Arterial and Mixed-Venous Differentials of Free Fatty Acid

— Free Fatty Acid Production in Pulmonary Circulation —

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THERE are two aspects of much interest in the study of lipid metabolism of the lung. One aspect is that alveolar stability is closely connected with a lipoprotein which interlines the alveolar surface as a surfactant. This lipoprotein seems to have a close interrelationship with lung tissue lipids and with blood lipids circulating in the lung. A review on this aspect was presented by FELTS¹ in 1964. The other aspect is that the lung might have other functions than its primary function of respiratory gas exchange. A comprehensive review of this aspect was presented recently by COMROE JR.², who tried, in his own words, "to correct the belief of some that the pulmonary circulation has one function". On the other hand, it has been postulated by many investigators that the lung has a role in lipid metabolism by trapping plasma lipids, synthesizing the tissue lipids, and hydrolyzing the circulating triglycerides,^{1,7-14} on the basis of in vitro and in vivo studies.

In this study, the aim was to investigate active fatty acid metabolism of the human lung by means of right heart catheterization. The effects of intravenous heparin injection upon the FFA difference in pulmonary circulation were studied, and the effects of an addition of linoleate to the diet was also investigated. For a better evaluation of the heparin effects upon the individual fatty acid differentials, the heparin responses of individual fatty acids of arterial FFA fraction were studied separately in systemic circulation.

MATERIAL AND METHODS

Twenty four patients cooperated in this study. The pertinent information concerning the patients is tabulated in Table I.

For the subjects who submitted to right heart catheterization, a routine diagnostic procedure was followed at first. Simultaneous sampling of arterial and mixed-venous blood was made through a catheter placed in the proximal pulmonary artery and through a Cournand's needle inserted into a brachial artery. The specimens were collected in a heparinized syringe, and the time span of a sampling was kept within 60 seconds or less for both arterial and mixed-venous samples. Postheparin samples were obtained in the same way before (zero time) and 5 or 15

TABLE I

Project number	Ages (average)	Clinical diagnosis and number of subjects	
1	15-64 (37.0)	Chronic pulmonary emphysema	2
		Bronchial asthma	1
		Mitral valvular disease	6
		Congenital heart disease	1
2	23-54 (44.3)	Congenital heart disease	1
		Uncomplicated syphilis	1
		Essential hypertension	1
		Cerebral thrombosis	1
3	19-65 (42.0)	Valvular heart disease	6
		Chronic pulmonary emphysema	3
		Essential hypertension	1
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Project No. 1	Study of arterial and mixed-venous FFA differentials before and after heparin injection.		
Project No. 2	Study of heparin response in systemic circulation of individual fatty acids of FFA fraction.		
Project No. 3	Study of arterial and mixed-venous FFA (individual fatty acids) differentials after the addition of linoleate to diet.		

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minutes after an intravenous heparin injection (20 mg of sodium heparin, Novo Industry, Copenhagen). In cases where heparin response in systemic circulation was observed, only arterial blood was collected at 0, 5, 10, and 30 minutes after heparin injection. In project 3 (see Table I) 10 grams of ethyl linoleate were added to breakfast for 3 or 4 days prior to catheterization. In the catheter studies (project 1 and 3, Table I) all blood samples were obtained 6 to 8 hours after eating.

Free fatty acid concentration was estimated by DOLE's method.⁵ Average coefficient of variation of FFA determinations in our laboratory was 3.56 per cent during the study. For the gas-chromatographic analysis of plasma FFA fraction, plasma lipids were extracted by BLOOR's procedure from 1 ml of plasma separated immediately after sampling. Column chromatographic separation was employed for FFA fraction following MCCARTHY's method.⁶ Purity of the fraction was checked by thin layer chromatography on a part of column eluents. Methylation of the separated fatty acids was performed with 2 per cent sulfuric acid in methanol, for 60 minutes at 55 degree centigrade in a warm bath with reflux. Gaschromatographic analysis was made with a Shimadzu GC-IIB gaschromatograph (Shimadzu Ind. Co., Kyoto) equipped with hydrogen flame ionization detector. Twenty five per cent di-ethylene glycol succinate polyester on

TABLE II VARIATION OF FFA DIFFERENTIALS

Differentials	before heparin	after heparin	
minimum	+ 7	+ 16	($\mu\text{Eq/L}$)
maximum	+130	+203	
mean	+ 37.6	+ 83.9	
(S.D.)	(38.5)	(55.5)	

S.D.: Standart Deviation

Shimalite, 60 to 80 mesh, in 3×2000 mm column was employed as a stationary phase.

RESULTS

Before heparin, FFA concentration of arterial plasma was higher than mixed-venous plasma (positive differential). Average FFA concentrations were 817.5 micro Eq/L in the arterial plasma and 779.9 micro Eq/L in the mixed-venous plasma giving a positive differential of 37.6 micro Eq/L. After heparin injection, this differential increased to 83.9 micro Eq/L, as average concentrations elevated to 1205.4 micro Eq/L in arterial sample and to 1121.5 micro Eq/L in mixed-venous

Fig. 1. Arterial and mixed-venous differentials in FFA concentration before (○) and after (●) heparin injection. Hatched lines show average levels of FFA concentration.

Fig. 2. Arterial and mixed-venous differentials in individual fatty acids of FFA fraction before (○) and 5 minutes after (●) heparin injection.

sample (Fig. 1. & Table II). All subjects showed positive differentials before and after heparin injection, though there were considerable variations in the degree of the differentials. The changes in arterial and mixed-venous differentials of individual fatty acids of FFA fraction at 5 minutes after heparin injection are shown in Fig. 2. At this early stage, individual fatty acids seemed to show an apparent response pattern in their differentials. Unsaturated fatty acids showed a tendency to shift up to positive differentials, and saturated acids to negative differentials as shown in the figure. At 15 minutes after heparin injection the changes in individual fatty acid differentials did not seem to show any apparent tendency, but to show a suggestively noticeable change that the initial differentials might be lost at the later stage after heparin injection.

The most marked change was observed at the early stage of 5 minutes after heparin injection also in systemic circulation. The response patterns of arterial FFA to heparin in systemic circulation are shown in Fig. 3. Some characteristic changes and facts were revealed. Generally speaking, unsaturated fatty acids increased and saturated fatty acids decreased most markedly in their percentage at this early stage. Unsaturated oleic and saturated stearic acid seemed to show a uniform response pattern, i.e. initial increase with

subsequent gradual decrease of slight degree in the former and initial marked decrease with subsequent gradual increase of moderate degree in the latter. In two cases the response pattern of linoleic acid was essentially same as that of oleic acid, but two others showed less marked changes in their increase. For palmitic acid, initial decrease with subsequent gradual increase was observed in all cases but one.

Individual fatty acid differentials after a dietary manipulation are shown in Fig. 4. Differentials of linoleic acid apparently deviated to the arterial side. The five other major acids of FFA fraction did not show such a definite tendency but seemed not to be effected, though for stearic acid there was a suggestive tendency toward negative differential.

DISCUSSION

As shown in Fig. 1, all cases dealt with in this study showed positive arterial and mixed-venous differentials in FFA concentration before and after heparin injection. However, the differentials observed here were not large enough to be accepted with validity, and additional evidences might be required to be evaluated as an established fact. Arterial and mixed-venous differentials in FFA concentration after heparin injection were also positive and were greater than before heparin (a

Fig.4. Effects of the addition of linoleate to diet upon arterial and mixed-venous differentials of individual fatty acids of FFA fraction.

ratio of 1 : 2.23). The minimum and maximum FFA differentials before heparin were plus 7 and 130 micro-Eq/L, and for after heparin injection they were plus 16 and 203 micro-Eq/L respectively. Therefore, certain contributing factors might be expected for such a variation. As listed in Table I, among 10 subjects of project No. 1, 7 cases were cardiac patients and other 3 were patients with chronic pulmonary disease. Free fatty acid differentials of cardiac patients were larger than patients with chronic pulmonary disease both before and after heparin injection (Table III), and this finding was in accord with the observations reported from our laboratory previously.^{3,4}

Heparin response of plasma FFA depends largely on plasma triglyceride concentration and the nature of plasma triglyceride carrier lipoprotein.²² However, all the subjects dealt with in this study showed no abnormal plasma triglyceride concentration (48–108 mg/dl, mean: 80 mg/dl, VANHANDEL-ZILVERSMIT's method¹⁵) and none of the plasma samples were turbid. Therefore it might be considered that substrate triglycerides of the subjects were essentially equal in nature, contributing only little to variations in arterial and mixed-venous FFA differentials after heparin injection.

The augmented arterial and mixed-venous FFA differentials after heparin seem to suggest

TABLE III ARTERIAL AND MIXED-VEINUS DIFFERENTIALS IN FFA CONCENTRATION AND UNDERLYING DISORDER

	Before heparin	After heparin
Cardiac patients*	+50.7	+95.0 (μEq/L)
Pulmonary patients**	+12.3	+42.7

* Average of 7 patients

** Average of 3 patients

strongly the increased release of FFA in the pulmonary circulation as a result of increased lipolysis which is facilitated by an injection of heparin. Such an increase in FFA production by the lung seems to be quite possible because it has been postulated with proof that pulmonary vasculature and/or the lung tissue contain lipoprotein lipase apoenzyme¹⁶ or a tissue factor⁸ which is believed to catalyze the conversion of a component in COHN's fraction IV-1 of plasma protein to a clearing factor lipase. It has been demonstrated that rabbit lung tissue has high lipolytic activity which is comparable to the activity of adipose tissue.¹⁴ Elevation of lipoprotein lipase activity in the perfusion fluid after an addition of heparin was demonstrated recently in normal fasting rabbits, and maximum activity was observed at 3 minutes after an addition of heparin to the perfusion fluid and it returned to the preheparin level within 20 minutes.¹⁷

Marked changes were observed in individual fatty acids at the early stage of 5 minutes after heparin injection both in systemic and pulmonary circulation. At this stage unsaturated fatty acids in systemic circulation increased their percentage, and in pulmonary circulation the differentials of unsaturated fatty acids tended to shift to arterial side. Contrary to unsaturated fatty acids, saturated fatty acids showed a decrease in systemic circulation and seemed to shift toward mixed-venous side in pulmonary circulation. On these findings, it is considered that a preferential release of triglyceride fatty acids takes place during early stage after heparin injection both in pulmonary and in systemic circulation probably with a common mechanism of intravascular lipolysis.

The addition of linoleate to the diet produce an increase of linoleic acid percentage both in the triglyceride fraction and FFA fraction of plasma lipids.²¹ Positive arterial and mixed-venous differentials of linoleic acid resulting from diet manipulation in this study suggest an increased release of linoleic acid in the pulmonary circulation; linoleate-rich plasma triglycerides are hydrolysed within pulmonary circulation.

On the basis of observations in this study and from evidences in the literature,¹⁸⁻²⁰ it might be concluded, tentatively at least, that the active FFA production is elaborated by the human lung, which is attributed mostly to the intravascular lipolysis in the pulmonary circulation. The meaning of the individual fatty acid response to heparin injection in pulmonary circulation, especially of the changes which appeared during later stage of lipolysis, is to be investigated further on a quantitative view point because an increase in the percentage of an acid is not to be attributed simply to the increased production of the acid.

SUMMARY

Arterial and mixed-venous differentials in concentration and individual fatty acids of free fatty acid across the human lung were investigated. The effects of intravenous injection of heparin and the addition of linoleate to the diet prior to catheter study were observed. And for the better evaluation of individual fatty acid differentials, the effect of heparin on the lipolysis in systemic circulation was studied in addition.

There are positive arterial and mixed-venous differentials (higher in arterial plasma) in free fatty acid concentration and these differentials are augmented noticeably during lipolysis facilitated by a heparin injection. Unsaturated fatty acids of

free fatty acid fraction showed a marked increase in their percentage in systemic circulation and a shift toward positive differentials in pulmonary circulation, while saturated fatty acids showed a decrease in systemic circulation and a shift toward negative differentials (higher in mixed-venous plasma) in pulmonary circulation during the early stage of the lipolysis facilitated by heparin. Addition of linoleate to the diet produced an apparent positive differential of linoleic acid of the free fatty acid fraction of plasma lipids.

These results seem to suggest strongly that active triglyceride degradation is elaborated in pulmonary circulation as well as in systemic circulation, and seem to give an evidence to the postulations based on animal experiments that the human lung might play an important role in lipid metabolism.

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