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THE EFFECT OF SYSTEMIC HEPARINISATION AND HAEMODIALYSIS ON PLASMA OCTADECA-9,11-DIENOIC ACID (9,11-LA′)

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Systemic heparinisation induces a sharp rise not only in plasma total free fatty acids but also in 9,11-LA′ concentration and in the 9,11-LA′/9,12-LA molar ratio. This "heparin effect" is enhanced by haemodialysis with cuprophan membranes but not with polycarbonate membranes.

KEY WORDS: Heparin, haemodialysis, diene conjugation, octadeca-9,11-dienoic acid.

INTRODUCTION

Regular haemodialysis is now well-established as a maintenance therapy for patients with chronic renal failure.[1] It involves the dialysis of blood over a 4–6 hour period 2–4 times a week. One of the complications of the procedure is a sharp decline in the numbers of circulating polymorphonuclear leukocytes within 15 min of starting the dialysis.[2] The count returns to the pre-dialysis level by 60 min. It has been suggested on considerable though not conclusive evidence that the initial fall reflects the sequestration of activated white cells in the alveolar capillaries.[3] A similar mechanism has been envisaged in the acute adult respiratory distress syndrome.[4] This has raised the possibility that increased free-radical activity may be responsible for some other immediate complications (e.g., the reduced oxygen tension).

The measurement of diene-conjugated compounds has long been recognised as an indicator of free-radical-induced damage to polyunsaturated lipids.[5] It has been shown in recent years that in human plasma, tissues and tissue fluids 95% of diene conjugation is due to octadeca-9,11-dienoic acid (9,11-LA′), a non-peroxide product of linoleic acid (9,12-LA).[6–9] A high-performance liquid chromatographic (HPLC) method has been established to measure in plasma both the diene-conjugated isomer and the parent fatty acid.[10] In the course of applying this method to patients on haemodialysis it became apparent that systemic heparinisation itself could lead to significant changes. It therefore became necessary to study the effect of heparin in non-dialysed control subjects as well as the combined effect of heparin and dialysis.

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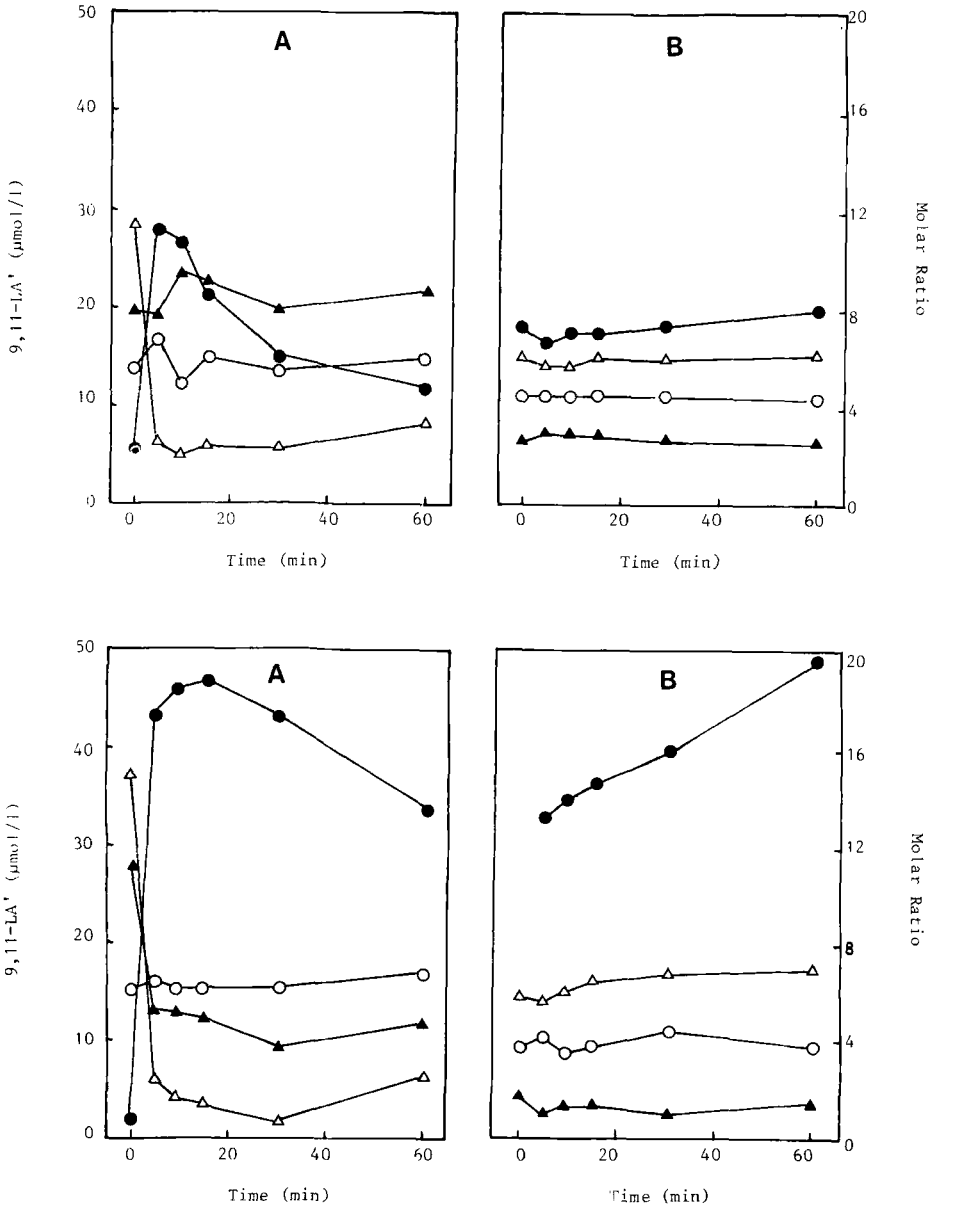
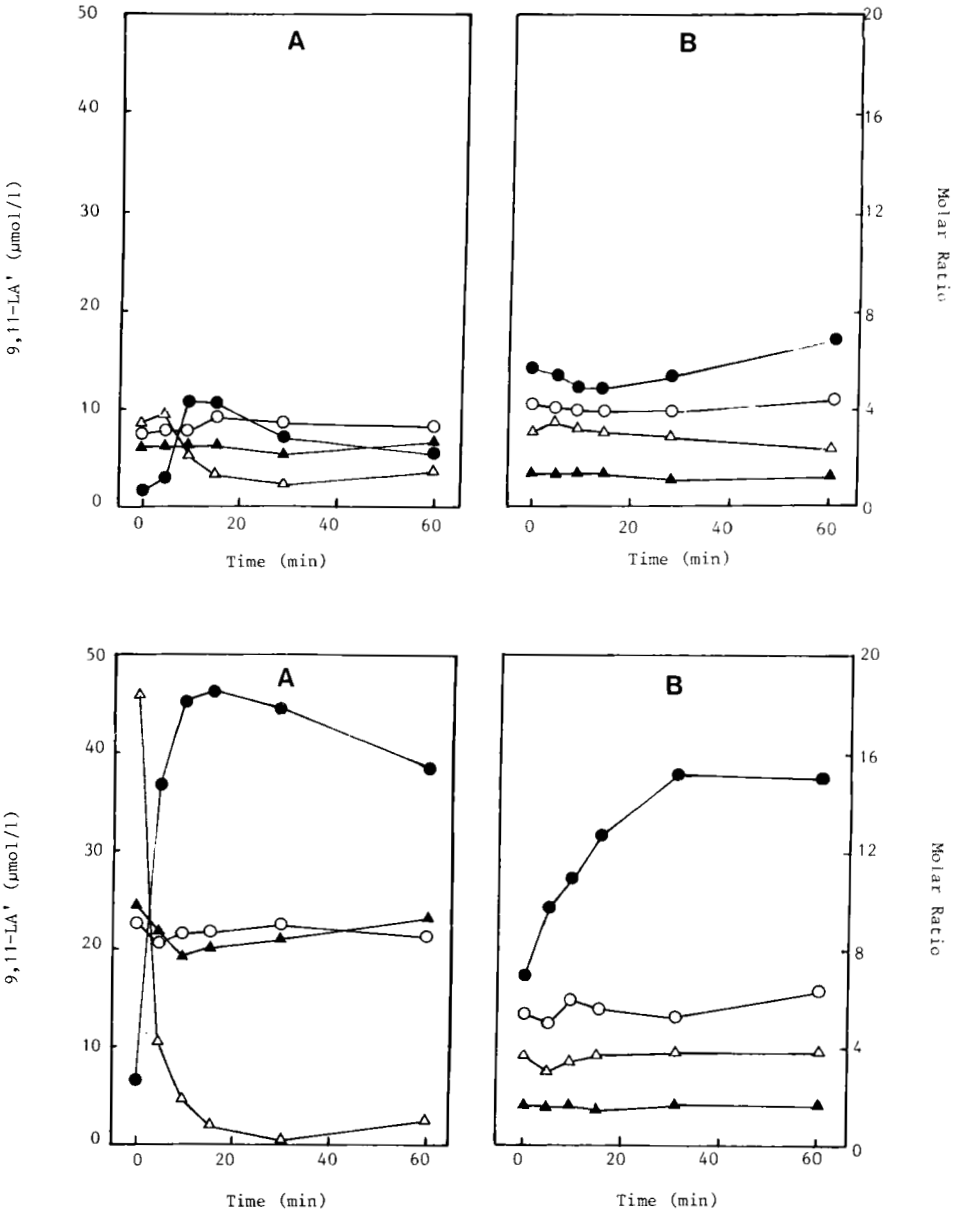


FIGURE 1 The response of the plasma 9,11-LA′ concentration and the 9,11-LA′/9,12-LA molar ratio in 4 normal subjects to a single injection of heparin, 5000 i.u. (A) Changes in the 9,11-LA′ concentration; and (B) changes in the 9,11-LA′/9,12-LA molar ratio in the four main plasma-lipid classes: (▲) esterified as phospholipids; (△) esterified as triglycerides; (○) esterified as cholesterol esters; (●) free fatty acids.



PATIENTS AND CONTROLS

The plasma 9,11-LA′ and 9,12-LA concentrations were measured and the molar ratio 9,11-LA′ × 100/9,12-LA (M%) was calculated in 51 patients (mean age 43 years) who had been on haemodialysis for chronic renal failure for several years (mean 5.8 years); and in 31 normal control subjects (mean age 28 years).

The effect of systemic heparinisation and dialysis was monitored on two occasions in 7 patients. Blood samples were collected before and 15, 30, 45, 60, 90, 120 and 240 min after starting dialysis. On the first occasion a cuprophan dialysis membrane was used (Gambro Lundia): on the second occasion a week later this was replaced by a polycarbonate membrane.

The effect of heparin alone was studied in 4 control subjects given 5000 i.u. lithium heparin intravenously. Blood samples were collected before and 5, 10, 15, 30 and 60 mir. after the injection.

METHODS

The HPLC method originally described by Iversen *et al.*[10] was designed to measure the molar concentration of 9,11-LA′ and 9,12-LA esterified as phospholipids, 9,11-LA′ and 9,12-LA present as free fatty acids (FFA) are included in this measurement; but in normal subjects and in most abnormal states previously studied the plasma concentration of FFAs is relatively low such that it can be ignored. Since this is not so in heparinised patients the method was modified by the omission of the phospholipase step to measure 9,11-LA′ and 9,12-LA in the FFA fraction. This was followed by the measurement as originally described. In the heparinisation experiments the 9,11-LA′ and 9,12-LA concentrations were also measured in the plasma cholesterol-ester and triglyceride fractions.[6] Total diene conjugation was measured spectrophotometrically as described by Wickens *et al.*[11]

RESULTS

There was no significant difference between the normal control group and patients who had been on regular dialysis for several years (Table I).

The effect of heparin alone was a sharp rise in the plasma FFA 9,11-LA′ concentration. Since the FFA 9,12-LA concentration rose less dramatically the M% also increased in this fraction. In the plasma triglycerides both the 9,11-LA′ and the 9,12-LA concentration declined. The plasma cholesterol-ester fraction showed no

TABLE I

Plasma (FFA & PL) 9,11-LA′, 9,12-LA and M% in haemodialysis patients and normal controls (mean ± SD)

	9,11-LA′ (μmol/l)	9,12-LA (μmol/l)	M%
Haemodialysis Patients (n = 51)	20.3 ± 10.1	1035 ± 309	2.1 ± 1.1
Controls (n = 31)	24.9 ± 7.4	1083 ± 319	2.1 ± 0.6

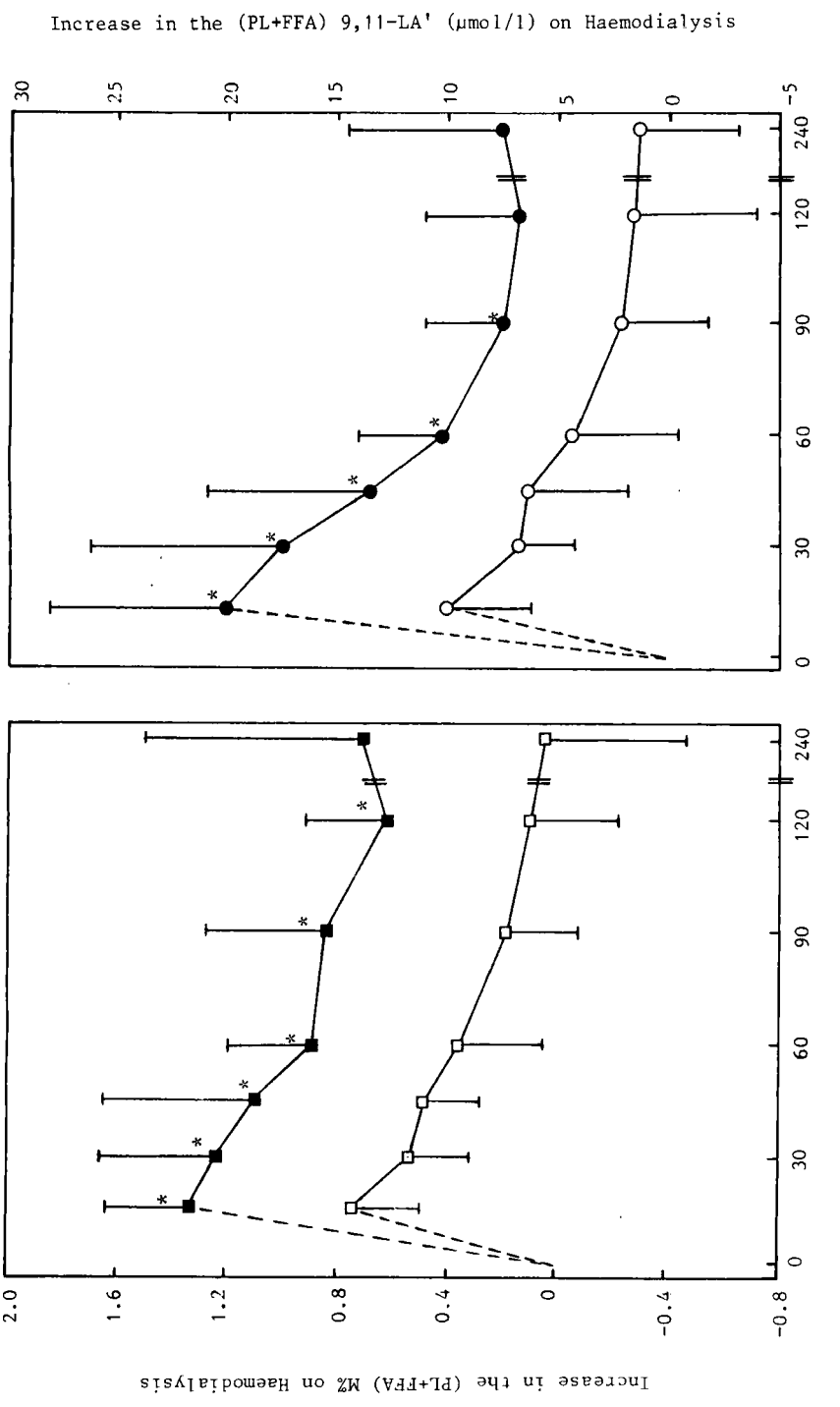


FIGURE 2 Effect of haemodialysis on the (PL & FFA) 9,11-LA′ (●, ○) and M% (■, □) on dialysis with cuprophan (●, ■) and polycarbonate (○, □) membranes in 7 patients with chronic renal failure. * indicates a statistically significant difference ($p < 0.05$) between the membrane materials. Data was analysed using a Student's paired t-test, error bars indicate 1 SD.

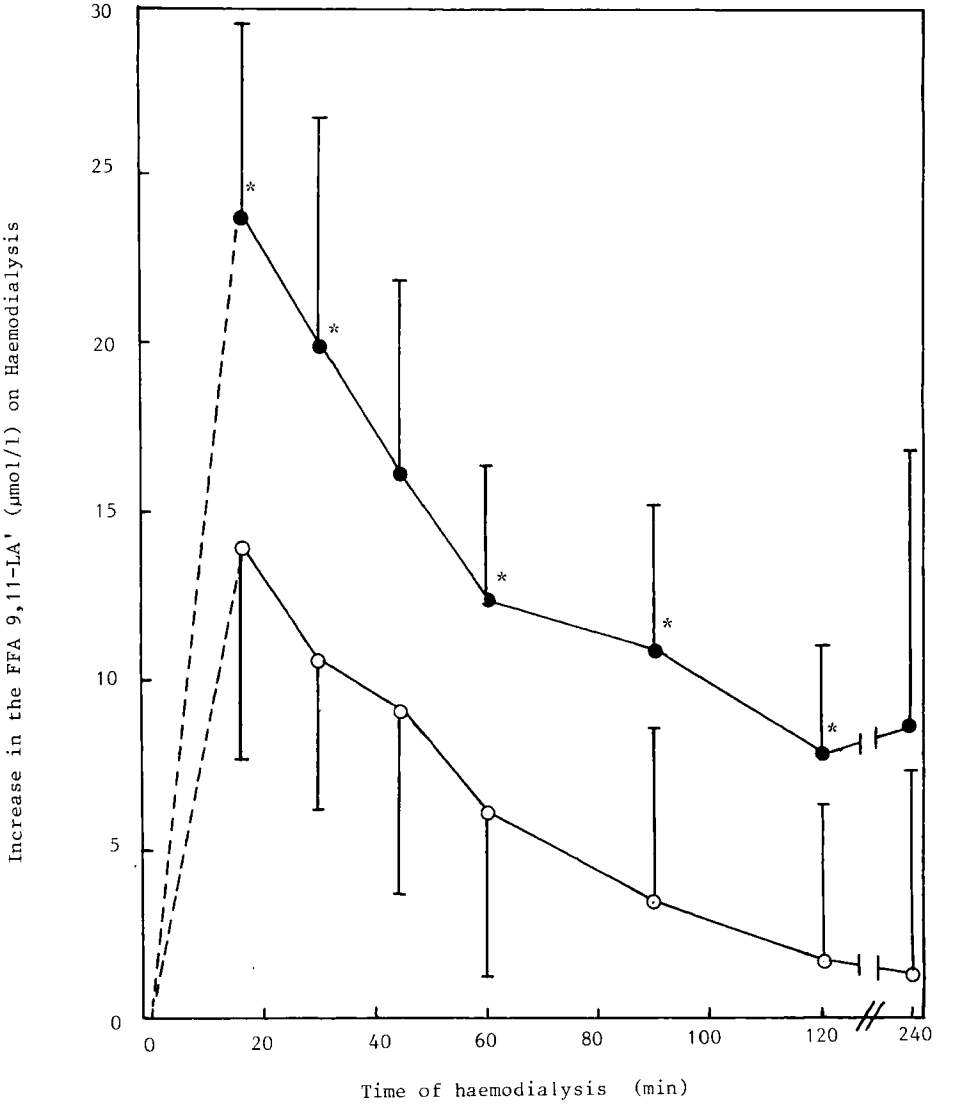


FIGURE 3 Effect of haemodialysis on the FFA 9,11-LA′ on dialysis with cuprophan (●) and polycarbonate (○) membranes in 7 patients with chronic renal failure. * indicates a statistically significant difference ($p < 0.05$) between the membrane materials. Data was analysed using a Student's paired t-test, error bars indicate 1 SD.

significant changes whilst 50% of the normals studied showed a decrease, and 50% no change in the plasma phospholipid concentrations of 9,11-LA′ and 9,12-LA (Fig. 1).

Dialysis with cuprophan membrane showed a significant increase in the "heparin effect". This was less marked with the polycarbonate membrane (Figs. 2 and 3).

None of the above changes affected the close correlation which exists between total

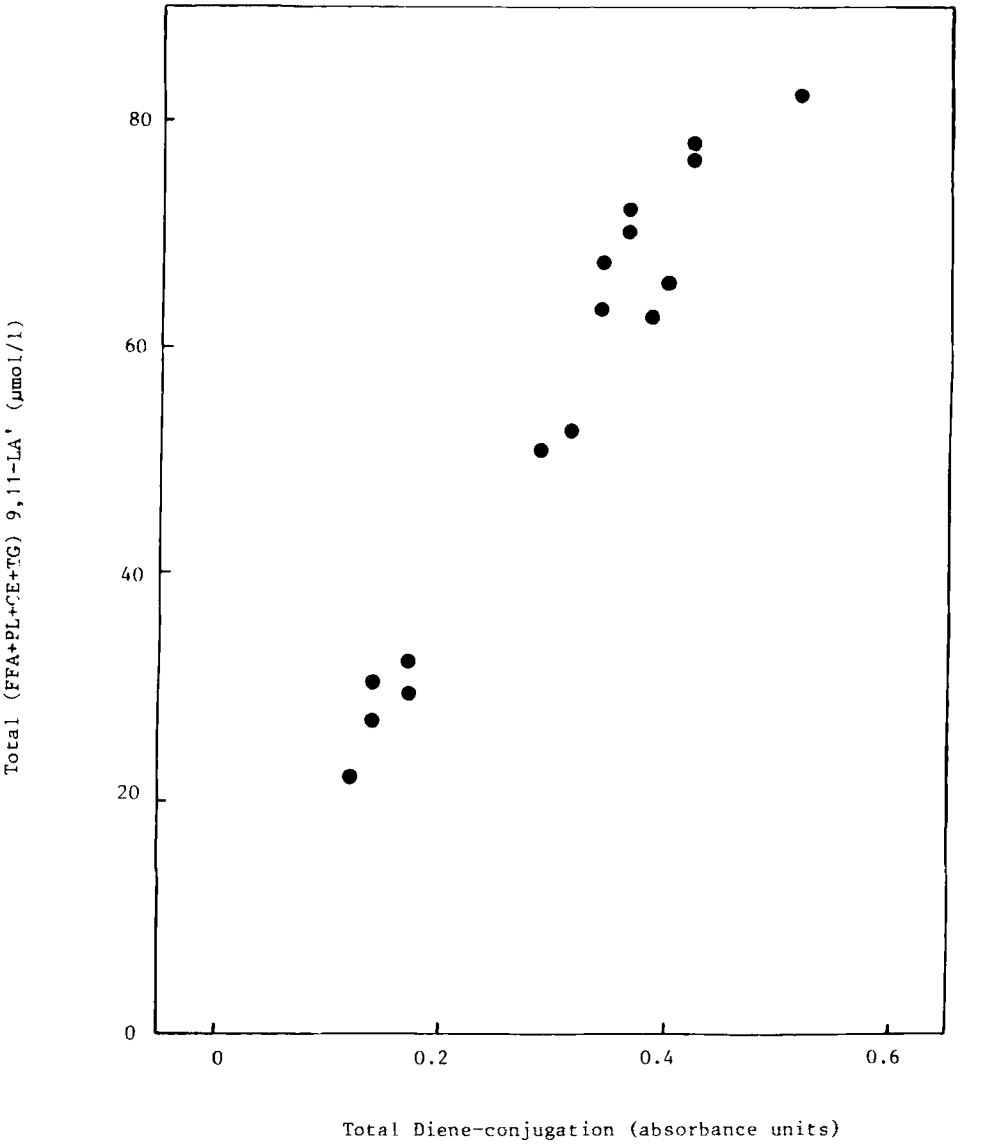


FIGURE 4 Correlation between the total diene conjugation measured spectrophotometrically (absorption at 240 nm) and the sum of the 9,11-LA′ concentrations measured by HPLC.

diene conjugation as measured spectrophotometrically and the sum of 9,11-LA′ concentrations measured by HPLC ($r = 0.962$) (Fig. 4).

DISCUSSION

Heparin is a well-recognised powerful lipolytic agent *in vivo*.[12] This is generally attributed to its activating effect on lipoprotein lipases. The action adequately ac-

counts for the sharp rise in total plasma FFAs. The sharp rise in the 9,11-LA′ concentration and in the M%, however, were unexpected. Several possible explanations may be envisaged.

1) The heparin-induced hydrolysis of triglycerides and phospholipids could entail the conversion of 9,12-LA to 9,11-LA′.

2) The ester bond of 9,11-LA′ could be hydrolysed more readily than the ester bond of 9,12-LA.

3) 9,12-LA could be more readily re-esterified (or eliminated) than 9,11-LA′. None of these effects is demonstrable when heparin is added to blood or plasma *in vitro*. However, they must be taken into account when investigating free-radical activity *in vivo* in heparinised subjects.

Several mechanisms may also account for the increased "heparin effect" during dialysis with cuprophan membranes (but not with polycarbonate membranes).

1) Blood passing over the cuprophan membrane could be sensitized to heparin.

2) An activating agent in the membrane could sensitize other tissues to heparin.

3) The release of a 'factor' by sequestrated white cells in the lung may sensitize other tissues to the action of heparin.

Although all lipid changes, including the rise in 9,11-LA′, return to normal (or near normal) by the end of each dialysis, their cumulative effect may be partly responsible for such well-recognised late complications as the increased incidence of myocardial infarction.[13]

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