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Lipoprotein lipase in hemodialysis patients and healthy controls

Effects of heparin

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ABSTRACT

Mortality from cardiovascular disease in patients on chronic hemodialysis (HD) is 10 to 20 times greater than in the general population. One major risk factor is renal dyslipidemia, characterised by an impaired catabolism of triglyceride (TG)-rich lipoproteins with accumulation of atherogenic remnant particles. A contributing factor may be derangement of the lipoprotein lipase (LPL) system, the major lipase in the catabolism of TG-rich lipoproteins. The functional pool of LPL is located at vascular surfaces, and is released by heparin into the circulating blood and extracted and degraded by the liver. Unfractionated heparin (UFH) is commonly used during dialysis to avoid clotting in the extracorporeal devices, but is increasingly replaced by various low molecular weight heparin (LMWH) preparations. Plasma LPL activity is usually lower after injection of LMWH which is therefore said to release less LPL and cause less disturbance of lipoprotein metabolism than UFH. However, animal studies have revealed that LMWH is as efficient as UFH in releasing LPL but is less efficient in retarding hepatic uptake.

The aim of this study was to explore the effects of UFH and a LMWH (dalteparin) on LPL activity and TG concentrations in HD-patients compared with healthy controls, matched for age and gender. A disturbed LPL system might contribute to an impaired lipoprotein metabolism, and hence, an aggravated cardiovascular condition.

An 8-hour primed infusion of UFH to controls gave rise to an initial peak of LPL activity within 30 minutes. The activity then dropped by almost 80% over the next two hours and levelled off to a plateau that corresponded to 15% of the peak level. When UFH was infused to HD-patients the curve for LPL activity resembled that for controls, but was reduced by 50% during the peak, while the plateau activities were comparable. The interpretation was that the functional pool, represented by the initial peak, was impaired in HD-patients, while the production of lipase molecules, reflected by the plateau, was only marginally reduced. During the peak of LPL activity TG decreased in both groups, but less in HD-patients, as was expected from the lower circulating lipase activity. During the plateau phase with low lipase activity, TG increased towards and beyond baseline values.

When dalteparin was infused, the same pattern of plasma LPL activity was observed, although remarkably reduced. In controls the peak was only 30% and the subsequent plateau 40% compared with the activities during the UFH infusion. A bolus of UFH given when the LPL

activity had levelled off to a plateau brought out about the same amount of activity, regardless of whether dalteparin or UFH had been infused. The conclusion was that both heparin preparations had reduced endothelial LPL to a similar extent, but that dalteparin less efficiently retarded the hepatic uptake of the enzyme. As a consequence to this, TG tended to reach higher levels after the dalteparin infusion. The LPL activities were further reduced in HD-patients during infusion with dalteparin, the peak was only 27% and the plateau 35% compared with the activities when UFH was infused. There was no decrease in TG, but rather a continuous increase, suggesting a profound depletion of functional LPL.

In another study in HD-patients, two anticoagulation regimes based on present clinical practice were compared, and the doses were adjusted to the respective manufacturers recommendation. UFH was administered as a primed infusion, whereas dalteparin was given only as a single bolus pre-dialysis, not followed by an infusion. The results were in line with those in the experimental studies and indicate that also in the clinical setting LMWH interferes with the LPL system as least as much as an infusion of UFH does, and temporarily impairs lipolysis of TG. This interference might, in consequence, contribute to an aggravated cardiovascular condition in HD-patients.

ORIGINAL PAPERS

This thesis is based on the following papers, which will be referred to in the text by their roman numerals.

- I Näsström B, Olivecrona G, Olivecrona T, Stegmayr BG: Lipoprotein lipase during continuous heparin infusion: Tissue stores become partially depleted. *J Lab Clin Med*, 138:206-213, 2001
- II Näsström B, Olivecrona G, Olivecrona T, Stegmayr BG: Lipoprotein lipase during heparin infusion: lower activity in hemodialysis patients. *Scand J Clin Lab Invest*, 63:45-53, 2003
- III Näsström B, Stegmayr BG, Olivecrona G, Olivecrona T: Lower plasma levels of lipoprotein lipase after infusion of low molecular weight heparin than after administration of conventional heparin indicate more rapid catabolism of the enzyme. *J Lab Clin Med*, 142:90-99, 2003
- IV Näsström B, Stegmayr BG, Olivecrona G, Olivecrona T: Lipoprotein lipase in hemodialysis patients. Depletion of tissue stores by a low molecular weight heparin, despite low plasma levels of the enzyme. *Submitted*
- V Näsström B, Stegmayr BG, Gupta J, Olivecrona G, Olivecrona T: A single bolus of a low molecular weight heparin to patients on hemodialysis depletes lipoprotein lipase stores and retards triglyceride clearing. *Submitted*

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ABBREVIATIONS

Apo	apolipoprotein
APTT	activated partial thromboplastin time
AUC	area under the curve
AV	arteriovenous
BMI	body mass index
CDC	central dialysis catheter
CETP	cholesterol ester transfer protein
CM	chylomicron
Da	Dalton
FFA	free fatty acids
GFR	glomerular filtration rate
HD	hemodialysis
HDL	high density lipoproteins
HL	hepatic lipase
HSPG	heparan sulphate proteoglycans
IDL	intermediate density lipoproteins
LCAT	lecithin cholesterol acyl transferase
LDL	low density lipoproteins
LDL-R	low density lipoprotein receptor
LMWH	low molecular weight heparin
LPL	lipoprotein lipase
LRP	low density lipoprotein receptor-related protein
PTH	parathyroid hormone
TG	triglyceride
TNF- α	tumor necrosis factor- α
UFH	unfractionated heparin
VLDL	very low density lipoproteins
VLDL-R	very low density lipoprotein receptor

INTRODUCTION

Background

The number of patients receiving renal replacement therapy (hemodialysis, peritoneal dialysis and kidney transplantation) in Sweden has continuously increased since documentation started in 1991. The prevalence in December 2002 was for the whole country, 756 cases per million and the incidence rate 125 cases per million and year. There has been a slight increase in incidence rate among patients older than 65 years, while the incidence rate among patients younger than 65 years has been stable. All modes of treatment increase and 6761 patients were treated in December 2002, 52% having a kidney transplant, 36% being on hemodialysis and 12% on peritoneal dialysis. The annual increase is approximately 5%. About two thirds of the patients are men, and one third are women. Glomerulonephritis is the most frequent disease causing uremia among the patients receiving renal replacement therapy. There is a great inflow from patients with diabetesnephropati and this is also the most common diagnosis among those initiating treatment. The average annual mortality rate is 14.3% for the whole uremia population, for transplanted 2.9% and for patients on dialysis 28.1%. Among patients on dialysis, death from uremia, i.e. discontinued dialysis treatment, has increased in later years. A gradual impairment in survival is explained by an ongoing increase in average age among the patients. Mortality from cardiovascular diseases dominates both among transplanted patients and patients on dialysis. (From the Swedish Registry for Renal Replacement Therapy 2003).

Cardiovascular disease is the leading cause of morbidity and mortality among dialysis patients and cardiovascular disease accounts for half or more of the deaths [1-3]. Approximately 22% of the deaths from cardiac causes are attributed to acute myocardial infarction. In patients who survive a myocardial infarction the mortality from cardiac causes is 41% at one year, 52% at two years and 70% at five years [4]. After stratifying for age, race and gender, mortality from cardiovascular disease in dialysis patients is 10 to 20 times greater than in the general population. The increase in risk ranges from 500-fold in individuals aged 25-34 years to 5-fold in individuals aged > 85 years [5]. The excess risk of vascular disease is due, at least in part, to traditional risk factors identified in the general population, including hypertension, diabetes mellitus, hyperlipidemia, tobacco use and physical inactivity. In addition, also hemodynamic and

metabolic factors related to the kidney disease are involved. These uremia related risk factors include dyslipidemia, high lipoprotein (a), prothrombotic factors, hyperhomocysteinemia, increased oxidant stress, hypoalbuminemia, inflammation, hemodynamic overload and anemia [6].

Lipoprotein metabolism

Lipids are an integral component in different cell structures and are also used in synthesis of biological active substances like steroid hormones, D vitamin and prostaglandins. Lipids serve also as the main source of energy for the body. As lipids are not directly soluble in an aqueous environment, the transport between organs is accomplished by specific particles, called lipoproteins. They are spherical particles consisting of lipids and specific proteins (apolipoproteins). The core is composed of insoluble lipids like triglycerides and cholesterol esters, whereas the surface consists of amphipathic compounds like phospholipids, unesterified cholesterol and apolipoproteins, which make the particle soluble. Each lipoprotein class has its characteristic apolipoprotein composition, in addition to its lipid content. Both the size as well as the composition changes as the lipoprotein particle is metabolised. In addition to their structural roles, the apolipoproteins also regulate lipoprotein metabolism and control the receptor-mediated uptake in different tissues. Lipoproteins are traditionally divided into five main classes and can be separated by density gradient centrifugation (Table I) [7, 8].

Table I: Characteristics of the main plasma lipoproteins

	Density (g/mL)	Diameter (nm)	Main apolipoprotein
Chylomicrons	<0.95	80-1200	B-48
VLDL	0.95-1.006	30-80	B-100
IDL	1.006-1.019	23-35	B-100
LDL	1.019-1.063	18-25	B-100
HDL	1.063-1.21	5-12	A-I

Lipids of dietary origin are transported by chylomicrons from the small intestine via the thoracic duct to the general circulation. The chylomicrons carry not only triglycerides, but also fat-soluble

vitamins, cholesterol and phospholipids as well. Chylomicrons, formed with apolipoprotein B-48 (apoB-48) and apoA, acquire apoE and the C-apolipoproteins by transfer from high density lipoproteins (HDL). In the capillaries, the chylomicron triglycerides are rapidly hydrolysed to free fatty acids and monoglycerides by the enzyme lipoprotein lipase (LPL). The lipolysis products are taken up locally by the tissue to be used for energy production (muscle and heart) or storage for future needs (adipose tissue). The continuing removal of triglycerides by LPL converts the chylomicron into a smaller particle, known as a chylomicron remnant, which is mainly taken up by the liver and degraded [7-10].

The liver continuously secretes very low density lipoproteins (VLDL), the main fasting triglyceride-rich lipoprotein. They are formed from endogenously synthesised lipids and lipids taken up from blood. Nascent VLDL contain apoB-100 and will later, like chylomicrons, acquire apoE and C-apolipoproteins by transfer from HDL. The triglycerides in VLDL are hydrolysed by LPL and gradually a smaller remnant particle is formed, intermediate density lipoprotein (IDL), mainly containing apoB-100 and apoE. A minor part of the IDL particles are cleared by receptor-mediated uptake in the liver, whereas the main part is further lipolysed by hepatic lipase (HL) into low density lipoproteins (LDL). LDL is the main plasma carrier of cholesteryl esters and contains only low amounts of triglycerides. The major apolipoprotein in LDL is apoB-100. LDL turn over relatively slowly and are removed by receptor-mediated endocytosis by binding of apoB-100 to the LDL receptor mainly expressed in the liver, adrenal glands and gonads. Not all LDL is removed by the LDL receptor. The scavenger receptor, primarily in macrophages and some endothelial cells preferably bind structurally modified LDL particles. Excessive uptake of LDL by this receptor may be of importance in the atherogenic process [7, 8, 10].

HDL plays an important role in the exchange of cholesterol, phospholipids and apolipoproteins between the different lipoproteins and in the reverse transport of cholesterol from extrahepatic tissues to the liver. Nascent HDL particles are synthesised by the liver and intestine, as small, phospholipid-rich discoid-like structures also containing cholesterol and apolipoproteins. HDL does not contain the B-apolipoproteins. By action of the enzyme lecithin-cholesterol acyl transferase (LCAT) the originally flat HDL rapidly transforms into spherical particles, HDL₃ and HDL₂ subfractions. Small and protein-rich HDL₃ particles assimilate cholesterol and phospholipids from degraded VLDL and chylomicrons, and are thereby converted into larger lipid-rich HDL₂ particles when LCAT catalyses the esterification of cholesterol. By action of

cholesterol ester transfer protein (CETP) the cholesteryl esters will be exchanged for triglycerides from VLDL and chylomicrons. HDL₂ is then converted back into HDL₃ through hydrolysis of triglycerides by HL [7, 8].

Lipoprotein lipase

LPL is the major enzyme responsible for hydrolysis of triglycerides present in circulating plasma lipoproteins, a process generating free fatty acids which can be utilised for storage or generation of energy [11, 12]. More than 60 years ago, Hahn noticed that intravenous heparin totally cleared diet-induced lipemia in dogs [13]. This effect could not be reproduced by adding heparin to plasma in vitro, suggesting that this “clearing factor” was released into plasma by heparin in vivo. This factor was later identified as a triglyceride lipase in 1955 [14] and isolated from human post-heparin plasma in 1977 [15].

Structural aspects

LPL belongs to the same gene family as HL and pancreatic lipase. These lipases are structurally related and especially the active site shows a high degree of conservation [16, 17]. Comparison of amino acid sequences, based on the molecular model of pancreatic lipase, indicates that all three enzymes exhibit a very similar three-dimensional structure [18, 19]. According to this, LPL is folded into two distinct domains, an amino-terminal and a carboxyl-terminal one [20, 21]. The larger amino-terminal domain contains the active site, which is covered by a loop that moves aside to provide access to the substrate. The loop structure differs between the lipases, and plays a crucial role in determining lipase substrate specificity [22]. The smaller carboxyl-terminal domain is important for binding to receptors and lipoproteins [23, 24]. LPL has high affinity for heparin [25] and both domains are probably involved in the binding of heparin [20, 23, 26-28]. The human LPL gene is located on chromosome 8 and is composed of 10 exons [16]. Catalytically active LPL is a noncovalent homodimer of about 100 kDa, composed of two identical subunits probably arranged in a head to tail configuration [29]. Active LPL is rather unstable and dissociation of the dimers into monomers leads to irreversible inactivation of the enzyme and a decrease in heparin affinity [30]. LPL requires the cofactor apoC-II for optimal activity, whereas apoC-III acts as an inhibitor. LPL is strongly inhibited by fatty acids, suggested

to be a feedback control mechanism. LPL is also inhibited by salt and protamine, and has an alkaline pH-optimum [12, 31].

Turnover

LPL is synthesised by parenchymal cells in many tissues, mainly in skeletal and cardiac muscles and in adipose tissue. Lesser amounts are detected in many other tissues including the nervous system, adrenals, ovaries, kidneys, lungs and macrophages. During lactation there is high LPL activity in the mammary gland [12, 32]. LPL is synthesised as an inactive proenzyme and undergoes intracellular processing for activation, including glycosylation. Some of the newly synthesised enzyme molecules are degraded within the cells and are not released [33]. The secreted fraction is transported through the extracellular matrix to endothelial cells in adjacent capillaries where it is anchored to heparan sulphate proteoglycans (HSPG) at the luminal surface [12, 34, 35]. LPL has a rather short lifetime at the endothelium [36-38] but the pathway for turnover is not well known. Some LPL is internalised and recycled to the cell surface [39] whereas some dissociates from the endothelial surface into blood [36, 37] and probably can bind to endothelial sites nearby, or transfers to tissues where it is not locally synthesised [12, 40]. LPL in plasma is not in equilibrium with endothelial LPL, but reflects a continuous release from sites of synthesis in peripheral tissues to sites for degradation in the liver [41, 42]. The main part of active LPL is located at the endothelium, whereas the concentration in plasma is very low [43, 44] due to rapid uptake and degradation in the liver [45-47]. The amount of catalytically inactive lipase protein, probably in monomeric form, in plasma is larger [42]. Most of both active and inactive LPL in plasma is bound to circulating lipoproteins [48, 49] and it has been suggested that LPL mediates binding of lipoproteins to cells [50].

Mechanism of action

LPL is anchored to HSPG at the vascular endothelium, which puts it in a position where it can freely interact with lipoproteins from circulating blood. LPL has broad substrate specificity, but the relevant substrates *in vivo* are triglycerides in chylomicrons and VLDL. The catalytic action yields fatty acids and monoglycerides for tissue utilisation [12, 51], and the lipoproteins are reduced to remnant particles and released back into the circulation.

LPL is an efficient enzyme, the turnover number for triglyceride hydrolysis being about 1000 per second. A chylomicron containing more than a million triglyceride molecules can be unloaded and transformed into a remnant particle in less than ten minutes. This implies that several LPL molecules must act simultaneously on the lipoprotein particle. VLDL are smaller particles and contain 10 000-15 000 triglyceride molecules. A VLDL could readily be hydrolysed by a single LPL molecule in about one minute, but in vivo it will take more than an hour to complete the delipidation, probably through short-lived interactions with individual LPL molecules [12].

LPL enhances binding of lipoproteins to cells, independent of its catalytic function. One site on LPL binds to the lipoprotein particle and another site binds to heparan sulphate, forming a bridge between the particle and the cell surface [12]. LPL also mediates binding of lipoproteins to a group of cell surface receptors in the LDL receptor family [50, 52, 53]. Both types of binding can lead to internalisation and degradation of the lipoprotein particle, the lipase or both [12].

Regulation

The regulation of LPL is complex and can occur at transcriptional, translational or post-translational levels in response to various physiological, nutritional, environmental and pathological situations [12]. LPL activity is regulated in a tissue-specific manner according to metabolic demands of individual tissues, which directs fatty acids to specific sites. The activity is relatively low in adipose tissue in the fasted state, but rises rapidly after a meal and directs an increased fraction of the blood lipids to adipose tissue for storage. Inversely, the activity in heart and muscle is higher in the fasted than in the fed state [51]. The regulation probably involves several factors, including insulin, cortisol, sex steroid hormones and sympathetic innervation [54].

The amount of LPL at the endothelium is also regulated by the amount of fatty acids released by the lipolysis reaction. Lipolysis is initially rapid and may exceed the rates for fatty acid transport and utilisation. Fatty acids will accumulate and form a complex with LPL that inhibits continued hydrolysis and causes dissociation of LPL from the endothelium [43, 55].

LPL deficiency

About 80 natural mutations in the LPL gene have been described in humans, the majority of which are missense. Most of these mutations are rare and lead to LPL deficiency if they are

present as homozygote or compound heterozygotes, resulting in the familial chylomicronemia syndrome, characterised by fasting chylomicronemia, marked hypertriglyceridemia and extremely low HDL cholesterol. Individuals with defects in apoC-II, the activator of LPL, have similar clinical features as those with defects in LPL, although less severe [32, 56]. Heterozygous mutations associated with reduced LPL activity may underlie some of the more common hyperlipidemic disorders found in the general population, including familial hypertriglyceridemia, familial combined hyperlipidemia and postprandial lipemia [57].

Acquired deficiency of LPL is more common than the hereditary disorders and is associated with hypertriglyceridemia and low levels of HDL cholesterol. LPL activity is often reduced in patients with diabetes mellitus, especially with severe insulin deficiency or resistance. Also other conditions like hypothyroidism, nephrotic syndrome and chronic renal failure are often accompanied by LPL deficiency [51].

Effects of heparin

LPL has higher affinity for heparin than for heparan sulphate [25], and an injection of heparin releases LPL from its endothelial binding sites into blood, forming an enzyme-heparin complex [58]. In this way, post-heparin plasma provides convenient access to the lipase for measurement. Most LPL in basal plasma consists of inactive LPL protein [48], whereas the amount of catalytically active LPL is low [44]. An injection of heparin increases active LPL by several hundred-fold but inactive LPL by only about two-fold [42]. Thus, heparin releases mainly the catalytically active dimeric form of LPL [48]. The amount of LPL activity in post-heparin plasma is assumed to reflect the pool of functional LPL available at the endothelial surface [51], and does not correlate with the amount before heparin [43, 59, 60]. This suggests that LPL in pre-heparin plasma is not in equilibrium with endothelial LPL, but reflects a continuous flow of lipase molecules from peripheral tissues via the blood to the liver, where the enzyme is degraded [47]. LPL mass and activity in pre-heparin plasma and the increase of LPL after the administration of heparin are separate parameters. Pre-heparin LPL mass and activity are not related to each other, whereas the correlation in post-heparin plasma is high [44]. The release of LPL leads to rapid hydrolysis of triglycerides in plasma because all of the lipoprotein particles have immediate access to the enzyme [61]. Heparin retards, but does not abolish, the uptake of the enzyme by the liver, and LPL activity in plasma is elevated as long as substantial amounts of heparin remain in

the circulation. Hence, high enzyme activity after injection of heparin is due to release from peripheral tissues combined with retarded uptake by the liver [62]. Then follows a period during which functional LPL is depleted [63, 64], and triglyceride clearing is temporarily decreased [61, 65], presumably because endothelial LPL has been depleted by accelerated transport to and degradation in the liver [62].

Plasma LPL activity is usually lower after an injection of low molecular weight heparin (LMWH) preparations which are therefore said to release less LPL [65, 66] and thereby cause less disturbance of lipoprotein metabolism [67]. Molecular studies on the lipase-heparin interaction do not support this view. Modelling shows that a heparin decasaccharide is enough to fill the heparin-binding groove on the lipase [19]. Direct studies of the LPL-heparin interaction have shown that the affinity increased with the length of the heparin chain as this was increased from tetra- to hexa- to octa- to decasaccharides, but after that there was only a marginal increase in affinity [25]. Also in biologic systems decasaccharides appear to be sufficiently long to exert full effect on LPL. When rat hearts were perfused it was found that heparin hexa- or octasaccharides released only relatively small amounts of LPL but that decasaccharides was more effective and actually released more LPL than unfractionated heparin (UFH) did [68]. Several groups have also found that the ability of LMWH or decasaccharides to release LPL from tissues in vitro is as high or higher than that of UFH [69-71]. The relatively low plasma LPL activity seen after injection of LMWH is probably not because less lipase is released, but rather because LMWH is less effective than UFH in preventing hepatic uptake and degradation of the lipase. This was directly shown in liver-perfusion experiments [72, 73]. It was also demonstrated that clearing of injected chylomicrons was more retarded after injection of LMWH or decasaccharides than after injection of UFH [61, 68], indicating a more pronounced depletion of LPL activity after LMWH.

Hepatic lipase

In 1970 it was found that post-heparin plasma contained another lipase, closely related to LPL, but differentiated by being resistant to inhibition by high salt concentration or protamine and by not requiring an apolipoprotein activator [31]. The knowledge of hepatic lipase (HL) turnover is less clear than that of LPL turnover. HL is synthesised by hepatocytes and is mainly located in the liver, adrenal glands and ovaries, but is absent in most other tissues. HL acts in the

degradation of lipoprotein remnants formed by LPL, and in the metabolism of LDL and HDL [12, 74]. Like LPL, also HL is able to mediate the binding and subsequent uptake of lipoproteins into cells [75, 76]. The activity of HL is regulated by steroid and other hormones, androgens, ACTH, HCG and thyroid hormones increases HL activity, whereas estrogens depresses it [77]. The clinical syndrome of HL deficiency is rare and difficult to identify, and only few cases have been described. Subjects with complete HL deficiency have elevated plasma cholesterol and triglyceride (TG) levels and some have premature atherosclerosis. The phenotype in heterozygotes is variable and can be modulated by secondary factors, both genetic and environmental [74, 78].

Like LPL, HL is released into blood when heparin is injected. A positive linear correlation in HL activity is found between pre- and post-heparin plasma [43, 60], which suggests that plasma HL activity is in equilibrium with HL activity in the liver. The curve for HL activity in plasma tends to follow the heparin concentration fairly closely [68], and as heparin is cleared from the circulating blood, HL returns to its binding sites in the liver. A second heparin injection releases a similar amount of HL activity as the first injection did, suggesting that heparin does not affect the turnover of HL [64].

Renal dyslipidemia

Progressive renal failure is associated with abnormal concentrations and composition of plasma lipoproteins. The dyslipidemia develops during the asymptomatic stages of renal insufficiency and becomes more pronounced as renal failure advances and continues to affect patients on long-term dialysis. At early stages of renal insufficiency these changes may not affect the lipid profile analysed by routine methods, but is rather reflected as a disturbance in the apolipoprotein profile [79]. The characteristic alterations detected early include reduced concentrations of apoA-I and apoA-II, and a marked elevation of apoC-III levels. ApoB and apoE are often normal in early renal insufficiency and moderately elevated in patients on hemodialysis (HD). ApoC-I and apoC-II are slightly elevated only in advanced renal failure. The apoC-II to apoC-III ratio is decreased. A highly significant reduction of the apoA-I to apoC-III ratio is detected early and seems to be a hallmark of the altered lipoprotein composition [80, 81]. The lipoprotein profile is characterised by the accumulation of intact and partially metabolised TG-rich apoB-containing lipoproteins with elevated levels of apoC-III and to a lesser extent apoE, resulting in a pronounced increase in

IDL and an enrichment of TG, apoC-III and apoE in LDL. The concentration of HDL is decreased with reduced contents of cholesterol, apoA-I and A-II and a decrease in the HDL₂ to HDL₃ ratio [79]. As renal failure advances, the characteristic lipid profile consists of moderate hypertriglyceridemia in combination with low HDL cholesterol, whereas levels of total and LDL cholesterol are usually normal [79, 82-89].

The pathogenesis of the renal dyslipidemia has only been partially elucidated, but it appears that a reduced catabolism and clearance of apoB-containing lipoproteins of hepatic and intestinal origin constitutes the main abnormality [79, 81, 90]. The impaired catabolism is related to reduced activities of the lipolytic enzymes, particularly LPL, HL and LCAT, detected in both patients and animal models [86, 89, 91-100]. Reduced LPL activity may account for a decreased catabolism of newly synthesised chylomicrons and VLDL, while reduced HL activity might retard further degradation and remodelling, resulting in an accumulation of remnant lipoproteins [78, 89, 92, 95, 99, 101]. Reduced LCAT activity in combination with low HL activity and decreased concentrations of apoA-I and apoA-II might contribute to a retarded conversion of HDL₃ to HDL₂ [79, 95, 97].

An altered composition of lipoproteins could interfere with their suitability as substrates for lipolysis [79, 99, 102], and recognition and uptake by receptor-mediated mechanisms [79, 103]. A defective hepatic clearance of postprandial chylomicron remnants has been described in dialysis patients resulting in an accumulation of these partly metabolised particles in the circulation [104]. In addition, several qualitative lipoprotein modifications like carbamylation, oxidation and glycation are present, which may generate small dense LDL (sdLDL) [105, 106]. Modified lipoproteins and remnants in varying degrees of degradation, will remain in the circulation for prolonged periods of time, resulting in increased clearance by alternative pathways, such as non-receptor-mediated uptake by macrophages [79, 107].

The renal dyslipoproteinemia has an atherogenic character and may contribute to an accelerated development of atherosclerosis [79, 108], and thereby constitute an important risk factor to the high cardiovascular morbidity and mortality in uremia [1, 109]. Except an atherosclerotic significance, it has also been suggested that the alteration in lipoproteins might accelerate the progress of renal failure [110-113].

LPL in uremia

In several studies, decreased LPL activity in post-heparin plasma has been reported in patients on HD [86, 91, 93, 95, 99]. A reduction of LPL activity has also been demonstrated in biopsies from adipose tissue in hypertriglyceridemic HD-patients [94] and in adipose tissue from uremic rats [114, 115]. The mechanism behind this reduction in LPL activity is not clear and several factors may contribute. The insulin resistance, frequently seen in uremic patients [116] may be involved by decreasing the synthesis of LPL [84, 114, 117]. When insulin was infused to uremic rats this reversed the abnormality in LPL activity and in lipid metabolism [96]. Uremic patients often develop secondary hyperparathyroidism [117] and in uremic dogs excess PTH may suppress LPL activity [118], probably not directly by enzymatic inhibition [86], but rather mediated by impeding the release of insulin from pancreas [119]. In uremic rats with secondary hyperparathyroidism, both reduced LPL protein and activity was reversed after parathyroidectomy, indicating that excess PTH causes depressed LPL production [120].

In recent years it has become obvious that various degrees of chronic inflammation often accompany uremia [121-123], resulting in elevated levels of cytokines such as TNF- α [124-126] which has been proved both to impede LPL synthesis [127], and increase its release from the endothelium [39]. Recently, it has been shown that TNF- α is involved in down-regulation of adipose tissue LPL in rats on food deprivation. This appears to occur by a rapid shift of newly synthesised LPL molecules towards an inactive form, and thereby shut down extraction of lipoprotein TG by the adipose tissue [128]. This may be of importance in uremic patients, as they often are affected by declined appetite [129, 130], and reduced food intake might contribute to further decrease in LPL activity.

Uremic plasma appears to contain inhibitors for the LPL reaction [86, 93, 131, 132]. Serum from uremic patients inhibits LPL activity in adipose tissue from rats [93], and in human postheparin plasma in vitro [86, 131]. The inhibitory activity was found mainly in the lipoprotein-free fraction of plasma and the inhibitor was nondialyzable [93]. Later, an inhibitor was isolated from the non-lipoprotein fraction of plasma, identified as a pre- β -HDL particle containing apoA-I and 3% phospholipid [132]. HD with more permeable high flux polysulfone membrane dialyzers have demonstrated an increase in LPL activity, suggesting removal of an inhibitor by dialysis [133-135], or by adsorption on the membrane [132]. It is of interest to note that the increased biocompatibility of polysulfone membranes compared with cellulose membranes might produce

less LPL inhibitory cytokines during dialysis [133]. The increased concentration of apoC-III may also add to further inhibition of the LPL-mediated lipolysis [102]. In one study, the rise in apoC-III was ameliorated by the use of high flux dialysis and LPL activity was improved [134].

Heparin, which is used to prevent coagulation during HD, displaces LPL molecules from the endothelium into the circulation [12], and hence, LPL is released into the circulation during every dialysis session. It has been suggested that repeated heparinisation may induce release and subsequent degradation of LPL that exceeds the rate of enzyme synthesis and thereby causes a depletion of LPL stores [67, 82, 136, 137].

Heparin preparations

Unfractionated heparin

Heparin was introduced as an anticoagulant in the late 1920s and has been used in the clinic since then. It has proved to be effective and relatively safe both in the prophylaxis and treatment of thrombosis as well as during hemodialysis [138, 139]. Heparin is an anionic mucopolysaccharide extracted commercially from porcine intestinal mucosa or bovine lung. The molecular weight of unfractionated heparin (UFH) is varying in size from 5 000 to 30 000 Dalton, with a mean molecular weight of 15 000 Dalton [138, 140]. About one-third of the molecules contain a pentasaccharide sequence which binds to antithrombin, thereby enhancing its ability to inhibit factor Xa of the clotting cascade. UFH also enhances thrombin inhibition by serving as a catalytic template to which both antithrombin and thrombin bind simultaneously. In addition, UFH releases tissue factor pathway inhibitor, a potent inhibitor of the initial step of the extrinsic coagulation system [138-140]. UFH appears to be cleared and degraded primarily by the reticuloendothelial system, a smaller amount of undegraded UFH also appears in the urine [138, 141]. The half-life of UFH in plasma depends on the dose administered, but in clinically adequate doses the half-life is usually about 60 minutes in normal individuals, but can be prolonged in patients with chronic renal failure [140]. Since the anticoagulant response to UFH varies, it is essential to evaluate the anticoagulant effect by measuring the time taken for clot formation and traditionally the activated partial thromboplastin time (APTT) is used [138-140]. The anticoagulant effect of UFH can be neutralised rapidly by a slow intravenous infusion of protamine, a cationic protein derived from fish sperm that binds strongly to UFH and neutralises the antithrombin activity [138, 140]. Complications associated with UFH include bleeding,

thrombocytopenia, dyslipidemia and abnormalities of hepatic function tests. More rare are allergic reactions, osteoporosis, alopecia and hyperkalemia [138-141].

Low molecular weight heparin

For some years, various low molecular weight heparin (LMWH) preparations have been progressively developed for clinical use. LMWH consists of fragments of UFH produced by enzymatic or chemical depolymerization processes that yield saccharide chains with a mean molecular weight of 4 500 to 5 000 Dalton, with a distribution of 1 000 to 10 000 Dalton [138-140]. The fragments bind to antithrombin to enhance inhibition of factor Xa, but are not long enough to provide the second binding site needed for inhibition of thrombin activity [139, 140]. Since LMWH are prepared by different methods of depolymerization, they differ to some extent in pharmacokinetic properties and anticoagulant profile, and may not be clinically interchangeable [138-141]. LMWH preparations have about twice as long duration of anticoagulant effect as UFH, when administered intravenously [141]. As LMWH is cleared principally by the renal route, their biological half-life can be further prolonged in patients with chronic renal failure [139-141]. For monitoring the anticoagulant effect traditional clotting time assays cannot be used, instead the bioactivity of LMWH is measured in terms of plasma antifactor Xa activity using a chromogenic substrate [139, 140]. In contrast to UFH, the anticoagulant effect of LMWH can not be completely reversed by protamine, as the cationic protein neutralises the antifactor Xa activity incompletely, because protamine exhibits reduced binding to low molecular weight components [140, 141]. LMWH are less associated with bleeding complications and thrombocytopenia compared with UFH, and might carry a lower risk of osteoporosis. The effects on blood lipids are suggested to be more favourable because of a supposed lesser influence on the lipolytic enzymes [138-140].

Hemodialysis

Dialysis refers to the diffusion of small molecules down their concentration gradient across a semipermeable membrane. In hemodialysis, blood is withdrawn from the patient body and passed by a membrane that separates the blood from a dialysate solution on the other side. The dialysate solution contains electrolytes and glucose. Small molecules such as urea, potassium and phosphorus diffuse down their concentration gradients from blood into dialysate solution. Small

molecules such as calcium and bicarbonate move down their concentration gradients from the dialysate solution into the blood. The effect is to remove low-molecular-weight toxins from the blood while, at the same time, increasing the plasma concentration of molecules that may be deficient in the patient with renal failure [142].

At the beginning of the 1960s HD was introduced for long-term or maintenance treatment of patients with uremia as major technical difficulties were solved. A relatively reliable access to the peripheral circulation was evolved and the use of heparin minimised the risk of clotting in the extracorporeal devices. It soon became evident that chronic dialysis treatment was able to sustain life in uremic patients, and in a relatively good condition for an extended period of time. Over the years the dialysis treatment has expanded and has been provided to an increasingly aged and ill population [143].

HD is a process that consists of two components, diffusion and ultrafiltration. Diffusion refers to the movement of small molecules down their concentration gradients and ultrafiltration to the removal of water. During the process of HD, diffusion and ultrafiltration occur at the interface between the dialyzer membrane and the blood. The membranes are either cellulose-based or, more commonly today, polymer-based. Polymer membranes are more biocompatible and do not cause complement activation to the same extent. The high porosity of some of these membranes, termed high-flux, may augment the convective removal of uremic toxins through increased “solvent drag” [142, 143].

The need for vascular access in patients with renal failure can be either temporary or permanent. Most chronic HD-patients have an arteriovenous (AV) fistula or graft. The AV fistula is a direct surgical anastomosis between an artery and superficial vein that causes the vein to dilate and develop a thickened wall. A well-developed AV fistula is the safest and the longest-lasting permanent vascular access. An AV graft involves the surgical interposition of a synthetic blood vessel between an artery and a vein, and is placed below the skin such that it can be repeatedly cannulated. The major complications associated with AV grafts or fistulas are stenosis, thrombosis and infection. When HD is required in patients who do not have a functioning AV fistula or graft, temporary or semipermanent vascular access is achieved by the use of a central venous catheter with two large-bore lumens. Such catheters are generally placed into the superior

vena cava through the internal jugular veins or into the inferior vena cava through the femoral veins [142, 144].

During HD, anticoagulation is essential to prevent clotting of the dialyzer and extracorporeal circuit. Heparin has been in widespread use for decades and is usually given as an initial bolus followed by an infusion administered up to the last hour of the dialysis treatment, or on an intermittent basis, where one or more boluses are given [142]. The pharmacodynamics of UFH varies widely between patients [138], and this makes an individual dosing schedule necessary. For routine anticoagulation the usual recommendation is a loading dose of approximately 50 IU/kg body weight, followed by a continuous infusion of 800-1500 IU/hour [145], or a slightly lower loading dose, 25-30 IU/kg body weight, followed by an infusion of 1500-2000 IU/hour [139]. It is essential to evaluate the anticoagulant effect by measuring the time taken for clot formation. Traditionally APTT is used, and a prolongation to 150% of the pre-dialysis value is recommended [139, 145].

For some years, various LMWH preparations have increasingly been used during HD. In addition to the ease of administration [146] also a less pronounced increase in LPL activity has been reported [147], suggesting less influence on lipid metabolism compared with UFH [67]. There is no established consensus regarding administration of LMWH. In early studies a bolus dose followed by continuous infusion was used [148-150]. As LMWH preparations have longer duration of the anticoagulant effect [141], a single bolus injection pre-dialysis is now often advocated [146, 151-154], also in dialysis sessions of five hours duration [155]. It has been suggested that LMWH should be dosed to give an antifactor Xa activity greater than 0.4-0.5 IU/mL at the end of dialysis [139]. This is based both on visual inspection of the extracorporeal bloodline [146, 156] and on measurement of biochemical markers of coagulation [151]. To achieve this anticoagulation effect when using a single bolus pre-dialysis, a dose between 50-100 IU/kg body weight is usually suggested [146, 157]. For administration of various LMWH, the prescriptions advocated by the individual manufacturers are recommended [145], and usually, individual dosing schedule and evaluation of the anticoagulant effect are not considered necessary.

AIMS

The general aim of the present study was to explore the effects of unfractionated heparin (UFH) and a low molecular weight heparin (dalteparin) on plasma LPL activity and TG concentrations in HD-patients and in comparison with healthy individuals. A disturbed LPL system might contribute to an impaired lipoprotein metabolism, and hence, an aggravated cardiovascular condition.

Specific aims:

To explore whether a prolonged infusion of UFH depletes the pool of functional LPL in healthy individuals.

To analyse the pattern of LPL activity and TG concentrations in HD-patients during an ordinary dialysis session using UFH as anticoagulant, and in comparison to healthy controls receiving a corresponding UFH infusion.

To compare the effects of dalteparin on LPL activity and on TG concentrations to the effects of UFH in healthy controls and in HD-patients.

To compare two different anticoagulant regimes during dialysis with respect to LPL activity and TG concentrations.

METHODS

Subjects and study design

Study I was designed to reflect the clinical protocol used for continuous anticoagulation, e.g. during HD. Unfractionated heparin (UFH) (Heparin-Leo[®], Leo Pharma, Malmö, Sweden) was administered as an initial bolus followed by a continuous infusion. Ten elderly volunteers, four women and six men, were included in the study, constituting the control group. One (MS) had undergone percutaneous transluminal coronary angioplasty and used a calcium channel blocker. All of the others were healthy and were taking no medication. No one displayed any signs of infection or other complaints. The median age was 72 years and the median BMI was 24.5. The control subjects reported to the study after an overnight fast. A peripheral venous access was established in each forearm, one for the UFH infusion and one for blood sampling. The latter was filled with saline solution between the blood sampling occasions. A baseline blood sample for lipids, lipase activity and APTT was drawn, and then the UFH loading was started with a bolus of 50 IU/kg body weight, followed by a continuous infusion of 1000 IU/hour for a total of eight hours. Additional blood samples were drawn at 15, 30, 60, 120, 180, 240, and 480 minutes. As the experiments were designed to reflect a clinical situation, meals were given at regular intervals. About one hour after the infusion was started, the control subjects received a small breakfast, and then, three hours later, a lunch including about 25g of fat. In four of the control subjects, during a later occasion, a new UFH loading was performed in exactly the same manner as previously. This time they were also given a second UFH bolus of 25 IU/kg body weight at 240 minutes. After this, no more UFH was given.

The design of study III was based on the same protocol used in study I, and all control subjects except one woman (MS) participated. The same regime used in study I was applied, except from the heparin preparation, this time a LMWH, dalteparin (Fragmin[®], Pharmacia, Stockholm, Sweden) was used. A bolus of 40 IU dalteparin/kg body weight was given, followed by a continuous infusion of 1000 IU/hour. In eight of the control subjects, at a later occasion, new dalteparin loading was performed in the same manner as previously, but the control subjects were each given a bolus of UFH (25 IU/kg body weight) at 240 minutes. The dalteparin infusion was continued for a total of 360 minutes.

Study II and IV included nine HD-patients (three women and six men) matched for age and gender with the control group. The median age was 73 years and the median BMI was 24.6. The diagnoses were chronic glomerulonephritis (n=3), polycystic kidney disease (n=2), diabetes nephropathy (n=1, non-insulin dependent, only treated by diet recommendations), chronic pyelonephritis (n=1), nephrosclerosis (n=1), and end stage kidney disease (n=1, not biopsied). They had been on maintenance dialysis for 2-35 months and were treated with bicarbonate HD either two or three times weekly, depending on residual renal function. All dialyses were performed with hemophan dialyzers (GFS+16, GAMBRO, Lund, Sweden) and Biosol dialysis solution (Pharmalink, Stockholm, Sweden). An AV fistula/graft was used as dialysis access in four of the patients and a central dialysis catheter (CDC) in five. To avoid clotting between dialysis sessions, the CDC was filled with a solution containing 5000 IU heparin/mL. The patients were treated with antihypertensive drugs (ACE-inhibitors, beta-blockers, calcium channel inhibitors), diuretics, sodium bicarbonate and phosphate-binding drugs. One patient, having a rejecting renal transplant, was treated with low doses of corticosteroids and cyclosporine. No one was treated with lipid lowering drugs. The experiments were carried out after an overnight fast, and 48-96 hours had passed since the previous HD. Blood samples for lipids, lipase activity and APTT was drawn before start, and then regularly at 15, 30, 60, 120, 180, and 240 minutes. According to existing routines, the patients had a combined breakfast/lunch, containing 25g fat, about two hours after the dialysis was started. In study II, UFH was used as anticoagulant, starting with a loading dose of 50 IU/kg body weight, followed by a constant infusion of 1000 IU/hour for four hours. In study IV, dalteparin was given as anticoagulant, 40 IU/kg body weight as loading dose, followed by a continuous infusion of 1000 IU/hour.

In study V a new group of nine HD-patients was recruited, four women and five men, none had participated in our earlier studies. The median age was 72 years and median BMI was 26. The diagnoses were chronic glomerulonephritis (n=3), nephrosclerosis (n=2), polycystic kidney disease (n=1), multiple myeloma in remission (n=1), medullary sponge kidney (n=1) and end stage kidney disease (n=1, not biopsied). They were treated with antihypertensive drugs (ACE-inhibitors, beta-blockers, calcium channel inhibitors), diuretics, sodium bicarbonate and phosphate-binding drugs. All received erythropoietin. No one was treated with lipid-lowering

drugs. They had been on chronic HD for 4-40 months, and all had an AV fistula/graft as access. No one displayed any overt signs of infection or inflammation (C-reactive protein < 10 mg/L). The patients were treated with bicarbonate HD either two or three times weekly. Eight patients had polysulphone dialyzers (F8HPS, Fresenius, Bad-Homburg, Germany). One used a polyamide dialyzer (PF14s, Gambro, Lund, Sweden). All dialyses were performed with Biosol dialysis solution (Pharmalink, Stockholm, Sweden). The experiments were carried out after an overnight fast and 48-72 hours had passed since the previous dialysis. The patients were given breakfast about two hours after the start of dialysis and lunch was served when the dialysis was completed, i.e. after four hours. Two dialysis-regimes were compared. The first was with UFH as anticoagulant and the second was with dalteparin. UFH was administered as a loading dose of 50 IU/kg body weight, followed by a continuous infusion of 800-1500 IU/hour. Infusion was discontinued about 30 minutes prior to cessation of dialysis in order to prevent bleeding from the AV fistulas after dialysis. Dalteparin was administered according to the manufacturers' recommendation, i.e. a single bolus dose of 5000 IU to all patients at start of dialysis, not followed by an infusion. All dialyses were completed after four hours. Blood samples for lipids and lipase activity were drawn at start and at 15, 30, 60, 180, and 240 minutes. Further samples for lipids were drawn at 7, 8, 10, 24, and 48 hours. The anticoagulation effect was evaluated by APTT and antifactor Xa activity. At a later occasion, eight of the patients participated in two further dialyses each, using UFH and dalteparin respectively, which was administered as during the first session for the first three hours. Then, at 180 minutes, a bolus of UFH (25 IU/kg body weight) was given to release remaining LPL into the circulating blood. After that no more heparin was infused. Everything else concerning the dialysis-regime was unchanged between the dialyses. The medical treatments and diet recommendations were kept constant.

In all studies, the local ethical committee approved the protocol, and informed consent was obtained from all individuals before participation.

Laboratory analyses

Lipase analyses

Blood samples for measurement of LPL and HL activity and LPL protein mass were collected in heparinised tubes. They were immediately chilled in ice water and centrifuged within 15 minutes. The plasma was frozen at -20°C and then stored at -70°C until analysis [158]. For HL we used a

gum arabic-stabilized emulsion of triolein containing [³H]-oleic acid-labelled triolein incubated at 1 mol/L NaCl. Under these conditions, LPL is inactivated. LPL activity was measured with an emulsion containing, per milliliter, 10 mg egg-yolk phospholipids, 100 mg soybean TG, and a trace amount of [³H]-oleic acid-labelled triolein. This emulsion was prepared by Fresenius-Kabi (Uppsala, Sweden). For the LPL assay, HL activity was inhibited by preincubation of the samples with immunoglobulins from rabbit antiserum to human HL. The LPL assay medium contained a relatively high concentration of heparin, and possible differences in the heparin concentration or type in the sample would not affect the activity. All assays were performed in triplicate and the mean value was used. A standard sample of human post-heparin plasma was run on each assay day and the value was used to calibrate for between-assay variations. LPL protein mass was determined with an enzyme-linked immunosorbent assay, as previously described [44], using immunoaffinity-purified chicken antibodies raised against bovine LPL for capture and the monoclonal antibody 5D2, also raised against bovine LPL, for detection (A gift of Dr J. Brunzell, Division of Metabolism, Endocrinology and Nutrition, University of Washington School of Medicine, Seattle, WA).

Lipid analyses

Blood samples for lipid determination were drawn in tubes without anticoagulant. They were immediately chilled in ice water, centrifuged within 15 minutes, and then frozen as described above. Total cholesterol, HDL cholesterol, and TG were determined by means of routine methods on a multianalyzer (Vitros 950 IRC; Johnson & Johnson Clinical Diagnostics Inc, New York, NY, USA). LDL cholesterol levels were calculated with the Friedewald formula [159].

Considering in vitro lipolysis of TG, this was checked out for two of the controls. In a duplicate sample, LPL was inactivated by adding 1 mol/L NaCl and the sample was then heated for 10 minutes at 50° C before being frozen. This sample was then thawed together with the regular sample and TG analysed on the same occasion. The regular samples did not show significantly lower TG concentrations than the pre-treated ones, demonstrating that no substantial lipolysis took place during the regular handling of the samples.

Anticoagulation analyses

Blood for the anticoagulation tests was drawn in citrate-containing tubes and was analysed for APTT by means of the clinical routine method. Antifactor Xa activity was determined with a chromogenic substrate (Coacut; Chromogenix AB, Mölndal, Sweden).

Statistical methods

Data are expressed in terms of median and range, due to small study populations and data not normally distributed. For the same reasons non-parametric statistics were used throughout the studies. Paired analyses were performed for significant differences using the Wilcoxon signed rank test. Simple linear regression and the Spearman rank correlation test were used to evaluate relationships between variables. Two-tailed P values below 0.05 were considered to be statistically significant.

RESULTS

Baseline data

There were no significant differences in baseline levels of lipid parameters between HD-patients and controls. Patients on HD often have moderate hypertriglyceridemia in combination with low HDL cholesterol. There was a tendency in this direction in our study but this did not reach statistical significance, probably due to small sample size. The median LPL activity was significantly higher in HD-patients, which was due to the five patients with CDC. Their catheters were filled with a heparin solution to avoid clotting between dialysis sessions. During preparation of the CDC pre-dialysis there is a leakage of small amounts of heparin from the CDC into the circulation, which increases LPL activity. In between dialysis sessions the basal level of LPL is low and comparable to that in patients without CDC or that in the controls (Paper II). Controls had significantly lower baseline values for total cholesterol (5.4 vs. 5.8 mmol/L) and LDL cholesterol (3.4 vs. 3.8 mmol/L) at the time for the dalteparin infusion compared with values at the time for the UFH infusion. Baseline values for TG and HDL cholesterol did not differ before the two infusions (Paper III). In the HD-patients there were no differences in baseline values before the dialysis with UFH and the dialysis with dalteparin, and there was no significant difference between the ultrafiltration rates during the two dialyses (Paper IV and Paper V).

A prolonged infusion of UFH to healthy controls depletes tissue stores of LPL (Paper I).

The pre-heparin LPL activity was low and increased more than 100-fold on injection of UFH (Fig1). In some controls the highest value was at 15 minutes, whereas in others the LPL activity continued to rise to 30 minutes (corresponding median values were 121 mU/mL, range 58-148, and 138 mU/mL, range 60-160, respectively). The activity then decreased rapidly to 31 mU/mL (range 15-72) at 120 minutes. Hence, the activity dropped by almost 80% from 30 to 120 minutes. After this, the activity decreased further to 180 minutes, and remained almost unchanged from 180 to 240 and 480 minutes, median values of 22, 21, and 26 mU/mL, respectively. The main impression of the curve is that after the initial peak, the activity dropped to a plateau that corresponded to around 15% of the peak level. There was a significant positive correlation between the LPL values at 15, 30, and 60 minutes, and between the values at 180, 240

and 480 minutes. In contrast, there was no significant correlation between the LPL activities during the initial peak and those during the subsequent plateau.

HL activities were maximal at 15 minutes (median value 186 mU/mL, range 107-388) in most of the controls (Fig 1). The levels remained about the same during the first hour and then decreased slowly during the remaining infusion period. The median had decreased by about 25% after 120 minutes (143 mU/mL, range 90-356) and by about 55% at the end of the study period, 480 minutes (80 mU/mL, range 58-208). There was a positive correlation between all activities measured from 15 to 480 minutes ($p < 0.004$). Hence, controls with high peak activity tended to remain high throughout the study, and vice versa for those with low activities.

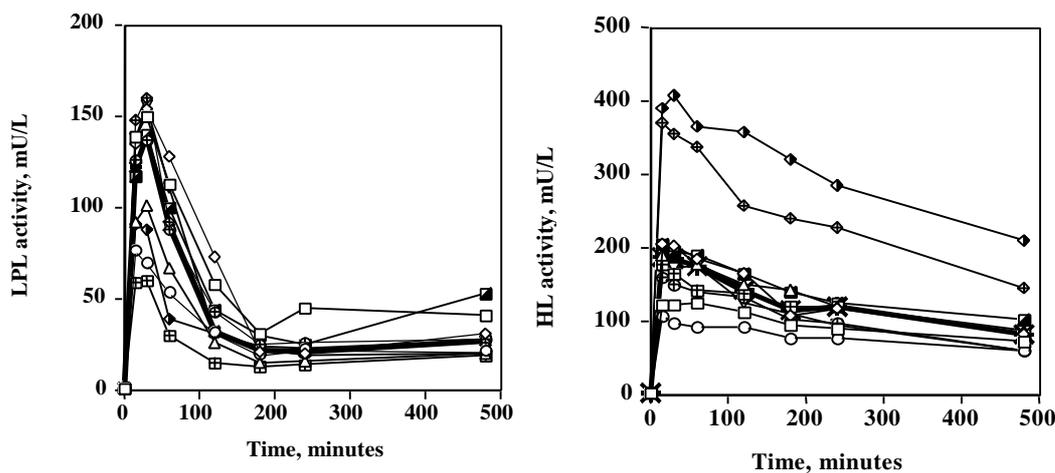


Fig 1. LPL activity and HL activity during infusion with UFH. Each curve represents data for one control subject. The bold line connects the median values.

Four controls were given a second bolus of UFH at 240 minutes, that is, after the LPL activity had subsided to the plateau level. The APTT returned to levels similar to those seen after the first bolus, indicating that the plasma UFH concentration was similar. The HL activity increased to about 75% of the first peak in the four subjects. The LPL activity also increased, but only to about 35% of the peak level. The UFH infusion was stopped when the second bolus was given and thereafter the APTT subsided towards pre-heparin values, and accordingly, the LPL activity decreased below the plateau value.

Lower LPL activity in HD-patients compared with healthy controls (Paper II).

When UFH was injected and the dialyses were started, LPL activity increased rapidly and peaked at 15 or 30 minutes (Fig 2). The activity then decreased rather sharply to 120 minutes and levelled off to a plateau from 180 to 240 minutes that was 23% of the peak value. During the peak phase the LPL activity was only about 50% of that in the controls. Median values for HD-patients versus controls were at 15 minutes 69 vs. 117 mU/mL ($p=0.008$), and at 30 minutes 74 vs. 137 mU/mL ($p=0.008$). During the plateau phase the activities tended to be lower in HD-patients than in the controls. Median values HD-patients versus controls were at 180 minutes 19 vs. 23 mU/mL ($p=0.214$), and at 240 minutes 17 vs. 22 mU/mL ($p=0.051$). The median area under the curve (AUC) for LPL activity was calculated. AUC for the HD-patients during the peak period (0-180 minutes) corresponded to 54% of that for the controls ($p=0.028$), and AUC during the plateau period (180-240 minutes) corresponded to 87% ($p=0.139$). In both HD-patients and controls the LPL activities for each individual correlated positively to each other during the peak (15-60 minutes, $p<0.005$) as well as during the plateau (180-240 minutes, $p<0.017$). In contrast, there was no correlation between the values during the peak and those during the plateau. There was no correlation between the heparin loading dose (IU/kg body weight) and the LPL activity during the peak or the plateau.

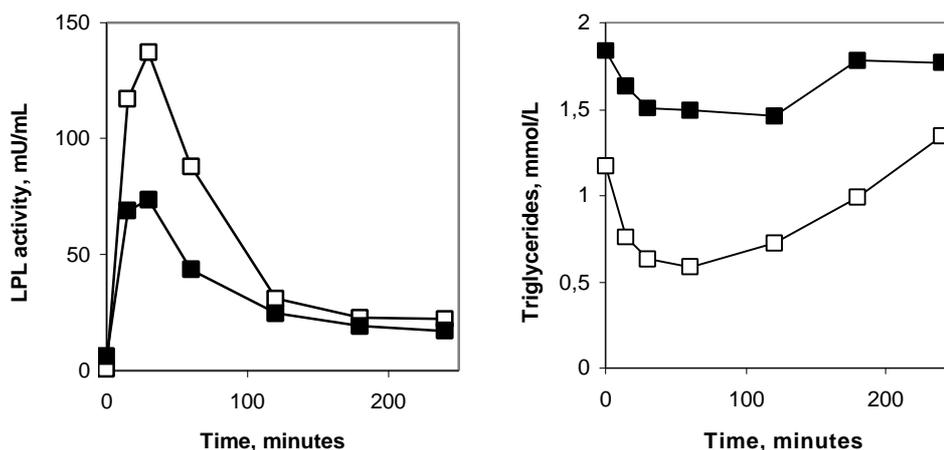


Fig 2. Median values of LPL activity and triglycerides during infusion with UFH to HD-patients (solid squares) and to controls (open squares).

In the basal blood sample HD-patients had a tendency towards higher TG levels, median value 1.84 mmol/L (range 0.96-3.49) compared with the controls, 1.17 mmol/L (range 0.64-2.46) ($p=0.139$) (Fig 2). TG decreased in both HD-patients and controls when the UFH infusions were started. In both groups the reduction was most pronounced at 60 minutes, but was more marked in the controls, 50% versus 24% for the patients. The TG then increased and returned to baseline values in both groups. In HD-patients there was no correlation between the levels of LPL activity and changes in TG, while in controls high enzyme activity correlated with lower TG values.

HDL-cholesterol increased in HD-patients from 1.09 mmol/L (range 0.67-2.06) at start to 1.19 mmol/L (range 0.67-2.31) at the end ($p=0.030$), while total cholesterol did not change. In controls there was a decrease in total cholesterol, at most 7% at 180 minutes ($p=0.007$), and an increase in HDL-cholesterol, at most 9% at 120 minutes ($p=0.038$).

More rapid catabolism of LPL after infusion of a LMWH than after UFH (Paper III).

Dalteparin was infused to healthy controls for 480 minutes, after an initial bolus. The LPL activity reached its highest values at 15 minutes (median 54 mU/mL, range 28-77), and then decreased rather sharply so that at 120 minutes the median was 14 mU/mL (range 10-20). After this, the activity decreased further to 180 minutes (11 mU/mL, range 8-14) and remained at a plateau to the end of the infusion, at 480 minutes (9 mU/mL, range 7-20). The shapes of the curves resembled that for the LPL activity during the earlier UFH infusions (Fig 3), but the activities were significantly lower throughout the dalteparin infusion ($p<0.012$). The AUC for LPL activity during the peak period (0-180 minutes) was only 30% of that for the UFH infusion, and AUC for the subsequent plateau (180-240 minutes) was only 40%.

To test whether the relatively low plasma LPL activities occurred because dalteparin released endothelial LPL less efficiently than UFH, a bolus of UFH was given at 240 minutes, i.e. after the LPL activity had levelled off to a plateau. The UFH bolus caused an equal increase in LPL activity compared with the increase during the earlier study with UFH. These data show that the UFH bolus brought out about the same amount of LPL, regardless of whether the controls had been infused with dalteparin or UFH, suggesting that dalteparin and UFH had reduced the peripheral stores of LPL to a similar extent during the preceding four hours of infusion. The LPL activities during the peak (15, 30, and 60 minutes) correlated with each other, as did the activities

during the plateau (180 and 240 minutes). In contrast there was no correlation between the activities during the peak and the plateau.

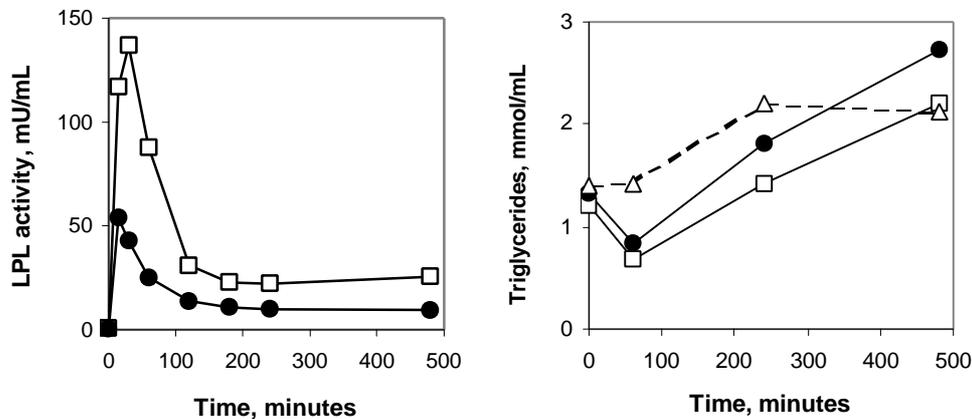


Fig 3. Median values of LPL activity and triglycerides in controls during infusion with UFH (open squares) and dalteparin (solid circles). Open triangles denote triglycerides during a control day with no infusion.

LPL mass in blood was measured in the experiment in which a bolus of UFH was given after 240 minutes infusion of dalteparin. The pattern followed closely that of LPL activity. The basal values represent an inactive form of LPL in plasma and UFH releases mainly or perhaps only the active form of the lipase [42]. To calculate the specific activity (LPL activity/LPL mass), the basal value was subtracted from the values obtained during the infusion. The specific activity was 0.37 mU/ng (range 0.27-0.45) for the initial peak after dalteparin (15 minutes) and 0.34 mU/ng (range 0.25-0.45) for the peak after the UFH bolus (255 minutes). The similarity of these figures ($p=0.116$) indicates that the material brought out by dalteparin and by UFH is the same form of LPL. This suggests that the differences between dalteparin and UFH were not because of a different ability to keep the enzyme in its active form but more likely due to a different influence on its turnover.

HL reached its highest value at 15 minutes (median 112 mU/mL, range 42-150), then decreased slowly during the remaining infusion period. The peak HL activity after dalteparin was about 60% of the peak activity during the earlier UFH infusion ($p=0.036$). After the UFH bolus at 240

minutes, HL activity increased (median 169 mU/mL, range 63-210) and the activity was significantly higher than the first peak ($p=0.018$).

TG decreased when dalteparin was administered but then increased to values greater than baseline. Fig 3 compares median TG levels during the dalteparin and UFH infusions and those during a control day with no infusion. On the control day, TG levels increased somewhat in most controls from the fasting value in the morning to the postprandial afternoon value. When the heparin preparations were infused, the TG concentration decreased rapidly, and fell significantly below baseline from 15 to 120 minutes with UFH and from 30 to 60 minutes with dalteparin. The TG concentration then increased again and was significantly greater than baseline at 480 minutes with UFH and at 240 and 480 minutes with dalteparin. At the last time, 480 minutes, the TG concentrations were significantly greater after infusion of dalteparin than on the control day ($p=0.028$), whereas after the UFH infusion the TG values were similar to those on the control day ($p=0.612$).

Only small, and statistically not significant, changes occurred in total cholesterol during the infusion of dalteparin, while HDL cholesterol decreased slightly at 480 minutes (19%, $p=0.038$).

Depletion of LPL stores in HD-patients during dialysis using a LMWH as anticoagulant (Paper IV).

The shape of the curve resembled that during the earlier dialysis with UFH, but the values were much lower during the dialysis with dalteparin ($p=0.008$) (Fig 4). The AUC for LPL activity during the peak period (0-180 minutes) was only 27% of that during the UFH infusion. For the plateau period (180-240 minutes) the AUC was 36% of the corresponding AUC during the dialysis with UFH.

Fig 4 compares the median values for LPL activities in all four studies, healthy controls given UFH or dalteparin, and HD-patients during dialysis with UFH or dalteparin. In both controls and HD-patients the values during dalteparin were lower than during UFH. The values in HD-patients were lower than in controls both with dalteparin and with UFH. Thus, the highest values were for controls given UFH and the lowest values were for HD-patients given dalteparin. The

differences were remarkably large. As these studies have been carried out on separate occasions over several years, the consistency of the values was checked.

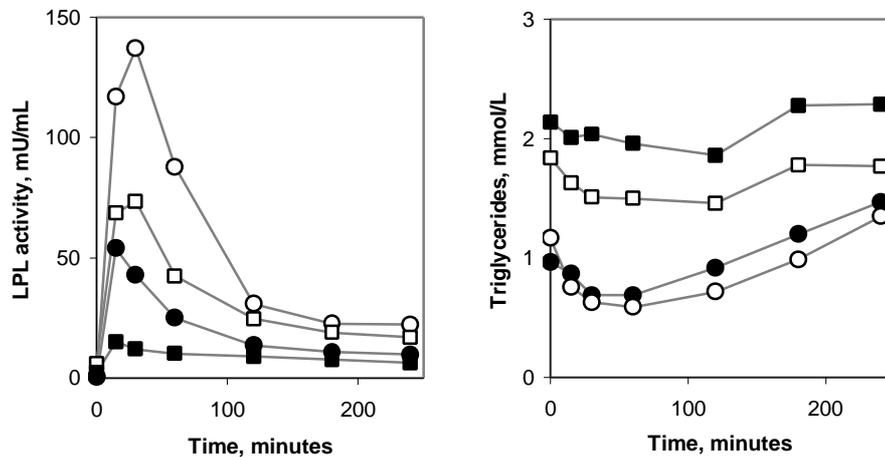


Fig 4. Median values of LPL activity and triglycerides. Solid squares denote HD-patients given dalteparin and open squares given UFH. Solid circles denote controls given dalteparin and open circles given UFH.

A major part of the saved duplicate samples was thawed and assayed for LPL activity and mass. There was good agreement between the activities from the earlier and the repeated assay for all four studies ($r=0.94$, $p=0.0001$). Hence, the large differences between the LPL activities registered for controls and HD-patients and for the two different heparin preparations were real. This conclusion was further supported by analysis of LPL mass in the samples. These values showed the same patterns and the same differences as the values for LPL activity.

During dialysis with dalteparin, TG increased continuously from 2.14 mmol/L (range 1.09-3.41) at start, to 2.59 mmol/L (range 1.49-5.04) at the end of dialysis at 240 minutes, representing a 21% increase ($p=0.008$). Compared to the values during the earlier dialysis with UFH, there was no difference at start, but from 30 minutes and through the remaining session TG values were significantly higher during the dialysis with dalteparin ($p<0.045$) (Fig 4).

Total cholesterol increased from 4.6 mmol/L (range 3.6-7.1) at start, to 6.1 mmol/L (range 3.8-8.4) at the end (32% increase, $p=0.021$), while HDL cholesterol did not change from baseline.

LDL cholesterol increased from 2.9 mmol/L (range 2.1-4.9) at start, to 3.75 mmol/L (range 2.1-5.6) at the end of dialysis (29% increase, $p=0.045$).

A single bolus of a LMWH pre-dialysis depletes LPL stores and retards TG clearing (Paper V).

This study compared two anticoagulation regimes based on present clinical practice, and the doses were adjusted to the respective manufacturers recommendation. UFH was administered as a primed infusion, whereas dalteparin was given only as a single bolus at start, not followed by an infusion. The effect of the respective heparin preparation on LPL activity and TG changes were evaluated and compared. TG values were also followed in several hours after the dialysis-session, because in our earlier studies (Paper II and IV) TG had increased to and above basal values when the dialyses were completed after four hours.

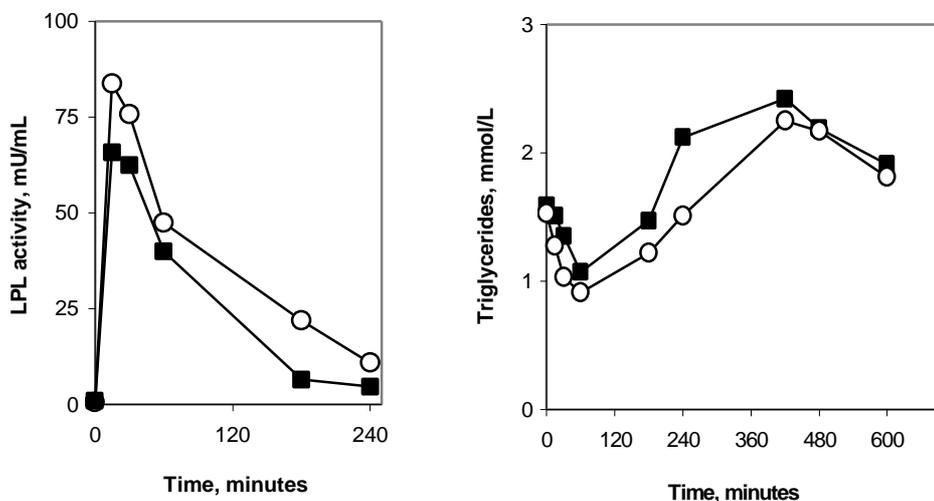


Fig 5. Median values of LPL activity and triglycerides during HD with dalteparin given as a bolus at start (solid squares) and with UFH given as a bolus followed by an infusion (open circles).

LPL activity prior to administration of UFH/dalteparin was low and did not differ between the two dialyses. When the heparin preparations were injected and the dialyses were started, LPL

activity increased and peaked at 15 or 30 minutes (Fig 5). During dialysis with UFH a median activity of 84 mU/mL (range 53-144) was reached at 15 minutes and 76 mU/mL (range 59-163) at 30 minutes. Corresponding values for the dialysis with dalteparin were 66 mU/mL (range 51-113) and 62 mU/mL (range 45-111), respectively. These values were not significantly different comparing UFH and dalteparin. After 30 minutes the activity decreased rather sharply. During the dialysis with UFH, the LPL activity was 22 mU/mL (range 9-43) at 180 minutes and 11 mU/mL (range 6-35) at 240 minutes. The rapid decrease during the last hour is probably explained by a decrease of the UFH concentration in blood, as the infusion, following clinical routines, was stopped after about 3.5 hours to prevent bleeding from the AV fistulas after dialysis. During the dialysis with dalteparin the LPL activity was 6 mU/mL (range 4-15) at 180 minutes and 5 mU/mL (range 1-10) at 240 minutes, significantly lower compared to the dialysis with UFH ($p=0.008$).

AUC for the peak LPL activity (0-60 minutes) during the dialysis with dalteparin corresponded to 80% of AUC during the dialysis with UFH ($p=0.066$). During the plateau (180-240 minutes), AUC for the dialysis with dalteparin was only 30% of that for the dialysis with UFH ($p=0.008$). As in the earlier studies, the peak LPL activities (15, 30, and 60 minutes) for each individual corresponded positively to each other, both during the dialysis with UFH ($p<0.006$) and with dalteparin ($p<0.017$). The plateau activities (180 and 240 minutes) correlated positively to each other during the dialysis with dalteparin ($p=0.002$) and tended to correlate during the dialysis with UFH ($p=0.050$). There was no association between the activities during the peak and the plateau in any of the dialyses.

To explore how much lipase activity remained at the endothelium, a bolus of UFH was given at 180 minutes, i.e. one hour before the dialyses were completed. In both dialyses, the bolus caused an increase of the LPL activity but the second peak was much lower than the initial peak ($p<0.018$, Fig 6). The peak values were similar for the dialyses with UFH and with LMWH, but the increase in activity from 180 minutes to the peak value was significantly higher in the dialysis with dalteparin, because the activity before the bolus was lower than in the dialyses with UFH.

HL activity prior to administration of the heparin preparations was low but increased rapidly when the dialyses were started and reached a peak at 15 or 30 minutes. During dialysis with UFH, the median HL activity was 189 mU/mL (range 83-354) at 15 minutes and 214 mU/mL

(range 79-340) at 30 minutes. The corresponding values for the dialysis with dalteparin were 173 mU/mL (range 57-255) and 152 mU/mL (range 49-218), respectively. During dialysis with UFH the activity remained high until 180 minutes, 167 mU/mL (range 42-222) but then decreased to 92 mU/mL (range 27-173) at 240 minutes. This decrease is probably explained by the decrease of the concentration of UFH in blood, as the infusion was stopped after about 3.5 hours to prevent bleeding from the AV fistulas after dialysis. During the dialysis with dalteparin, HL activity decreased rather sharply already after 30 minutes and reached 21 mU/mL (range 5-59) at 180 minutes and 11 mU/mL (range 1-36) at 240 minutes.

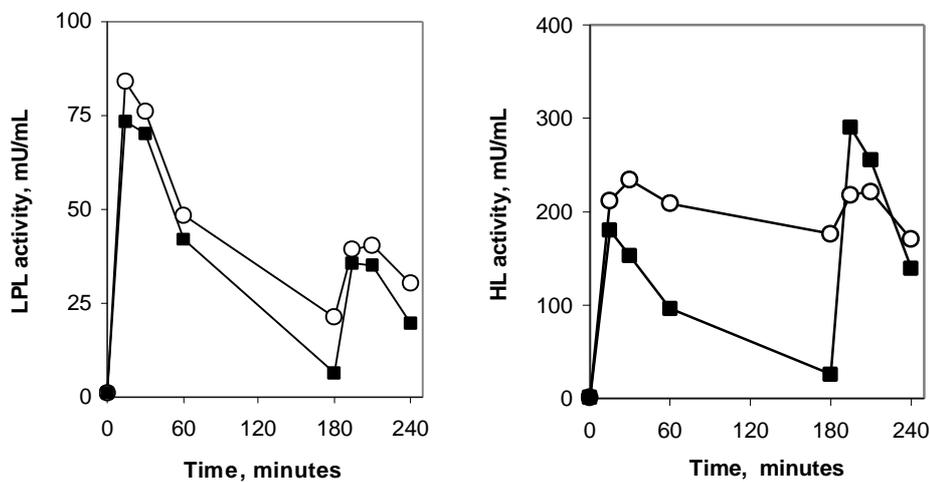


Fig 6. Median values of LPL activity and HL activity in HD-patients during dialysis with UFH (open circles) and dalteparin (solid squares), and the response to a bolus of UFH given after three hours of dialysis.

The HL activity was significantly lower during the dialysis with dalteparin compared to the dialysis with UFH ($p < 0.012$), except at 15 minutes. The median AUC during 0-240 minutes for the dialysis with dalteparin corresponded to 35% of that for the dialysis with UFH. After the UFH bolus at 180 minutes, the HL activity increased to a second peak in both dialyses (Fig 6). The peak value after the bolus was significantly higher than the initial peak during the dialysis with dalteparin ($p = 0.012$), but not during the dialysis with UFH ($p = 0.161$).

The pre-dialysis TG values did not differ between the two dialyses, the median values before the dialysis with UFH was 1.53 mmol/L (range 0.81-2.24) and before dalteparin 1.59 mmol/L (range 1.00-2.65). TG decreased during the first hour in both dialyses, then began to increase and reached values higher than baseline (Fig 5). The lowest values were at 60 minutes, when the reduction was 40% during the dialyses with UFH and 30% during the dialysis with dalteparin. Then, TG values increased and were significantly higher during the dialysis with dalteparin at 180 and 240 minutes compared to the dialysis with UFH ($p < 0.025$). The maximal TG values were reached at 7 hours, 2.25 mmol/L (range 1.47-4.79) for the dialysis with UFH and 2.42 mmol/L (range 1.35-4.37) for the dialysis with dalteparin. At 10 hours TG values had decreased to 1.81 mmol/L (range 1.34-3.23) for the dialysis with UFH and to 1.91 mmol/L (range 1.15-3.18) for the dialysis with dalteparin. At 24 hours TG was 1.56 mmol/L (range 1.10-2.93), and at 48 hours 1.40 mmol/L (range 0.89-2.62) after dialysis with UFH. Corresponding values after dialysis with dalteparin were 1.83 mmol/L (range 1.07-2.62) and 1.58 mmol/L (range 1.09-2.56), respectively. The median AUC for TG was significantly lower during the dialysis with UFH from start and until 10 hours, representing about 80% of that for the dialysis with dalteparin ($p = 0.038$).

The baseline HDL cholesterol levels did not differ between the two dialyses, but increased from 1.25 mmol/L (range 0.64-2.13) at start to 1.42 mmol/L (range 0.80-2.30) at 24 hours when UFH was used during dialysis. Corresponding values for the dialysis with dalteparin were 1.20 mmol/L (range 0.76-2.08) and 1.37 mmol/L (range 0.79-2.10), respectively. The increase was significantly higher during the dialysis with UFH compared to the dialysis with dalteparin ($p = 0.033$).

DISCUSSION

Anticoagulation

It is not obvious how one should compare doses of UFH and LMWH, since they differ both in their pharmacokinetic properties and in their mechanism of action [141]. The doses given during HD are chosen to give sufficient anticoagulation for the duration of the dialysis. The anticoagulation properties of different heparin preparations have no bearing on the interaction with LPL, which mainly depends on the degree of sulphation [35, 160, 161]. LMWH preparations differ in composition [138], and the mass needed to give a certain number of anti-Xa units varies. Approximately 1.5 to 2 times the mass is needed for a LMWH than for UFH. For a given mass there are more molecules in a LMWH preparation and clinically equivalent doses may mean 3 or 4 times as many molecules of LMWH. Furthermore, all heparin preparations are polydisperse, both in size and sulphation, and different molecular species turn over at different rates, so certain species may accumulate [162]. Our comparisons here are based on clinically relevant doses during HD, as recommended from manufacturers and clinical guidelines, not on a molecule-for-molecule basis.

When UFH was infused the highest APTT values were reached at 15 minutes and thereafter the values decreased slowly. The concentrations were well within the range needed for effective anticoagulation throughout the study period, and no statistically significant differences were found between HD-patients and controls. During the dalteparin infusions, the antifactor Xa activity was within the range needed for effective anticoagulation, as recommended by the manufacturer, both in controls and HD-patients throughout the study period (Paper I-IV).

When dalteparin was given as a single bolus at start of dialysis, the antifactor Xa activity was well above the recommended values during the initial part of the session. At the end of dialysis however, the antifactor Xa activity had decreased to values below recommended in five of the patients. This indicates that it is important to check that sufficient anticoagulation is maintained also when using LMWH as anticoagulant during dialysis, especially when administered as a single bolus. The APTT values during the dialysis with UFH were within recommendations throughout the sessions (Paper V).

Lipoprotein lipase and triglycerides

This study shows that during continuous UFH administration, LPL activity in plasma first rises to an initial peak, then decreases over the next two hours and levels off towards a stable, but much lower plateau of activity. The initial rise is well known [11, 12]. The new observation here concerns the decrease and the plateau. Our interpretation is that UFH releases the functional pool of LPL that is present at or near the endothelium. This release is reflected by the initial peak but the lipase is rather rapidly cleared by the liver [45-47]. After less than one hour, the activity decreases when the stores of lipase have been exhausted. Then the activity levels off to a plateau, which presumably represents a steady state where the delivery of newly synthesised LPL molecules balances the continued uptake and degradation in the liver.

When UFH was infused to healthy controls, LPL peaked within 30 minutes, then dropped by almost 80% from 30 to 120 minutes, and levelled off to a plateau that corresponded to 15% of the peak level. Earlier studies have shown that plasma LPL does not reflect a static equilibrium with tissue binding sites but rather a continuous flux of the enzyme through plasma to the liver [43, 60, 163, 164]. Release of the lipase into the circulating blood by UFH accelerates this transport [47, 64]. In the present study a bolus of UFH was given after four hours of infusion, i.e. after the activity had subsided to the plateau level. LPL activity increased to only 35% of the initial peak, demonstrating that there had been a large loss of LPL from the system. This agrees with studies in both animals [63, 64] and human subjects [66, 67, 136, 137]. The present study also shows that this loss of lipase occurs rapidly and is extensive, as reflected by the drop of almost 80% in plasma LPL activity during continued UFH infusion.

When LPL activity was followed in HD-patients during an ordinary dialysis session using UFH as anticoagulant, the time course of the activity was found to resemble that for the controls. There was an initial peak followed by a decrease to a much lower plateau, which exhibited a distribution that has not been described in HD-patients before. Compared with controls, the initial peak was reduced by 50% while the plateau activity was almost comparable. Our interpretation was that the functional pool of LPL activity, represented by the initial peak, was impaired in HD-patients, while the production of lipase molecules, reflected by the plateau, was only marginally reduced.

TG decreases when UFH is injected, this illustrates the well-known clearing reaction caused by LPL [13]. Before UFH is administered the lipase is attached to endothelial binding sites, whereas most of the lipoproteins are in the circulation. Hence, most of the substrate is not in contact with the enzyme. After UFH has released the lipase, all lipoproteins are exposed to lipase action and the reaction therefore accelerates. During the peak of LPL activity, TG decreased in both HD-patients and controls, but less in the patients. This can be ascribed partly to the lower lipase activity, but the activity was still high enough for a larger decrease in TG to have been expected than was observed. Four of the controls had LPL activities at 30 minutes that overlapped with five of the patients within the range of 65-101 mU/mL. In these four controls TG decreased between 40 to 45 %, while in the five patients the reduction was only between 13 to 23%. This supports the view that the lower lipase activity cannot fully explain the lesser decrease in TG. There are probably other factors involved in HD-patients, like inhibitors for the lipase reaction [93, 132] and altered substrate properties, which render their lipoproteins less favourable as substrates for LPL [102].

During the plateau phase with low lipase activity, TG increased towards baseline values, both in controls and HD-patients. The plateau level of LPL activity was only marginally reduced in the patients, median value was about 80% compared to that of controls. This indicates that there was only a relatively small difference in lipase production in the patients. A decrease is expected since it has been reported that adipose tissue LPL activities are reduced in HD-patients [94] and reduced LPL mRNA levels have been demonstrated in uremic rats [115]. This suggests that the decrease in LPL activity is mainly due to an impaired pool of functional LPL at the endothelium and to a lesser extent to impaired production of lipase molecules. Thus, some additional factors must be in play to cause the more extensive reduction in endothelial LPL, as reflected by the 50% reduction of peak-level LPL activity in HD-patients. One explanation might be an alteration at the endothelial surface resulting in reduced ability to retain the LPL molecules. Another possibility could be that the repeated infusions of UFH during the dialysis sessions two to three times a week exceed the individual's capacity to fully replenish the lipase stores in time for next dialysis. Anyhow, it is clear that during and following each dialysis session there is a period when LPL activity becomes depleted to a level that is limiting for normal lipoprotein metabolism.

During infusion with dalteparin, the same pattern of plasma LPL activity was observed, although remarkably reduced. In controls the peak was only about 30% and the subsequent plateau 40% compared with the activities during the infusion with UFH. The activities were further reduced in HD-patients, the peak corresponded to only 27% and the plateau 35% of the activities during the dialysis with UFH. Earlier studies have found that the peak of LPL in blood is lower after injection of LMWH preparations than after injection of UFH [65, 69, 165-167]. The lower LPL activity has been attributed to reduced ability to release the enzyme from its binding sites [147]. Animal experiments and direct studies on the interaction between heparin and LPL do not support this [19, 25, 68, 69, 71], but indicate that the difference is due to a more rapid extraction of LPL by the liver [68, 72]. To test this assumption, a bolus of UFH was given to the controls after four hours of infusion, a procedure comparable to the earlier study when UFH was infused. This brought out relatively small and similar amounts of LPL, regardless of whether dalteparin or UFH had been infused. Hence, the LMWH preparation had not left a larger amount of LPL in the tissues. Our interpretation was that the lower LPL activity observed throughout the dalteparin infusion resulted from similar release of LPL from peripheral tissues but more rapid hepatic clearance of the circulating LPL.

TG decreased in controls immediately after dalteparin was administered, but the reduction was somewhat less than after UFH. This is to be expected as the circulating LPL activity was lower after dalteparin. After one hour, plasma TG started to increase and in some controls the TG concentration was more than two times higher than the baseline value. There was no significant decrease of plasma TG in HD-patients and the subsequent rise was marked and more pronounced than during the dialysis with UFH. This can probably not be explained by hemoconcentration, as the ultrafiltration rate did not differ between the two dialyses. Thus, the increase in TG indicates that dalteparin caused a profound depletion of functional LPL even though the plasma levels of LPL activity were relatively low throughout the dialysis session. This is in accordance with results from animal experiments when injection of decasaccharides to rats resulted in delayed clearance of chylomicron TG and a marked decrease in functional LPL [68].

The peak level of LPL in plasma after injection of dalteparin was less than half of that after UFH, both in healthy controls and in HD-patients, and this is probably due mainly to a difference in how much the respective heparin preparation retards the uptake of LPL by the liver. The peak level was lower in HD-patients than in controls both after dalteparin and after UFH, and this is

probably a consequence of the kidney disease but the detailed mechanism is not known. These two effects compound to an almost ten-fold difference in peak LPL activity comparing the HD-patients given dalteparin to the healthy controls given UFH. This indicates that both heparin preparations reduce endothelial LPL to a similar extent, but that dalteparin less efficiently retards the hepatic uptake of the enzyme. As a consequence to this, TG tends to reach higher levels during dialysis with dalteparin.

Because of the convenience, various LMWH preparations are increasingly administered as a single bolus at start of dialysis, not followed by an infusion. When this was tested in comparison to UFH given as an ordinary bolus and continuous infusion, LPL activity was significantly lower during the dialysis with dalteparin. To explore the remaining activity at the endothelium a bolus of UFH was given after three hours of dialysis. The bolus brought out a somewhat larger increase in LPL activity during the dialysis with dalteparin indicating that slightly more LPL remained at the endothelium. On the other hand, the accumulated LPL activity after the bolus did not differ between the dialyses. This indicates that the liver had cleared the same amount of the enzyme during the preceding three hours of dialysis with both heparin preparations. The initial decrease in TG was less marked, and the subsequent increase more pronounced with dalteparin than with UFH. This indicates that also a single bolus of dalteparin pre-dialysis interferes with the LPL system as much or more than an infusion of UFH does, and temporarily impairs lipolysis of TG-rich lipoproteins.

Hepatic lipase

When UFH was infused to controls the time course of HL activity differed markedly from the peak - rapid decrease - plateau pattern seen for LPL. There was an initial peak and then a slow gradual decrease. This is in accordance with the hypothesis that UFH causes a redistribution of HL but no major loss from the system [64]. Less is known about HL turnover than about LPL turnover. In contrast to LPL, HL is mainly present in the liver, and this suggests that the binding sites must be different. When HL is injected, most, or all, binds in the liver and can be released back into plasma by UFH [168, 169]. Hence, there must be binding sites for HL in the liver separate from the binding sites that mediate binding, internalisation and degradation of LPL. This is expected, because liver sinusoids are the site of action for HL, and HL is turned over relatively

slowly [170]. The overall activity was lower during infusion with dalteparin than during infusion with UFH. A bolus of UFH after four hours caused an increase in HL activity during both infusions. This second peak was higher than the initial one during the infusion with dalteparin, whereas the second peak during the infusion with UFH was lower or comparable to the initial one. When dalteparin was given as a single bolus to HD-patients pre-dialysis, the initial peak of HL activity was similar to the peak when UFH was infused. After the peak, the activity remained essentially unchanged during the dialysis with UFH but decreased with time during the dialysis with dalteparin. This was probably a consequence of the decreasing concentration of dalteparin rather than due to any large difference in the ability of dalteparin to release HL compared to UFH. This conclusion is in concert with a study in rats, where it was found that a LMWH preparation released HL as efficiently as UFH did [64]. In further support of this conclusion, the decrease in HL activity in this study correlated to the decrease in antifactor Xa activity during the later part of the dialysis. Thus, the different patterns of HL activity in blood probably reflect the fact that dalteparin was given as a bolus and its concentration decreased with time whereas UFH was infused. When a bolus of UFH was given after three hours of dialysis, the HL activity increased to a second peak. The peak-value after the bolus was higher than the initial peak during the dialysis with dalteparin, but not during the dialysis with UFH, and hence, the total amount of accessible HL in the system was essentially unchanged. It appears that the level of the heparin preparation in blood determinates the distribution of HL between binding sites in the liver and the circulating blood. In contrast to effects of the heparins on LPL, the heparins did not seem to have any major consequences for the turnover of HL. It is not known what effects, if any, the redistribution of HL from liver to blood has for lipoprotein metabolism.

Anticoagulation during hemodialysis

Various LMWH are increasingly used during HD, and is suggested to release endothelial-bound LPL less efficiently and thereby cause less derangement of lipid metabolism compared to UFH. In our studies the increase of plasma TG at later times tended to be more pronounced during and after dialysis with dalteparin. This indicates that the depletion of functional LPL was as large as, or larger, with dalteparin than with UFH. This is in line with earlier observations [171] and animal experiments [61, 68]. A number of studies in HD-patients have reported that during chronic administration of heparin, e.g. in conjunction with dialyses, the use of a LMWH results in

a more favourable plasma lipid profile compared to UFH [150, 154, 172-176]. These data refer to the fasting lipid levels a few days after the administration of the heparin preparations. The interpretation in several of these studies is that LMWH exerts a lesser influence on the LPL system by preserving lipase molecules at the endothelial surface [172, 173, 176], but conflicting results exist [149, 153, 177-179]. In contrast to most other studies we analysed TG variations during but also after a dialysis session. Our data indicate that TG clearance was more, not less, disturbed during dialysis with dalteparin in comparison to UFH. This implies that during some hours at the end of and following dialysis there is an increased number of partially metabolised chylomicron and/or VLDL remnants in the blood, although the nature and composition of the lipoproteins was not analysed in our studies. It is of interest to note that a defect in the hepatic clearance of postprandial lipoproteins has been reported in HD-patients, and on this basis it has been suggested that elevated concentrations of remnant lipoproteins may be an important pathogenic factor in the accelerated atherosclerosis seen in HD-patients [104]. These data, and our current observations, raise the question if accumulation of atherogenic remnant particles during dialysis with heparin may accelerate atherosclerosis. Our study demonstrates that a single bolus of dalteparin pre-dialysis interferes with the LPL system as much or more than an infusion of UFH does. Both heparin preparations have the same ability to release endothelial LPL, but dalteparin is less effective in preventing uptake and degradation of LPL by the liver. As a result, the depletion of LPL stores and the temporarily impaired lipolysis of TG-rich lipoproteins was as large or even larger using the anticoagulation protocol with a single bolus of dalteparin compared to the traditional protocol with infusion of UFH.

CONCLUSIONS

During continuous infusion of UFH to healthy controls, LPL activity in plasma first rises to a peak, then decreases over the next two hours and levels off towards a stable, but much lower plateau. UFH depletes the functional pool of LPL available at the endothelium and the loss of lipase occurs rapidly and is extensive. After less than one hour, the stores of LPL have been exhausted, and recruitment of lipase into plasma depends on a slow but stable delivery of newly synthesised molecules.

When UFH is used as anticoagulant during dialysis, there is an initial peak of LPL activity as well as a reduction in TG during the first hour. Thereafter LPL decreases towards a plateau, while TG increases towards baseline. The peak of LPL activity is only half of that in healthy controls, while the plateau is comparable. The interpretation is that the functional pool of LPL activity, represented by the initial peak, is impaired in HD-patients, while the production of lipase molecules, reflected by the plateau, is only marginally reduced. This indicates that the heparinisation during an ordinary dialysis session leads to depletion of LPL stores, such that the lipase activity becomes critically low in relation to TG transport rate.

During continuous infusion of dalteparin to healthy controls, both the peak and the plateau activity of LPL is reduced to almost one-third of the activities during a corresponding infusion of UFH. These results are in concert with animal experiments that have indicated that both heparin preparations have the same ability to release endothelial LPL, but dalteparin is less effective in preventing rapid uptake and degradation of LPL by the liver. As a consequence of this more rapid loss of LPL from the system, TG tends to reach higher levels during the infusion with dalteparin. This indicates that dalteparin depletes LPL stores at least as efficiently as UFH does and temporarily retards the metabolism of TG-rich lipoproteins.

When dalteparin is used as anticoagulant during dialysis, the LPL activity is reduced to almost one-third of the activity during dialysis with UFH, both during the peak and the plateau. There is no initial decrease in TG and the subsequent increase is marked and more pronounced than during the dialysis with UFH. This suggests that dalteparin causes a profound depletion of

functional LPL, even though the plasma levels of LPL activity are relatively low throughout the dialysis session. This indicates that dalteparin, used for anticoagulation during dialysis, disturb the LPL system as much or more than UFH does.

When a single bolus of dalteparin is used as anticoagulant pre-dialysis in comparison to an infusion of UFH, the LPL activity is lower but similar amounts of the enzyme seem to be cleared by the liver, and in addition, TG tends to increase more. This indicates that also a single bolus of dalteparin pre-dialysis interferes with the LPL system as much as an infusion of UFH does, and temporarily impairs lipolysis of TG-rich lipoproteins.

The main conclusion is that the functional pool of LPL available for lipoprotein metabolism becomes reduced during an infusion of heparin. Immediately after heparin, LPL activity is high and catabolism of TG-rich lipoproteins is accelerated. Then follows a period when the stores of LPL are depleted and lipoprotein metabolism is retarded. This depletion is at least as marked after dalteparin as after UFH. The results indicate that dalteparin, used for anticoagulation during dialysis, interfere with the LPL system as much or more than UFH, and temporarily impair lipolysis of TG-rich lipoproteins. This interference might, in consequence, contribute to an aggravated cardiovascular condition in HD-patients.

SAMMANFATTNING PÅ SVENSKA

Mortaliteten i kardiovaskulära sjukdomar är 10 till 20 gånger högre hos patienter i kronisk hemodialys (HD) jämfört med befolkningen i övrigt. En viktig riskfaktor är den s.k. renala dyslipidemin, d.v.s. de lipidförändringar som uppkommer vid njurinsufficiens. Den karakteriseras av en försämrad nedbrytning av triglycerid (TG)-rika lipoproteiner med ansamling av atherogena s.k. rest partiklar. En bidragande orsak kan utgöras av en rubbning i lipoprotein lipas (LPL) systemet, ett viktigt enzym i nedbrytningen av TG-rika lipoproteiner. Det funktionella lagret av LPL finns lokaliserat vid kärlväggen och frisätts av heparin ut i det cirkulerande blodet och tas upp i levern där det bryts ned. Ofraktionerat heparin (UFH) används ofta vid dialys för att förhindra koagulering i dialysator och slangpaket, men ersätts allt mera av olika lågmolekylära hepariner (LMWH). LPL aktiviteten i plasma är vanligen lägre efter en injektion av LMWH och sägs därför frisätta mindre LPL ut i blodbanan och därmed orsaka mindre störning i lipoprotein metabolismen än UFH. Djurstudier har emellertid visat att LMWH frisätter LPL lika effektivt som UFH men förhindrar ej upptaget i levern lika effektivt.

Syftet med denna studie var att undersöka effekten av UFH och ett LMWH preparat (dalteparin) på LPL aktiviteten och TG koncentrationen hos HD-patienter i jämförelse med ålders- och könsmatchade friska kontroller. En påverkan på LPL systemet skulle kunna bidra till en försämrad lipoprotein metabolism, och därmed ett förvärrat kardiovaskulärt tillstånd.

En åtta timmars infusion med UFH till kontroller gav upphov till en initial topp av LPL aktivitet inom 30 minuter. Aktiviteten sjönk sedan med nästan 80% under nästföljande två timmar och planade ut mot en plåtå som motsvarade 15% av toppvärdet. När UFH infunderades till HD-patienterna så liknade kurvan för LPL aktiviteten den för kontrollerna, men aktiviteten under toppen var reducerad med 50%, medan aktiviteten under plåtån var jämförbar. Tolkningen var att det funktionella lagret, representerat av den initiala toppen, var reducerad hos HD-patienterna, medan produktionen av LPL molekyler, representerat av plåtån, endast var marginellt reducerad. Under toppen av LPL aktiviteten minskade TG i båda grupperna, men mindre hos HD-patienterna, vilket var förväntat eftersom LPL aktiviteten i blodet var lägre. Under plåtå fasen med låg LPL aktivitet ökade TG upp till och hos flertalet även över utgångsvärdet.

När dalteparin infunderades observerades samma förlopp hos LPL aktiviteten, dock anmärkningsvärt reducerad. Hos kontrollerna var toppen bara 30% och den efterföljande platån 40% i jämförelse med aktiviteterna under infusionen med UFH. En bolus med UFH gavs när LPL aktiviteten hade planat ut till en platå vilket medförde en likvärdig ökning av aktiviteten oberoende om dalteparin eller UFH hade infunderats under de föregående timmarna. Slutsatsen blev att båda heparin preparaten hade reducerat endotelbundet LPL i samma utsträckning men att dalteparin förhindrade upptaget i levern mindre effektivt. Som en konsekvens av detta tenderade TG att nå högre nivåer under infusionen med dalteparin. LPL aktiviteten var ytterligare reducerad hos HD-patienterna under infusionen med dalteparin, toppen var bara 27% och platån 35% i jämförelse med när UFH infunderades. TG sjönk inte alls utan ökade istället under hela infusionsperioden, vilket tyder på att det funktionella lagret av LPL till stor del hade förbrukats.

I en annan studie på HD-patienter jämfördes två regimer med avseende på antikoagulation under dialys baserad på nuvarande klinisk praxis och doserna var avpassade enligt respektive tillverkarens rekommendation. UFH gavs på vanligt sätt som infusion medan dalteparin endast gavs som en bolus vid start. Fynden var i linje med resultaten i de mera experimentella studierna och indikerar att LMWH också i kliniken påverkar LPL systemet negativt i minst samma utsträckning som UFH, och temporärt hindrar lipolys av TG. Som en konsekvens kan detta bidra till ett förvärrat kardiovaskulärt tillstånd hos HD-patienterna.

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