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Adipose Tissue Cholesterol Storage: The Effect of Essential Fatty Acid Deficiency[1] (40041)

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Production of essential fatty acid[2] deficiency in the rat is associated with increased cholesterol ester content in a number of extrahepatic tissues, most notably skin (1), adrenal (2), and ovary (3). In skin, accumulation of sterol esters may account in part for the abnormal keratinization, tail necrosis and scaliness which represent the classical identifying features of EFA deficiency (4). It has been suggested that the increased content of skin cholesterol esters is due to stimulation of cholesterol esterifying enzymes with simultaneous inhibition of cholesterol ester hydrolase activity, all ascribed to the lack of prostaglandin synthesis from arachidonic acid (5). Unlike skin, the adrenal and ovary normally contain large amounts of cholesterol esters. In these tissues, absence of the preferred substrate, cholesterol arachidonate, for steroid hormone synthesis induces utilization of other cholesterol esters which normally serve only in a storage capacity (2, 3).

It has been demonstrated that rat adipose tissue also contains cholesterol esters (6–8), which presumably may be hydrolyzed enzymatically for mobilization (9). This tissue likewise can synthesize prostaglandins *in vitro* from essential fatty acids (10). Such findings as well as the results obtained in the other tissues mentioned above prompted us to examine the effect of essential fatty acid deficiency on the storage of free and esterified cholesterol in isolated fat cells of the rat. A preliminary report has appeared (11).

Methods. Twenty male weanling Sprague-Dawley rats three weeks of age were divided into four dietary groups as shown in Table I. Group I rats received a fat-free diet (experimental I) or a control diet containing corn oil (control I). Group II experimental rats were fed hydrogenated coconut oil in order to produce a more rapid and severe form of EFA deficiency than the fat-free group (4). Control group II received corn oil together with hydrogenated coconut oil. Thus, all animals except the fat-free group received 7% lipid, 20% protein and equivalent amounts of carbohydrate. In addition to the dietary components listed in Table I, all diets contained 4% fiber as cellulose, 4% salt mix, 0.7% vitamin mix and 0.3% choline chloride. All of these semi-synthetic diets were provided *ad lib* for a period of 16 weeks.

Following decapitation of all animals, isolated fat cells were prepared from epididymal adipose tissue by the method of Rodbell (12) and both cells and plasma were extracted using an isopropanol–Zeolite system (13). Cell size was calculated as previously described by us (14) and others (15). Free and esterified cholesterol from extracted adipocytes were separated using lipophilic Sepahdex LH-20 gel filtration according to a method recently developed in our laboratory.[3] The Student *t* test was utilized to determine significant differences between means (16). All statistical analyses were done using a Wang Model 500 Programming Calculator (Wang Laboratories, Inc., Tewksbury, MA).

Results and Discussion. Retardation of animal growth has been used in early studies on EFA deficiency both as a criterion for the state of deficiency and in bioassays for determining the essentiality of various fatty acid derivatives (17, 18). As shown in Table II, both groups of essential fatty acid deficient rats in the present study also showed

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[2] Abbreviation: essential fatty acid (EFA).

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TABLE I. COMPOSITION OF CONTROL AND ESSENTIAL FATTY ACID DEFICIENT DIETS.

Group	Lipid	Protein	Carbohydrate
I			
Control	7% corn oil	20% casein	64% sucrose
Experimental	zero	20% casein	71% sucrose
II			
Control	2% corn oil 5% HCO[a]	20% casein	64% sucrose
Experimental	7% HCO	20% casein	64% sucrose

[a] Hydrogenated coconut oil.

TABLE II. AUTOPSY DATA FROM RATS FED CONTROL AND ESSENTIAL FATTY ACID DEFICIENT DIETS.[a]

Group	Body weight (g)	Fat pad weight (g)	Cell size[b]
Control I	503.6 ± 25.1	7.1 ± 0.7	73.4 ± 4.6
Control II	500.4 ± 25.3	6.5 ± 0.5	59.9 ± 6.7
Pooled control	502.0 ± 16.8	6.8 ± 0.5	66.7 ± 6.2
Experimental I	383.6 ± 10.1[c]	5.4 ± 0.5	50.7 ± 5.7
Experimental II	401.6 ± 8.2[c]	6.2 ± 0.8	50.1 ± 5.9
Pooled experimental	392.6 ± 6.8	5.8 ± 0.4	50.4 ± 5.7[d]

[a] Values are means ± SE of 6 rats/group.
[b] Cell size is expressed as μg triglyceride/cell $\times 10^{-2}$.
[c] Significantly less than respective control at 5% level.
[d] Significantly less than the pooled control values at 5% level.

significantly less weight gain during the feeding period than respective control animals. The wet weight of epididymal fat pads in the EFA-deficient groups showed an average nonsignificant decrease of 16%, while mean cell size differed by 24% when compared to controls. However, differences in cell size between experimental and control rats did reach statistical significance when all control rats were pooled and compared to all EFA deficient animals as a whole (Table II). These tendencies toward change in adipose tissue triglyceride storage, which may largely contribute to the body weight retardation, are consistent with the original description of EFA deficiency by Burr and Burr in which it was reported that "storage fat can be almost entirely eliminated" when young rats are deprived of fat for several months (19). Such decreases in cell size were also not surprising in view of the fact that others have reported increased *in vitro* lipolysis in EFA-deficient rats (10, 20).

As expected, EFA deficient rats as a whole had significantly lower plasma total cholesterol levels than controls (Table III). This agrees with the work of Alfin-Slater *et al.* (21). The significant decrease in plasma cholesterol esters, resulting in an increased percentage of free cholesterol, has also been reported and has been ascribed to decreased liver cholesterol esterification (22).

On the other hand, the levels of total cholesterol in adipocytes, as shown in Table IV, were higher than those previously reported for Sprague-Dawley rats of similar body weight fed normal rat chow (7, 14). It is possible that this is due to the semisynthetic nature of the diets, as well as to the high sucrose content, both of which increase plasma, liver, skeletal muscle, and adipose tissue lipid levels (7, 18, 23). Although cell size was not different between experimental groups (Table II), deficient animals receiving lipid in their diets (experimental II) had significantly less adipocyte total as well as free cholesterol compared to rats receiving no dietary fat (experimental I). This would seem to support the contention that triglyceride content or cell size is not the only determinant of adipocyte cholesterol accumulation (14, 24). The largest adipocytes did not have the greatest content of stored cholesterol, which also suggests dissociation of cholesterol storage from that of triglyceride.

Adipocyte total cholesterol in control rats was approximately 36% cholesterol ester. These data for the percent of total cholesterol as esters agree most closely with that

TABLE III. EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON PLASMA CHOLESTEROL LEVELS.[a]

Group	Free cholesterol	Cholesterol esters	Total cholesterol	% Free cholesterol
Controls	75.5 ± 5.5	71.4 ± 6.5	145.9 ± 11.1	51.2
Experimentals	73.9 ± 6.8	45.2 ± 3.8[b]	119.2 ± 10.5	61.9[b]

[a] Expressed as mg/dl, $n = 4$ for each group.
[b] Significantly different from control value at 5% level.

TABLE IV. EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON ADIPOCYTE CHOLESTEROL STORAGE.[a]

Group	Free cholesterol	Cholesterol esters	Total cholesterol	Percent cholesterol esters
Control I	1353.9 ± 132	779.5 ± 81	2133.5 ± 173	36.7
Control II	1195.5 ± 75	680.7 ± 56	1876.2 ± 128	36.2
Experimental I	2011.8 ± 100[b]	847.9 ± 238	2859.8 ± 329[b]	29.9[b]
Experimental II	1327.7 ± 214[c]	673.9 ± 151	2001.7 ± 363[c]	31.5[b]

[a] Expressed as $\mu g/10^6$ cells ± SE, $n = 5$ for all groups.
[b] Significantly different than respective control at 5% level.
[c] Significantly less than experimental I, $P < 0.1$.

of Farkas *et al.* (6) who found that isolated fat cells of 400 g rats contained 24% of total cholesterol as esters. Other laboratories (7, 8) have reported much lower percent-ester values. As discussed previously,[3] these discrepancies arise most likely because of technical difficulties inherent in determination of free and esterified cholesterol in the presence of larger quantities of neutral lipid, a problem unique to adipose tissue.

Adipose tissue does not appear to respond to EFA deficiency like other peripheral tissues. A significant increase in adipocyte free and hence total cholesterol occurred in rats deprived of dietary lipid, which resulted in a significant decrease in the percentage of total cholesterol as cholesterol esters (Table IV). Stability of the adipocyte ester pool under conditions of EFA deficiency was apparent despite the pronounced alterations in triglyceride (25) and carbohydrate metabolism (26) known to occur in EFA deficiency. A probable mechanism for increased free cholesterol storage in fat cells may be related to the increased percentage of free cholesterol in plasma of EFA rats also reported in this study, or to changes in membrane structure due to alteration of phospholipid fatty acids in EFA deficiency (17). More work and possibly the use of other models are required to reveal the regulatory mechanisms controlling cholesterol fluxes in adipose tissue.

Summary. Essential fatty acid deficiency was induced in one group of rats by feeding a fat-free diet, and in another group by supplying hydrogenated coconut oil. All deficient animals had significantly reduced weight gain and tended to have decreased epididymal fat cell size compared to controls. Rats in the fat-free group had significantly more adipocyte free cholesterol than control animals, but no significant alteration in stored free sterol occurred in rats made essential fatty acid deficient with hydrogenated coconut oil. Between experimental essential fatty acid deficient groups, the fat-free group had greater quantities of adipocyte free, and hence, total cholesterol. No alterations in ester content due to essential fatty acid deficiency could be demonstrated, although the percentage of total cholesterol as esters decreased significantly in both experimental groups. It is suggested that adipose tissue responds differently than other peripheral tissues to essential fatty acid deficiency.

1. Ziboh, V. A., and Hsia, S. L., J. Lipid Res. **13,** 458 (1972).
2. Walker, B. L., and Carney, J. A., Lipids **6,** 797 (1971).
3. Carney, J. A., and Walker, B. L., Comp. Biochem. Physiol. **41B,** 137 (1972).
4. Aaes-Jorgensen, E., Physiol. Rev. **41,** 1 (1961).
5. Ziboh, V. A., and Dreize, M. A., Biochem. J. **152,** 281 (1975).
6. Farkas, J., Angel, A., and Avigan, M. I., J. Lipid

Res. **14,** 344 (1973).
7. Kovanen, P. T., Nikkila, E. A., and Miettinen, T. A., J. Lipid Res. **16,** 211 (1975).
8. Schriebman, P. H., and Dell, R. B., J. Clin. Invest. **55,** 986 (1975).
9. Pittman, R. C., Khoo, J. C., and Steinberg, D., J. Biol. Chem. **250,** 4505 (1975).
10. Christ, E. J., and Nugteren, D. H., Biochim. Biophys. Acta **218,** 296 (1970).
11. Krause, B. R., Alam, S. Q., and Hartman, A. D., Physiologist **20** (1977).
12. Rodbell, M., J. Biol. Chem. **239,** 375 (1964).
13. Kessler, G., and Lederer, H., *in* "Automation in Analytical Chemistry" (Skeggs, L. T., ed.) p. 341, Mediad, Inc. (1965).
14. Krause, B. R., and Hartman, A. D., Biochim. Biophys. Acta **450,** 197 (1976).
15. Hirsch, J., and Gallian, E., J. Lipid Res. **9,** 110 (1968).
16. Sokal, R. R., and Rohlf, F. J., Biometry **367** (1969).
17. Guarnieri, M., and Johnson, R. M., Adv. Lipid Res. **8,** 115 (1970).
18. Stein, O., and Stein, Y., Biochim. Biophys. Acta **84,** 621 (1964).
19. Burr, G. O., and Burr, M. M., J. Biol. Chem. **82,** 345 (1929).
20. Bizzi, A., Veneroni, E., Garattini, S., Puglisi, L., and Paoletti, R., Europ. J. Pharmacol. **2,** 48 (1967).
21. Alfin-Slater, R. B., Aflergood, L., Wells, A. F., and Devel, H. J., Arch. Biochem. Biophys. **52,** 180 (1954).
22. Sugano, M., and Portman, O. W., Arch. Biochem. Biophys. **109,** 302 (1965).
23. Shiff, T. S., Roheim, P. S., and Eder, H. A., J. Lipid Res. **12,** 596 (1971).
24. Angel, A., and Farkas, J., J. Lipid Res. **15,** 491 (1974).
25. Solyom, A., Muhlbachova, E., and Puglisi, L., Biochim. Biophys. Acta **137,** 427 (1967).
26. Hazinski, T. A., Barr, M., and Hertelendy, F., Nutr. Metabol. **18** (1975).

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