et al. 1996).

The high concentration of plasma glucose in diabetic patients leads to increased oxidation of lipoproteins. Oxidized LDL particles may have an increased atherogenic potential (Evans and Orchard 1994). Lipoproteins are more readily oxidized in non-diabetic uraemic patients than in normal controls. A slight reduction in LDL oxidation is seen after initiation of CAPD, but not after initiation of hemodialysis (Maggi et al. 1994).

4. Insulin resistance in non-uraemic and uraemic diabetic patients

4.1. What is insulin resistance?

In insulin resistance normal amounts of insulin produce a subnormal biological response in a target organ (muscle, liver, fat). In healthy subjects male gender (Nuutila et al. 1995), genetic factors, physical inactivity (Endre et al. 1994), obesity and regional adiposity (Ross et al. 2000) may induce insulin resistance. Hyperlipidemia, hypertension, proteinuria and certain drugs (corticosteroids, diuretics, β-blockers) may also affect insulin resistance (Ekstrand 1992, Samuelsson et al. 1994).

At the cellular level, insulin resistance is a postreceptor defect affecting the transport, metabolism, and intracellular storage of glucose (DeFronzo and Alvestrand 1980, Pedersen et al. 1985). Glucose uptake by peripheral tissues has two metabolic pathways: 1) oxidative glucose metabolism includes glycolysis and the Krebs cycle, 2) non-oxidative glucose storage as glycogen. Defects in either of these metabolic pathways may induce insulin resistance. Cellular factors exerting an influence on insulin resistance include the guanosine triphosphatase Rad (Ras gene associated with diabetes)(Reynet and Kahn 1993), the membrane glycoprotein PC-1 (Kumakura et al. 1998), tumor necrosis factor α (Hotamisligil et al. 1994), peroxisome proliferator activated receptor γ (Park et al. 1997), and an increased glucose flux through the hexosamine pathway in skeletal muscle (Yki-Järvinen et al. 1996).

4.2. Measurement of insulin resistance

Insulin resistance in NIDDM can be studied using a variety of techniques. They include the intravenous glucose tolerance test, the intravenous insulin tolerance test, the combined intravenous insulin-oral glucose tolerance test, the forearm perfusion technique, the quadruple infusion protocol, the somatostatin modification of the quadruple infusion protocol, the radioactive glucose infusion techniques, and the insulin clamp technique (DeFronzo et al. 1982b). When studying uraemic insulin resistance, oral or intravenous glucose tolerance tests provide insufficient information. In order to evaluate and quantify insulin resistance, the clamp method has been developed (DeFronzo et al. 1979). The clamp method can be used to study insulin resistance in the absence of endogenous insulin secretion in IDDM patients (DeFronzo et al. 1982b). In this method endogenous glucose production is completely

inhibited by an intravenous infusion of insulin, which results in hyperinsulinaemia. At the same time the quantity of exogenous glucose required to maintain euglycaemia (the M value) is a reflection of the net sensitivity of target tissues (mainly skeletal muscle) to insulin.

4.3. Insulin resistance in diabetes mellitus

By using an insulin clamp method it has been shown that even IDDM patients with fairly low daily insulin requirements (~35 U/day) have impaired insulin action. IDDM is associated with insulin resistance primarily in peripheral tissues (DeFronzo et al. 1982b, Yki-Järvinen and Koivisto 1984b). Glucose removal is regulated by plasma glucose (Schmitz et al. 1988) and insulin concentration (DeFronzo et al. 1978). Insulin can increase glucose uptake in skeletal muscle by increasing its blood flow in healthy subjects (Baron et al. 2000). At all glucose levels 75-95% of insulin mediated glucose uptake occurs in skeletal muscle (Baron et al. 1988). Insulin induced glucose uptake by splanchnic tissue (10%) is of minor importance (DeFronzo et al. 1985). There is an inverse relationship between fasting glucose concentrations and glucose clearance in normal, NIDDM, and IDDM subjects (DeFronzo et al. 1982b). In comparison with healthy subjects, fasting plasma glucose clearance is reduced (32%) in IDDM patients (DeFronzo et al. 1982a, Ekstrand 1992). The reduction is even more pronounced (~55%) when corrected for plasma glucose concentration (DeFronzo et al. 1982b).

Hyperglycaemia *per se* may induce insulin resistance (Unger and Grundy 1985). This is supported by the finding of decreased peripheral insulin resistance in response to correction of hyperglycaemia by insulin therapy in IDDM patients (Yki-Järvinen

and Koivisto 1984a). In healthy subjects and NIDDM patients with physiologic plasma insulin concentrations and hyperglycaemia, the glucose disposal rate is substantially accounted for by splanchnic tissue. This is not seen in the hyperinsulinaemic state (DeFronzo et al. 1983, DeFronzo et al. 1985).

In non-diabetic laboratory test animals, endogenous glucose production is largely (~80%) attributable to liver gluconeogenesis and glycogenolysis (Sindelar et al. 1998, Cersosimo et al. 1999b). Hepatic glucose production is controlled by the peripheral insulin concentration in non-diabetic laboratory test animals (Ader and Bergman 1990). By increasing the plasma insulin concentration, hepatic glucose production is suppressed. In adipose tissue insulin has an antilipolytic effect, leading to decreased release of free fatty acids. Decreased plasma free fatty acid concentration is an important suppressor of hepatic glucose production in non-diabetic laboratory test animals (Sindelar et al. 1997). Both IDDM and NIDDM patients have increased endogenous glucose production. In these patients, impaired insulin-mediated suppression of hepatic glucose production is the principal determinant of fasting plasma glucose. The rate of glucose production is inappropriately high for the associated degree of hyperglycaemia and hyperinsulinaemia (DeFronzo et al. 1982b).

Although basal hepatic glucose output is significantly elevated in IDDM patients, it can be normally suppressed by hyperinsulinaemia (DeFronzo et al. 1982a). The relative portal hypoinsulinaemia and peripheral hyperinsulinaemia are suggested to explain, at least in part, the insulin resistance seen in IDDM patients (DeFronzo et al. 1982b, Yki-Järvinen and Koivisto 1984b). Hepatic glucose production has been shown to be very sensitive to small changes in portal insulin concentration (DeFronzo et al. 1979, Ferrannini et al. 1983). This effect could be mediated through the peripheral insulin concentration. In NIDDM patients, even the normal rate of basal

hepatic glucose production is resistant to the inhibiting effects of insulin and hyperglycaemia (DeFronzo et al. 1982b).

The human kidney makes a substantial contribution to endogenous glucose turnover (20%) in fasting healthy subjects. Renal glucose production is persistently elevated in diabetic patients. Insulin suppresses renal glucose production and stimulates renal glucose uptake (Meyer et al. 1998a, Cersosimo et al. 1999a). In healthy subjects, in a mild to moderate state of hypoglycaemia, about 36% of the endogenous glucose is produced by the kidneys (Cersosimo et al. 1999b). The effect of exogenous insulin on renal glucose production is not well defined (Meyer et al. 1998b).

4.4. Insulin resistance in uraemia

In uraemia a number of factors, such as anaemia (Mak 1996), hyperinsulinaemia (Rizza et al. 1985), metabolic acidosis (DeFronzo and Beckles 1979), uraemic toxins (Maloff et al. 1983), hyperosmolality (Bratusch-Marrain and DeFronzo 1983), and hormonal aberrations (Kautzky-Willer et al. 1995) can modify insulin sensitivity. Some of these are at least partly reversible by dialysis treatment, which improves insulin sensitivity.

Non-diabetic uraemic patients have impaired glucose tolerance. B-cell function may be impaired in a fashion similar to what is seen in NIDDM (DeFronzo and Alvestrand 1980). These findings are in accord with the insulin resistance found in all uraemic patients (DeFronzo 1978).

The degree of insulin resistance in IDDM is similar to that seen in non-diabetic uraemic patients. In IDDM, either the insulin deficiency *per se* or the metabolic

derangements secondary to insulin deficiency, would be the major determinant of insulin resistance. Non-dialysed uraemic subjects with IDDM have extreme insulin resistance in peripheral tissues. The severe insulin resistance as determined by a clamp method in non-dialysed uraemic IDDM patients nearly equalled the sum of insulin resistance seen in non-diabetic uraemic patients and non-uraemic IDDM patients combined (Schmitz 1985). In IDDM, initiation of dialysis improves insulin mediated glucose uptake after 3-14 weeks (Schmitz et al. 1984). CAPD is associated with insulin mediated glucose uptake similar to that seen in in non-diabetic patients on hemodialysis (Kobayashi et al. 2000).

Hepatic glucose production, insulin-mediated suppression of glucose production and glucose uptake are normal in non-diabetic uraemic patients (DeFronzo and Alvestrand 1980, DeFronzo et al. 1981). Muscle tissue glucose metabolism accounts for 75-90% of the disposal of an infused glucose load. Skeletal muscle is therefore the most likely primary site of insulin resistance in uraemia (DeFronzo and Alvestrand 1980, DeFronzo et al. 1981, Baron et al. 1988).

In muscle, a major effect of insulin is to suppress protein degradation. Insulin-stimulated protein metabolism is abnormal in the muscle tissue of chronically uraemic laboratory test animals (May et al. 1987). The mechanism for this change in insulin action could be related to insulin resistance (Bailey et al. 1997).

The mechanism of the extreme insulin resistance seen in uraemic IDDM patients is not understood. Insulin binding to its receptor, major glucose transporter expression, insulin receptor phosphorylation (Friedman et al. 1991), and kinase activation (Bak et al. 1989b) are all normal in uraemia, despite insulin resistance. Therefore, uraemic insulin resistance is probably a postbinding defect. In a study using a clamp method with indirect calorimetry, it was shown that non-oxidative, but

not oxidative glucose uptake, is impaired in uraemic patients (Castellino et al. 1992). Adipocyte (Pedersen et al. 1985) and skeletal muscle (Bak et al. 1989a, Bak et al. 1989b) models suggest that the impaired intracellular insulin action in uraemia may be different from the impaired insulin action observed in IDDM. It is suggested that the blunted insulin action is caused by defects located in separate intracellular events.

5. Leptin in non-uraemic and uraemic diabetic patients

Leptin is the protein product (16 kDa) of the obesity (OB) gene. It was found, identified, and sequenced from obese mutant *ob*/*ob* mice in 1994 (Zhang et al. 1994). It controls body composition largely, if not entirely, via hypothalamic receptors, which regulate food intake and body weight (Caro et al. 1996). Leptin receptor isoforms, however, are found widely throughout the body, e.g. liver, pancreas and skeletal muscle (Shimabukuro et al. 1997). In addition to affecting energy expenditure and appetite (Keim et al. 1998), leptin also may play a role in the activation of the sympathetic nervous system (Satoh et al. 1999), insulin sensitivity and secretion (Fernandez-Real et al. 2000), sodium management (Jackson and Li 1997), and control of vascular tone (Lembo et al. 2000). The location of leptin and the leptin receptor in subcutaneous and visceral human fat cells suggests an autocrine and paracrine role for leptin (Bornstein et al. 2000).

The concentration of plasma leptin is elevated in obesity, and reduced during fasting. Leptin levels are higher in females than in males (Caro et al. 1996). In IDDM and NIDDM, leptin concentrations are not different from those of control subjects, and correlate to body mass index (BMI) (Haffner et al. 1996). The kidney participates in the disposal of several polypeptide hormones, such as insulin, glucagon, and

parathyroid hormone (Katz and Emmanouel 1978). Accordingly, leptin is principally cleared by the kidney (Cumin et al. 1996). Plasma leptin levels increase more than would be expected with respect to body fat mass when renal function becomes compromised in ESRD (Heimbürger et al. 1997). Neither hemodialysis nor CAPD treatment are able to restore normal plasma leptin levels (Johansen et al. 1998, Heimbürger et al. 1999, Pérez Fontán et al. 1999). CAPD is associated with even higher plasma leptin concentrations than is hemodialysis or the conservative treatment of uraemia (Pérez Fontán et al. 1999). The importance of the kidney in leptin metabolism is emphasized by the fact that renal transplantation normalises leptin levels (Kokot et al. 1998).

Plasma leptin levels are increased by sustained hyperinsulinaemia in both healthy and uraemic patients (Utriainen et al. 1996, Zavaroni et al. 2000). Insulin has displayed a stimulatory effect on leptin synthesis and/or OB gene messenger ribonucleic acid in various cell cultures and in laboratory test animals (Cusin et al. 1995, MacDougald et al. 1995). One suggested reason for elevated plasma leptin in ESRD is chronic inflammation (Heimbürger et al. 1997). Cytokines such as TNF-α and interleukin 1 induce both an increase in leptin messenger ribonucleic acid concentrations and anorexia in laboratory test animals (Grunfeld et al. 1996). Elevated leptin concentration may be associated with uraemic anorexia (Johansen et al. 1998).

6. Hepatic steatosis in non-uraemic and uraemic diabetic patients

Fatty change of the liver has been described in alcoholic liver disease, obesity, NIDDM, malnutrition, and after exposure to certain drugs and toxins. This process is often diffuse involving the entire organ. Focal fatty change of the liver is also fairly

common (Jain and McGahan 1993) and is associated with a number of etiological factors (Grove et al. 1991). Intra-abdominal accumulation of fat is seen after the initiation of CAPD (Fernström et al. 1998). A unique fatty change situated under the liver capsule has been found at autopsy in patients receiving IP insulin during CAPD (Wanless et al. 1989, Grove et al. 1991, Burrows and Jones 1994). The thickness of these patchy, rim-like lesions measured up to 12 millimetres. Wanless and associates (1989) found a correlation tendency between the amount of subcapsular steatosis and duration of CAPD. Patients on SC insulin had no steatotic areas.

7. Subcutaneous versus intraperitoneal insulin treatment in non-uraemic and uraemic diabetic patients

7.1. General considerations

Patients on intermittent PD treatment received IP insulin for the first time in 1971 (Crossley and Kjellstrand 1971). In 1979 it was reported as advantageous to use IP insulin in IDDM patients on CAPD (Flynn et al. 1979). An implantable pump system for IP insulin delivery has been developed for use in non-uraemic diabetic patients (Selam et al. 1989, Saudek et al. 1996).

SC insulin injections often cause pain. IP insulin is introduced into the dialysate bag. This may improve compliance to treatment in patients switching from SC to IP insulin. In IP insulin therapy, a short-acting, soluble insulin is used.

7.2. Absorption mechanisms of intraperitoneal insulin

IP insulin administration is the most physiologically correct method available for treatment of diabetes. IP insulin treatment partly mimics normal pancreatic insulin secretion (Figure 2). Insulin diffuses passively through the peritoneum. This absorption is suggested to occur mainly by transcapillary diffusion (Rubin et al. 1986, Wideröe et al. 1996). In a study on healthy laboratory test animals, a single bolus of insulin was introduced into the empty peritoneum. Half of the IP insulin was absorbed via the capillaries of the visceral peritoneum with a subsequent drainage into the portal vein. The other half of the IP insulin avoided first-pass liver metabolism by entering the systemic circulation via the abdominal lymphatics or through absorption via the vasculature of the parietal peritoneum. Insulin absorption via the capillaries of visceral peritoneum was faster than absorption through both the lymphatics and the capillaries of parietal peritoneum, because a significant amount of the insulin bypassing the liver has to first traverse the lymphatics and the right lymphatic duct before reaching the systemic circulation (Radziuk et al. 1994). In another study on non-uraemic diabetic laboratory test animals, it was calculated that virtually all IP insulin was absorbed by the portal circulation (Selam et al. 1990). Insulin can also pass from the peritoneal space straight into the liver via the liver capsule (Ersoy et al. 1995). Because of passive diffusion, the amount of insulin absorbed directly through the liver capsule could be proportional to the ratio of liver surface area to the peritoneal surface area in humans (11-16%) (Rubin et al. 1988, Kuzlan et al. 1997).

In non-uraemic IDDM patients on IP insulin infusion the diurnal peripheral plasma insulin concentration and area under the curve are lower than with patients on SC insulin infusion (Schade et al. 1980a, Lassmann-Vague et al. 1996). In

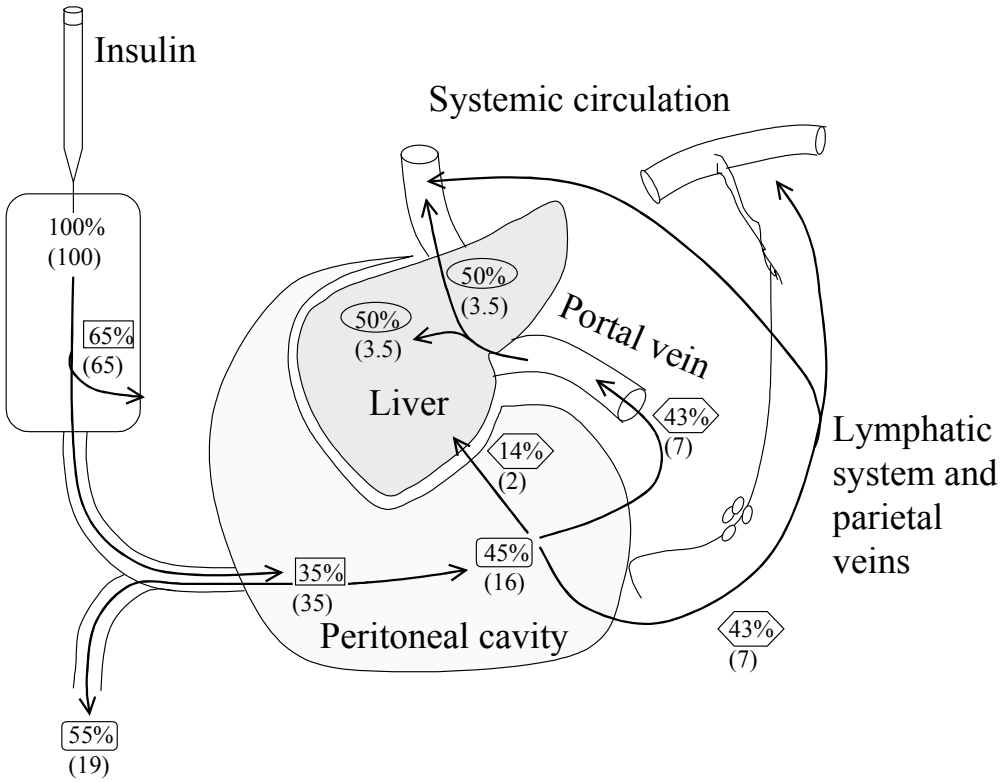


Figure 2. Intraperitoneal insulin administration and absorption kinetics during CAPD treatment. The amount of insulin entering each step is given as a percentage of the previous step (each step is represented with a different geometrical shape), and units of insulin with respect to an initial daily 100 U dose in parentheses. The maximal daily amounts of insulin loss are shown. Insulin is absorbed from the peritoneal cavity via the capillaries of the visceral peritoneum, the capillaries of the parietal peritoneum, the lymphatic system, and directly through the hepatic capsule. From the visceral peritoneal capillaries, insulin is transported via the portal vein to the liver, which removes 50% of the insulin before it reaches the systemic circulation (numbers are given according to studies by Rubin *et al.* 1988, Radziuk *et al.* 1994, Wideröe *et al.* 1996 and Kuzlan *et al.* 1997).

laboratory test animals, the portal insulin concentration has been reported to be either equal to (De Vos et al. 1996) or higher (34-72%) than peripheral insulin concentration one hour after IP insulin injection (Nelson et al. 1982, Radziuk et al. 1994). With SC insulin, no difference between portal and peripheral insulin concentration is detected (Nelson et al. 1982, Radziuk et al. 1994).

As the dwell time progresses, dialysate fluid volume and osmolality increase, and the IP insulin absorption rate decreases. This does not, however, change the net bioavailability of insulin. Total insulin absorption increases with dialysate dwell time. Accordingly, in order to avoid nocturnal hypoglycaemia, about 20% less insulin is instilled in the evening dwell (Wideröe et al. 1996). During CAPD, IP insulin can be administered into the empty peritoneal cavity to induce a more rapid absorption of insulin to peripheral blood (30-45 minutes) when compared to injecting insulin into the dialysate bag before introducing dialysate into the peritoneal cavity (90-120 minutes) (Shapiro et al. 1979, Schade and Eaton 1980).

7.3. Factors affecting absorption of intraperitoneal insulin

When using implanted SC or IP pumps the dose of insulin required for glycaemic control is the same (Micossi et al. 1986, Selam et al. 1989, Ruotolo et al. 1990). When patients are on CAPD, the IP insulin doses are 1.3-4.8 times higher than the SC insulin doses (Beardsworth et al. 1988). When insulin is introduced into the dialysate bag, 3 to 65% is adsorbed into the apparati (Johnson et al. 1983, Twardowski et al. 1983, Wideröe et al. 1983). Contact time plays a significant role. Adsorption into the tubing is negligible, presumably due to high flow rates (Wideröe

et al. 1983). Adsorption rises as dialysate temperature increases (Twardowski et al. 1983). As dialysate insulin concentration increases, the percentage of adsorbed insulin decreases (Rottembourg et al. 1986). The maximal amount of insulin adsorbed during one exchange is 18 U (Twardowski et al. 1983). The percentage of insulin delivered to the peritoneal space via a line port (76-80%) greater than when insulin is delivered via a bag port (65%)(Rottembourg et al. 1986). During an eight hour dwell, 45% of the initial insulin dose is absorbed from the dialysate (Wideröe et al. 1996). Most of the insulin not absorbed is lost by dialysate drainage. Peritoneal wall tissues may degrade some insulin. It has been calculated that of the total insulin initially introduced into the dialysate bag, only some 13 to 30% is actually absorbed into the patient (Johnson et al. 1983, Wideröe et al. 1983). The increase in splanchnic blood flow induced by ingesting a meal does not change the absorption of IP insulin (Scavini et al. 1993). When long-term insulin absorption was studied in non-uraemic IDDM patients whose IP insulin was administered by an implantable pump, no change of absorption during 30 months of treatment was found (Scavini et al. 1995). IP insulin administration has not been associated with any changes in the rate of dialysate glucose absorption (Rubin et al. 1986).

7.4. Effects on glycaemic control

In nonrandomised studies of diabetic patients without nephropathy, glycaemic control with respect to IP and SC insulin administeration by an implanted pump has been equally good (Selam et al. 1989, Bagdade et al. 1994, Dunn et al. 1997) or better (Micossi et al. 1986, Monnier et al. 1989, Ruotolo et al. 1994, Hanaire-Broutin et al. 1995, Bagdade et al. 1997) in favour of IP insulin administration. In two randomised

studies comparing IP insulin to intensive SC insulin treatment, an equally good (Selam et al. 1992) or better (Saudek et al. 1996) glycaemic control was attained with IP insulin. Two comparative studies on diabetic CAPD patients have shown equal (Selgas et al. 1989, Scarpioni et al. 1994) and two studies better (Balaskas et al. 1994, Lahtela et al. 1995) glycaemic control favouring IP to SC insulin administration. Decreased hepatic glucose production during IP insulin treatment could partly explain the better glucose control in both non-uraemic IDDM patients (Robert et al. 1993) and IDDM patients on CAPD (Lahtela et al. 1995). It has not been studied whether the route of insulin administration influences renal glucose production.

In IDDM patients with frequent hypoglycaemic events, CAPD is preferable to hemodialysis treatment, as the later is associated with a higher incidence of hypoglycaemic episodes (Tzamaloukas et al. 1992). IP insulin is associated with less blood glucose variation and fewer episodes of severe hypoglycaemia than SC insulin treatment in non-uraemic IDDM patients (Hanaire-Broutin et al. 1995, Dunn et al. 1997). Not all studies agree with this finding (Selam et al. 1992). Three studies have attempted to clarify the mechanism responsible for the observed better control of hypoglycaemic episodes in IDDM patients with implantable IP insulin pumps. One study found a less negative glucose balance (glucose production - glucose utilisation) when patients were on IP as opposed to SC insulin (Selam et al. 1995). Two other studies comparing IP to SC insulin pump therapy in non-uraemic IDDM patients, found an improved glucagon response during exercise-induced hypoglycaemia (Oskarsson et al. 1999, Oskarsson et al. 2000b) and during hypoglycaemia induced by a bolus of intravenous insulin at rest (Oskarsson et al. 1999, Oskarsson et al. 2000b) during IP but not SC insulin. High peripheral insulin concentrations inhibit glucagon release (Oskarsson et al. 2000a). These findings may contribute in explaining why IP

insulin is associated with a lower incidence of hypoglycaemia than SC insulin in spite of similar or better glycaemic control (Selam et al. 1992, Saudek et al. 1996). Other hormonal (cortisol, adrenaline, noradrenaline, growth hormone) counterregulation of hypoglycaemia is similar during IP and SC insulin treatment (Selam et al. 1995, Oskarsson et al. 1999, Oskarsson et al. 2000b).

7.5. Potential disadvantages of intraperitoneal insulin

IP insulin treatment has been suggested to be associated with an increased incidence of peritonitis in IDDM patients on CAPD (Selgas et al. 1989). There is increasing evidence, however, against this allegation (Amair et al. 1982, Madden et al. 1982, Lindblad et al. 1988, Wikdahl et al. 1997).

Although the peritoneum is an immunologically active organ, the administration of IP insulin promotes no more insulin antibody production than intensive SC insulin treatment in either IDDM patients without nephropathy (Olsen et al. 1994) or IDDM patients on CAPD (Groop and Bonsdorff 1985). In non-uraemic IDDM patients with high insulin antibody levels, the maximum plasma concentration of free insulin after IP insulin administration is reduced when compared to patients with low antibody levels (Lassmann-Vague et al. 1996).

VI AIM OF THE STUDY

The aim of this study was to evaluate the metabolic effects of SC and IP insulin treatment in diabetic ESRD patients on CAPD. Special attention was given to effects on:

1. Glycaemic control and insulin sensitivity,
2. Plasma lipids and lipoproteins,
3. Plasma leptin,
4. Hepatic steatosis.

VII SUBJECTS OF THE STUDY

1. Patients

A total of 26 (14 females, 16 males) patients were studied. Some patients participated in 2 or more studies. All patients had diabetic ESRD (Wirta et al. 2000). The clinical characteristics of the patients are presented in Table 2. The subjects in studies **I-IV** all had IDDM and were C-peptide negative. In studies **V-VI** eleven patients had IDDM and five had NIDDM (World Health Organization 1985). In studies **V-VI**, all except one patient required insulin before the initiation of CAPD.

Table 2. Clinical characteristics of the patients (mean±SEM).

Study number	Number of subjects	Sex (F/M)	Age (years)	DM duration (years)	BMI (kg/m^2)	DM type (I/II)
I	8	6/2	48.1±5.4	31.0±5.3	24.9±1.0	8/0
II & III[a]	11	7/4	42.9±2.9	31.4±3.4	24.0±0.9	11/0
IV[b]	12	8/4	42.9±2.9	31.4±3.4	23.8±0.8	12/0
V & VI[d]	16	5/11	42.9±7.3	27.8±6.6	25.8±1.1	11/5

[a] Includes 7 patients of study I, [b] Includes 11 patients of studies II & III, [d] Includes 3 patients of study IV. (BMI=body mass index, DM=diabetes mellitus)

In studies **I-IV**, all IDDM patients on CAPD treatment in the Tampere University Hospital district participated. In study **II**, six patients underwent metabolic

evaluations before the initiation of CAPD. In studies **V-VI**, all IDDM and NIDDM patients undergoing insulin treatment during CAPD therapy in the Tampere University Hospital district were included.

Before CAPD, the recommended daily protein intake was 0.8 g/kg/day, and during CAPD 1.2 g/kg/day. In addition, the daily diet consisted of >200 g/day carbohydrate with moderate phosphate, sodium, and potassium restriction. Individual adjustments were made as necessary. CAPD treatment was conducted as four daily exchanges using 1.5-2.5-litre bags containing 1.36% or 2.27% glucose. During the course of studies **I-IV**, it became necessary to enhance dialysis in two patients. During the studies patients used antihypertensive drugs, nitrates, recombinant human erythropoetin, and a vitamin D analogue. No antihyperlipidemic agents were used.

2. Ethical aspects of the study

All patients gave written informed consent and the Ethical Committee of Tampere University Hospital approved the study protocols.

VIII METHODS

1. Study protocol

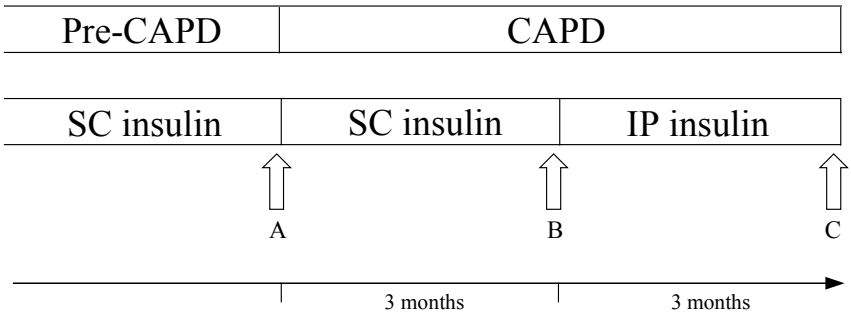
During SC insulin treatment patients administed regular insulin (Actrapid®, NovoNordisk, Gentofte, Denmark) three times per day before meals and NPH insulin (Protaphane®, NovoNordisk, Gentofte, Denmark) in the evening. During IP insulin therapy regular insulin was introduced into the dialysis bags four times per day just

before the dialysate exchange. IP insulin was divided into three equal doses during the daytime and one bedtime dose that was 20% smaller than the others. Blood glucose home monitoring was carried out prior to insulin administration at least four times per day once a week and as necessary. Blood glucose readings were recorded in a journal. The insulin dose was adjusted individually both by either the physician or the patients according to blood glucose readings. The aim was to keep pre-meal blood glucose values between 4 and 7 mmol/l.

Six patients participated in metabolic evaluations before the initiation of CAPD (**II**, Figure 3). During CAPD all patients were first treated with SC and then with IP insulin. In study **II**, the SC treatment period for six of the 11 patients (3-phase study) was 3.0±0.0 (median 3.0) months in duration and for the IP insulin period 3.1±0.1 (median 3.0) months. In studies **I-IV**, the 2-phase study mean duration of CAPD with SC insulin was 5.6±1.4 (median 3.0) months and with IP insulin 2.9±0.2 (median 3.0) months. The timing of the studies was uniformly scattered throughout the calender year.

Studies **V-VI** were carried out in a cross-sectional manner to evaluate the presence and degree of hepatic steatosis. The dimensions of hepatic subcapsular steatosis were correlated with peritoneal equilibration test (PET) results, weekly KT/V_{urea}, demographic and serologic data. Information was gathered from routine treatment follow-up and the patients underwent hepatic ultrasound scan once during a routine control visit.

Figure 3. Study protocol. Arrows represent timing of the metabolic evaluations. In study **II**, six patients participated in metabolic evaluations three times (A, B and C) and 5 patients twice (B and C). In studies **I**, **III** and **IV**, patients participated in metabolic evaluations twice (B and C) during stable CAPD treatment.



In study **V**, throughout CAPD treatment all routine glycated haemoglobin A fraction 1c (HbA_{1c}) and lipid values were recorded and the means calculated. The median number of serial measurements for HbA_{1c} was 5 (range 3-17) and for lipid values 4 (range 1-12). The mean values for haemoglobin, alanine aminotransferase, alkaline phosphatase and serum albumin were recorded. Insulin dosage and administration route, dose of dialysis, composition of dialysate, and episodes of peritonitis were recorded. The mean amount of insulin introduced into the dialysate (U/l) per day and the daily amount of instilled dialysate glucose (g/day) were calculated.

PETs (Twardowski et al. 1987) and 24-hour dialysate and residual urine collections were performed every six months (median 3, range 1-7 tests per patient).

2. Clamp study

For the measurement of insulin sensitivity, patients were admitted to hospital one day prior to the clamp study. Routine dialysis and insulin regimen were continued up until the evening prior to the study. The peritoneal cavity was drained at 22.00 hours. During the SC treatment period regular insulin was given SC according to the evening blood glucose level in order to avoid early morning hyperglycaemia and uneven absorption of NPH insulin, which could exert an adverse effect on the reliability of the evaluations. During the IP insulin period no extra insulin was necessary, due to absorption of residual insulin from the peritoneal cavity, except in one case where an IP bolus of regular insulin was given. Patients fasted for 12 hours and metabolic evaluation started at 07.00. Two indwelling cannulae were placed, one into a forearm arteriovenous shunt for blood sampling, and the other into a contralateral antibrachial vein for infusions. The euglycaemic hyperinsulinaemic clamp study was done according to the method described by DeFronzo and associates (1979), where an intravenous priming dose of crystalline human insulin (Actrapid®, NovoNordisk, Denmark) is used. The rate of the priming infusion was initially 3.3 times greater than the constant infusion rate. This rate was decreased every two minutes until a constant intravenous insulin infusion of 80 mU/m^2/min (480 pmol/m^2/min) was attained ten minutes after starting the infusion. This led to plasma insulin concentrations of 180±3, 197±5 and 197±9 mU/l (multiply by 6.0 to get

pmol/l) during the predialysis, SC, and IP insulin periods, respectively (P=NS). Initially, plasma glucose was allowed to reach about 5.3 mmol/l and was kept stable for 120 minutes by adjusting the infusion rate of a 20% dextrose solution. Samples for plasma glucose determination were drawn at 5-15 minute intervals. Plasma insulin was measured from fasting pretest samples and samples drawn during the steady-state at 10 minute intervals. Plasma steady-state glucose concentrations were 5.6±0.2, 5.4±0.1 and 5.3±0.4 mmol/l during pre-CAPD, SC, and IP insulin treatment periods, respectively (NS).

3. Peritoneal equilibration test and dialysis adequacy

PETs were carried out according to the method described by Twardowski and associates (1987). The dialysate dwell time preceeding the equilibration test should be 8-12 hours in duration. The pretest dialysate is completely drained, the test dialysate is agitated and a sample of both the dialysate and blood are collected. Two litres of 2.27% dialysis solution (Dianeal® 2.27%, Baxter Healthcare, Castlelbar, Ireland) are infused over 10 minutes. The patient is in the supine position during infusion and rolls from side to side after each 2 minutes for better mixing. When the infusion is completed (0 dwell time) a 10 ml sample of dialysate is taken concurrently with a blood sample. The patient is ambulatory during the dwell period. Samples of dialysate and blood are taken at 120 minutes. After 4 hours, the dialysate is drained with the patient sitting. Total dialysate volume is measured and both dialysate and blood samples are taken again.

Dialysate and plasma creatinine are measured from dialysate and blood samples taken after 10 minutes (D/Pcrea0), two (D/Pcrea2) and four (D/Pcrea4) hours, and the

dialysate to plasma ratios are calculated. Dialysate glucose concentration is measured after two (D/D0gluc2) and four (D/D0gluc4) hours, and the ratios of these measured values to pre-test glucose concentrations are calculated.

Weekly KT/V_{urea} and PCR samples are from 24-hour dialysate and residual urine collections.

4. Analytical methods

Plasma cholesterol (coefficient of variation (CV) 1.0-1.8%) and triglycerides (CV 0.9-1.8%) were measured with automated enzymatic procedures (Kodak Ektachem 700, Eastman Kodak Company, Rochester, NY, USA). Serum HDL-cholesterol (CV 1.6-4.5%) was measured using the enzymatic colorimetric dry chemistry method after precipitation of other lipoproteins with dextran sulphate/magnesium chloride reagent (Dextralip50®, Genzyme Diagnostics, Cambridge, USA, molecular weight 50000 Daltons, 10 g/l / $MgCl_2 \times H_2O$, 101.6 g/l, respectively). LDL-cholesterol was calculated according to the Friedewald formula (LDL-cholesterol = total cholesterol – HDL-cholesterol – (triglycerides ÷ 2.2) when the plasma triglyceride concentration was < 5 mmol/l) (Friedewald et al. 1972). Plasma HDL subclass (at 2°C, densities of 1.063-1.125 g/mL for HDL_2 and 1.125-1.210 g/mL for HDL_3, centrifugation at 35000 rpm/130000 × *g*, 48 hours for each, using a Beckman Ti 50.4 rotor, CV 3.8-4.8%) fractionation was carried out by sequential flotation in an ultracentrifuge (Beckman L 8-70, Beckman Inc., Palo Alto, CA, USA) using a modification of the original method (Havel et al. 1955) as described in detail by Taskinen and associates (1988). Plasma Apo A-I (CV 3.5%)

and Apo A-II (CV 3.7%) concentrations were determined by immunoturbidometric methods using commercially available kits (Boehringer Mannheim, Germany), and Apo B levels (CV 4.4%) by an immunochemical assay (Orion Diagnostica, Espoo, Finland).

Plasma glucose was analysed with a glucose oxidase method using routine application (CV 0.6-2.0%, Epos Analyzer 5060, Hamburg, Germany). Plasma free insulin was determinated by radioimmunoassay after separating bound and free insulin by double antibody suspension (CV 5.8-9.8%, Phasedeph®, Pharmacia, Uppsala, Sweden). HbA_{1c} (CV 1.9-2.3%, reference range 4.5-6.4%) was determined by high-performance liquid chromatography (Mono-S, Pharmacia Biotech, Uppsala, Sweden). Plasma leptin concentration was measured by human leptin radioimmunoassay (CV 3.4-8.3%, Human Leptin RIA kit, Linco Research Inc., St. Charles, MO, USA). Serum creatinine (CV 0.6-0.9%) and other analytical procedures were carried out with an automated selective chemistry analyser using routine procedure (Kone-C, Helsinki, Finland).

5. Imaging methods

The ultrasound imaging in studies **V-VI** was performed with an Acuson Sequoia 512 ultrasonic scanner (Mountain View, CA, USA) using a 4 MHz sector transducer (4V2) with the patient lying in left lateral position with dialysate in the peritoneal cavity. Each subcapsular steatosis was measured for maximal thickness and two diameters at 90-degree angles and steatosis area was calculated. All scans were performed by the same radiologist, who was unaware of the history or the current treatment of the patients.

In study **VI**, magnetic resonance imaging was performed on one patient (Signa 1.5 T, General Electrics, Milwaukee, IL, USA). T1-weighted and fat suppression imaging techniques were used to differentiate between fatty areas and liver parenchyme.

6. Calculations

In the clamp study (**I-III**), the glucose disposal rate (M) equals the glucose infusion rate required to maintain a steady state plasma glucose level, and was calculated as the mean rate of the final 60 minutes of the clamp. Insulin sensitivity is expressed as M/ΔIRI, and in calculations M/ΔIRI × 1000 where M is equal to glucose disposal rate and ΔIRI is the difference between the steady state immunoreactive insulin (IRI) concentration and the basal level multiplied by 1000.

In study **V** the calculations for the PETs, KT/V_{urea} and PCR were performed with PD Adequest (version 1.3, Baxter, Deerfield, IL, USA), a computer-based kinetic modeling program for peritoneal dialysis.

Dialysate weekly KT/Vurea is calculated using the equation:

$$((C_P \div C_S) \times (V_d \times T)) \div V$$

where C_P is the dialysate urea concentration, C_S is the serum urea concentration, V_d is the 24-hour dialysate drain volume, T is the number of days, and V is the patient's total body water. For calculating V, the PD Adequest program uses the following equations:

female: $-9.926 + 0.17003 \times \text{height} + 0.21371 \times \text{weight}$

male: $-14.249 + 0.19678 \times \text{height} + 0\ 29571 \times \text{weight}$

For those patients who maintain renal function, their residual function is added to this calculated clearance. Residual renal clearance is calculated as:

$$(((V_U \times C_U) \div C_S \times 1440 \text{ minutes}) + ((V_U \times U_{Cr}) \div S_{Cr} \times 1440 \text{ minutes}))) \div 2$$

where V_U is 24-hour urine volume, C_U is the urine urea concentration, U_{Cr} is the urine creatinine concentration, and S_{Cr} is the serum creatinine concentration.

PCR is expressed as normalized PCR. In a stable PD-patient with balanced nitrogen metabolism PRC is equal to dietary protein intake and is calculated using the equation

$$((5.02 \times G) + 3.12) \div \text{body weight}$$

where G is the urea clearance rate (mg/minute), and G is the total amount of urea removed by both the dialysate and kidneys during a specific time period.

In study **V** the area of hepatic subcapsular steatosis was calculated from either a solitary or all distinct subcapsular steatosis areas observed. The area of each lesion was calculated by equations for the area (A) of a circle or an ellipse:

$$A = \pi \times (r^2) \qquad \text{or} \qquad A = \pi \times a \times b,$$

where r is the radius of a circle, and a and b are the two perpendicular diameters of an ellipse.

7. Statistical methods

Student's two-tailed *t* test for paired data was applied in the comparison of means in studies **I, II**, **III**, and **IV**. For skewed distributions of parameters, the Wilcoxon rank sum test (**I**, **II, III**) or the Mann-Whitney U-test (**V**) was used for paired comparison. In study **IV** a logarithmic transformation was used for variables which were not normally distributed. Differences between means of continuous variables were analyzed with one-way analysis of variance. In study **V** linear correlation analysis was used to describe the association between logarithmic-transformed plasma leptin and other variables. Intercorrelations of the variables were described by Pearson's product-moment correlation coefficient and Spearman's nonparametric rank test. In studies **I** and **II** the statistical analyses were carried out by BMDP Solo software (version 4.0, Los Angeles, CA, USA), SPSS software was used in studies **III** and **V** (version 7.0, Chicago, IL, USA) and GraphPad Prizm software package in study **IV** (version 2.01, San Diego, CA, USA).

IX RESULTS

1. Glycaemic control and insulin sensitivity

In study **II** patients first participated in metabolic evaluations while on SC insulin, before the initiation of CAPD. After the initiation of CAPD, the SC insulin dose increased from 30.7±2.4 to 35.2±3.6 U/day (P=0.069). An insignificant increase in HbA_{1c}, from 8.85±0.54% to 9.58±0.66%, was seen at the same time (**II**, Table 3).

Although insulin administration route was unchanged (SC) at the initiation of CAPD therapy, the glucose disposal rate (M) rose from 5.88±0.95 to 8.16±1.38 mg/kg/min (P=0.053, Table 3). Insulin sensitivity (M/ΔIRI) values were increased from 5.57±1.13 to 7.98±1.95 mg/kg/min/pmol/l (NS) between the pre-CAPD and CAPD periods with SC treatment (**II,** Table 3).

During CAPD therapy (**I-II**) insulin was first administered SC and then IP. Insulin dose increased from 39.9±4.2 U/day during SC insulin to 90.9±10.9 U/day during the IP insulin period (P=0.0004, Table 3). Fasting plasma free insulin concentration decreased from 104.8±15.8 pmol/l with SC insulin to 87.9±16.3 pmol/l during IP insulin treatment (P=0.052). After SC insulin treatment, HbA_{1c} was significantly higher than after IP insulin treatment (9.49±0.43 and 8.13±0.39%, respectively, P=0.004).

Glucose disposal rate (M) increased from 7.10±0.84 mg/kg/min during SC insulin to 8.12±0.83 mg/kg/min during IP insulin treatment (P=0.002, Figure 4). The insulin sensitivity (M/ΔIRI) was 6.61±0.93 mg/kg/min/pmol/l during SC and 7.45±1.00 mg/kg/min/pmol/l during IP insulin treatment (P=0.001, Table 3, **I-II**).

Table 3. Clinical characteristics relating to insulin treatment and glycaemic control (**II**, mean ± SEM).

	N=6		N=11	
	pre-CAPD + SC insulin	CAPD + SC insulin	CAPD + SC insulin	CAPD + IP insulin
Insulin dose (U/day)	30.7 ±2.4	35.2 ±3.6	39.9 ±4.2	90.9 † ±10.9
Fasting plasma insulin (pmol/l)	90.0 ±15.5	100.2 ±18.7	104.8 ±15.8	87.9 ±16.3
HbA_{1c} (%)	8.85 ±0.54	9.58 ±0.66	9.49 ±0.43	8.13 † ±0.39
M (mg/kg/min)	5.88 ±0.95	8.16 ±1.38	7.10 ±0.84	8.12 † ±0.83
M/ΔIRI (mg/kg/min/pmol/l)	5.57 ±1.13	7.98 ±1.95	6.61 ±0.93	7.45 ±1.00

† $P<0.01$ CAPD + SC insulin vs CAPD + IP insulin (paired *t* test). SC = subcutaneous, IP = intraperitoneal, M = glucose disposal rate, IRI = immunoreactive insulin concentration, M/ΔIRI = insulin sensitivity.

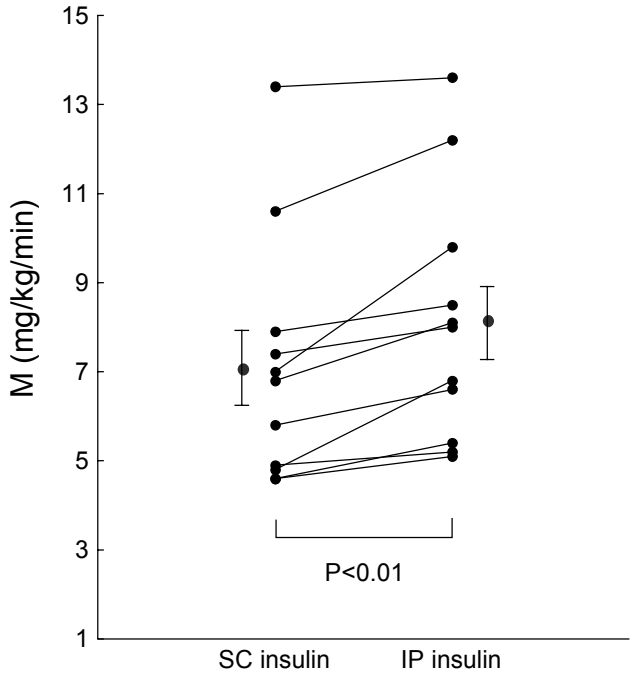


Figure 4. Glucose disposal rate (M) in 11 insulin-dependent diabetic patients on CAPD therapy after subcutaneous (SC) and intraperitoneal (IP) insulin treatment (paired *t*-test).

2. Lipids and apolipoproteins

The initiation of CAPD did not change plasma lipid profiles significantly (**II**, Table 4). Switching from SC to IP insulin administration increased LDL-cholesterol by 16% (from 3.22±0.33 to 3.73±0.42 mmol/l, P=0.085, **I-II**). Plasma cholesterol rose 4% and triglycerides 19% (NS). The serum HDL-cholesterol concentration decreased by 33% from 1.35±0.15 to 0.90±0.04 mmol/l (P=0.009, Figure 5), and the LDL/HDL-cholesterol ratio increased significantly (from 2.70±0.34 to 4.31±0.58, P=0.005, Table 4).

Switching from SC to IP insulin decreased plasma HDL_3-cholesterol from 0.81±0.06 to 0.64±0.04 mmol/l (P=0.007) and HDL_2-cholesterol from 0.48±0.10 to 0.32±0.04 mmol/l (NS). The HDL_3/HDL_2-cholesterol ratio did not change (2.15±0.37 after SC and 2.23±0.34 after IP insulin, **III**).

Table 4. Lipoprotein changes in study **II** (mean ± SEM).

	N=6		N=11	
	pre-CAPD + SC insulin	CAPD + SC insulin	CAPD + SC insulin	CAPD + IP insulin
Cholesterol (mmol/l)	5.43 ±0.47	5.43 ±0.36	5.38 ±0.33	5.60 ±0.49
LDL-cholesterol (mmol/l)	3.31 ±0.41	3.09 ±0.51	3.22 ±0.33	3.73 ±0.42
HDL-cholesterol (mmol/l)	1.27 ±0.22	1.48 ±0.24	1.35 ±0.15	0.90 ∗ ±0.04
LDL/HDL ratio	2.93 ±0.55	2.49 ±0.56	2.70 ±0.34	4.31 ∗ ±0.58
Triglycerides (mmol/l)	1.91 ±0.24	1.91 ±0.45	1.75 ±0.28	2.09 ±0.35

∗ P<0.05 CAPD + SC insulin vs CAPD + IP insulin (paired *t* test). SC = subcutaneous, IP = intraperitoneal.

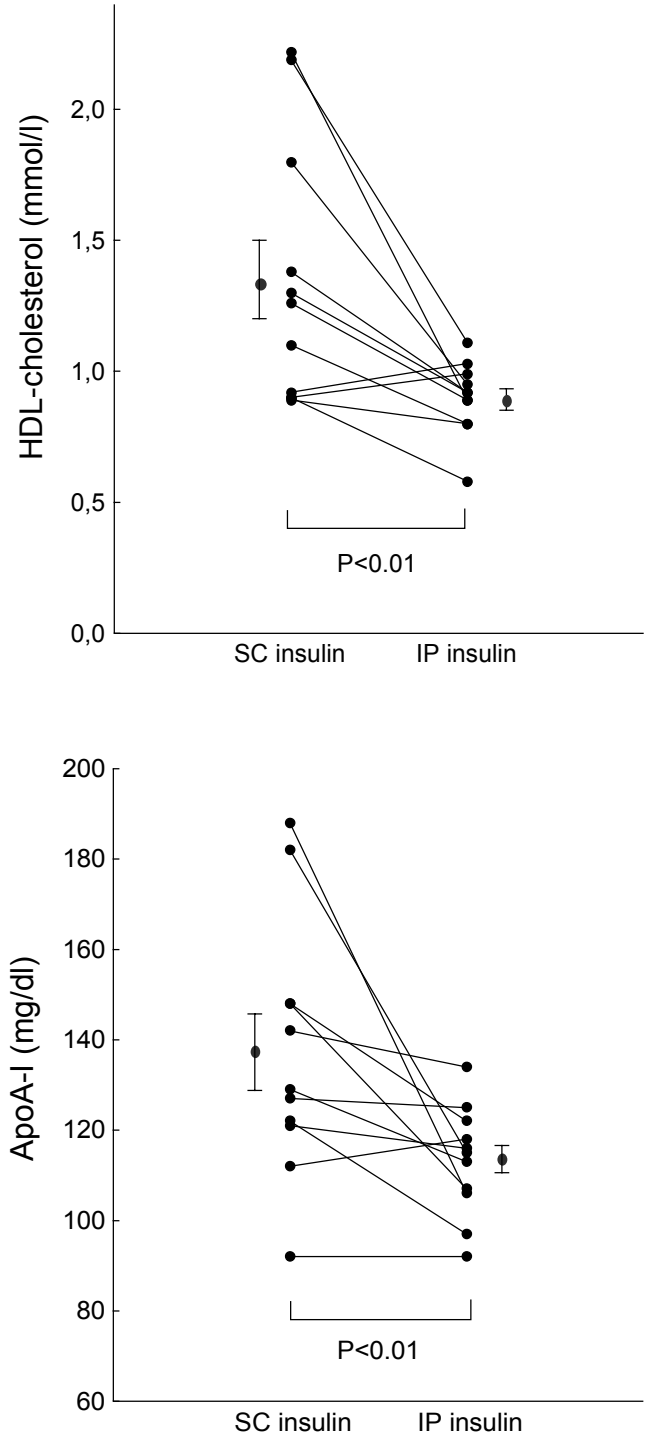


Figure 5. Serum HDL-cholesterol (upper graph) and plasma apoprotein A-I (lower graph) in 11 insulin-dependent diabetic patients on CAPD therapy receiving first subcutaneous (SC) and then intraperitoneal (IP) insulin treatment (paired *t*-test).

After SC insulin administration plasma Apo A-I was 137±9 mg/dl, and after IP insulin the corresponding figure was 113±4 mg/dl (P=0.018, Figure 5, **III**). Apo B and Apo A-II levels did not change. The ratio of Apo B/Apo A-I was lower after SC insulin than after IP insulin (0.53±0.05 and 0.62±0.05, P=0.001). The Apo A-I/Apo A-II ratio decreased from 4.32±0.22 to 3.86±0.13 (P=0.007). The HDL-cholesterol/Apo A-I ratio decreased during IP insulin administration (from 0.37±0.02 to 0.31±0.01, P=0.011). The LDL-cholesterol/Apo B ratio remained stable.

Table 5. Plasma HDL-cholesterol subfractions and apoproteins in 11 IDDM patients on CAPD therapy (means±SEM).

	SC insulin	IP insulin
HDL_3-cholesterol (mmol/l)	0.81±0.06	0.64±0.04[b]
HDL_2-cholesterol (mmol/l)	0.48±0.10	0.32±0.04
ApoA-I (mg/dl)	137±9	113±4[a]
ApoA-II (mg/dl)	30.4±1.7	29.6±1.5
ApoB (mg/dl)	68.5±6.7	71.1±6.4
ApoA-I/ApoA-II	4.32±0.22	3.86±0.13 [b]
ApoB/ApoA-I	0.53±0.05	0.62±0.05 [b]
HDL-cholesterol/ApoA-I	0.37±0.02	0.31±0.01 [a]
LDL-cholesterol/ApoB	1.84±0.14	2.08±0.16

SC = subcutaneous, IP = intraperitoneal, Apo = apoprotein.

[a] = $P<0.05$, [b] = $P<0.01$, (paired *t* test).

3. Plasma leptin

Mean body weight (without dialysate) increased from 63.7±2.8 to 66.0±2.5 kg (P=0.058, **II**) after initiation of CAPD. When insulin treatment was switched from SC to IP during stable CAPD, the mean body weight decreased by 2.1±0.8 kg (P=0.035, **II**).

In study **IV** the mean plasma leptin concentration was 2.4 times higher in females than in males, whereas the mean leptin concentration for females in the control group was only 1.8 times higher. A correlation between plasma leptin and BMI were seen in both sexes (females R=0.803, P=0.016 and males R=0.678, P=0.049). No significant correlation was detected between plasma leptin and fasting plasma insulin concentration, insulin sensitivity (M), HbA_{1c}, serum creatinine, serum urea or plasma lipids.

Changing insulin therapy from SC to IP for three months significantly reduced mean plasma leptin concentrations (from 19.8±5.9 ng/ml to 12.8±6.2 ng/ml, P<0.001, Figure 6, **IV**). This change in plasma leptin concentration did not correlate to the changes in body weight, fasting plasma insulin levels, or glucose disposal rate. A tendency towards negative correlation was seen between the changes of logarithmic plasma leptin concentration and HbA_{1c} (R=-0.577, P=0.063). The calculations were also performed gender specifically. Plasma leptin correlated with BMI both during SC insulin and IP insulin therapy in males and in females. The correlation between leptin and BMI is significant (R=0.489, P=0.015), if the results after SC and IP insulin treatment are combined. No relationship was seen between leptin concentration and plasma insulin levels, insulin dose, HbA_{1c} or glucose disposal rate.

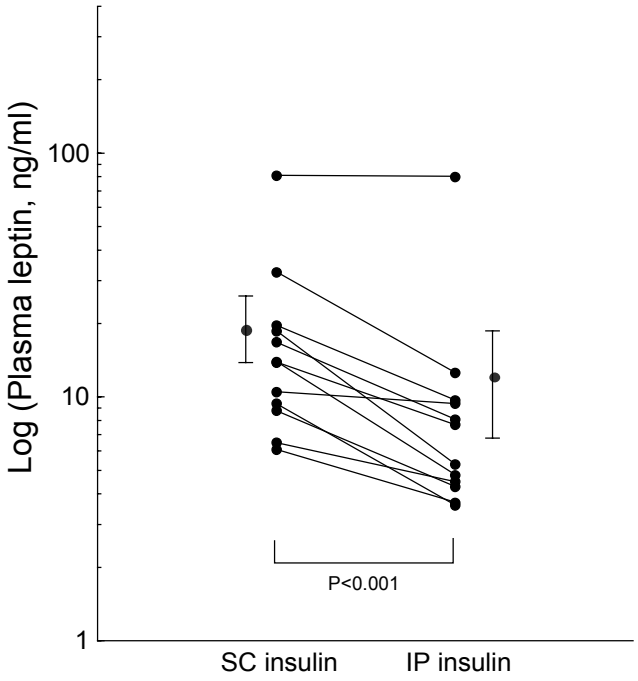


Figure 6. Logarithmic plasma leptin concentration in 12 insulin-dependent diabetic patients on CAPD therapy receiving subcutaneous (SC) and intraperitoneal (IP) insulin treatment (Wilcoxon rank sum test).

4. Hepatic subcapsular steatosis

Ultrasound scan showed hepatic subcapsular steatosis as a bright echogenic rim on the surface of the liver. Steatosis was seen in 7 of 8 patients belonging to the IP insulin group, and in none belonging to the SC insulin group (**V**, **VI**). The maximal thickness and area of the steatosis ranged from 6-17 mm (**VI**) and 7-96 cm^2 (**V**), respectively. Some patients showed several discontinuous steatotic lesions in the subcapsular liver. Most frequently the steatotic areas were situated on the cranial segments of the liver under the diaphragm. Subcapsular steatosis neighbouring the gallbladder fossa, an area usually spared from steatosis, was seen in two patients using

IP insulin (**VI**). Minor periportal hepatic steatosis was seen in four IDDM and one NIDDM patients. Two of the former were on IP insulin and they also had hepatic subcapsular steatosis (**V**). In one patient magnetic resonance imaging showed that the area of hepatic subcapsular steatosis was much greater than how it appeared in the ultrasound examination. All the liver surface area in contact with the dialysate was steatotic (**VI**).

Ultrasound visibility of the liver structures was better if the examination was performed during the dialysate dwell period as opposed to through an empty peritoneum. Intraperitoneal dialysate provides a window for enhanced visibility. The use of the native tissue harmonic imaging mode seemed to accentuate the contrast between normal liver tissue and the rim of a fatty liver change. Movements of the liver caused by respiration sometimes facilitated visibility of the demarcation between subcapsular steatosis and extrahepatic echogenic structures. The localisation of a steatotic area does not markedly hinder the measurement of maximal thickness. Measuring the diameter of steatotic liver surface areas with ultrasound scan is more difficult in dorsal and caudal areas than in anterior and right lateral areas (**VI**).

In one patient fat suppression images in magnetic resonance imaging yielded a hypointense signal and T1-weighted images yielded a hyperintense signal in the subcapsular liver region (Figure 7). The presence of hepatic subcapsular steatosis was verified post mortem in two patients. Yellowish macroscopic subcapsular islets were seen in the middle of areas of liver tissue. Presence of steatosis was confirmed microscopically. No intraparenchymal steatosis was found.

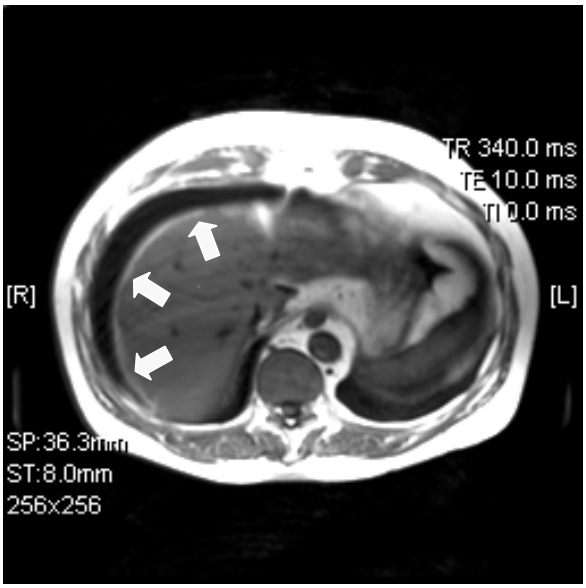


Figure 7. T1-weighted axial image of the liver in a 44-year-old male with extensive subcapsular steatosis (steatotic area shown by arrows).

In study **V** patients on IP insulin had lower total weekly KT/V_{urea} than patients on SC insulin (2.07±0.35 vs 2.59±0.52, P=0.039), but no significant difference was observed in either dialysate weekly KT/V_{urea} (1.84±0.32 vs 1.60±0.52) or PCR (0.92±0.18 vs 0.91±0.20 g/kg/day). Four patients using IP insulin had no residual renal function. PET results did not differ between patients on IP and SC insulin. In study **V**, mean PET results for all patients during dialysis treatment were: D/D_{0gluc2} 0.57±0.07, D/D_{0gluc4} 0.41±0.07, D/P_{crea0} 0.10±0.03, D/P_{crea2} 0.52±0.10 and D/P_{crea4} 0.68±0.10.

The maximal thickness of the lesions correlated directly with creatinine parameters (D/Pcrea2: R= 0.80, P= 0.033; and D/Pcrea4: R= 0.73, P= 0.060) and inversely with glucose parameters in the PETs (D/D0gluc2: R= -0.89, P= 0.007;

D/D0gluc4: R= -0.80, P= 0.030, Figure 8) and with PCR (R= -0.82, P= 0.024). The area of subcapsular steatosis correlated directly with body weight (R= 0.80, P= 0.031) and inversely with total weekly KT/V_{urea} (R= -0.90, P= 0.005, **V**).

No correlations were found between the area or maximal thickness of hepatic subcapsular steatosis and duration of CAPD, BMI, HbA_{1c}, daily insulin dose, instilled dialysate glucose load, serum albumin, plasma cholesterol, LDL-cholesterol, triglycerides or serum HDL-cholesterol.

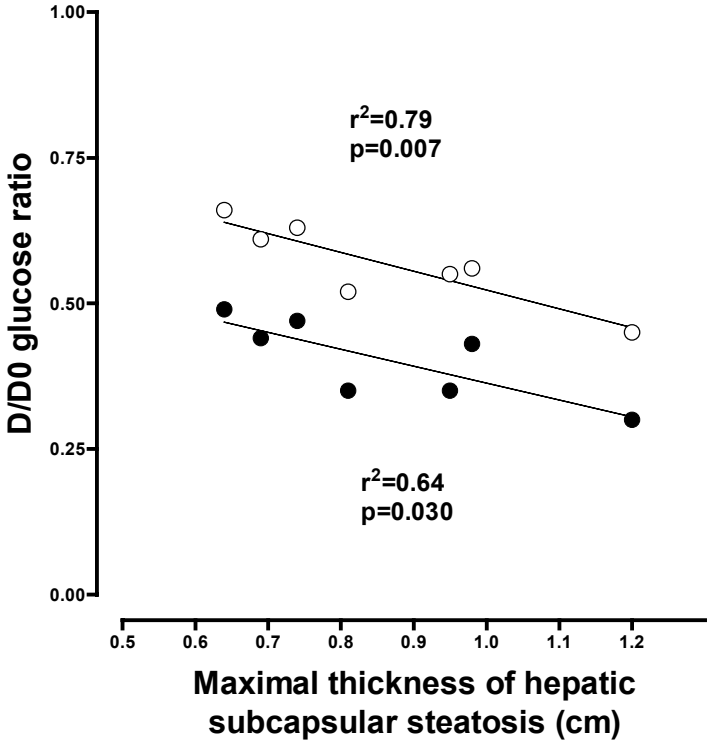


Figure 8. Regression of mean glucose concentration ratios (D/D0) in the peritoneal equilibration tests (PET) as a funtion of maximal thickness of hepatic subcapsular steatosis in diabetic patients on intraperitoneal insulin during continuous ambulatory peritoneal dialysis. ○ = 2-hour PET results, ● = 4-hour PET results.

X DISCUSSION

1. General considerations

In this study all insulin-treated diabetic patients on CAPD in the Tampere University Hospital district participated. The mean age of the study subjects and the duration of IDDM were similar to those of diabetic CAPD patients reported in the Finnish Registry of Renal Diseases at the time (Finnish Registry for Kidney Diseases 1998). Therefore, the study group was a representative sample of IDDM patients on CAPD in Finland. There were no NIDDM patients in studies **I-IV**, and only five in studies **V-VI**, which is a reflection of increasing number of NIDDM patients on dialysis. The diagnosis of diabetic ESRD is often made on a clinical basis. When the duration of diabetes exceeds ten years, a slowly progressive ESRD, with findings of heavy proteinuria, elevated blood pressure, and the presence of proliferative retinopathy, is assumed to be of diabetic origin (Wirta et al. 2000). The study population of the present study met these criteria.

Treatment with β-blocking agents or recombinant erythropoetin can exert an influence on serum lipid and lipoprotein profiles (Kontessis et al. 1993, Pollock et al. 1994). No significant changes were made to these or other pharmacological treatments during the studies.

A before-and-after evaluation protocol was used in studies **I-IV**, and each patient served as their own control. The follow-up period lasted six months. Due to the fact that a decreasing residual renal function for example, with a potential need for a significant change in dialysis treatment, could have obscured the metabolic findings,

a more prolonged study seemed inappropriate. Conversely, in this group of patients the drop out rate may be high because of renal transplantation or high cardiovascular mortality. The cross over study design would have given more strength to the findings. Nevertheless, the setting of the current study and the number of patients was considered adequate for statistical purposes. In a similar study by Lahtela and associates (1995), all but one patient chose to continue on IP insulin administration, when patients were presented with the option to switch from IP to SC insulin. Therefore, patients might have resisted returning to SC insulin in a cross over study.

2. Methods

The clamp method is unique in determining insulin sensitivity because it can be used in the absence of endogenous insulin production. It is a laborious and time-consuming method unsuitable for routine clinical work. The method is highly reproducible. An insulin dose-response curve can be generated (DeFronzo et al. 1979). In the present study, a euglycaemic hyperinsulinaemic clamp was performed with an insulin infusion rate of 80 mU/m^2 × minutes, which is twice that used in most studies (DeFronzo et al. 1982a, Schmitz 1985). Infusion rates up to 400 mU/m^2×min have been used in studies on uraemic insulin resistance (Smith and DeFronzo 1982). In IDDM patients with normal kidney function, endogenous glucose production is almost completely suppressed with insulin concentrations 100 mU/l above basal levels (DeFronzo et al. 1982a). In the present study maximal suppression of endogenous glucose production was pursued in a group of severely insulin resistant patients. The high infusion rate was used to avoid insulin clamp prolongation, which is associated with falsely enhanced glucose utilisation (Schmitz 1985). The patients of

the present study had endogenous glucose production maximally suppressed (98%) with an insulin concentration of 160-180 mU/l over the basal level (Lahtela et al. 1995). In clamp study insulin antibodies can prolong the time needed for reaching both the steady-state plasma level of free insulin and the steady-state glucose infusion rate. Prolonged insulin clamp is associated with enhanced glucose utilisation (Schmitz 1985). In this study patients served as their own controls to avoid the problem with insulin antibodies. Insulin antibody production does not change when patients switch from SC to IP insulin (Groop and Bonsdorff 1985).

PET is a simple, approximate measure of peritoneal dialysis performance. It is a complementary study for clearance measurements used to guide the dialysis prescription in routine clinical work (Twardowski et al. 1995). In this study PETs and clearance measurements were performed regularly. Information on dialysis adequacy, nutritional status, and the peritoneal transport rate and its changes were provided.

Haemoglobin carbamylation in uraemia, acetaldehyde adduct in alcohol abuse, acetylation in acetylsalicylic acid use, and structural haemoglobin variants are all known to interfere with HbA_{1c} determination. Most analytical methods give false results owing to this interference. In this study high performance liquid chromatography was used, which excluded interference from these factors except for negligibly increased HbA_{1c} in uraemia (Koskinen et al. 1998).

Serum/plasma lipids and apolipoproteins were measured in the fasting state. Because of the continuous dialysate glucose supply, CAPD patients are never in a true fasting state. Predialytic patients in study **II** naturally had empty peritonea during the blood sampling. The peritonea of the same patients were drained the evening prior to morning blood sampling during both SC and IP insulin periods while on CAPD. The protocol for emptying the peritoneum has been used by other researchers in the study

of metabolic features of uraemic IDDM patients (Schmitz 1985). Having an empty peritoneum may resemble the fasting state very closely, but it is the normal status of a CAPD patient to have dialysate intraperitoneally.

The measurement of plasma leptin is rather artifact-free. Plasma leptin concentration, however, may be increased by inflammation, a frequent event in chronic PD treatment (Heimbürger et al. 1997). In this study, plasma C-reactive protein levels did not significantly change when the insulin administration route was switched from SC to IP. In this study neither tumor necrosis factor α nor interleukin 1, both infammatory cytokines associated with increased leptin production (Heimbürger et al. 1997), were measured.

Ultrasound study is non-invasive and readily available. Although it gives information on relatively small linear changes (±0.2-0.4 mm), one can easily underestimate the area of steatotic lesions. With magnetic resonance imaging, an acceptable estimate of the grade of diffuse hepatic steatosis can be obtained (Levenson et al. 1991). Focal liver changes, with or without fatty change, may appear different on ultrasound and magnetic resonance imaging (Monzawa et al. 1999). Only post mortem studies provide precise information concerning microscopic liver changes (Wanless et al. 1989).

3. Glycaemic control and insulin sensitivity

In study **II** the insulin administration route (SC) was not changed at the initiation of CAPD; and CAPD did not improve glycaemic control as measured by HbA_{1c}. Other before-and-after studies in uraemic IDDM patients have used a protocol where patients switch from SC to IP insulin when CAPD treatment is initiated. In

these studies, glycaemic control change has ranged from none (Rottembourg et al. 1983) to substantial improvement when HbA_{1c} served as the indicator(Madden et al. 1982, Wikdahl et al. 1992).

In studies **I-IV** the administration route of insulin was changed from SC to IP during stable CAPD therapy in a group of uraemic IDDM patients. As a result of this change, glycaemic control improved. This finding is supported by studies on uraemic (Balaskas et al. 1994, Lahtela et al. 1995) and non-uraemic (Micossi et al. 1986, Monnier et al. 1989, Ruotolo et al. 1994, Hanaire-Broutin et al. 1995, Saudek et al. 1996, Bagdade et al. 1997) IDDM patients who achieved a better glycaemic control with IP insulin than with SC insulin. IP insulin was also advantageous in that it reduced glycaemic variability and episodes of clinical hypoglycaemia (Selam et al. 1992, Lahtela et al. 1995, Saudek et al. 1996).

One reason for the better glycaemic control with IP insulin as opposed to SC insulin is the decreased basal hepatic glucose production found in non-uraemic (Robert et al. 1993) and uraemic IDDM patients (Lahtela et al. 1995). Secondly, study results on healthy subjects suggest that a decreased peripheral insulin concentration could substantially decrease basal renal glucose production (Cersosimo et al. 1999a). Thirdly, a better recognition of hypoglycaemic symptoms with less fear of severe hypoglycaemic episodes can be suggested in patients using IP as opposed to SC insulin (Lahtela et al. 1995, Selam et al. 1992, Saudek et al. 1996). Hypoglycaemic episodes are more frequent with SC than with IP insulin treatment (Selam et al. 1992, Saudek et al. 1996). In a diabetic laboratory test animal model, the responses of endogenous glucose production and glucagon to hypoglycaemia have been more increased during IP than SC insulin administration (Shi et al. 1995). In non-uraemic

IDDM patients on IP insulin, an increased glucagon response to hypoglycaemia has also been observed (Oskarsson et al. 1999, Oskarsson et al. 2000b).

Peripheral hyperinsulinaemia caused by SC insulin administration can induce insulin resistance in healthy subjects (Rizza et al. 1985). In the present study SC insulin dose and peripheral insulin concentration increased slightly but not significantly when CAPD was initiated in IDDM patients. At the same time insulin sensitivity markedly improved, a finding also observed by other investigators (Schmitz 1985). The improvement of insulin sensitivity could be explained by the correction of uraemic state through dialysis.

When insulin administration was switched from SC to IP during CAPD, insulin sensitivity improved 13% (**II**). The results of Schmitz (Schmitz 1985) are in concordance with this observation. Patients on IP insulin treatment during CAPD have better insulin mediated glucose uptake than patients on hemodialysis and SC insulin (Schmitz 1985). The clamp method measures peripheral glucose uptake, of which muscle is 75-95% responsible (Baron et al. 1988). In non-diabetic laboratory test animals, hyperinsulinaemia has been shown to decrease glucose utilisation in skeletal muscle tissues (Cusin et al. 1990). The findings in this study could be partially explained by decreased peripheral hyperinsulinaemia (Schade et al. 1980a, Nelson et al. 1982). Hyperglycaemia may impair peripheral insulin sensitivity (Unger and Grundy 1985). The higher HbA_{1c}–level seen in patients on SC, when compared to IP insulin, could partly explain the decrease of insulin resistance after switching to IP insulin.

In non-uraemic IDDM patients, high hepatic glucose production is a marker of hepatic insulin resistance. Even normal rates of glucose production are inappropriately high for the prevailing hyperinsulinaemia and hyperglycaemia (DeFronzo et al.

1982b). In contrast to SC insulin therapy, the direct hepatic insulinisation and greater portal versus peripheral insulin concentration observed during IP administration decreases hepatic insulin resistance and hepatic glucose output in non-uraemic IDDM patients (Robert et al. 1993) and IDDM patients on CAPD (Lahtela et al. 1995).

Since the treatment of uraemia by dialysis decreases insulin resistance (Schmitz 1985) it is conceivable that insulin resistance may increase when the efficacy of CAPD is decreased by other factors, e.g. decreasing residual renal function. In this study no major changes in dialysis treatment were made. Therefore, no major change of residual renal function was expected during the six-month follow-up period.

4. Lipids and apolipoproteins

Most studies in non-uraemic diabetic patients suggest better lipoprotein profiles during IP than SC insulin therapy and possibly an improved cardiovascular survival rate (Ruotolo et al. 1994, Selam et al. 1994, Bagdade et al. 1997). Some studies in non-uraemic IDDM patients have, however, revealed decreased HDL-cholesterol (Micossi et al. 1986, Selam et al. 1989, Ruotolo et al. 1990, Dunn et al. 1997) and increased triglyceride levels (Micossi et al. 1986, Selam et al. 1989, Dunn et al. 1997) during IP insulin administration, as was the finding in the present study. These are the changes suggested to be associated with increased cardiovascular morbidity and mortality (Pekkanen et al. 1990, Manninen et al. 1992).

Selam and associates (1994) found that in comparison with SC insulin therapy, IP insulin is associated with a more enhanced reverse cholesterol transport and cholesteryl ester transfer in non-uraemic IDDM subjects (Figure 1). After this process, HDL_2 is a good substrate for hepatic lipase. Another study in non-uraemic IDDM

patients comparing IP to SC insulin treatment found increased activity of hepatic lipase in patients on IP insulin (Ruotolo et al. 1994). In laboratory test animals, hepatic lipase activity renders HDL-cholesterol more susceptible to hepatic uptake (Marques-Vidal et al. 1994).

In diabetic laboratory test animals (Kazumi et al. 1986) and IDDM patients on IP insulin without renal disease (Bagdade et al. 1994), lipoprotein lipase activity is lower than during SC insulin therapy. Lipoprotein lipase activity correlates with plasma HDL-cholesterol concentration. In this study the decreased lipoprotein lipase activity, a reflection of the lower peripheral insulin concentration observed during IP insulin administration when compared to SC insulin, may partly explain decreased plasma HDL-cholesterol (Taskinen 1990).

HDL loss through the peritoneal membrane may provide one explanation for the serum HDL-cholesterol change observed in this study. In non-diabetic CAPD patients, continuous loss of HDL to the peritoneal cavity and the hypertriglyceridemia have both been suggested to contribute strongly to the persistently low plasma levels of HDL (Kagan et al. 1990b). Insulin is known to be a peripheral vasodilator (Steinberg et al. 1994). The effect of supraphysiologic insulin concentration on peritoneal vasculature could be increased synthesis and release of nitric oxide (Baron et al. 1991, Steinberg et al. 1994, Zeng and Quon 1996). By increasing nitric oxide locally, insulin may mimic the action of nitroprusside. Nitroprusside is a nitric oxide donor, which has been shown to increase peritoneal permeability in diabetic CAPD patients (Douma et al. 1997).

In patients on dialysis, residual renal function deteriorates as a function of time. Plasma cholesterol, LDL-cholesterol, Apo B, and Apo A-I all decrease when residual renal function is lost, but there is no change in plasma HDL-cholesterol in CAPD

patients (Kagan et al. 1997). Studies **I-IV** were unidirectional as insulin administration was switched from SC to IP in all patients. At the same time residual urine output showed a tendency to decrease. In this study neither the increases in plasma cholesterol, LDL-cholesterol and Apo B, nor the decrease in serum HDL-cholesterol can be explained by decreased residual renal function. These changes are diametrically opposed to those observed by Kagan and associates (1997).

In contrast to SC insulin therapy serum triglycerides are increased in laboratory test animals (Kazumi et al. 1986), non-uraemic (Dunn et al. 1997), and uraemic (**I**) IDDM patients on IP insulin. Free fatty acids are the substrate for triglyceride synthesis in the liver. In the insulin resistant state there is increased free fatty acid release from adipose tissue (Abbasi et al. 2000). A lower peripheral insulin concentration after switching from SC to IP insulin treatment (Schade et al. 1980a) may further enhance peripheral free fatty acid release and its uptake by the liver. Enhanced insulinisation of the liver could augment this process, since higher insulin concentration directs the metabolic fate of free fatty acids in hepatocytes from β-oxidation to esterification into triglycerides (Kilworth et al. 1985, Wanless et al. 1989).

There is no previous data available concerning the effects of IP insulin administration on plasma HDL subgroups or Apos in diabetic patients on CAPD. In study **III,** reduction of plasma HDL_3-cholesterol was the main finding, but HDL_2-cholesterol also decreased. In non-uraemic IDDM patients, two studies have found increased HDL_3-cholesterol or decreased HDL_2-cholesterol during IP insulin treatment (Micossi et al. 1986, Ruotolo et al. 1994). Compared to non-uraemic patients, the subjects in the present study differ in that lipoproteins can be lost through the dialysate (Kagan et al. 1990b). During IP insulin therapy both cholesteryl ester

transfer and hepatic lipase activity are higher than during SC insulin therapy. The action of these enzymes normally transforms HDL_2 to HDL_3, which is utilised by reverse cholesterol transport (Clay et al. 1992). HDL_3 is a smaller particle than HDL_2 and is therefore potentially subject to greater loss through the peritoneum (Kagan et al. 1990b). The loss could be enhanced by local action of IP insulin on the peritoneal lining cells and vasculature (Baron et al. 1991, Steinberg et al. 1994, Zeng and Quon 1996).

Studies on the metabolism and significance of Apos in diabetic renal failure are scarce. In this study patients on IP insulin had lower levels of plasma Apo A-I than patients on SC insulin. One reason for decreased Apo A-I could be the commonly observed accelerated HDL Apo A-I removal in non-diabetic patients with high plasma triglycerides and low HDL-cholesterol (Brinton et al. 1991). During IP insulin administration, cholesteryl ester transfer from HDL to triglyceride rich lipoproteins was increased when compared to SC insulin administration (Selam et al. 1994). This increase may reduce plasma HDL-cholesterol concentration by both removing cholesteryl ester from HDL-particles, and by reducing the number of HDL particles in the circulation. Secondly, lipoprotein lipase activity is associated with the transfer of Apo C-III from VLDL to HDL. The Apo C-III content of HDL correlates inversely with the fractional catabolic rate of Apo A-I (Le et al. 1988). When compared with SC insulin administration, the decreased lipoprotein lipase activity is observed during IP insulin treatment (Kazumi et al. 1986, Bagdade et al. 1994) could partly explain the decreased Apo A-I concentration observed in this study. Thirdly, Apo A-I could be either lost in excess through dialysate, or by being coupled to HDL particles due to the local effect of insulin on peritoneal or vascular permeability when SC insulin is switched to IP insulin. This is supported by a finding in which more HDL Apo A-I

particles were present in an easily exchangeable form in healthy patients with low plasma HDL-cholesterol (Ginsberg et al. 1993). When HDL_2 particles are modulated to HDL_3 by hepatic lipase *in vitro*, Apo A-I is more readily dissociated from HDL_3 (Clay et al. 1992). Ruotolo and associates (1994) showed that the Apo A-I content in non-uraemic IDDM patients on IP insulin was increased in the HDL_3 fraction, *i.e.* in the more diffusible form. Free Apo A-I, thereafter, can be catabolised by the kidney (Glass et al. 1983) or lost through dialysate during CAPD (Kagan et al. 1990b). Increased fractional catabolic rate of Apo A-I is a major determinant of plasma HDL-cholesterol concentration in healthy subjects (Brinton et al. 1994).

5. Plasma leptin, nutrition and body weight

Due to reduced renal elimination capacity, plasma leptin concentration in ESRD patients on CAPD is high (Landt et al. 1999). Increased body fat and female gender are also associated with high plasma leptin concentration (Parry et al. 1998). These findings were supported by this study (**IV**). Plasma leptin increases markedly during the first three months of CAPD treatment (Kim et al. 1999). Concurrent intra-abdominal fat accumulation (Fernström et al. 1998) could partly explain increased plasma leptin. This is supported by the correlation between plasma leptin concentration and the amount of visceral fat (Tsujimoto et al. 1999) or total body fat mass (Parry et al. 1998), and opposed by lack of correlation between plasma leptin concentration and BMI (Kim et al. 1999) in CAPD patients in general. Other factors than increase of body mass, e.g. hyperinsulinaemia, are suggested to contribute to the inappropriately high plasma leptin concentration seen during CAPD (Kim et al. 1999).

Leptin is a product of adipose tissue. The weight reduction observed after switching from SC to IP insulin could partly explain the resulting decrease in plasma leptin. There was no correlation, however, between plasma leptin and BMI, indicating that factors other than amount of adipose tissue exert an influence on plasma leptin concentration.

Leptin production is stimulated by insulin in healthy subjects (Saad et al. 1998), IDDM (Luna et al. 1999) and CAPD patients (Kagan et al. 1999). When insulin is given IP, a higher than expected dialysate leptin concentration is observed. Reasons for this are suggested to be increased peritoneal clearance and local production of leptin (Heimbürger et al. 1999, Tsujimoto et al. 1999). IP insulin could influence peritoneal permeability and therefore increase peritoneal clearance of leptin. On the other hand, high local leptin concentration can induce vasodilatation in mesenteric vessels of non-diabetic laboratory test animals (Lembo et al. 2000). High local leptin concentration in the peritoneal cavity could potentiate the vasodilatatory action of insulin. These mechanisms could also partly explain the lower peripheral plasma leptin seen during IP with respect to SC insulin administration in this study. Another explanation for the observed low plasma leptin could be an increase in the lipolytic activity of subcutaneous adipose tissue (Fisher et al. 1999), because of the relatively lower peripheral insulin concentration during IP when compared to SC insulin administration (Schade et al. 1980a). In other words, the higher the subcutaneous adipose tissue lipolysis, the lower the plasma leptin in non-diabetic subjects (Fisher et al. 1999).

In vitro studies indicate that leptin may impair the metabolic actions of insulin and induce insulin resistance (Cohen et al. 1996). In healthy subjects with similar adiposity, insulin resistance may contribute to the observed variation in plasma leptin

levels (Maffei et al. 1995). Insulin resistance in IDDM (Tuominen et al. 1997) or NIDDM patients (Mohamed-Ali et al. 1997) has not been shown to correlate with plasma leptin. Plasma leptin increases after initiation of CAPD (Kim et al. 1999). In this study insulin resistance somewhat diminished after the initiation of CAPD observed. This suggests that plasma leptin does not exert a major influence on uraemic insulin resistance. In the present study, a further reduction of insulin resistance was seen after transition from SC to IP insulin treatment. Decreased plasma leptin observed with the change in insulin administration route could explain improved insulin sensitivity, but there was no correlation between plasma leptin and insulin sensitivity or plasma insulin in this study. These findings suggest that other mechanisms in addition to plasma leptin regulate insulin resistance in IDDM patients on CAPD.

Malnutrition is significant clinical problem in dialysis patients. In a study by Young and associates (1991), 51% of diabetic patients on CAPD suffered from malnutrition. Serum albumin is a simple but non-specific indicator of nutritional status. It is lower in diabetic than in non-diabetic ESRD patients. Serum albumin increases during the first year on CAPD and remains relatively elevated although subnormal (Blake et al. 1993). In this study serum albumin decreased slightly after the initiation of CAPD, which could be the result of volume expansion (Jones et al. 1998). After switching from SC to IP insulin, serum albumin increased. At the same time serum creatinine, plasma cholesterol, and LDL-cholesterol all increased, suggesting a better nutritional status (Young et al. 1991, Johansen et al. 1998) despite decreased body weight. Hypoalbuminemia in CAPD patients is associated with an increased extracellular volume (Jones et al. 1998) and increased serum albumin is seen in volume contraction. CAPD patients frequently present with fluid overload (Takeda et

al. 1998, Jones, 1998 #420), which makes volume contraction an unlikely explanation for decreased body weight in this study.

Lipoprotein lipase activity leads to plasma triglyceride breakdown and uptake by adipocytes. During IP insulin therapy in laboratory test animals and non-uraemic IDDM patients the activity of lipoprotein lipase is lower than during SC insulin treatment (Kazumi et al. 1986, Bagdade et al. 1994). A decreased uptake and storage of plasma triglycerides in adipose tissue could explain some of the observed weight reduction in this study. In an opposite situation, induced peripheral hyperinsulinaemia, such as is seen during SC insulin treatment in laboratory test animals, has resulted in insulin resistance, enhanced adipose tissue lipogenesis, and increased body weight (Cusin et al. 1990).

In non-uraemic patients a better weight control is achieved with IP insulin than with SC insulin (Saudek et al. 1996, Dunn et al. 1997) and even weight reduction in IDDM patients on CAPD (**I-IV**). IP insulin yielded lower peripheral plasma insulin than SC insulin in non-uraemic IDDM patients (Schade et al. 1980a) and may cause less inhibition of lipolysis by adipocyte hormone sensitive lipase. Increased hormone sensitive lipase activity favours depletion of triglycerides from adipocytes and weight reduction. Adipose tissue triglyceride breakdown results in free fatty acid release. During IP insulin treatment the plasma free fatty acid concentration increased insignificantly in a small group of IDDM patients on CAPD when compared to SC insulin (Lahtela et al. 1995). In the present study both body weight and plasma leptin decreased after transition from SC to IP insulin (**IV**). There was not , however, any correlation between the two. The lower plasma leptin observed during IP insulin administration could be a marker of increased lipolysis. When adipocytes are most lipolytic, leptin production is found to be at its lowest in healthy subjects (Fisher et al.

1999). In one study involving non-uraemic NIDDM patients, a single dose of IP insulin was shown to maintain suppression of lipolysis for a shorter duration than an equivalent dose of SC insulin (Kelley et al. 1996). The better weight control observed in CAPD patients on IP insulin, might be explained by a potentially higher rate of lipolysis.

6. Hepatic subcapsular steatosis

Almost all CAPD patients on IP insulin developed hepatic subcapsular steatosis, which was not the case in patients on SC insulin (Wanless et al. 1989) - a finding confirmed by the present study (**V-VI**). In Wanless' study, the maximal thickness of the steatotic lesions was 0.05-12 mm, while the corresponding figure in this study was 6-17 mm (**VI**). In the histologic study by Wanless and associates (1989), the smallest subcapsular lesions were only a few cells thick. With ultrasound such minute changes are indetectable. Hepatic subcapsular steatosis involves 5 to 80% of the liver surface area.

In this study, fatty lesions were most often seen on the cranial segments of the liver. The steatosis was difficult to detect if the patient was in an upright position. In the supine position, however, the liver is surrounded by dialysate, which enhances visibility and may partly explain the location of most lesions.

In the present study, various approaches were utilised to confirm that the subcapsular lesions were actually steatotic. Assurance was increased by the typical ultrasonic echo pattern, the characteristic magnetic resonance signal intensities on T1 and fat suppressing sequences in one patient, and the post mortem verification in two patients.

Both local and systemic factors contribute to the development of subcapsular steatosis. The subcapsular distribution of steatosis suggests an alteration of hepatocyte metabolism by supraphysiological concentrations of insulin (400 to 6300-fold) and glucose (10 to 30-fold) locally. High insulin concentration directs the hepatic metabolism of free fatty acids towards esterification into triglycerides, thus exceeding hepatocyte triglyceride secretion rate. Free fatty acids are accumulated within the liver (Wanless et al. 1989). The finding of increased plasma triglycerides during IP insulin treatment supports this.

A local mechanism for the fatty change formation could be the high osmolarity of the dialysate. In this study, a correlation between the amount of hepatic subcapsular steatosis and elevated peritoneal transfer rates was found. Increased glucose concentration in the subcapsular space of the liver may be utilised in energy metabolism, leading to the increased esterification of free fatty acids into triglycerides in high transporters. Alternatively the increased glucose absorption in high transporters could result in a more hyperosmolar medium in subcapsular liver tissue and concurrent shrinkage of hepatocytes associated with inhibition of triglyceride secretion (Zammit 1995).

Malnutrition is a frequent problem in dialysis patients. During CAPD, energy intake is fundamentally changed due to the substantial amount of energy uptake from carbohydrates. Protein malnutrition, with relatively high energy intake from carbohydrates, is seen in kwashiorkor, which is also associated with hepatic steatosis (Doherty et al. 1992). In laboratory test animals, a high carbohydrate – fat free diet produces a dysequilibrium between triglyceride synthesis and secretion (Delzenne et al. 1997). In this study, low PCR, suggesting protein malnutrition, correlated with the amount of hepatic subcapsular steatosis. Also, the amount of steatosis correlated

inversely with KT/V_{urea}, which could be explained by the association between KT/V_{urea} and malnutrition (Lindholm and Bergström 1994). The amount of subcapsular steatosis increased in patients with high body weight. Body weight is used in calculating both PCR and KT/V_{urea}, and it could be inferred that low PCR and KT/V_{urea} are only a reflection of higher body weight. No correlation, however, was found between the amount of steatosis and BMI.

7. Significance of the study

Tight control of blood glucose is crucial in the prevention of diabetic complications in non-uraemic IDDM patients (DCCT Research Group 1996). In patients on dialysis, however, the role of blood glucose control in the development of diabetic microvascular and macrovascular complications remains unknown. This study suggests that IP insulin offers better glycaemic control than SC insulin during CAPD. IP insulin administration results in lower plasma insulin levels than SC insulin, and could be advantageous by virtue of being less atherogenic (Lamarche et al. 1998, Glueck et al. 1999). Improved insulin sensitivity is also beneficial to the patient.

In comparison with SC insulin, the increased LDL/HDL cholesterol ratio and decreased HDL-cholesterol seen during IP insulin treatment are proatherogenic changes. High LDL-cholesterol, LDL/HDL cholesterol ratio, and Apo B are all associated with increased cardiac mortality in diabetic patients on hemodialysis (Tschöpe et al. 1993).

The significance of decreased plasma HDL_3 and Apo A-I in this study remains unresolved, which is partly due to the fact that the association between these

lipoproteins and atherosclerosis is still under debate (Buring et al. 1992, Smuts et al. 1994, Lamarche et al. 1997). One study, however, dealing with IDDM and NIDDM patients on either hemodialysis or CAPD, found decreased serum Apo A-I to be an independent predictor of both cardiac and noncardiac death (Koch et al. 1997).

Whether or not the liver steatosis seen in patients using IP insulin affects their survival, is unclear. The most modern CAPD protocols employ combination therapies with dialysates containing glucose polymers and aminoacids together with glucose based dialysate. Whether or not hepatic fatty changes can be decreased by reducing dialysate glucose load warrant further studies.

XI SUMMARY AND CONCLUSIONS

The metabolic effects of administering insulin either intraperitoneally or subcutaneously in diabetic patients on CAPD treatment can be summarised as follows:

I IP insulin is associated with better glycaemic control and insulin sensitivity than SC insulin.

II The effect of IP insulin on serum lipid profiles is disadvantageous when compared to SC insulin. The ratio of LDL/HDL-cholesterol increases, serum HDL-cholesterol decreases and there is a tendency for plasma LDL-cholesterol and triglycerides to increase. During IP insulin therapy, plasma Apo A-I and HDL_3-cholesterol are significantly decreased and there is a tendency for HDL_2-cholesterol to decrease.

III Plasma leptin concentration and patient weight are lower during IP insulin therapy than during SC insulin therapy. The change in body weight does not appear to be the result of malnutrition.

IV IP insulin treatment and dialysate glucose cause hepatic steatosis, which is situated subcapsularly. The amount of hepatic subcapsular steatosis increases when the peritoneal transfer rate and body weight are high.

In conclusion, IP insulin treatment facilitates better blood glucose control than SC insulin, but makes plasma lipoprotein levels potentially more atherogenic. IP insulin induces the formation of hepatic subcapsular steatosis. Both direct intense insulinisation of the liver and low peripheral insulin concentration are implicated as causes for the metabolic changes induced by IP insulin treatment. Weight reduction and decreased plasma leptin after transition from SC to IP insulin are potentially positive changes since they do not seem to be associated with malnutrition. According to these results, both SC and IP insulin therapy are acceptable from clinical standpoint. IP insulin treatment is, however, preferable to SC insulin treatment.

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Pasi Nevalainen

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