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Lipoprotein Lipase Inhibitor Removal During Ultrafiltration of Renal Failure Patients

Alisa Minear

Abstract

LIPOPROTEIN LIPASE INHIBITOR REMOVAL
DURING ULTRAFILTRATION OF RENAL FAILURE PATIENTS

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Plasma samples were obtained from seven hypertriglyceridemic (> 150 mg/dl) chronic renal failure patients before and after regular hemodialysis. The effect of the plasma on the lipolytic activity of bovine milk lipoprotein lipase was investigated. The lipoprotein lipase activity, as measured by release of C^{14}-labeled free fatty acid from triglyceride, was decreased 8-67% when incubated with uremic plasma as compared to the control plasma (44 mg/dl triglyceride). The inhibition noted prior to hemodialysis did not appear to be alleviated by the dialysis but subsequent treatment with an isolated ultrafiltration technique did appear to remove an inhibitory substance as evidenced by a 22-66% inhibition of lipolytic activity by the ultrafiltrate. It is proposed that the triglyceride-lowering effect observed by others subsequent to hemofiltration treatments is a result of the removal of one or more inhibitors of lipoprotein lipase from the plasma of hypertriglyceridemic renal failure patients.

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LIPOPROTEIN LIPASE INHIBITOR REMOVAL DURING ULTRAFILTRATION OF RENAL FAILURE PATIENTS

by

Alisa Minear

A Thesis in Partial Fulfillment
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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

James W. Blankenship, Chairman
James W. Blankenship, Professor of Nutrition

Stewart W. Shankel, M.D., Chief of Renal Unit, Veterans Administration Hospital

Kathleen K. Zolber, Professor of Food Administration

Irma B. Vyhmeister, Professor of Nutrition

Winston J. Craig, Assistant Professor of Nutrition

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TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF TABLES

INTRODUCTION

The advent of hemodialysis in 1960 and subsequent technological improvements have enabled patients with chronic renal failure to experience a longer life span as a result of better control over the physiological manifestations of uremia and fewer complications arising from the dialysis procedure itself. However, now many of the deaths associated with renal failure seem to be related to cardiovascular and cerebrovascular complications in those patients undergoing maintenance hemodialysis.

Linder, et. al., (1) studied 39 patients receiving long-term hemodialysis with reference to mortality from arteriosclerotic cardiovascular complications and found that at the end of a 13 year follow-up period, overall mortality was 56.4%. More than half of the deaths could be attributed to myocardial infarction, strokes, or refractory congestive heart failure; the incidence of these was many times higher than for normal and hypertensive groups of comparable age.

Accelerated atherosclerosis appears to be a major risk factor in the survival of maintenance hemodialysis patients. Whether the dialysis procedure itself is responsible for accelerated atherosclerosis is questionable (2). Hypertension, cardiac output, carbohydrate metabolism, and hyperlipidemia have been proposed to play significant roles in the prevalence of cardiovascular disease in renal failure

patients (3).

Frequency, Kinetics, and Proposed Mechanisms of Hypertriglyceridemia

Bagdade, et. al., (4,5) sought to determine the frequency and possible mechanism of plasma triglyceride (TG) elevation in chronic renal failure which could be contributing to the cardiovascular diseases in these patients. In 13 undialyzed and 25 dialyzed patients with non-nephrotic uremia, basal plasma TG concentrations were found to be 164 ± 62 mg per 100 ml and 276 ± 250 mg per 100 ml, respectively, while the levels for nonuremic controls were 68 ± 42 mg per 100 ml. Basal immunoreactive insulin levels of both groups were also higher than in nonuremic subjects and thus it was suggested that these increased levels may be related to increased hepatic synthesis of TG-rich lipoprotein. Peak post-heparin lipolytic activity (PHLA), an indirect measurement of tissue lipoprotein lipase and TG removal capacity, was subnormal in both dialyzed and undialyzed patients. Lipoprotein lipase (LPL) is an enzyme associated with the capillary endothelial cells of adipose tissue and is responsible for liberating fatty acids from very low density lipoproteins (VLDL); the free fatty acids can then be used for the production of energy in a variety of tissues or can be stored as fat in the adipocyte (6). The enzymatic activity of the lipoprotein lipase has been shown to be directly proportional to the triglyceride uptake by adipose tissue (7).

Gutman and coworkers (8) wanted to clarify the frequency and characteristics of hyperlipidemia in chronic renal failure and consequently examined a larger population of uremic non-nephrotic patients than previous researchers. Their study included 7 normal males and 42 male patients with renal failure. Results revealed approximately 70% of both undialyzed and hemodialyzed uremic patients had elevated fasting plasma TG levels and the majority also had low plasma PHLA. The authors support the thesis that abnormal triglyceride clearance mechanisms, as reflected by low plasma PHLA, may play a major role in the pathogenesis of the hypertriglyceridemia.

In an attempt to further define the means by which triglycerides become elevated in uremia, Cattran (9) investigated plasma lipid abnormalities as well as plasma triglyceride turnover in 78 patients undergoing different types of chronic dialysis - 26 hospital hemodialysis, 30 home hemodialysis, and 14 peritoneal dialysis patients. The injection of labeled glycerol-2-^{3}H and an activity disappearance curve generated from this was used to calculate the rate of triglyceride turnover. Elevated TG concentrations were observed in all groups regardless of the type of dialysis employed and an electrophoretic analysis of the triglycerides revealed a predominence of the type IV lipoprotein pattery (increased VLDL). Triglyceride concentrations throughout the period of turnover remained constant indicating a steady state, however

a plot of TG concentration against turnover displayed that all patients regardless of age, sex, or type of dialysis were below and to the right of normal curves indicating a TG removal defect. Although Cattran recognizes increased insulin levels have been suggested as an explanation for uremic hypertriglyceridemia (by inducing hepatic synthesis), he believes the low TG turnover rates in relation to triglyceride levels in this study point towards an impaired TG removal mechanism.

Ibels, et. al., (10) also examined the nature of hypertriglyceridemia in chronic renal failure by performing fasting serum triglyceride and cholesterol measurements and lipoprotein characterizations in four groups of patients with kidney disease - uremic, short- and long-term hemodialysis, and renal transplant recipients - and comparing these results with those of control subjects. The predominant lipid abnormalities were hypertriglyceridemia and increased VLDL in both uremic and hemodialysis patients; however, following renal transplantation, hypercholesterolemia became more common and both low density and/or very low density lipoproteins were increased with types IIa, IIb, and IV hyperlipoproteinemia occuring equally frequently. Basal insulin levels were not elevated in any patient group. On the evidence that the excess triglyceride appeared in the VLDL fraction, it was postulated that elevated TG in uremia is a specific metabolic abnormality and that hyperinsulinism does

not contribute importantly to the hypertriglyceridemia in uremic or dialysis patients.

Another team of researchers (11) studied lipids and lipoproteins in a group of chronic renal failure patients, some of whom were on regular hemodialysis. Compared with normals, there was a significant increase in plasma TG and VLDL concentrations in both dialyzed and undialyzed patients. There was no correlation between insulin and triglyceride values. Peak PHLA was significantly decreased however no correlation between TG and PHLA was found. Regular hemodialysis for 32 weeks did not improve the elevated state of triglycerides.

Significant hypertriglyceridemia was first found to occur when the creatinine clearance fell to 50 ml per min by Frank, et. al., (12). Furthermore, the prevalence of hypertriglyceridemia continued to rise as creatinine clearance fell progressively with the highest rate developing at a clearance of less than 10 ml per min. In addition, it was noted that the incidence of hypertriglyceridemia in the hemodialysis population decreased from 33% for those patients on therapy for less than five years to 16% for those treated for more than five years. However, the authors stated the decline in the prevalence of hypertriglyceridemia may reflect a selection process in which those who have hyperlipidemia and vascular disease die early in the course of dialysis treatment. Since the metabolism of triglyceride is influenced by insulin, glucagon, and growth hormone, the

relationship of these hormones to TG levels in renal failure patients was examined. Plasma glucagon correlated with plasma triglyceride levels; however, no correlation was found between insulin and plasma TG (even though insulin levels were elevated) or between growth hormone and plasma triglycerides.

Norbeck, Orö, and Carlson (13) determined the concentrations of triglycerides and cholesterol in three major serum lipoprotein classes--very low, low, and high density lipoproteins (VLDL, LDL, and HDL)--in 39 patients with chronic uremia. Triglyceride concentrations were raised in VLDL and LDL but concentrations of cholesterol were low in LDL and HDL. Type IIa, III, and IV hyperlipidemic patterns were observed in respectively 6, 9, and 30% of the patients. The ratio between cholesterol and triglycerides was higher in VLDL lipoproteins but lower in LDL lipoproteins compared to controls. The authors propose that these abnormal triglyceride and cholesterol concentrations in VLDL and LDL may result from a deranged lipoprotein metabolism.

The effect of acute uremia in TG kinetics in the rat was studied by Gregg, et. al., (14) in order to elucidate more clearly the means by which triglycerides become elevated in patients with chronic renal failure. After the production of acute uremic in rats, plasma TG levels became elevated. The three methods utilized for examining triglyceride production included determining the rate of TG accumulation

following Trition WR 1339 inhibition of lipoprotein removal, measuring hepatic TG secretion rate during in situ liver perfusion, and quantifying hepatocyte VLDL content with the electron microscope. The results from all three approaches indicated that triglyceride synthesis and secretion were decreased in acute uremia, suggesting that the elevated plasma levels had to result from a removal defect. In accordance with this hypothesis, prelabeled VLDL-triglycerides were injected into acutely uremic and control rats and their rate of disappearance was measured. The half-time of removal in acutely uremic rats was found to be approximately twice that of the controls, thus providing evidence for the notion that the increased plasma TG levels are due to a defect in the removal of VLDL-triglycerides from the plasma. The researchers also pointed out that increased plasma levels of insulin do not necessarily lead to increased TG production.

Heuck and associates (15) also examined the possible contributing factors in the promotion of hyperlipoproteinemia in rats with experimental chronic renal insufficiency. In uremic rats, fasting glucose concentrations were normal, immunoreactive serum insulin levels were decreased, and immunoreactive glucagon levels were increased, while plasma triglycerides were elevated 10-fold above control values. Upon analysis, it was observed that fatty acid composition of serum lipoproteins was unchanged and concomitantly, in liver tissue, there was no change in lipid content and fatty

acid composition. Adipose tissue revealed a decrease in TG content. These findings argue against glucose- or insulin-mediated hepatic overproduction as a major cause of hypertriglyceridemia and point to peripheral underutilization of lipoproteins.

Triglyceride turnover was examined in 12 undialyzed patients with chronic renal failure and 8 control subjects by Verschoor (16) through the use of labelled free fatty acids. The patients showed significantly increased fasting triglyceride concentrations and absolute plasma TG turnover rates (mg/hr/kg BW), while the fractional removal rate (hr^{-1}) was significantly decreased. Again, this would indicate an impaired removal mechanism as the major cause of the hypertriglyceridemia. Like Ibels, Frank, and Gregg, no relationship was found between parameters of triglyceride metabolism and fasting insulin levels.

Diet and Abnormally Elevated Triglycerides in Renal Failure

A sufficient caloric intake and a good nutritional status resulting in adequate triglyceride synthesis seem to be a necessary condition for hypertriglyceridemia. It has been shown that the majority of undernourished undialyzed and dialyzed patients had normal triglyceride levels (8). The proportion of carbohydrate in the diet has been suggested to play a role in the genesis of hypertriglyceridemia in uremic patients (10), however a comprehensive examination

did not reveal a correlation between TG levels and total energy intake nor the amount of carbohydrate in the diet (11).

On the other hand, Sanfelippo, et. al., (17) observed a reduction of plasma triglycerides in 7 undialyzed hypertriglyceridemic (> 150 mg/ml) subjects with chronic renal failure in response to changes in the composition of meal formula diets. The proportion of carbohydrate was reduced from 50 to 35% of the total daily calories and the polyunsaturated to saturated fat ratio was increased from 0.2 to 2.0 in an isocaloric diet. The triglyceride production rate was significantly lower on a diet low in carbohydrate and higher in polyunsaturated fat and thus it was concluded that dietary modification can promptly reduce triglyceride levels. The authors emphasize their support of the notion which attributes increased triglyceride levels to a defective clearance system, yet they stress the importance of dietary modification when possible to effect a decreased production rate and thus contribute to an improved lipid profile.

In a subsequent study, Sandelippo (18) investigated the triglyceride-lowering effect of a low carbohydrate diet with 12 renal failure patients on hemodialysis. The dietary modification was the same as the previous experiment, however results showed a reduction of plasma triglycerides with the low carbohydrate diet regardless of the type of fat ingested. An analysis of the triglyceride kinetics revealed lower triglyceride production rates in the dialyzed patients than

the controls, despite higher plasma triglyceride concentrations, thus indicating a defect in the removal of triglycerides. It was also observed that conventional hemodialysis did not effect a reduction in triglycerides in these patients. As with the previous study, it is recommended that dietary treatment be a feature of the total care of chronic renal failure patients to aid in the reduction of triglyceride levels even though this would not produce any fundamental change in the actual mechanism responsible for the abnormal TG kinetics. Placing these patients on a low carbohydrate diet before end-stage renal disease (when the hypertriglyceridemia becomes most prominent) may offset the advent of elevated triglycerides and thus the physiologically detrimental consequences.

In contrast to Sandelippo, Gokal, et. al., (19) performed a study with renal failure patients to examine the consequences of reducing the total fat content of the diet. Twenty dialysis patients with hyperlipoproteinemia (10 with type IV, 10 with type IIb) were given diets for one month planned to reduce their intake of fat and alter the ratio of saturated to polyunsaturated fat from 4:1 to 1:1; carbohydrate intake was increased to replace the calories. Plasma levels of triglyceride fell significantly in patients with type IV and type IIb hyperlipoproteinemia. Of particular interest was the fact that 12 patients upon resuming their original diets, reverted towards their pretreatment lipid abnormalities.

Others (20) have concluded that serum triglycerides can be lowered by both a low-carbohydrate diet or a low-fat diet. Fourteen hypertriglyceridemia patients on a diet low in fat and high in polyunsaturated fat displayed a significant decrease in triglycerides, cholesterol, and B-lipoproteins (LDL), while a low-carbohydrate diet given to 10 other hypertriglyceridemic patients produced a significant fall in the levels of triglycerides and pre-B-lipoproteins (VLDL). It should be noted that although these were normal renal function hypertriglyceridemic patients, the low-carbohydrate diet was responsible for altering the VLDL levels which is important since the lipid abnormality in chronic renal failure patients occurs in the VLDL fraction.

Lipoprotein Lipase and Serum Triglyceride Clearance

The pronounced role of lipoprotein lipase in triglyceride lipolysis has promoted investigations into the activity of this enzyme in relation to hypertriglyceridemia in both normal and impaired kidney function subjects. Worthy of mention is the fact that lipoprotein lipase (LPL) activity in adipose tissue was unaltered during induced hypertriglyceridemia (21). Needle biopsies of adipose tissue and blood samples were obtained before and at short intervals after a "bolus" injection of 10% intralipid after which lipoprotein lipase activities were measured in acetone-ether extracts of the tissue samples. No significant changes were observed in the activities of LPL in adipose tissue during the time

interval so it was concluded that the enzyme is probably not depleted as a secondary feature of an elevated plasma triglyceride level. This suggests that, in the relationship between serum triglycerides and tissue lipoprotein lipase, it is the latter which plays the determining role in the development of hypertriglyceridemia.

The presence of a defect in LPL has been inferred as the cause for hypertriglyceridemia in uncontrolled diabetes. Low adipose tissue LPL activity has been demonstrated in untreated diabetic man (22). Brunzell, et. al., (23) were interested in discerning whether or not defects in LPL-mediated endogenous TG removal could be related to elevated triglycerides. They found, in untreated diabetes, an abnormality in the interaction of LPL with circulating plasma triglycerides; an increased K_M for plasma triglyceride-lipoprotein lipase interaction (substrate-enzyme) and a decreased maximal removal capacity (V_{max}) for triglycerides was observed. No evidence for an increase in triglyceride production due to diabetes was found. It is proposed that V_{max} could be lowered as a result of insulin deficiency and K_M increased because of a change in VLDL apoprotein cofactors necessary for LPL activation or a change in lipoprotein lipase structure itself.

Persson (24) determined the <u>adipose tissue</u> LPL activity, serum TG, and free fatty acid (FFA) levels in healthy men and women and subjects with atherosclerotic disease, diabetes and

impaired glucose tolerance, and uremia. Results showed a relationship between LPL activity and serum lipids in controls and the majority of all subjects; thus, it is believed that an average level of enzyme activity in any of the disease conditions can be correlated to the average serum lipid levels of that condition. A direct comparison including an age, sex, and weight match was made between the uremic group and a group of healthy subjects; patients with uremia were characterized by higher serum triglycerides, but lower adipose tissue lipoprotein lipase activity and plasma FFA than the controls. The low enzyme activity in uremia is consistent with the findings by Bagdade, et. al., (4).

The mechanism of hyperlipidemia in relation to LPL-mediated TG removal in chronic renal failure was scrutinized in greater detail by Bagdade and coworkers (25). Male Sprague-Dawley rats demonstrated a stable state of renal insufficiency following a 5/6 nephrectomy in conjunction with significantly increased TG levels. Hepatic TG secretion rates and the activity of acetyl-Co-A carboxylase, the rate-limiting enzyme in hepatic lipogenesis, were both reduced making it unlikely that the TG elevation observed in the uremic animals resulted from increased VLDL production. Triglyceride removal was assessed by measuring the LPL activity released by heparin in three tissues--adipose, heart, and diaphragm; but this activity was not reduced in any of the tissues. The authors felt that although these in vitro

estimates of TG removal failed to reveal any quantitative defect in LPL, they could not be taken to indicate that this process was normal in vivo since normal tissue uptake of TG from the plasma requires the integration of LPL synthesis, activation, transport, and secretion.

The mechanisms in the pathogenesis of hypertriglyceridemia in uremia and hemodialysis were also studied by Ibels, et. al., (26). Plasma PHLA was significantly reduced in uremic and hemodialysis patients, both of which displayed elevated serum triglycerides. Triglyceride clearance was also reduced as determined from an impaired removal during infusions of triglyceride in patients and control subjects.

Inhibition of LPL and Elevated Serum Triglycerides

Others have provided evidence for decreased LPL activities (8, 11, 27) in uremic and hemodialysis patients and many viewpoints have been presented as to why these activities may be low. Heparin is thought to deplete tissue stores of LPL by some (27, 28) while a possible inhibitor of the enzyme has also been proposed (9, 13).

Bagdade, et. al., (29) were interested in determining whether there might be a substance in the serum of uremic animals which inhibited LPL action. Serum was obtained from rats which had been chronically uremic for five months and was added in increasing concentrations to an LPL-assay system which had been maximally activated with serum from control subjects. A significant reduction of LPL activity

was observed indicating the presence of an inhibitory factor in the serum of the uremic rats. To determine whether the inhibitory effects of serum were related to the duration of uremia, studies were performed utilizing the serum from rats which underwent nephrectomy only 24 hours earlier. Again inhibition of LPL was found and thus the authors hypothesize the accumulation of an inhibitory factor within 24 hours after the onset of renal failure. The results of this study indicate a functional defect in LPL, rather than a quantitative one, which could be responsible for the inadequate TG removal mechanism in uremic man and animals.

Murase, et. al., (30) were also interested in a possible inhibitory effect of LPL by uremic plasma which could explain the concomitant hypertriglyceridemia in chronic renal failure. In their experiment, the effects of the addition of plasma from 6 uremic dialyzed patients and 13 normals on the activity of lipoprotein lipase from rat epididymal adipose tissue were examined. Adding increments of normal and uremic plasma increased the LPL activity to maximal levels when 0.1 ml of plasma was added; larger amounts of uremic plasma produced a significant inhibition of LPL activity which was not observed with the normal plasma. In addition, combining larger amounts of uremic plasma with an incubation mixture already maximally activated with 0.1 ml normal plasma resulted in the inhibition of lipoprotein lipase. This inhibition was still present in uremic plasma which had been dialyzed against cold

saline in an effort to remove an inhibitory substance. When uremic and normal plasmas were divided into lipoprotein and lipoprotein-free fractions, it was observed that, upon incubation with a LPL-activated system, the lipoprotein-free fraction produced the inhibition. Protein precipitation of uremic plasma with trichloroacetic acid (TCA) resulted in a supernatant which displayed no inhibitory effect and thus the researchers feel the inhibitor is a protein and may play a very significant role in the pathogenesis of uremic hypertriglyceridemia.

Possible Inhibitor Removal by Ultrafiltration vs. Conventional Hemodialysis

The significance of an inhibitor of lipoprotein lipase in uremic plasma which might be removed becomes apparent upon observing the differences between a rather new blood-cleansing modality, referred to as predialytic isolated ultrafiltration, and conventional hemodialysis. In ultrafiltration, water and solutes are pressed through a semi-permeable membrane, the driving force being a pressure gradient which is achieved by applying a positive pressure on the blood side (with a blood pump) and a negative pressure on the dialysate side (with a negative pressure pump or gravity) (31). In regular hemodialysis therapy, ultrafiltration is generally applied continuously or intermittently while the dialysis procedure is going on, the main aim of the ultrafiltration being not to remove an excess of uremic waste products but to rid the patient of

excess water and extracellular electrolytes (31). However, detrimental side-effects of this process, such as hypotension, may be eliminated or minimized by sequential treatment; i.e., ultrafiltration followed by dialysis, or predialytic isolated ultrafiltration.

Conventional hemodialysis relies on diffusion down concentration gradients for the removal of undesirable solutes from the blood and this removal, in turn, depends on the size of the solute; i.e., smaller solutes are removed more readily than larger solutes. Henderson, et. al., (32) first realized the limitations of regular hemodialysis in removing larger toxic substances and thus undertook a study to examine the benefits of ultrafiltration. The process was found to have the advantage of removing solutes through the ultrafilter in proportion to their plasma concentration rather than their concentration gradient (as with diffusion), thus facilitating the removal of larger undesirable solutes. Quellhorst and coworkers (33) also observed that toxic substances with different molecular weights could be eliminated at the same rates during ultrafiltration in contrast to hemodialysis in which the removal of toxic compounds is dependent on their relative sizes.

The porosity and chemical nature of the artificial membranes used in ultrafiltration decide what size molecules will pass the membrane (34). Suitable membranes can be worked into either two-dimensional sheets or hollow fibers.

The hollow fiber filtration device XM 50 (Amicon) was one of the first developed and clinically applied to result in significantly desirable progress with ultrafiltration; it is considered a high-flux dialyzer with a rate of ultrafiltration in the neighborhood of four or more liters per hour (35). Medium-flux dialyzers only reach a flow rate of 1.5-2.0 liters per hour.

The inability of conventional hemodialysis to remove significant quantities of intermediate molecular weight compounds ranging up to 5,000 molecular weight in size has been a focus of considerable interest since it is speculated that these compounds may be preventing the complete correction of uremia in the well-dialyzed patient (36). It seems that the ultrafilter can remove solutes efficiently even if they are low in concentration in the plasma, large in size, and slow in diffusion rate. Ultrafiltration has been shown to be superior to hemodialysis in the removal of solutes having molecular weights of several hundred to several thousand, but the converse is true for smaller solutes; i.e., smaller solutes such as urea and creatinine are removed less with ultrafiltration (36). Furthermore, it appears that dialyzable solutes of "middle" molecular weight are associated with the severity and perhaps the duration of uremia (37).

Associated with predialytic ultrafiltration is a similar technique in which the ultrafiltrate removed is replaced with an intravenous electrolyte solution and dialysis follows.

The process, termed hemofiltration, is different than the former in that the entire dialysis process is one of sequential ultrafiltration, fluid replacement, and dialysis; ultrafiltration and dialysis facilitate fluid removal while ultrafiltration is also used as a means of more efficient removal of uremic solutes (31). Of particular significance is the observation that hemofiltration was responsible for an appreciable decrease in the serum triglycerides of renal failure patients (33). Henning and Balusek (38) investigated this in greater detail and provided additional support implicating the efficacy of hemofiltration in lowering plasma triglycerides. Whereas long-term hemodialysis was unable to improve hypertriglyceridemia, patients undergoing regular hemofiltration showed a prolonged decrease in plasma triglycerides. In fact, excessive hypertriglyceridemia in one case which still persisted after five years of chronic hemodialysis could be normalized by hemofiltration.

In conjunction with the preceding discussion it is desirable to summarize the important points:

(1) hypertriglyceridemia is a prevalent metabolic consequence of chronic renal failure;

(2) elevated triglycerides are common in both dialyzed and undialyzed patients;

(3) a defective removal mechanism for VLDL-TG appears to be more responsible for the increase in serum lipids than an overproductive synthetic system;

(4) the defect in this degradative mechanism apparently involves a functional impairment in adipose tissue lipoprotein lipase;

(5) an inhibitor may be responsible for this impairment; and

(6) hemofiltration seems to contribute to a reduction in serum TG.

Assuming the aforementioned ideas bear credence, it appears reasonable to present the supposition that an inhibitor of adipose tissue LPL could be removed during the process of ultrafiltration and thus account for the improved state of hypertriglyceridemia in renal failure patients who have undergone treatment with hemofiltration. Therefore, this study was designed to discern whether inhibition of LPL does indeed exist in the serum of uremic patients and, if so, whether a possible inhibitor might be removed during the course of ultrafiltration with a large pore-size ultrafilter.

Control and uremic plasmas as well as ultrafiltrate (fluid containing toxic substances drawn off during dialysis and ultrafiltration) was incubated with a lipoprotein lipase purified from bovine milk and a radio-labelled triglyceride emulsion in order to investigate the effects each have on the lipolysis of FFA from triglycerides by LPL.

MATERIALS AND METHODS

Subjects

Seven male renal failure patients on chronic hemodialysis at the Veterans Administration Medical Center in Loma Linda, Ca. between the ages of 35 and 62 years were selected. Four of the seven patients had been on dialysis for at least four years, the remaining three began treatment close to the time of experimentation. Seventy gm protein, 2 gm Na^+, and 2 gm K^+ were prescribed for dietary control of their conditions. Serum triglycerides ranged from 170 to 446 mg/100 ml; triglyceride levels at the time of blood withdrawal were noted. A control serum of 44 mg/100 ml from a subject with normal kidney function was used for subject comparison in the lipoprotein lipase assay. All blood samples were drawn into tubes containing EDTA followed by immediate centrifugation, plasma removal, and storage at -20°C until needed.

Lipoprotein Lipase Preparation

A lipoprotein lipase enzyme was purified from bovine milk according to the method of Egebrud and Olevercrona (39) with slight modifications and the exclusion of the final purification step by affinity chromotography on Sepharose 4B.

Unpasteurized bovine milk was obtained from a local dairy and the preparatory work was started immediately. In order to remove the fat, 3.6 liters of whole milk were centrifuged for 60 min at 5000 RPM in small plastic centrifuge

tubes containing 250 ml each. The skim milk (ca. 3000 ml) was filtered through glass wool and 30 ul of B-mercapto-ethanol (a preservative; Sigma Chemical Co., St. Louis, Mo.) and 0.21 gm of crude rennet powder (Sigma) were added to it. The milk was then heated slowly in a large beaker (4000 ml) under stirring on an electrical heating unit. When the temperature of the milk was 30°C it was kept at room temperature without stirring until a clot had formed (about 15 min) and was then centrifuged in the plastic tubes for 30 min at 5000 RPM in a refrigerated centrifuge set at 4°C. All further operations were performed at 0-4°C and all buffers and water contained 0.001% (v/v) B-mercaptoethanol. The volume of the supernatant (the whey) was measured and the precipitate was homogenized in the same volume of 1.16 M NaCl in a Waring Blender. The suspension was stirred overnight (16-18 hrs) in a refrigerator and then centrifuged for 30 min at 5000 RPM. This supernatant was filtered through glass wool and 300 gm per liter of solid $(NH_4)_2SO_4$ were slowly added under stirring. After 1 hr the precipitate was collected by centrifugation for 60 min at 5000 RPM; floating material was discarded. The precipitate was dissolved in a minimal volume (200 ml) of deionized ice water and dialyzed in approximately 3 ft of 1-in tubing against 3 liters of deionized ice water for 7 hr with 3 changes and then against 0.154 M NaCl for 10 hrs (dialysis was carried out in a refrigerator). After dialysis the total volume was 400 ml. The

dialysate was centrifuged for 10 min at 3000 RPM to remove small amounts of heavy insoluble material. Subsequently 135 ml (=1/3 volume of dialysate) of cold acetone were added dropwise under stirring, while being kept cold. Fifteen min after the last addition of acetone the precipitate was collected by centrifugation for 30 min at 5000 RPM. The precipitate was dissolved in 50 ml of 0.05 M NH_4OH-NH_4CL, pH 8.5, and reprecipitated by the addition of 750 ml of acetone. The precipitate was collected on a Büchner funnel and washed with 250 ml of ethyl ether (commercial grade). The acetone-ether powder was stored at -20°C up to several weeks while being used for the enzyme assay. This purification process was done a total of four times in order to maintain relatively fresh samples for the assays.

The powder lipoprotein lipase was brought into solution according to the method by which Murase, et. al., (30) brought acetone-ether dried powder from the epididymal fat pads of rats into solution except that homogenization was carried out for only 5 min. Ninety milligrams of this milk-derived acetone-ether powder was homogenized in 6.0 ml of .05 M NH_4OH-NH_4CL buffer, pH 8.5, containing .5 U/ml heparin, for 5 min. Care was taken not to allow the homogenizing unit to become too hot for protection of the enzyme. The suspension was centrifuged and the supernatant was used as the enzyme source.

Hemodialysis and Ultrafiltration Treatments

The first third of the study involved dialyzing the subjects with a small pore (300-600 Mol Wt) dialyzer, CDAK 1.8, according to their normal schedule for four hours. Pre-dialysis and post-dialysis blood samples were drawn and a 20 ml sample of ultrafiltrate was collected and saved for further analysis. The second third again involved a regular hemodialysis treatment but with a larger pore (2000-3000 Mol Wt) dialyzer, CDAK 3500; pre- and post-dialysis blood samples were drawn and 20 ml of ultrafiltrate was retained. With both the CDAK 1.8 and CDAK 3500, 2000-3000 U of heparin were administered at the beginning of dialysis followed by 1000 U per hour. Lastly, the patients underwent a 30-40 min period of ultrafiltration with a large pore (5,000-50,000 Mol Wt) hollow-fiber dialyzer, the Amicon, followed by hemodialysis. Fluid was replaced if needed, depending on the blood pressure of the patients. Pre- and post-dialysis as well as post-filtration blood samples were drawn and a 20 ml sample of ultrafiltrate was saved. With the Amicon ultrafilter, the patients received 4000-5000 U of heparin at the start of hemo-filtration. The samples of plasmas and ultrafiltrates were used in an assay system to observe their effects on lipoprotein lipase.

Lipoprotein Lipase Substrate Preparation

The substrate for the assay was prepared according to Whayne and Morelli (40). Triolein (Sigma), 0.133 gm, was

weighed into a small flask and .525 ml of .01 M PO_4-.005 M ethylenediamine tetraacetate (EDTA) buffer, pH 7.5, was added. Subsequently, 5.25 ml of 15% bovine serum albumin (Sigma; adjusted to pH 8.6 served as FFA acceptor), 2.25 ml of 0.025 M NH_4Cl buffer (pH 8.6), 0.9 ml of 1.35 M Tris-HCl buffer (pH 8.6), and 0.675 ml of 1.5% Triton X-100 (New England Nuclear, Boston, Mass.) were added. This mixture had a final pH of 8.6. One microcurie (10 ul) of glycerol tri[1-^{14}C] oleate (54 mCi/mmole, 50 miCi/500ul; Amersham Corporation, Arlington Heights, Ill.) was added to the pure triolein emulsion and the mixture was sonicated on ice for 4 min at maximum power output. The [1-^{14}C] triolein was a guaranteed purity of 99% and is prepared by the esterification of [1-^{14}C] oleic acid with glycerol.

Radioactive Assay for Lipoprotein Lipase Activity

The methods for assays developed by Murase, et. al., (30) and Wayne and Morelli (40) were combined for use in the determination of LPL activity. For each patient undergoing a specific type of treatment, .01 ml each of control, pre-dialysis, post-filtration, and post-dialysis plasmas were added individually to test tubes (in triplicate) containing .4 ml of triglyceride substrate. The total volume in the test tubes was brought to .8 ml with a .01 M PO_4-.005 M EDTA buffer (pH 7.5). Subsequently, 0.01 ml of .25 M $CaCl_2$ and 0.2 ml of the solubilized lipoprotein lipase acetone-ether powder were added. Incubation was carried out for 30 min at 37°C.

The effects of the different plasmas on the LPL lipolysis of the triglyceride substrate were to be determined from the above-mentioned test tubes. Accompanying these were incubation mixtures similar in content but with the following modifications: a standard with 0.1 ml control plasma and no radiolabelled triglycerides; 0.1 of ultrafiltrate without any plasma to determine the effect of this alone on LPL activity; 0.1 ml of ultrafiltrate in addition to 0.1 ml of control plasma to observe the combined effect of these and the plasma on the enzyme activity; test tubes with control, pre- and post-dialysis, and post-filtration plasmas, yet lacking in the enzyme preparation to discern the activity of heparin-released endogenous LPL; and, a test tube which contained no plasma or ultrafiltrate in that the effect of the enzyme on triglyceride lipolysis alone may be examined; (see Appendices B, C, D for detailed descriptions of incubations).

One assay was performed per day for each patient undergoing either hemodialysis of ultrafiltration including an assay of the control plasma; thus, three assays were done for each patient according to the particular type of treatment (CDAK 1.8 and CDAK 3500 dialyzers or Amicon ultrafilter), the control being inherent in the assay since each assay for each patient was accompanied by a comparable assay with control plasma.

Following incubation of the test tubes the reaction was

terminated by the addition of 5 ml of isopropanol-2N H_2SO_4 in H_2O-heptane (40:1:10). Lipids were extracted by adding 2.0 ml of H_2O and 3.0 ml of heptane. The test tubes were rotated on a mechanical revolver for 10 min and then centrifuged for 5 min at 1000 RPM. Three milliliters of the heptane phase, containing FFA and any unhydrolyzed TG, were removed and placed in clean test tubes. Three milliliters of 0.1 N KOH were added and the heptane-KOH mixtures were rotated for 10 min and centrifuged for 5 min at 1000 RPM. Two milliliters of the KOH phase (bottom layer) containing FFA were removed via a syringe attached to a 7-inch 17-gauge needle and placed in polyethelene counting vials. To these were added 15 ml of Instagel (Packard Instrument Co., Downers Grove, Ill.) and radioactivity was counted by a liquid scintillation counter in counts per minute. The relative effects of the various uremic plasmas and ultrafiltrates on the activity of LPL in comparison to a control plasma could then be quantitatively assessed by the concomitant radioactivity due to the C^{14}-labelled FFA released by enzymatic lipolysis.

RESULTS AND DISCUSSION

Pre-dialysis Plasma Inhibition of Lipoprotein Lipase

During the course of the study each patient remained in a stable condition except patient #7 who was deceased prior to treatment with the Amicon ultrafilter. Plasma samples from blood drawn ante-hemodialysis produced a marked inhibition of bovine milk lipoprotein lipase (8-67%) in contrast to the control plasma (Tables 1, 2, 3). It has been shown that in the absence of any added plasma, the enzyme always displayed a low amount of lipolysis (30). Thus, the counts per minute (cpm) from FFA released by the enzyme with no added plasma was subtracted from the cpm due to FFA released by the enzyme in conjunction with the plasma in order to correct for basal LPL activity.

The degree of inhibition was in reference to the amount of activity of the enzyme as affected by various plasmas. Replacing 0.1 ml of control plasma with 0.1 ml of uremic plasma in the incubation medium resulted in inhibition of LPL for all patients. Likewise Murase, et. al., (30) also found uremic plasma to inhibit LPL, however in their study this effect was not observed until greater amounts ($>$ 0.1 ml) of uremic plasma had been added to the triglyceride substrate; i.e., uremic and normal plasma both activated the enzyme when 0.1 ml of plasma was added, but additional quantities of uremic plasma produced a 60% inhibition of LPL. This difference may arise from their use of an acetone-ether lipoprotein

Table 1

Per cent inhibition* and activation** of lipoprotein lipase by uremic plasma and ultrafiltrate from patients dialyzed with CDAK 1.8 (n=7).+

	Patient						
	1	2	3	4	5	6	7
TG (mg/100 ml)	405	189	177	189	446	295	170
x/y							
pre-dialysis/ control plasma	-33%	-63%	-27%	-	-34%	-31%	-21%
post-dialysis/ pre-dialysis plasma	+15%	+119%	+25%	-	+109%	+26%	+64%
post-dialysis/ control plasma	-23%	-20%	-9%	-57%	+37%	-14%	+30%
ultrafiltrate + control/control plasma	-37%	+15%	-1%	+4%	+21%	+25%	+117%

*-% = 100 - (x/y)100; x,y are cpm values (Appendix B).
**+% = (x/y)-1)100; x,y are cpm values.
+Inhibition and activation are in reference to x effects on y.

Table 2

Per cent inhibition* and activation** of lipoprotein lipase by uremic plasma and ultrafiltrate from patients dialyzed with CDAK 3500 (n=7).+

	Patient						
TG (mg/100 ml)	1 445	2 362	3 177	4 216	5 446	6 235	7 170
x/y							
pre-dialysis/ control plasma	-66%	-67%	-46%	-36%	-35%	-8%	-41%
post-dialysis/ pre-dialysis plasma	+140%	+83%	+43%	+34%	+65%	+6%	+45%
post-dialysis/ control plasma	-19%	-39%	-23%	-14%	+7%	-3%	-14%
ultrafiltrate + control/control plasma	+3%	+72%	-5%	+6%	+12%	+33%	+28%

* % = 100 - (x/y)100; x,y are cpm values (Appendix C).
** % = ((x/y)-1)100; x,y are cpm values.
\+ Inhibition and activation are in reference to x effects on y.

Table 3

Per cent inhibition* and activation** of lipoprotein lipase by uremic plasma and ultrafiltrate from patients ultrafiltrated with the Amicon (n=6).+

	Patient				
	1	2	4	5	6
TG (mg/100 ml)	230	288	238	446	295
x/y					
Pre-dialysis/ control plasma	-14%	-45%	-67%	-26%	-44%
post-filtration/ pre-dialysis plasma	+15%	+71%	+134%	+4%	+19%
post-dialysis/ pre-dialysis plasma	+32%	+127%	+150%	+34%	+19%
post-dialysis- post-filtration plasma	+14%	+32%	+7%	+28%	+0%
post-filtration/ control plasma	-1%	-6%	-23%	-22%	-34%
post-dialysis/ control plasma	+13%	+24%	-18%	-1%	-34%
ultrafiltrate + control/ control plasma	-22%	-40%	-63%	-38%	-45%

* % = 100 - (x/y)100; x,y are cpm values (Appendix D).
** % = ((x/y)-1)100; x,y are cpm values.
\+ Inhibition and activation are in reference to x effects on y.

lipase powder derived from rat epididymal adipose tissue with a different degree of purification and/or sensitivity. Bagdade and coworkers (29) observed a 48% inhibition of LPL with plasmas from acutely and chronically uremic rats at every concentration studied.

According to the data presented (Tables 1, 2, 3), LPL activity is not directly related to the levels of triglycerides of the patients at the time blood was drawn for the individual assays. Also other investigators were not able to correlate plasma post-heparin lipolytic activity (PHLA) with lipid levels in hemodialysis patients (11, 27, 28).

The majority of studies performed in relation to defective TG removal mechanisms have utilized PHLA as a means of examining lipoprotein lipase activity in both normals and uremics. In these cases, blood is drawn subsequent to intravenous heparin administration which facilitates endogenous LPL release and activation and is incubated with a triglyceride emulsion. Gutman, et. al., (8) found that all but 1 of 22 chronic renal failure patients examined had low plasma PHLA. Others have observed significantly lower PHLA values in both dialyzed and undialyzed patients (4, 11, 26, 27, 28, 41). In this study an exogenous lipoprotein lipase has been used in order to observe plasma inhibitory effects on it. Although lower PHLA in uremia is of importance in that it supports a removal defect as the contributing factor in the hypertriglyceridemia associated with renal failure, it does not help to clarify the specifics of this defect; i.e., a low

PHLA could mean either a quantitative or functional impairment of lipoprotein lipase. On the other hand, a specific quantity of a lipoprotein lipase purified from bovine milk in an assay with plasma may reveal possible functional defects.

Persson (24) noted the limitations of PHLA assays in respect to the differences in the activity pattern of the organs from which PHLA is derived and the idea that different organs may release qualitatively different enzymes. Consequently, he determined adipose tissue lipoprotein lipase activity (LPLA) specifically by a heparin-eluate method since adipose tissue is a major source of the enzyme. Thirty uremic patients were characterized by low LPLA which was expressed relative to the weight of the adipose tissue - the author assumed this activity expression may reflect the enzyme concentration at the functional site. But the wide range in size of the adipose tissue makes it difficult to express the true activity and this technique also does not provide information as to possible functional impairments.

Applebaum-Bowden, et. al., (27) attribute PHLA to both hepatic triglyceride lipase (HTGL) and LPL; HTGL has been found to represent about 70% of the total PHLA. Thus, the reported low PHLA in uremic patients could be due to a depression in either post-heparin plasma LPL or HTGL, or both. These researchers found both to be low in both elevated TG and normal TG hemodialysis patients. The role of LPL in the

hypertriglyceridemia of renal failure is also not clear in this study.

Garfinkel and Schotz (42) explored the possibility of different types of lipoprotein lipase which may have different properties and functions in adipose tissue. Upon applying extracts of rat adipose acetone-ether powder to agarose gel chromatography columns, two peaks of lipoprotein lipase were eluted - the first activity corresponded to a protein of molecular weight approximately five times that of the second and the addition of heparin to the eluted fractions markedly stimulated activity of the first but suppressed that of the second. Both lipases did have the characteristics that distinguish lipoprotein lipase from other tissue lipases: a requirement for serum for substrate activation, inhibition by 1 M NaCl, and an alkaline pH optimum (pH 8.0). These observations emphasize the difficulty in interpreting data derived from experiments with crude extracts from adipose tissue and, for that matter, human PHLA data.

Due to the previously mentioned drawbacks, the method of lipolytic activity assessment in this study seems to be superior to PHLA determinations and LPL assays with crude adipose tissue extracts. Daubresse, et. al., (11) concluded that PHLA alone is not a particularly useful method for studying the mechanism of hypertriglyceridemia associated with renal failure and that PHLA may only partially reflect the disturbance in TG transport. Observing the effects of plasmas

on a purified skim milk lipoprotein lipase have helped to disregard the different types of triglyceride lipases and have provided information as to why LPL activity is low. The data from this study do indeed support the notion of lipoprotein lipase inhibition by uremic plasma.

Effect of Post-filtration and Post-dialysis Plasmas on LPL Activity

A 6% to 150% increase in lipolytic activity was observed in post-dialysis plasma (Tables 1, 2) and post-filtration plasma (Table 3) when compared to pre-dialysis plasma. The effects of the heparin received by the patients before and during all three treatment modalities (CDAK 1.8), CDAK 3500, Amicon) make it difficult to extrapolate this data since heparin does activate and release endogenous lipoprotein lipase. Thus, the incubation mediums containing plasmas drawn after heparin administration would display an increase in lipolytic activity deriving from both milk-derived (exogenous) LPL and endogenous lipoprotein lipase (or PHLA).

Gutman, et. al., (8) observed a significant increase in the PHLA of all patients on general heparinization at the end of the hemodialysis session; in addition PHLA was found to be in the same low range as before dialysis 12 to 36 hr after the end of the hemodialysis session (when the heparin received should have been cleared (43)). The authors attribute plasma PHLA increases in hemodialysis patients to the large doses of

heparin received. However, in contrast to normal controls given a large dose of heparin, the achieved enzyme levels were lower.

Assays performed without exogenous LPL in this study (Tables 4, 5) show that post-filtration and post-dialysis plasma have as high as a 5-, 7-, and 12-fold increase in PHLA than the normal control, but this control was not given heparin. Excluding the milk enzyme allowed the activity due to endogenous LPL only to be measured. Post-dialysis and post-filtration plasmas from the same subject produced a 1- to 5-fold increase in endogenous lipolytic activity (Tables 4, 5). These findings are consistent with data by others who recognize the role of heparin in the release and activation of endogenous enzyme during hemodialysis and thus its contribution to increased PHLA levels (9, 23, 26, 27).

There is conflicting data as to whether elevated lipolytic activity due to heparin infusions during hemodialysis result in lower triglyceride levels. Bagdade (5) and Gutman (8) showed that an increase in PHLA at the end of hemodialysis was coincident with a fall in triglycerides while Tsaltas (44) found both PHLA and plasma triglycerides to increase during dialysis. On the other hand, Daubresse (11) observed low PHLA in dialyzed patients with no change in TG levels after a 32 week period. The differences between these studies may be due to variances in the experimental designs of the researchers. Bagdade achieved lower TG levels with more frequent

dialysis (40 hrs/wk) and although triglycerides were also lowered in Gutman's study, it must be noted they returned to pretreatment values 12 to 36 hr after dialysis. The lack of change in TG levels shown by Daubresse should be considered in light of the fact dialysis was only performed 24 hrs/wk and the change in triglycerides was in reference to pre- and post-study levels, not pre- and post-dialysis levels as with the previous two experiments. Although TG levels were not determined during and after dialysis in our study, the possible alteration of triglycerides as a result of hemodialysis is of significance.

The observation by Bagdade, et. al., (4) of a decrease in both PHLA and plasma triglycerides in dialyzed patients, conflicts the data to an even greater degree. It was suggested that prolonged and repeated heparin infusions during hemodialysis could have depleted the tissues of lipoprotein lipase that subsequently led to the low PHLA. Huttunen and coworkers (28) have demonstrated that in patients with venous thrombosis continuous heparin administration can in fact exhaust the tissue stores of both lipoprotein and hepatic lipases. However, this depletion hypothesis seems unlikely since undialyzed uremics and peritoneal dialysis patients produced low PHLA levels in spite of the fact they did not receive heparin (8). Another argument against a depletion theory has to do with the observation by Bagdade (5) that an increment in the number of hours on hemodialysis from

Table 4

Per cent differences between incubations with no exogenous lipoprotein lipase--CDAK 1.8 and CDAK 3500 (n=7).+

	Patient						
	1	2	3	4	5	6	7
x/y							
CDAK 1.8							
pre-dialysis/ control plasma	+56%	+112%	+25%	+41%	+15%	+1%	+47%
post-dialysis/ control plasma	+523%	+409%	+493%	+357%	+71%	+53%	+241%
post-dialysis/ pre=dialysis plasma	+298%	+140%	+376%	+224%	+49%	+52%	+131%
CDAK 3500							
pre-dialysis/ control plasma	+61%	+114%	+6%	+8%	-1%	+95%	+50%
post-dialysis/ control plasma	+723%	+1200%	+226%	+365%	+48%	+252%	+332%
post-dialysis/ pre-dialysis plasma	+412%	+509%	+207%	+332%	+50%	+80%	+189%

Table 5

Per cent differences between incubations with no exogenous lipoprotein lipase for the Amicon (n=6).+

	1	2	4	5	6
x/y					
Amicon					
pre-dialysis/ control plasma	+2%	+6%	7%	-17%	+0%
post-filtration/ control plasma	+149%	+217%	+76%	+295%	+463%
post-dialysis/ control plasma	+134%	+203%	+7%	-17%	+193%
post-filtration/ pre-dialysis plasma	+144%	+200%	+65%	+379%	+464%
post-dialysis/ pre-dialysis plasma	+130%	+187%	+54%	+126%	+194%
post-dialysis/ post-filtration plasma	-6%	-4%	-6%	-53%	-48%

18 to 40 hr/wk led to a 2-fold increase in PHLA. Data presented here (Tables 4, 5) also do not lend support to depletion of LPL during hemodialysis through the influence of heparin since post-dialysis plasma lipolytic activity was much enhanced compared to pre-dialysis plasma with the CDAK 1.8, CDAK 3500, and Amicon. The decrease in post-dialysis plasma in reference in post-filtration plasma in patients ultrafiltrated with the Amicon (Table 5) is probably due to the fact heparin was only administered at the beginning of this treatment modality and thus endogenous LPL release became less as dialysis proceeded. When comparing and extrapolating data related to heparin activation of LPL, it is crucial that the amount of heparin and frequency of administration be taken into consid tion. This might explain low PHLA levels in some cases ($r = 0.65$) trast to higher ones in other observations.

It is of interest to note the apparent higher activity of endogenous LPL in pre-dialysis vs. control plasma (Tables 4, 5) despite the lack of heparin in both cases (recall heparin is cleared within 12 to 36 hrs and pre-dialysis blood samples in these patients are drawn at least 48 hr after their last dialysis since dialysis is only performed three times per week). When this finding is compared to the low levels of activity of exogenous LPL incubated with pre-dialysis plasma in reference to control plasma (Tables 1, 2, 3) in all patients, the significance of a possible inhibitor is revealed; i.e., although endogenous LPL activity

is as much as 70, 80, 90, and 100% higher in many of the subjects compared to the control plasma activity, when their plasma is incubated with an exogenous source of LPL, the lipolytic activity of this enzyme is reduced to as much as 40, 50, and 60% of that of the control. Thus, it does appear as though a substance in the blood of the uremic subjects might indeed inhibit lipoprotein lipase. It is questionable as to why the endogenous LPL does not seem to be inhibited by its own plasma. Perhaps a possible inhibitor is specific for the site of LPL location before release or, in light of the contribution HTGL makes to plasma lipolytic activity, a possible inhibitor may be extremely specific for lipoprotein lipase only and thus, the true extent of its actions overwhelmed at first glance by HTGL.

Ultrafiltrate Activation and Inhibition of Lipoprotein Lipase

Due to the action and concomitant effects of heparin, the combined activity of exogenous and endogenous LPL must be taken into consideration in the analysis of the data presented in the previous section. Therefore, although lipolytic activity is increased subsequent to dialysis and ultrafiltration, it cannot be confidently suggested from this evidence that an inhibitor of LPL is possibly being removed during the dialysis and ultrafiltration procedures which consequently effects the increase in lipolysis. However, examination of the properties of the ultrafiltrate removed

during dialysis with the CDAK 1.8 and 3500 and ultrafiltrate removed during ultrafiltration with the Amicon help clarify the dilemma. Incubating equal volumes of ultrafiltrate and control plasma in an assay system with the milk enzyme resulted in an increase in lipolysis in 5 out of 7 patients dialyzed with the CDAK 1.8 (Table 1) and 6 out of 7 patients dialyzed with the CDAK 3500 (Table 2). This ultrafiltrate may contain activators of the exogenous lipoprotein lipase which account for the increased percentage of lipolysis compared to an assay with control plasma only. Although ultrafiltrate from a few of the patients only minimally induced LPL lipolysis (3, 4, 6%), ultrafiltrate from the majority of patients produced a marked increase in the lipolysis of triglyceride (28, 33, 37, 72, 117%).

In distinct contrast to these observations, incubation of equal volumes of ultrafiltrate and control plasma in the LPL assay system produced a 22 to 63% inhibition of TG lipolysis in 5 out of 6 patients ultrafiltrated with the Amicon (Table 3). Because the sixth patient (patient #3) went into shock shortly after commencement of ultrafiltration with the Amicon and, thus, very little ultrafiltrate was collected, the data from this patient has been discarded for this particular treatment modality.

The consistent inhibitory influence of the ultrafiltrate on the lipolytic activity of the milk enzyme supports our hypothesis that the process of ultrafiltration, which

utilizes a large-pore membrane, facilitates the removal of a substance in uremic plasma which impedes the maximal activity of endogenous lipoprotein lipase. Moreover, this uremic substance could be the responsible factor in the hypertriglyceridemia of renal failure, or more specifically, the cause of the defective removal mechanism resulting in elevated triglycerides in uremia. A summary of the inhibition of LPL by pre-dialysis plasma and ultrafiltrate removed by the Amicon and LPL activation by ultrafiltrate removed during dialysis with CDAK 1.8 and CDAK 3500 is shown in Table 6.

Studies have shown that lipoprotein lipase does require certain serum factors which regulate crucial steps for proper functioning. Apo C-II, an apolipoprotein which is transferred from circulating high-density lipoproteins to newly secreted chylomicrons and VLDL, is probably the sole activator of lipoprotein lipase (45). Erkelens, et. al., (46) were interested in observing whether the availability of apo C-II for binding to infused TG-rich particles might be a quantitative determinant of triglyceride removal, but results showed the amount of apo C-II bound did not control the rate of triglyceride removal from the plasma. It is possible that some other substances mediate the relationship between apo C-II and circulating triglycerides. Nonetheless, the requirement of apo C-II for maximal lipoprotein lipase activity has been established (47, 48, 49). In addition, La Rosa, et. al., (50) have found apo-LP-Glu to be a prime lipoprotein lipase activator.

Table 6

Per cent inhibition* and activation** of lipoprotein lipase by pre-dialysis plasma and control plasma plus ultrafiltrate with CDAK 1.8 (n=7), CDAK 3500 (n=7), and the Amicon (n=6).+

	1	2	3	4	5	6	7 (deceased)
x/y : pre-dialysis/ control plasma							
CDAK 1.8	-33%	-63%	-27%	-	-34%	-31%	-21%
CDAK 3500	-66%	-67%	-46%	-36%	-35%	-8%	-41%
Amicon	-14%	-45%	-	-67%	-26%	-44%	-
x/y : filtrate + control/ control plasma							
CDAK 1.8	-37%	+15%	-1%	+4%	+21%	+25%	+117%
CDAK 3500	+3%	+72%	-5%	+6%	+12%	+33%	+28%
Amicon	-22%	-40%	-	-63%	-38%	-45%	-

* % = 100 - (x/y)100; x,y are cpm values.
** % = ((x/y)-1)100; x,y are cpm values.
\+ Inhibition and activation are in reference to x effects on y.

On the other hand, the apoproteins C-I, C-III-1, and C-III-2 seem to have an inhibitory effect on lipoprotein lipase. Schrecker and Greten (47) investigated the kinetics of triolein hydrolysis by purified human plasma lipoprotein lipase utilizing different amounts of apo C-I, C-III-1, and C-III-2. While increasing amounts of C-II were found to stimulate the activity of the enzyme, increasing amounts of C-I, C-III-1, or C-III-2 without C-II caused a significant inhibition of triolein hydrolysis. The authors suggest that apo C-II may have both binding sites for triglycerides and sites which interact with the enzyme protein to facilitate proper orientation. The inhibitory effect of apo C-I, apo C-III-1, and apo C-III-2 could then possibly be explained by their ability to bind with lipid substrates, thus preventing the formation of lipid-substrate complexes. Apo A-I, apo A-II, and apo E appear to have this ability to interact with lipids also. Because the inhibitory effects of these apoproteins can be reversed by apo C-II, these same researchers think it unlikely that the proteins play a role as physiological inhibitors during lipoprotein catabolism.

In light of this data, it seems reasonable to speculate that specific circulating inhibitors and activators of lipoprotein lipase in the plasma mediate substrate-enzyme interactions and thus maintain an influential effect on the TG-removal mechanism. Chylomicrons and VLDL, the substrates for LPL, are very large compared to the enzyme molecule and are

reacted on only at the surface where the enzyme-substrate interaction occurs (47). Thus, at this surface it is possible that other factors affect the lipolytic reaction taking place between lipoprotein lipase and triglyceride molecules. This hypothesis if true, would help explain our results. The dialyzing membranes (CDAK 1.8 and CDAK 3500) could have been removing lipoprotein lipase activating factors from the blood of the patients but restricted the removal of inhibitory factors due to the small size of the pores. This would account for the activating influence of this ultrafiltrate in the LPL assay with control plasma. In contrast, the large pore size of the Amicon ultrafilter may have not only allowed the passage of circulating activators, but also may have facilitated the flux of certain inhibitory substances characterized by a larger molecular weight. In this case, the inhibitory influence of this ultrafiltrate on the activity of the milk enzyme would be due to the removal of components exhibiting LPL inhibition during the process of ultrafiltration. If this concept is indeed valid, the fact that hemofiltration does effect a reduction in triglyceride levels in hemodialysis patients would be partially if not totally explained.

Suggestions for Inhibitor Characterization

Although Gutman, et. al., (8) could find no evidence involving the presence of a dialyzable inhibitor from regular hemodialysis patients, Lutz (51) was successful in isolating

a strongly basic uremic peptide from the dialysis fluid of renal failure patients who had peritoneal dialysis. The peptide did not have an effect on the liberation of lipoprotein lipase activity from human adipose cells but it did inhibit stimulation of liberation by insulin. (Glucose uptake and thus insulin are necessary for the transport of LPL from adipose cells to the walls of the capillary vessels (52)). In addition, stimulation of liberation caused by heparin was unaltered in the presence of the uremic peptide. It is doubtful that this particular peptide is responsible for the inhibition of lipoprotein lipase observed in our study and others since (1) we are concerned with a functional rather than a quantitative defect in LPL which is the consideration in the study by Lutz, and (2) hemodialysis and ultrafiltration patients are heparinized. However, the fact that a uremic peptide was isolated specifically from the dialysis fluid supports the proposition that ultrafiltrate may well contain many substances with unrecognized properties.

Exactly what these substances might be remains a mystery. The familiar uremic toxins such as urea and creatinine can probably be excluded since both hemodialysis and ultrafiltration adequately remove concentrations of these in the plasma. In addition, Murase, et. al., (30) showed that urea, at a concentration of 100 mg/dl, and creatinine, at a concentration of 10 mg/dl, when incubated with lipoprotein lipase

activated by normal plasma, only produced 16% and 13% inhibitions, respectively - uremic plasma produced up to a 68% inhibition in this same assay system.

Babb, et. al., (53) are responsible for formulating the "middle" molecule hypothesis which proposes the existence of certain 500 to 5,000 molecular weight toxic substances in uremia. Funck-Bretano and coworkers (54) have related specific clinical symptoms of renal failure patients to the presence of higher molecular weight (middle molecule) substances in uremic plasma. Mignone, et. al., (55) performed a middle molecule study with uremic and normal serum, uremic and normal urine, and dialysis fluid. Through the use of gel filtration, they demonstrated the accumulation in uremic serum of substances having a molecular weight of 500 to 3,500 which were mostly peptides, however no specific compound was identified. Successful removal of uremic substances of "middle" molecular weight through ultrafiltration and an approximation of their size was done by Henderson, et. al., (56). In comparison to hemodialysis in which the capacity to remove solutes was rapidly diminished when molecular weights of 100 were approached (urea, creatinine, and uric acid acid approximate this value), ultrafiltration efficiently removed larger substances up to 10,000 molecular weight.

One or more of these middle molecules may contribute to the inhibition of endogenous lipoprotein lipase and subsequently effect an elevation of VLDL. Then again, a possible

inhibitor of the enzyme may be of smaller molecular weight (not necessarily a "middle" molecule) but exist in the plasma in too low a concentration to be removed by regular hemodialysis. As mentioned earlier, ultrafiltration with a large pore membrane not only removes larger molecular substances but also is capable of removing smaller substances at lower plasma concentrations since removal is not based on a concentration gradient. Whatever the case may be, the clinical significance of blood-cleansing by means of filtration through larger pore membranes is obvious. More and larger uremic toxins can be removed utilizing this treatment with renal failure patients and thus, improved physiological conditions may be realized.

SUMMARY

Plasma from seven hypertriglyceridemia chronic renal failure patients drawn prior to regular hemodialysis therapy was found to inhibit the lipolytic activity of bovine milk lipoprotein lipase by 8-67% as compared to plasma from a control with triglycerides in the normal range. The inhibition did not appear to be alleviated by dialysis utilizing two different small pore-size membranes (CDAK 1.8 and CDAK 3500), however treatment with an isolated ultrafiltration technique with a large pore-size membrane (Amicon) did appear to remove an inhibitory substance as evidenced by a 22-66% inhibition of lipolytic activity by the ultrafiltrate. This substance may interact with endogenous lipoprotein lipase and prevent an optimal degradation of triglycerides resulting in the hypertriglyceridemia so commonly associated with chronic renal failure.

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APPENDIX A

Patient Data

Table A

Sex, age, weight, and length of time on hemodialysis* for seven patients with chronic renal failure

	Patient						
	1	2	3	4	5	6	7
sex	M	M	M	M	M	M	M
age (yr)	55	35	43	60	45	62	57
weight (kg)	70	61	86	77	85	71	68
Δt (mo)	52	64	24	5	3	60	84

*length of time on hemodialysis = Δ t

APPENDIX B

Incubation Mixtures and CPM Values for Patients Dialyzed with CDAK 1.8

Table B-1

Incubation components of test tubes

ml: test tubes	TG*	Plasma	Ultrafiltrate	EDTA	$CaCl_2$	LPL**
1. standard	-	0.1 control	-	0.7	0.01	0.2
2. control	0.4	0.1 control	-	0.3	↓	↓
3. pre-dialysis	↓	0.1 pre-dialysis	-	↓	↓	↓
4. post-dialysis	↓	0.1 post-dialysis	-	↓	↓	↓
5. no plasma	↓	-	-	0.4	↓	↓
6. ultrafiltrate	↓	-	0.1	0.3	↓	↓
7. ultrafiltrate + control	↓	0.1 control	0.1	↓	↓	↓
8. control, no LPL	↓	0.1 control	-	0.5	↓	-
9. pre-dialysis, no LPL	↓	0.1 pre-dialysis	-	↓	↓	-
10. post-dialysis, no LPL	↓	0.1 post-dialysis	-	↓	↓	-

*radiolabelled triglycerides
**solubilized acetone-ether powder

Table B-2

Radioactivity in cpm of incubation mixtures

	Patient						
	1	2	3	4	5	6	7
TG (mg/100 ml)	405	189	177	189	446	295	170
test tube				cpm			
1	749	37	831	382	1095	1056	217
2	10282	6535	8521	2969	6071	6545	7895
3	9615	5421	8165	1170	5595	6104	7597
4	9816	6186	8407	2064	6588	6356	8333
5	8271	4778	7204	1371	4688	5142	6448
6	7611	5745	7524	1699	4913	5758	6781
8	270	159	199	157	1095	1269	268
9	422	338	248	221	1262	1274	395
10	1682	810	1181	717	1877	1941	914

APPENDIX C

Incubation Mixtures and CPM Values for Patients Dialyzed with CDAK 3500

Table C-1

Incubation components of test tubes

test tubes	ml: TG*	Plasma	Ultrafiltrate	EDTA	$CaCl_2$	LPL**
1. standard	-	0.1 control	-	0.7	0.01	0.2
2. control	0.4	0.1 control	-	0.3		
3. pre-dialysis		0.1 pre-dialysis	-			
4. post-dialysis		0.1 post-dialysis	-			
5. no plasma		-		0.4		
6. ultrafiltrate		-	0.1	0.3		
7. ultrafiltrate + control		0.1 control	0.1			
8. control, no LPL		0.1 control	-	0.5		-
9. pre-dialysis, no LPL		0.1 pre-dialysis	-			-
10. post-dialysis, no LPL		0.1 post-dialysis	-			-

*radiolabelled triglycerides
**solubilized acetone-ether powder

Table C-2

Radioactivity in cpm of incubation mixtures

	Patient						
	1	2	3	4	5	6	7
TG (mg/100 ml)	445	362	177	216	446	235	170
test tube				cpm			
1	506	790	82	88	1171	397	360
2	8648	6183	3235	6486	5867	5215	6968
3	6056	5077	2298	4905	5403	5073	5699
4	7889	5533	2762	5857	5962	5162	6539
5	4743	4525	1211	2097	4550	3517	3849
6	6825	5350	2148	2658	4884	4254	3902
7	8771	7370	3139	6726	6029	5784	7848
8	291	197	133	118	1113	208	149
9	468	421	141	127	1099	406	223
10	2394	2565	433	549	1648	733	644

APPENDIX D

Incubation Mixtures and CPM Values for Patients Ultrafiltrated with Amicon

Table D-1

Incubation components of test tubes

test tubes (ml:)	TG*	Plasma	Ultrafiltrate	EDTA	$CaCl_2$	LPL
1. standard	-	0.1 control	-	0.7	0.01	0.2
2. control	0.4	0.1 control	-	0.3	↓	↓
3. pre-dialysis	↓	0.1 pre-dialysis	-	↓	↓	↓
4. post-filtration	↓	0.1 post-filtration	-	↓	↓	↓
5. post-dialysis	↓	0.1 post-dialysis	-	↓	↓	↓
6. no plasma	↓	-	-	0.4	↓	↓
7. ultrafiltrate	↓	-	0.1	0.3	↓	↓
8. ultrafiltrate + control	↓	0.1 control	↓	↓	↓	↓
9. control, no LPL	↓	0.1 control	-	0.5	↓	↓
10. pre-dialysis, no LPL	↓	0.1 pre-dialysis	-	↓	↓	↓
11. post-filtration, no LPL	↓	0.1 post-filtration	-	↓	↓	↓
12. post-dialysis, no LPL	↓	0.1 post-dialysis	↓	↓	↓	↓

*radiolabelled triglycerides
**solubilized acetone-ether powder

Table D-2

Radioactivity in cpm of incubation mixtures

	Patient					
	1	2	3	4	5	6
TG (mg/100 ml)	230	288	177	238	446	295
test tube			cpm			
1	1435	952	1906	2324	637	364
2	7983	7078	6684	7938	7185	7712
3	7711	6600	6105	6789	6770	6440
4	7968	7010	6611	7545	6820	6742
5	8243	7329	5486	7634	7180	6749
6	6037	6025	6267	6225	5558	4846
7	6458	5682	5899	6876	5758	5860
8	7561	6658	7130	6850	6568	6410
9	1483	1076	1543	2308	781	518
10	1509	1139	2854	2464	644	517
11	3688	3412	3073	4055	3086	2916
12	3473	3264	2754	3794	1457	1520