

Fractionation of Plasma Non-Esterified Fatty Acids. (22808)

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Previous studies(1,2) have shown that non-esterified fatty acids in plasma (NEFA) fall sharply after administration of glucose or insulin, and rise after an injection of epinephrine. On the other hand, fat meals caused no significant rise of NEFA, even during the phase of hyperlipemia or in the subsequent clearing of plasma. The detailed composition of this fraction has remained undefined, except for indirect evidence that it is composed mainly of long chain fatty acids loosely coupled to albumin. Chromatographic analysis, used in the present study, shows that palmitic and oleic acids predominate in NEFA, as they do in plasma triglycerides and adipose tissue(3), and that fall in concentration of these acids accounts for the main part of the fall in concentration of NEFA after injection of insulin.

Methods. NEFA was extracted from

plasma and titrated into 90% ethanol as previously described(2), then re-extracted into petrol ether (BP 50-60°C) and concentrated at 55°C under a stream of nitrogen to an acidity of 15 to 25 $\mu\text{eq./cc.}$ After addition of a small amount of C^{14} -palmitic acid* to serve as a marker, the concentrate was fractionated on an octane-methyl cellosolve-water partition column(4), and alternate tubes of collected effluent were titrated for acidity or bromine uptake(5). Since palmitic and oleic acid overlap in this fractionation, a more accurate measure of their relative proportions is obtained by preliminary bromination of the extract at -20°C , thus moving the oleic acidity to a relatively empty place in the plasma spectrum. Both methods were used.

Results. Fig. 1 shows the fractionation of plasma NEFA obtained from a normal fasting subject. Fig. 2 and 3 show the changes in

TABLE I. Distribution of Fatty Acids in NEFA before and after Injection of Insulin.

Time (min.)	Plasma sample	NEFA ($\mu\text{Eq/l}$)	Relative proportions (%)			
			Stearic	Palmitic	Oleic	Not identified
-2	1	1068	6	34	54	6
0			(Insulin, 0.1 u/kg, inj. i.v.)			
45	2	527	8	36	54	2
90	3	468	10	29	42	19

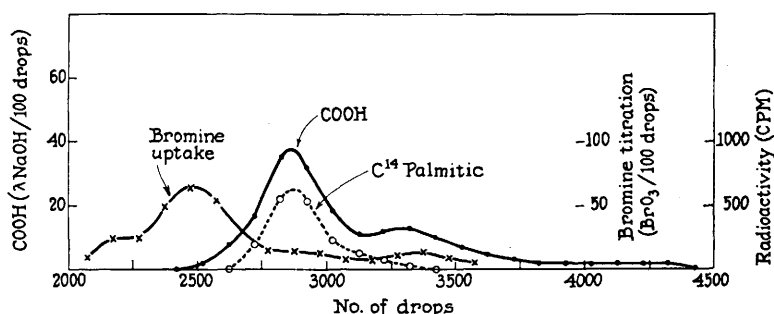


FIG. 1. Chromatographic fractionation of plasma extract. Titration of acidity (●) and uptake of bromine (×). The C^{14} -palmitic acid, added as a marker (○), contributed negligible acidity. The two partially separated peaks of acidity appear to represent palmitic and oleic acids.

* Obtained from Tracerlab, Inc., Boston.

pattern after intravenous injection of insulin. The total NEFA and proportions of major fatty acids in one experiment are given in the table.

Summary. Palmitic and oleic acids are the predominant non-esterified fatty acids of human plasma. Their concentrations fall proportionately after an injection of insulin.

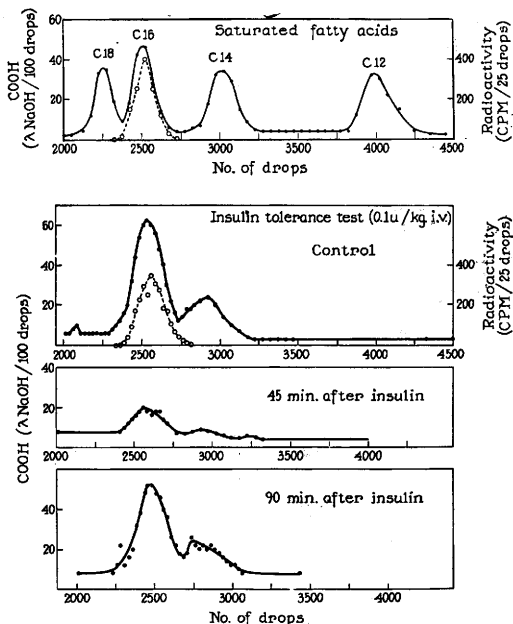


FIG. 2. Fractionation of plasma taken before and after an inj. of insulin. The upper curve shows a calibration of the column with 4 standard saturated fatty acids (solid curve) and a C^{14} -palmitic acid marker (dotted curve).

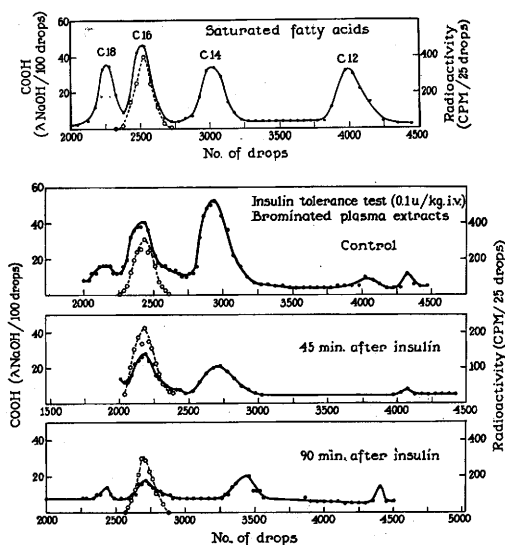


FIG. 3. Same kind of exp. as that shown in Fig. 2. The extracts were brominated before chromatography in order to increase the polarity of oleic acid and thus move the peak away from that of palmitic acid.

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