

## Analysis of Free Fatty Acids in Blood of Healthy Person and that of Hepatitis Patient

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### Introduction

Polyunsaturated fatty acids (PUFA) are highly susceptible to oxidation and their levels can rapidly decrease under oxidative stress [1]. A concomitant increase in monounsaturated fatty acids such as oleic and palmitoleic acids is often observed probably as a result of the activation of  $\Delta^9$ -desaturase [1]. Analysis of fatty acid profiles is therefore of importance. Here we describe the analysis of plasma free fatty acids (FFA) only, because the measurement of fatty acids in neutral lipids, phospholipids, and total lipids has already been well documented. Plasma FFA can, furthermore, be produced by oxidatively damaged tissues because hydrolysis of phospholipids is stimulated by oxidative stress [2].

### Experimental

- Mix plasma (50  $\mu$ L) with methanol (200  $\mu$ L) containing margaric acid (12.5  $\mu$ M; internal standard) and centrifuge at 12,000 rpm for 3 min.
- Dry the supernatant (50  $\mu$ L) under a stream of  $N_2$  and mix the residue with a solution of monodansylcadaverine in  $N,N$ -dimethylformamide (2 mg/mL, 50  $\mu$ L) and with diethyl phosphorocyanidate (1  $\mu$ L).
- Stand for 20 min at room temperature in the dark.

- Analyse aliquots (5  $\mu$ L) by HPLC with fluorescence detection. Column: 3.3 cm $\times$ 4.6 mm i.d., 3  $\mu$ m, octadecylsilyl (Supelco) and 25 cm $\times$ 4.6 mm i.d., 5  $\mu$ m, pKb-100 (Supelco) in series. Mobile phase: 17.5:65.0:17.5 (v/v) acetonitrile-methanol-water. Flow rate: 1.5 ml/mL. Column oven: 40°C. Excitation: 320 nm. Emission: 520 nm.

### Calculation

Concentrations of plasma FFA can be calculated by use of the following equation.

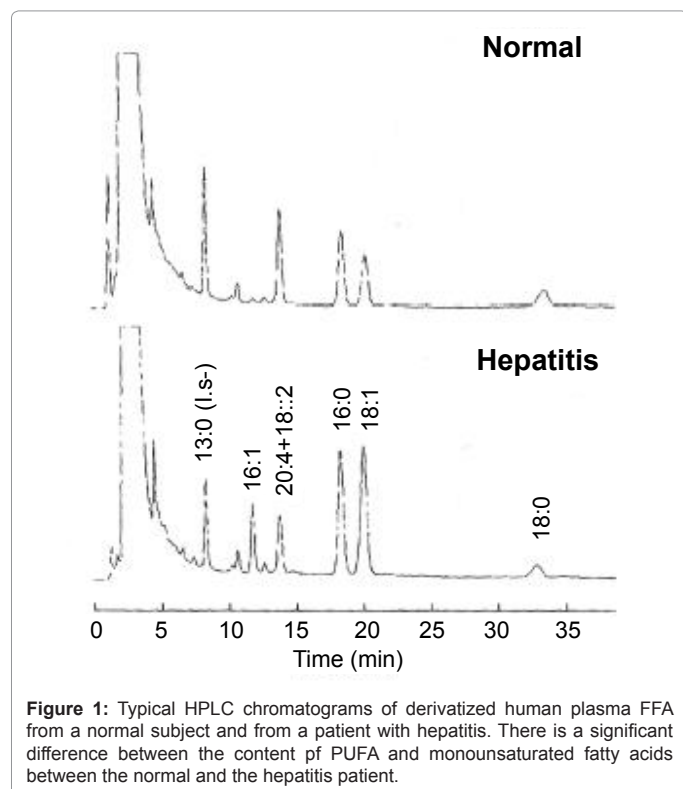
[Plasma FFA] in  $\mu$ M = (50  $\times$  peak area for FFA)/(peak area for margaric acid).

### Discussion

Only FFA in blood of healthy person and that of hepatitis patient can be measured and compared because fatty acids in phospholipids and neutral lipids are not derivatized (Figure 1).

### References

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2. Yamamoto Y, Nagata Y, Katsurada M, Sato S, Ohori Y (1996) Changes in rat plasma-free fatty acids composition under oxidative stress induced by carbon tetrachloride: Decrease of polyunsaturated fatty acids and increase of palmitoleic acid. *Redox Report* 2: 121-125.



**Figure 1:** Typical HPLC chromatograms of derivatized human plasma FFA from a normal subject and from a patient with hepatitis. There is a significant difference between the content of PUFA and monounsaturated fatty acids between the normal and the hepatitis patient.

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