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Correlation Between Release of Individual Free Fatty Acids and Fatty Acid Composition of Adipose Tissue.* (31381)

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The control of free fatty acid (FFA) release from adipose tissue has been the subject of many studies. Much less attention has been given to the factors influencing the individual FA composition of the mobilized lipid from adipose tissue. The present study attempted to analyze the quantitative relationship between the individual FA released from adipose tissue when incubated *in vitro,* and the total FA composition of the same tissue.

Materials and methods. Young (50-100 g) and adult (more than 400 g) male rats of the Wistar strain were used after an overnight fast under ether anesthesia. Seven experiments were performed, 3 employing adult, and 4 using young rats. Epididymal fat pads of the adult rats were removed, divided into approximately equal parts, and used for the various incubation vessels. Fat pads of several young rats were pooled and aliquots from this pool were incubated.

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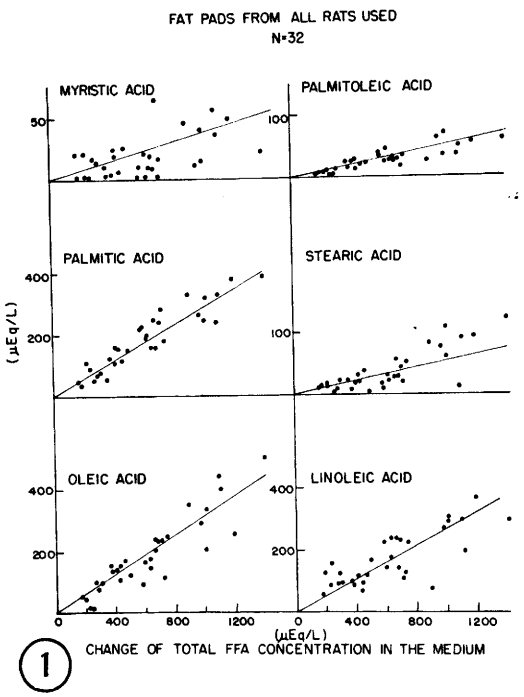
Tissues were incubated according to the method of Gordon and Cherkes(1) in Krebs-Ringer phosphate buffer at pH = 7.3-7.4 in the presence of 5% bovine albumin. No glucose or hormones were added to the incubation medium. In 2 experiments, half of the tissues were incubated in the presence of albumin previously treated according to Goodman(2) to remove most of the FFA. In the other half untreated bovine albumin was used. The results obtained with young or adult rats were very similar, as were the ones using untreated or pre-extracted albumin. Therefore data of all experiments were pooled.

To supply a wide range of concentration of the released FFA without employing hormones, in most of the experiments tissue aliquots from the same pool were incubated separately for 15, 45, and 90 minutes. At the beginning and end of incubation, FFA content of the medium was determined by the method of Dole and Meinertz(3) and the



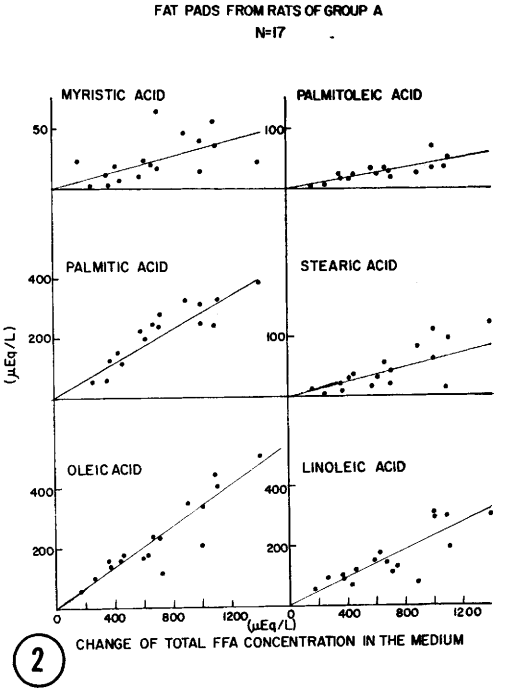


FIG. 1. Correlation between FFA released in incubation medium and concentration of individual fatty acids. All experiments are included in this Figure.

FIG. 2. Correlation between FFA released in incubation medium and concentration of individual fatty acids. Only experiments of Group A are included.

released FFA calculated. The composition of the released FFA and that of the total FA of adipose tissue were analyzed by gas-liquid chromatography(4).

Results. In Fig. 1, the concentration of each of the 6 individual FFA is plotted against the respective total FFA concentrations in the medium. The regression lines appear to be linear for these components.

In an effort to relate the rate of release of any one of the individual FA to the relative concentration of the same acid in adipose tissue, the relative rate of release was determined for each acid employing the following formula:

$$\frac{\text{slope of regression of one particular FA}}{\text{sum of slopes of regression for all FFA}} \times 100.$$

The relative rates of release thus obtained were then compared to the percentage composition of FA in the adipose tissue. This comparison is indicated in Table I.

In examining the data of FA composition

TABLE I. Individual Fatty Acid Release and Fatty Acid Composition of Rat Adipose Tissue (*in vitro* Study).

Fatty acids	Slopes (%) of individual FFA regression lines	Fatty acid composition of adipose tissue mean % & range
C14=0	3.1	2.7 (.1~ 7.2)
C16=0	28.3	26.5 (21.2~32.7)
C16=1	5.0	5.1 (.8~ 8.8)
C18=0	5.2	4.6 (1.4~ 7.5)
C18=1	31.4	32.4 (25.3~39.9)
C18=2	27.0	28.6 (16.8~39.8)
	N=32	N=32

of adipose tissue, a fairly wide range was noted for most of the individual FA (as can be observed in Table I). This was considered to be an advantage under the present experimental conditions and was one of the reasons for employing the several variables in the experimental design (*i.e.*, young and adult rats, untreated and pre-extracted albumin, various incubation times). The widest range was observed in linoleic acid content. Therefore the data were subdivided into 2 experimental groups: Group A containing

TABLE II. Individual Fatty Acid Release and Fatty Acid Composition of Rat Adipose Tissue (*in vitro* Study).

Fatty acids	Slopes (%) of individual FFA regression lines		Fatty acid composition of adipose tissue mean % & range	
	Group A	Group B	Group A	Group B
C14=0	3.5	2.5	3.1 (.2~ 7.2)	2.3 (.1~ 4.9)
C16=0	28.5	27.5	27.6 (22.1~32.7)	25.2 (21.2~31.6)
C16=1	4.0	5.0	4.7 (.8~ 6.6)	5.6 (3.3~ 8.8)
C18=0	6.1	5.2	5.2 (3.0~ 7.5)	3.9 (1.4~ 6.8)
C18=1	34.2	26.1	35.7 (27.4~39.9)	28.7 (25.3~34.8)
C18=2	23.2	33.7	23.7 (16.8~28.4)	34.2 (29.2~39.8)
	N=17	N=15		

adipose tissues with lower than the mean (28.6%) linoleic acid content, and Group B containing those with higher linoleic acid content than the mean for the whole group. This separation also subdivided adipose tissues with regard to their oleic acid content. All tissues from Group B had lower oleic acid content than the mean for Group A (35.7%) and all but one tissue from Group A had higher oleic acid content than the mean for Group B (28.7%). The concentrations of the 6 individual FA were plotted against the respective total FFA concentrations in the medium for each subgroup, as shown in Fig. 2 and 3. Rate of release of the individual FFA was calculated and the 2 subgroups compared on this basis (Table II). A close correspondence is shown in both subgroups between rate of release of an individual fatty acid and its content in adipose tissue.

Discussion. The dissimilarity of individual FFA composition of the plasma and total FA composition of adipose tissue has puzzled many investigators(5-8) and raised the question of whether the release of FFA from adipose tissue is a random process, or a release favoring certain individual acids.

The latter view was expounded by Hollenberg(7,9) based on data obtained from incubated rat epididymal fat pads in the presence of hormones that are known to enhance lipase activity. Under those conditions, the ratio of the amount of each FFA in the medium to that in the tissue varied depending on chain length and saturation of the FA. These ratios showed similar trends for each individual FFA (with the exception of stearic acid) between 1 and 3 hours of incubation. Due to different ways of analyzing

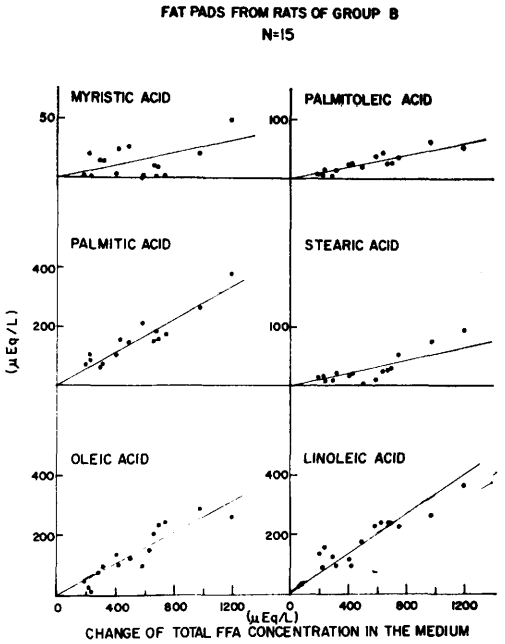


FIG. 3. Correlation between FFA released in incubation medium and concentration of individual fatty acids. Only experiments of Group B are included.

the data and to the presence of lipase stimulators, these results cannot be easily compared to those of the present communication.

Meinertz(10) observed a differential release of individual FFA from rat adipose tissue in the presence of epinephrine. The ratio of palmitic acid to oleic in the tissue did not change during 2 hours of incubation (11), but their relative distribution between released FFA and tissue glycerides was different. Oleate accumulated in the tissue diglycerides, while the released FFA mixture was low in oleic acid(11). Meinertz has also shown that "as lipolysis continues, the pat-

tern becomes quite similar to that of bulk lipids in the tissue"(12). Because of the presence of epinephrine and the unavailability of detailed data, it is difficult to compare the results of the above abstracts with those of the present studies. The presence of lipase stimulators is known to enhance reesterification in adipose tissue(13), a process that may show rate-differences regarding the different individual fatty acids(9).

Stein and Stein(14) using an *in vivo* incubation technique found that fasting induced mobilization of palmitic, oleic and linoleic acids at the same rate. Adipose tissue fatty acid composition remained the same. FA composition of adipose tissue was also found to be unaltered during starvation(8,14,15).

It has been demonstrated recently(16) that in patients the relative rate of release of each individual FFA depends on its respective concentration in the adipose tissue. Using similar methods of analysis for the present data, obtained under *in vitro* conditions, yielded the same conclusion, *i.e.*, rate of individual FFA release correlates well with the respective concentrations of the individual acids in adipose tissue. Two aspects of the employed methodology are worth mentioning: a) no hormone-stimulus was employed, yet a considerable range of medium FFA levels was elicited by varying the length of incubation, and b) although duration of incubation was varied, very short periods (less than 15 minutes) or very long periods (more than 90 minutes) were avoided. This was done to eliminate as much as possible the interference of the process of equilibration between tissue FFA and medium FFA, on the one hand, and to exclude the process of equilibration of released FFA with that of the tissue acids on the other hand. The latter process is likely to take place after the more active binding sites of albumin become almost saturated. Similar conclusions were reached from unpublished data obtained in dogs after administration of catecholamines, where the rate of release of the individual FFA was found to be proportional to the FA composition of adipose tissue.

Summary. Rat epididymal fat pads from young and adult rats were incubated for varying lengths of time without addition of glucose or hormones to the incubation medium. Fatty acid composition of medium and adipose tissue was determined and rates of release of the 6 major individual fatty acids were compared to their respective concentrations in the adipose tissue. The rate of release of each individual fatty acid was proportional to the concentration of the same acid in the adipose tissue.

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