Relationship of Whole Blood Clotting Time to Physiological Variations in Circulating Saturated Free Fatty Acids.* (30245)

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(Introduced by Roslyn B. Alfin-Slater)
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Saturated long chain fatty acids when added in minute amounts to blood *in vitro* (1) or given intravenously(2) have been shown to have a profound procoagulant effect; in the latter case, death occurs as a result of intravascular thrombosis. Furthermore, when rabbits were given huge doses of ACTH, the ensuing elevation of plasma free fatty acids (FFA) was associated with evidence of intravascular hypercoagulability(3). The physiological significance of these interesting observations has been uncertain because FFA are thought to exist in the blood stream at all times(4) bound to albumin, and in that state the saturated acids have no effect on clotting(5,6).

The present study was initiated to determine whether any alteration in rate of clotting could be found in a given individual when concentration of saturated FFA was reduced from the relatively high fasting level by physiological means—*i.e.*, by ingestion of carbohydrate. This study is an extension of previous work(7) in which no correlation between whole blood clotting time and serum saturated FFA levels was found in a large group of elderly men in the fasting state.

Methods. The 9 subjects were adult human volunteers in good health (age range: 20-50 years; 7 men and 2 women). On each of 2 mornings, usually one week apart, venous blood was obtained after an overnight fast. On one of the 2 days the subject was given 50 g dextrose dissolved in 360 ml of unsweetened grapefruit juice, and on the other day he received 360 ml of water. A second blood sample was obtained 90 minutes later from the other arm. The intake of the subject was unknown to the investigator when the whole blood clotting times were done.

Whole blood clotting time. After a careful venipuncture in the antecubital fossa, blood was obtained under gravity flow without stasis through a #18 siliconized needle to which was attached a plastic adaptor and a 4″ length of polyvinyl tubing. At least 10 ml of blood for other purposes were collected through this system before blood for clotting times was taken. One ml of blood was then introduced directly into each of 4 new 12 × 75 mm calibrated tubes of polystyrene. The 4 tubes were used as duplicate sets, each set consisting of a pair of tubes. The tubes were placed into a water bath at 37°C immediately after collection. After an interval of 10 minutes, the first tube of each set was tilted just sufficiently to determine fluidity. This was repeated every minute until the tube could be inverted without flow of blood, after which the second tube was tilted in a similar manner. The clotting time as reported here refers to that of the second tube. Of the duplicates, the set with the longer final time is used. As reported previously(7), standard error of measurement in comparison of such duplicate pairs is ± 2.4 minutes.

Free fatty acid analyses. Concentration of total FFA in serum was determined in duplicate by the Schotz modification(8) of the method of Dole. FFA were also isolated from a heptane-isopropanol extract of serum lipids by thin-layer chromatography and converted to their methyl esters by the method of Metcalfe and Schmitz(9). Relative abundance of the individual FFA of each sample was determined by gas-liquid chromatography (GLC) and serum concentrations of individual FFA were calculated. Saturated FFA concentration refers to the sum of concentration of palmitic (16:0) and stearic (18:0) acids. This sum comprised 33.5% and 39.8% of

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TABLE I. Saturated FFA Concentrations and Whole Blood Clotting Times, Fasting and After Carbohydrate.*

	Fasting	After CHO	Fasting	After water
Saturated FFA conc (μEq/l)	152 (±30)	77 (±25)	159 (±24)	192† (±87)
Whole blood clotting times (min)	31.9 (±10.9)	30.3 (±9.2)	30.7 (±4.8)	34.2 (±11.0)

* Data are given as mean, with standard deviation in parentheses.

† Mean and standard deviation include one atypically high value, 427 μEq/l. When this value is omitted, mean equals 162.4 μEq/l and standard deviation, ±27.6.

total FFA in the fasting state and after carbohydrate, respectively. Myristic acid (14:0)—the only other saturated FFA that appeared in serum—was present infrequently and then, in small amounts. Details of these procedures are described elsewhere(7).

Results. Although the saturated FFA concentration was reduced 50% by carbohydrate, the whole blood clotting times were identical (Table I). The slight increase of saturated FFA concentration after water was not significant. Fig. 1 shows the data analyzed as paired observations(10). Each bar is a plot of the mean of individual changes. Thus, the small negative value for whole blood clotting times in the carbohydrate experiment indicates, if anything, that clotting tended to be shorter after carbohydrate, when the saturated FFA concentration was reduced by approximately 75 μEq/l. Similarly, clotting times after water tended to be longer, although the saturated FFA concentration was slightly higher. The changes in clotting times did not reach statistical significance. The change in saturated FFA concentration after carbohydrate is highly significant.

Discussion. Saturated FFA are thought to accelerate clotting by activation of Hageman factor (factor XII)(1) and/or of plasma thromboplastin antecedent (factor XI)(11). Recently, it has been shown that certain FFA also induce platelet agglutination *in vitro* (12) and thrombocytopenia when given intravenously(13). However, fatty acids most active on platelets are relatively inactive in accelerating coagulation. The reactions of clotting in which Hageman factor and plasma thromboplastin antecedent are involved are especially prolonged when clotting is measured without glass contact. It may be expected, then, that whole blood clotting time without glass contact would serve as an adequate test for activating effects of FFA. More pertinent is the fact that the *in vitro* effects of saturated FFA upon whole blood clotting times are as dramatic as the effects seen by any other test of coagulation.

The findings of the present study indicate that within the range of physiological variations of saturated FFA concentration encountered here, whole blood clotting time is not altered. This observation suggests that saturated FFA native to the blood behaves differently in the clotting mechanism than saturated FFA added to it. Many of Connor's experiments(1) with the Chandler wheel involved addition of relatively large amounts of sodium acylates, raising plasma concentrations by approximately 500 μEq/l (assuming that the added material all remained in the plasma). However, he obtained the same profound shortening of thrombus-formation time when the plasma level was raised by about 100 μEq/l, and some effect when it was raised by only 5 μEq/l. The FFA concentration in the original blood is not known, but since it was post-prandial

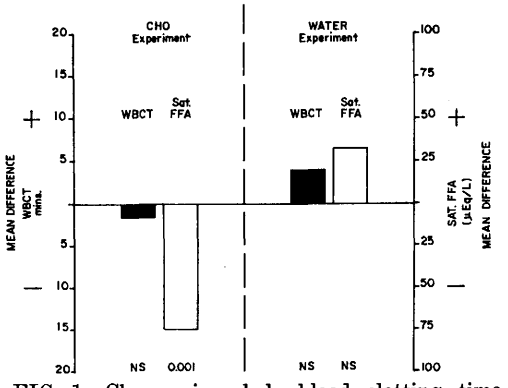

FIG. 1. Change in whole blood clotting time and serum saturated FFA concentration, from fasting, after carbohydrate and after water. Data are analyzed as paired observations (see text). Mean difference of saturated FFA in water experiment includes one atypically large value 258 μEq/l.

blood its plasma FFA concentration was presumably rather low.

Similarly, we have reported experiments (7) in which sodium stearate was added to post-carbohydrate blood to increase plasma saturated FFA concentrations by 44 μEq/l from a mean starting level of 61 μEq/l. Clotting time fell from a mean value of 30 minutes to 11 minutes, the lowest possible result by the procedure used. The experiments described here were done fasting and post-carbohydrate; however, they may be legitimately viewed in reverse, as reflecting the effect of a fast which added 75 μEq of saturated FFA per liter to the post-carbohydrate level of 77 μEq/l. The major finding in the present study is that this physiological increment of 75 μEq/l has no effect on clotting whereas addition of only 44 μEq/l as sodium salt(7) had a profound effect. Preincubation of saturated FFA with albumin abolishes their clotting effects (*vide supra*). Therefore, it is reasonable to conclude that the albumin-bound state of saturated FFA native to the blood prevents activation of coagulation during physiological mobilization of fat from adipose tissue.

The possibility cannot be excluded that the FFA-albumin bond may be altered under some pathological conditions so that saturated FFA may activate coagulation. This may be the explanation for the findings, cited above, in ACTH-toxic rabbits. It is theoretically possible that FFA may be dissociated from albumin locally in the vascular tree, under abnormal conditions, in sufficient quantities to promote thrombosis. Although this cannot be excluded, it remains to be demonstrated. However, the results of the present study render it unlikely that physiological fluctuations in FFA concentrations induce significant changes in coagulation.

Summary. Whole blood clotting times without glass contact and saturated free fatty acid concentrations of serum were determined in 9 normal human subjects in the fasting state and 1½ hours after ingestion of dextrose. The mean concentration of saturated free fatty acids after carbohydrate fell to one-half the fasting value. The mean whole blood clotting times did not change.

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1. Connor, W. E., J. Clin. Invest., 1962, v41, 1199.
2. Connor, W. E., Hoak, J. C., Warner, E. D., ibid., 1963, v42, 860.
3. Hoak, J. C., Poole, J. C. F., Robinson, D. S., Circulation, 1963, v28, 660.
4. Fredrickson, D. S., Gordon, R. S., Jr., Physiol. Rev., 1958, v38, 585.
5. Connor, W. E., Poole, J. C. F., Quart. J. Exp. Physiol., 1961, v46, 1.
6. Hoak, J. C., Connor, W. E., Warner, E. D., Fed. Proc., 1962, v21, 60.
7. Tompkins, M. J., Dayton, S., Pearce, M. L., J. Lab. Clin. Med., 1964, v64, 763.
8. Schotz, M. C., Masson, G. M. C., Page, I. H., Proc. Soc. Exp. Biol. and Med., 1959, v101, 159.
9. Metcalfe, L. D., Schmitz, A. A., Analyt. Chem., 1961, v33, 363.
10. Dixon, W. J., Massey, F. J., Jr., Introduction to Statistical Analysis, McGraw-Hill, New York, 1957, 124.
11. Botti, R. E., Ratnoff, O. D., J. Clin. Invest., 1963, v42, 1569.
12. Shore, P. A., Alpers, H. S., Fed. Proc., 1963, v22, 504.
13. Zbinden, G., J. Lipid Research, 1964, v5, 378.

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