Effect of Odd and Even Numbered Medium Chain Fatty Acids on Glucose Uptake by Adipose Tissue[1] (34276)

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(Introduced by William H. Sebrell, Jr.)

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Whereas studies of the influence of fatty acids on glucose uptake by muscle have been made (1–3), few are available as regards adipose tissue. Enhanced uptake of glucose by rat epididymal fat pad in the presence of high levels of long chain free fatty acid has been demonstrated and attributed to increased incorporation of fatty acid into new tissue triglyceride of which the glycerol moiety is derived from glucose (4, 5). The effect of medium chain fatty acids (MCFA) on glucose uptake by adipose tissue has not been investigated. Previous work has shown that infusion into animals of certain MCFA induces striking hypoglycemia (6, 7), and that even and odd numbered MCFA can be incorporated into adipose tissue triglycerides *in vitro* (8) and *in vivo* (9). This study reports the effect of even and odd numbered MCFA on glucose uptake by rat adipose tissue using an epididymal fat pad incubation system.

Materials and Methods. Animals. Male Wistar rats fed Purina chow diet *ad libitum* were used. For each experiment, five animals weighing 120–160 g were decapitated and their epididymal fat pads were separated and washed in oxygenated Krebs–Ringer–bicarbonate buffer. Pieces, each weighing approximately 7–12 mg, were excised from the peripheral parts of fat pads and added directly to the incubation medium so that all flasks contained one piece of tissue from each animal.

Incubation medium. The basic medium was Krebs–Ringer–bicarbonate buffer to which 165 mg/100 ml of *d*-dextrose and 250 mg/100 ml of fatty acid-poor bovine albumin[5] were added. The albumin was dialyzed for 12 hr against distilled water before use. The contribution by albumin of fatty acid to the medium was less than 0.03 μeq/liter. A stock solution of crystalline beef insulin[6] of low glucagon content (less than 0.0003%) was freshly diluted on the morning of each experiment with Krebs–Ringer albumin glucose (KRAG) buffer to yield concentrations up to 100 μU/ml.

Sodium salts of MCFA (octanoate,[7] nonanoate[8] and decanoate[7]) and a three-carbon fatty acid, propionate,[7] were analyzed by gas liquid chromatography and were found to be 98–99% pure. These were added in appropriate amounts to the incubation medium.

Incubations. The basic technique of incubation by Steelman *et al.* (10) was used. Adipose tissue segments were incubated for 3 hr in flasks containing 2 ml of KRAG buffer in a Dubnoff metabolic shaker under 95% O_2 and 5% CO_2, at pH 7.4 and temperature 37°. The fatty acids in concentrations of 1600 μeq/liter were tested in this system to determine their effect on glucose uptake by adipose tissue. In each experiment duplicate samples containing fatty acids and insulin (0–100 μU/ml) were compared with duplicate controls using the same buffer and insulin concentrations. Also, varying concentra-

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TABLE I. Effect of Even and Odd Numbered Fatty Acids (Octanoate, Nonanoate, Decanoate, and Propionate) on Glucose Uptake (mg of glucose per g of wet tissue/3 hr) with and without Insulin, by Rat Epididymal Fat Tissue.[a]

Insulin (μU/ml)	(μeq/liter)							
	Octanoate		Nonanoate		Decanoate		Propionate	
	0	1600	0	1600	0	1600	0	1600
0	3.7 ± 0.4	3.3 ± 0.7	4.5 ± 0.4	7.1 ± 0.5	4.2 ± 0.4	3.9 ± 0.3	4.1 ± 0.4	3.9 ± 0.6
	NS		$p < 0.0005$		NS		NS	
10	5.1 ± 0.7	5.8 ± 0.9	5.3 ± 0.4	7.9 ± 0.6	4.9 ± 0.4	4.3 ± 0.6	4.7 ± 0.7	4.8 ± 0.3
	NS		$p < 0.005$		NS		NS	
50	7.6 ± 0.6	7.5 ± 0.9	8.6 ± 0.6	11.7 ± 0.9	7.8 ± 0.5	6.3 ± 1.1	8.3 ± 0.8	8.0 ± 0.7
	NS		$p < 0.005$		NS		NS	
100	8.7 ± 0.5	8.3 ± 0.7	9.9 ± 0.9	12.3 ± 0.8	9.7 ± 0.9	6.9 ± 0.6	9.3 ± 0.8	9.0 ± 1.1
	NS		$p < 0.025$		$p < 0.025$		NS	

[a] For each set of experiments, the effects of simultaneously run controls were compared statistically with those of their respective fatty acids. Data are expressed as mean $\pm$ SE for sets of 9, 9, 6, and 4 experiments for each of the four fatty acids, respectively.

tions (400–3200 μeq/liter) of nonanoate, octanoate, and propionate were tested as regards their effect on glucose uptake in the absence of insulin. Validity of each assay was judged by the slope of the control glucose uptake curve. The glucose uptake was measured by the change in glucose concentration of the medium. Glucose was determined by a ferricyanide method (11) using a Technicon AutoAnalyzer. Sensitivity of glucose measurement was increased by changing the concentration of alkaline potassium ferricyanide reagent (12).

Results. In control series of 28 experiments, the glucose uptake, expressed as milligrams of glucose per gram of wet tissue per 3 hr, was determined at various insulin concentrations. The mean $\pm$ SE glucose uptakes were as follows: 0 insulin (basal): 4.1 $\pm$ 0.2; 10 μU/ml: 5.1 $\pm$ 0.3; 50 μU/ml: 8.0 $\pm$ 0.3; 100 μU/ml: 9.4 $\pm$ 0.4. Thus, a linear relationship was established between glucose uptake and log insulin concentrations, allowing for statistical comparisons of glucose uptake in the presence of various fatty acids.

The effects of MCFA (1600 μeq/liter) on glucose uptake with and without insulin are shown in Table I. The results indicate that octanoate exerted no significant effect on glucose uptake by adipose tissue. Also, decanoate had no significant influence on glucose uptake except that at an insulin concentration of 100 μU/ml an inhibitory effect was found. The odd numbered short chain fatty acid, propionate, exerted no significant influence on glucose uptake by the adipose tissue. In contrast, the presence of the odd numbered MCFA, nonanoate, was associated with significant enhancement (Table I) of glucose uptake in the absence and presence of insulin. The nonanoate-induced enhancement of glucose uptake did not appear to be influenced by insulin, since the increment in the uptake associated with nonanoate remained constant at all concentrations of insulin tested.

The effects of nonanoate, propionate, and octanoate were tested further, without insulin, using concentrations of the fatty acids varying from zero to 3200 μeq/liter. The results (Fig. 1) show that with increasing amounts of nonanoate in the medium, there was a proportionate increase in glucose uptake by adipose tissue so that, at a concentration of 3200 μeq/liter, there was a twofold increase over base line in glucose uptake. In contrast, propionate did not influence glucose uptake significantly ($p > 0.1$) in four experiments. Also, glucose uptake in the presence of octanoate at similar concentrations did not differ significantly ($p > 0.1$) from that of

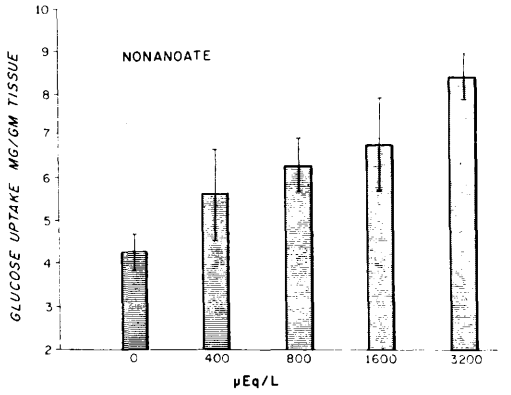

FIG. 1. Effect of varying concentrations of nonanoate ($N = 8$) on mean ± SE glucose uptake by rat epididymal fat tissue. The enhancement of glucose uptake in the presence of nonanoate is directly proportional to the amount of fatty acid in the medium.

simultaneously run controls in five experiments.

Discussion. The results indicate that the MCFA differ appreciably with respect to their effects on glucose uptake by adipose tissue. Octanoate, decanoate and the short chain propionate did not have an enhancing effect on glucose uptake either in the presence or absence of insulin. In contrast, nonanoate had a significant enhancing effect on glucose uptake which appeared to be independent of insulin. Moreover, glucose uptake was directly proportional to the concentration of nonanoate in the medium. The mechanism of nonanoate-induced enhancement of glucose uptake is not clear, although certain inferences can be made. One possibility may relate to the glycerogenic potential of this fatty acid via its oxidation to propionate. Recent studies indicate that adipose tissue is endowed with an enzyme system capable of glyceride glycerol synthesis from pyruvate by way of the dicarboxylic acid shuttle (13). The propionate moiety of nonanoate could be expected to participate in this pathway. However, other studies have shown that, in contrast to liver (14), adipose tissue is virtually devoid of several gluconeogenic enzymes (15, 16). In the present study, propionate itself had no effect on glucose uptake by adipose tissue. Thus, it is unlikely that the enhanced glucose uptake is mediated by oxidation of nonanoate to propionate. An alternative mechanism may relate to a direct nonanoate effect on glucose uptake by adipose cells. The present data do not provide evidence for this effect, since all the fatty acids used in this study are of similar molecular configuration, and are, on this basis, not likely to exert a differential influence on glucose entry into the adipose cell.

Previous studies (4, 5) showed that high concentrations of palmitate exerted an enhancing effect on glucose uptake by rat adipose tissue. This effect was attributed to greater incorporation of free fatty acids into triglycerides and therefore greater need for glucose carbons for glycerophosphate production. This mechanism would obtain if the rate of incorporation of nonanoate into adipose tissue glycerides were greater than that of octanoate or decanoate. There is evidence to suggest that the rate of incorporation of nonanoate into adipose tissue triglycerides is greater than that of octanoate (9). Thus, it is possible that nonanoate may create a greater need for glucose-derived intermediates for new triglyceride synthesis resulting in enhanced glucose uptake.

Summary. The effect of odd and even numbered medium chain fatty acids (MCFA) on glucose uptake by rat adipose tissue was studied *in vitro*, in the absence and presence of varying concentrations of insulin. The MC FA studied were octanoate (C8), nonanoate (C9), and decanoate (C10), and a three-carbon fatty acid, propionate. The effect of the odd numbered fatty acid, nonanoate, differed strikingly from that of an equimolar amount of the even homologues. The presence of nonanoate in the incubation medium was associated with a significant increase in glucose uptake with and without insulin, while octanoate, decanoate, and propionate exerted no such enhancing effect on glucose uptake. Incubation studies of adipose tissue with varying amounts of nonanoate indicate that the glucose uptake varied directly with the concentration of the fatty acid in the medium. Enhanced glucose uptake in the presence of nonanoate is not likely to be related to the glucogenic potential of the propionate moiety

of the odd numbered fatty acid and has been ascribed to possible increased incorporation of this fatty acid into triglyceride of adipose tissue.

1. Randle, P. J., Newsholme, E. A., and Garland, P. B., Biochem. J. **93**, 652 (1964).

2. Shipp, J. C., Metabolism **13**, 852 (1964).

3. Buse, M. G., McMaster, J., and Buse, J., Diabetes **15**, 388 (1966).

4. Lebouef, B. and Cahill, G. F., Jr., J. Biol. Chem. **236**, 41 (1961).

5. Westman, S. and Hellman, B., Med. Exptl. **7**, 324 (1962).

6. Campbell, R. G., Hashim, S. A., and Van Itallie, T. B., Clin. Res. **13**, 547 (1965).

7. Sanbar, S. S., Hentenyi, G., Jr., Forbath, N., and Evans, J. R., Metabolism **14**, 1311 (1965).

8. Knittle, J. L. and Hirsch, J., J. Lipid Res. **6**, 565 (1965).

9. Zurier, R. B., Campbell, R. G., Hashim, S. A., and Van Itallie, T. B., Am. J. Physiol. **212**, 291 (1967).

10. Steelman, S. L., Oslapas, R., and Busch, R. D., Proc. Soc. Exptl. Biol. Med. **105**, 595 (1960).

11. Hoffman, W. S., J. Biol. Chem. **120**, 51 (1937).

12. Hasagawa, K., Ph.D. thesis, Columbia Univ., 1965, p. 113.

13. Ballard, F. J., Hanson, R. W., and Leveille, G. A., J. Biol. Chem. **242**, 2746 (1967).

14. Siegel, I. and Lorber, V., J. Biol. Chem. **189**, 571 (1951).

15. Weber, G., Hird, H. J., Stamm, N. B., and Wagle, D. S. *in* "Handbook of Physiology," (A. E. Renold and G. F. Cahill, Jr., eds.), Sect. 5, p. 225. Am. Physiol. Soc., Washington, D. C. (1965).

16. Young, J. W., Shrago, E., and Lardy, H. A., Biochemistry **3**, 1867 (1964).

Received May 26, 1969. P.S.E.B.M., 1969, Vol. 132.