Plasma concentrations of non-esterified fatty acids in chronic renal failure in the United Arab Emirates

Michael P. Gillett, BSc, PhD, Enyioma N. Obineche, MD, Safina T. Khan, BSc, MSc, Abdishakur M. Abdulle, BSc.

ABSTRACT

Objective: In end-stage renal failure, dyslipoproteinemia is linked to risk of cardiovascular disease. Increased concentrations of triacylglycerol-rich, very low density lipoproteins (VLDL) and decreased concentrations of high density lipoproteins (HDL) are usual, whilst total cholesterol and low density lipoprotein (LDL) concentrations are not increased. Non-esterified fatty acids (NEFA) are not transported by lipoproteins, but increased concentrations may also be associated with cardiovascular disease risk. In this study, plasma concentrations of NEFA and other lipids were compared in healthy subjects and patients with end-stage chronic renal failure who were either undialyzed or undergoing peritoneal dialysis or hemodialysis.

Methods: Fasted blood samples for measurement of albumin, total, free and HDL-cholesterol, triacylglycerols and NEFA were taken from 56 apparently healthy subjects and from 48, 28 and 46 patients from the United Arab Emirates during 2002 who were either untreated or on peritoneal or hemodialysis. Hemodialysis subjects were studied immediately before and after a single treatment session.

Results: For all groups of patients, total, and LDL-cholesterol were unchanged, triacylglycerols and free cholesterol were raised and HDL-cholesterol concentrations and the percentage of esterified cholesterol were significantly decreased compared to controls. Plasma NEFA concentrations for untreated patients were similar to controls, but were decreased in peritoneal dialysis patients and markedly increased both before and, even more so, after dialysis in hemodialysis patients.

Conclusion: Patients with end-stage renal failure share common features of dyslipoproteinemia irrespective of whether they are untreated or on peritoneal dialysis or hemodialysis. However, only hemodialysis patients show significantly increased concentrations of NEFA.

Saudi Med J 2004; Vol. 25 (11): 1611-1616

Patients with end-stage renal disease, whether or not they are undergoing dialysis therapy, frequently present evidence of dyslipoproteinemia[1-4] and have an increased risk of developing cardiovascular disease and of suffering from its complications. The frequent findings are increased concentrations of triacylglycerols and very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL,)[5-8] and decreased concentrations of high density lipoproteins (HDL).[4,6,9] By contrast, total cholesterol and low density lipoproteins (LDL) appear to be little affected by renal disease, except in nephrotic subjects.[10,11] The main features of renal dyslipoproteinemia have been linked to impaired catabolism of lipoproteins by lipoprotein and

From the Department of Biochemistry (Gillett, Khan), Department of Internal Medicine (Obineche, Abdulle), Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, *United Arab Emirates*.

Received 10th February 2004. Accepted for publication in final form 5th June 2004.

Address correspondence and reprint request to: Dr. Michael P. Gillett, Associate Professor, Department of Biochemistry, Faculty of Medicine and Health Sciences, UAE University, PO Box 17666, Al Ain, *United Arab Emirates*. Tel. +971 (3) 7039500. Fax. +971 (3) 7672033. E-mail: m.gillet@uaeu.ac.ae

hepatic lipases[12-14] and by lecithin: cholesterol acyltransferase (LCAT).[4,15-18] Lipoproteins, however, are not the only lipid transport system in the blood since, unlike triacylglycerols and cholesterol, non-esterified fatty acids (NEFA) are carried in the plasma bound to albumin.[19] Although plasma concentrations of NEFA are normally very low compared to other plasma lipids, NEFA are metabolically significant and have a high metabolic turnover rate. Even in healthy individuals, plasma concentrations of NEFA are very variable, being influenced by hormonal, metabolic and nutritional status. Abnormally high plasma concentrations of NEFA are implicated in increased risk of ventricular fibrillation[20] and sudden cardiac death[21] and more controversially of coronary heart disease.[22-24] The importance of NEFA in renal disease seems to have been neglected in recent years; nevertheless, many studies have in the past, established that NEFA concentrations in end-stage renal failure patients are increased following treatment by hemodialysis.[25-28] Heparinization of patients during hemodialysis and the consequent release into the circulation of lipoprotein and hepatic lipases has been thought to be related to raised post-dialysis NEFA concentrations, but so too, have other factors such as carnitine deficiency[29,30] and the presence of acetate in the dialysis buffer solutions.[31] Due to the importance of raised NEFA concentrations as predictors of the risk of sudden cardiac death and possibly also of coronary heart disease, the present study was undertaken to compare plasma NEFA concentrations in apparently healthy control subjects and in three groups of patients with end-stage renal failure who were either undialysed or receiving peritoneal dialysis or hemodialysis in the United Arab Emirates. The patients on hemodialysis were studied both before and after a single dialysis session. The results show that not only are NEFA concentrations significantly raised post-hemodialysis, but they are also significantly very much higher pre-hemodialysis than those of controls and untreated or peritoneal dialysis patients.

Methods. This study was approved by the Research Ethics Committee of the Faculty of Medicine and Health Sciences, UAE University and all subjects included had given their informed consent to participate. The control population included 36 men and 20 women from Al Ain city, who were apparently healthy and had no history of kidney, liver or heart disease or diabetes. The patients who were studied were from Al Ain and Dubai Hospitals. The untreated group of near-end-stage renal failure patients comprised 33 men and 15 women yet to begin any dialytic therapy. Residual creatinine clearance in this group was in the range of 10-23 ml.min-1. The second group of patients included 18 men and 10 women who had been undergoing peritoneal dialysis for between 2 and 5-months with lactate-containing exchange solutions. For this group, 1000 units of low molecular weight heparin were added to the exchange solution for each cycle. The third group consisted of 46 patients (30 men and 16 women) who for between 6 months and 2-years were on thrice-weekly maintenance hemodialysis with acetate-containing buffer and adequate heparinization using low molecular weight heparin. The mean age (± SEM) of the control group was 41 ± 1 years and for the untreated, peritoneal dialysis and hemodialysis patients 49 ± 2, 47 ± 5 and 52 ± 2 years. Ethnically, both the control group and the three groups of end-stage renal patients were composed of approximately equal numbers of Arabs and South Asians. All subjects had fasted overnight before giving a blood sample, which was anti-coagulated with Na_2EDTA and immediately placed on ice. Since raised plasma NEFA concentrations can result from in vitro lipolysis if samples are left standing or kept at room temperature, or both,[32] all blood samples in the present study were immediately chilled on ice and kept at 4°C and during centrifugation to separate plasma within 2 hours of collection. Plasma was aliquoted into several plastic microcentrifuge tubes. One aliquot was used fresh for analysis of HDL-cholesterol, and the others were immediately frozen at -80°C, until thawed out for the other analyses; once thawed, samples were not refrozen and were discarded after one day. For HDL-cholesterol determination, VLDL, IDL and LDL were first removed from plasma by polyanion precipitation with phosphotungstic acid and $MgCl_2$ followed by centrifugation. Cholesterol in the supernatant was measured by the same enzyme-colorimetric test (Roche Diagnostics, Mannheim Germany) used to determine plasma total cholesterol. Plasma triacylglycerols, free cholesterol (both Roche Diagnostics) and NEFA (WAKO Chemicals, Richmond, United States of America) were also determined enzymo-colorimetrically using kits. Cholesteryl esters and LDL-cholesterol were calculated as already described.[4] Plasma albumin was measured by binding to bromocresol green using a kit supplied by Sigma-Aldrich Co., Poole, United Kingdom Molar ratios of NEFA/albumin were calculated. Unless otherwise stated, the results from this study are presented as mean ± standard error of the mean. The Statistical Package for the Social Sciences (SPSS) Version 10.0 for Windows (SPSS, Chicago, USA) was used for all statistical analyses. The significance of the difference between mean values was assessed using the independent-samples t test. A value of $p<0.05$ was considered to be

Table 1 - Fasting plasma concentrations of lipids and lipoproteins in apparently healthy control subjects and in chronic renal failure patients either untreated or undergoing peritoneal dialysis or hemodialysis

Lipid or lipoprotein	Controls (N=56)	Untreated (N=48)	Peritoneal dialysis (N=28)	Hemodialysis (pre-dialysis) (N=46)	Hemodialysis (post-dialysis) (N=46)
Total cholesterol (mmol.L^{-1})	4.78 ± 0.14	4.68 ± 0.36	4.81 ± 0.14	4.81 ± 0.14	4.51 ± 0.15
Free cholesterol (mmol.L^{-1})	1.31 ± 0.04	1.56 ± 0.08	1.59 ± 0.12	1.59 ± 0.12	1.44 ± 0.04
Esterified cholesterol (%)	72.5 ± 0.3	64.5 ± 1.4***	66.8 ± 1.2 ***	66.8 ± 1.2 ***	67.4 ± 0.8***
LDL-cholesterol (mmol.L^{-1})	3.06 ± 0.11	3.16 ± 0.33	3.20 ± 0.29	3.20 ± 0.29	2.79 ± 0.12
HDL-cholesterol (mmol.L^{-1})	1.02 ± 0.04	0.68 ± 0.04***	0.71 ± 0.06***	0.71 ± 0.06***	0.94 ± 0.06
Triacylglycerols	1.53 ± 0.16	1.85 ± 0.13 **	1.89 ± 0.17**	1.89 ± 0.17**	1.92 ± 0.36*

LDL - low density lipoprotein, HDL - high density lipoprotein
values significantly different from the controls *p<0.05, **p<0.01, ***p<0.001

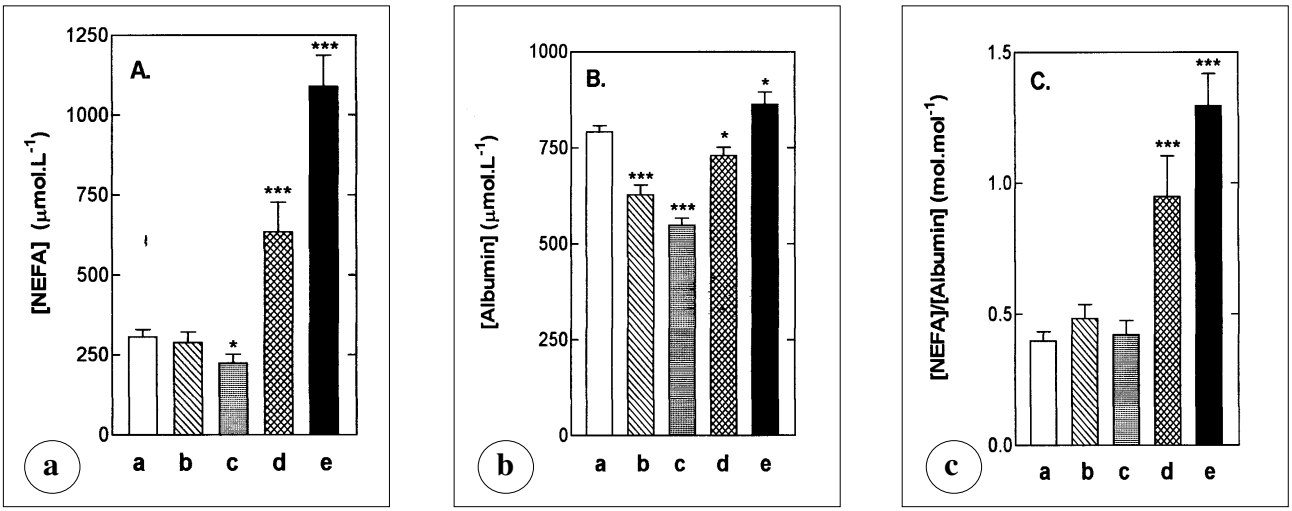

Figure 1 - Comparison of plasma concentrations of NEFA and albumin and of the NEFA-albumin molar ratio for apparently health control subjects **(a)** Chronic renal failure patients who were untreated. **(b)** Maintained by peritoneal dialysis **(c)** Or hemodialysis (pre-dialysis) or post dialysis). **(a)** Plasma concentrations of NEFA with values significantly different from controls shown *p<0.05 and values significantly different from controls and other groups of patients shown ***p<0.001. **(b)** Plasma concentrations of albumin with values significantly different from controls shown *p<0.05, *** p<0.001 **(c)** Molar ratio of NEFA/albumin with values significantly different from controls and other groups of patients shown ***p<0.001. NEFA - non-esterified fatty acids

statistically significant. Multivariate linear regression analysis was performed on all of the measured parameters and values for the Pearson correlation coefficient with p<0.05 were considered significant.

Results. The plasma concentrations of total, free and esterified cholesterol, triacylglycerols and HDL- and LDL-cholesterol for healthy subjects and for the three groups of end-stage renal disease patients are shown in **Table 1**. Compared to the controls, the plasma concentrations of total cholesterol for patients were unchanged or slightly lower and free cholesterol was higher, but significantly so only for untreated and peritoneal dialysis patients. Furthermore, the proportion of total cholesterol in the esterified form for each patient group was significantly lower than for controls as too were the concentrations of HDL-cholesterol. This was most marked for the untreated group of patients. In **Figure 1**, the plasma concentrations of NEFA and albumin and the NEFA/albumin molar ratio are compared for healthy subjects, untreated, peritoneal and hemodialysis patients. The plasma concentrations of NEFA for untreated subjects were similar to those of controls and in both were significantly higher than for peritoneal dialysis patients. By contrast, the

concentrations of NEFA, both pre- and post-dialysis, in the hemodialysis group were markedly and very significantly higher than for controls, untreated and peritoneal dialysis patients **(Figure 1a)**. Furthermore, post-dialysis NEFA concentrations were also significantly higher than pre-dialysis values. However, 9 out of the 46 individual patients studied actually showed a significant decrease in plasma NEFA following dialysis and these included 6 patients who had NEFA concentrations above 1000 μmol.l^{-1} before dialysis (not shown). In neither, the control nor the patient groups were any significant correlations apparent between NEFA concentrations and the concentrations of other lipids and lipoproteins. Plasma albumin concentrations were significantly lower than those of the controls for untreated, peritoneal dialysis and pre-hemodialysis patients, but were significantly higher than normal for post-hemodialysis patients **(Figure 1b)**. The molar ratio of NEFA/albumin reflected these differences and the ratio was significantly higher, both pre- and post hemodialysis compared to controls, untreated and peritoneal dialysis patients **(Figure 1c)**.

Discussion. The alterations in plasma lipid and lipoprotein concentrations recorded for untreated patients with end-stage renal failure and for patients undergoing either peritoneal dialysis or hemodialysis are similar to those previously noted for patients that have been studied in the United Arab Emirates and the significance of the decreased concentrations of cholesterol esters and HDL-cholesterol has been discussed.[4] The major focus of the present study, however, was to measure plasma concentrations of NEFA in patients with end-stage renal failure and to relate this to their treatment. The patient data clearly show 2 differences from the healthy controls. In the first, the patients on peripheral dialysis were shown to have slightly, but significantly lower concentrations than controls whilst untreated patients showed no difference. The second difference was the dramatically increased plasma concentrations of NEFA found in hemodialysis patients. These were measured in both pre-and post-hemodialysis samples and in both, the concentrations were not just very significantly different from those of control samples, but also from those of untreated and peritoneal dialysis patients. Previous studies have reported the rise in NEFA following hemodialysis,[26-31] but none of them seems to have documented that NEFA concentrations in these patients, before dialysis, are already twice as high on average as those of controls and non-hemodialysis patients. The most likely factor influencing NEFA concentrations in the hemodialysis patients is heparinization during the dialysis session, but this has been questioned in some studies[26,27,31] and NEFA have been shown to increase in some patients hemodialyses without heparin.[28] Certainly in the present study, NEFA concentrations increased following hemodialysis in the majority of patients, but in 9 patients, they actually decreased. Heparinization, therefore, does not seem to be the only factor involved in generating increased concentrations of NEFA in hemodialysis and it does not explain the high NEFA concentrations that were recorded in patients before hemodialysis, unless the lipases released by heparinization persist in the blood for several days. This seems unlikely since recent studies suggest that lipoprotein lipase activity peaks very soon after heparinization and then decreases rapidly to a plateau of approximately a fifth of peak activity,[33] although peak hepatic lipase activity may decrease more slowly.[34] Depletion of free carnitine in hemodialysis[29] could theoretically account for more persistent increases in plasma NEFA, but concentrations of this molecule that is required to transport fatty acyl-CoA into mitochondria are also depleted in peritoneal dialysis[35] even though the present study shows that NEFA concentrations in these patients are lower than for controls. At physiological pH, NEFA are ionized and are toxic to cells.[36] To counteract this, NEFA concentrations, both intracellularly and in the plasma, are generally kept low and NEFA are usually bound to proteins, which essentially act as buffers for them. Intracellularly, the proteins that do this are specific fatty acid-binding proteins, but in plasma, NEFA are bound to albumin. As the most abundant plasma protein, albumin has a remarkable capacity to bind all sorts of hydrophobic ligands,[37] including fatty acids. Albumin is also remarkable in that it has multiple high affinity binding sites for NEFA. There appear to be 7 binding sites for NEFA of chain length 12-18 carbon atoms,[38] but the number of binding sites decreases for NEFA as the chain length increases further.[39]

In the present study, plasma albumin concentrations were lower in untreated, peritoneal dialysis and pre-hemodialysis samples compared to controls, but were higher in post-hemodialysis samples, probably as of hemoconcentration. The molar ratios of NEFA to albumin were, therefore, also very significantly higher in hemodialysis patients both before and after dialysis. The actual ratios recorded, even post-hemodialysis, are not much greater than one mol.mol-1, and are, therefore, far short of saturation of the binding capacity of albumin. Nevertheless, the increased ratio may significantly shift the distribution of NEFA to a different set of binding sites of lesser overall affinity from those occupied at lower NEFA/albumin ratios.[19,40] This in itself may be significant, since it may make it easier for albumin to give up NEFA to tissues such as heart muscle.

In conclusion, the results of the present study have shown that in hemodialysis patients there are significant increases in NEFA concentration and in the NEFA/albumin molar ratio both before and after dialysis. Further, studies will be needed to determine what causes the increased NEFA concentrations in pre-hemodialysis patients in the absence of heparinization.

Acknowledgment. This study was supported by a research grant from the FMHS Research Committee, United Arab Emirates University. The authors would also like to acknowledge the kind help of Dr. M. Mangalore of Al Ain Hospital and Dr. M. El-Rokhaimi and Dr. M. Sulaiman, Dubai Hospital for allowing us to study patients under their care. Thanks are also due to Mr. Chandrasekharan Chathanath for expert secretarial assistance during the course of this study.

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