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ALTERATION OF RAT ADIPOSE TISSUE LIPOLYTIC RESPONSE TO NOREPINEPHRINE BY DIETARY FATTY ACID MANIPULATION[1]

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SUMMARY: The present study clearly shows that, by feeding rats a semi-synthetic diet of known composition enriched with saturated fatty acids, the epididymal fat pad responsiveness to norepinephrine in vitro can be abolished relative to fat pads from animals fed a similar diet but enriched with polyunsaturated fatty acids. Addition of varying concentrations of norepinephrine to the incubation medium produced a clear dose-response relationship in fat pads from animals fed diet enriched with polyunsaturated fatty acids while no effect of norepinephrine was apparent at any dose level in fat tissue from animals fed saturated fatty acids. These changes in lipolytic responsiveness were concurrent with alterations in fatty acid compositions of adipose tissue phospholipids and triglycerides as well as in total tissue contents of phospholipids and cholesterol.

It has been well established that alterations of cellular lipid contents and fatty acid composition can alter a variety of cellular functions such as enzyme activity and nutrient uptake (1-4). This has been most widely studied with respect to alteration of various membranes (natural and artificial). The majority of these studies, however, have been performed using artificially prepared membranes, thus eliminating the opportunity to observe how such changes in cellular lipids might affect the physiology of the tissues in question. The present paper reports on a study designed to determine how dietary fatty acid manipulation might affect the responsiveness of adipose tissue to the lipolytic activity of norepinephrine (NE).

MATERIALS and METHODS: Male Wistar weanling rats (National Lab. Animals Corp., O'Fallon, MO) were divided into two groups and placed on semi-synthetic diets of the following composition: corn starch, 30%; sucrose, 17.3%; casein, 26%; Celufil c (United States Biochemical Corp., Cleveland, OH) 6%; mineral mix (Teklad, Madison, Wis.), 4%; vitamin mix (5), 0.2%; choline chloride, 0.1%; and DL-methionine, 0.4%. This diet was enriched with either polyunsaturated fatty acids through the inclusion of 16% (w/w) safflower

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oil (64% polyenoic - ω6 fatty acids) (diet 1) or saturated fatty acids (diet 2) through the addition of 16% (w/w) coconut oil (93% saturated fatty acids with only 1% polyenoic - ω6 fatty acids). The animals were placed on their respective diets upon attaining a body weight of 80-90 gms and allowed free access to both food and water for 4 weeks. At the end of this four week period, the animals were killed by cervical dislocation and the epididymal fat pads were removed and placed in ice-cold saline for subsequent biochemical analysis.

For measurement of adipose tissue lipolysis, epididymal fat pad tissue was finely minced and incubated at 37°C in Krebs-Ringer phosphate buffer (pH 7.4) containing 2.5% fatty acid-poor bovine serum albumin and $CaCl_2$ (11mM). This incubation system also included NE at varying concentrations (10^{-7}, 10^{-6}, 10^{-5} or 10^{-4} M) or vehicle (saline) only (no NE). Incubated systems contained 150-250 mg of minced adipose tissue per incubation flask. Aliquots of the incubation medium were taken for measurement of free fatty acids (FFA) by the automated titration method of Lorch and Gey (6) after 5 minutes of equilibration to 37°C and after 60 additional minutes of incubation. Net lipolytic rate was expressed as μEq of FFA released/gm tissue (wet weight)/hour.

Measurements of epididymal fat pad cholesterol (7) and phospholipid (8) contents were performed following separation of these fractions by thin layer chromatography (9). Total lipid content was estimated by gravimetrical analysis on aliquots from Folch extracts (10). The analyses of phospholipid and triglyceride fatty acid compositions were performed as described previously (11).

Results were anlayzed statistically by the use of Student's t-test.

RESULTS: Feeding the experimental diets for four weeks resulted in similar weight gain in both groups. Animals receiving diet 1 (safflower oil) gained an average of 115.0 $\pm$ 8.2 gm over the four week period, while those receiving diet 2 (coconut oil) gained an average of 122.0 $\pm$ 4.6 gm. There was no mortality exhibited in either group during the experimental period and the animals appeared healthy. Similar results were obtained in the second experiment. Food consumption did not differ between groups in each experiment.

Figure 1 shows the *in vitro* activity of NE on lipolysis in epididymal fat pads from animals receiving either diet 1 (safflower Oil) or diet 2 (coconut oil). The results from two separate experiments are shown since the magnitude of response differed in each experiment. In Figure 1A it can be seen that upon addition of varying doses of norepinephrine to the incubation medium, the fat pads from animals fed diet 1 were responsive in a clear dose-response manner while the fat pads from animals fed diet 2 were unresponsive to the hormone. In Figure 1B it can be seen that a pattern of hormone responsiveness similar to that observed in Figure 1A occurs in Figure 1B, eg. fat pads from animals fed diet 1 were sensitive to the lipolytic activity of NE while fat pads from animals on diet 2 were virtually unresponsive to this

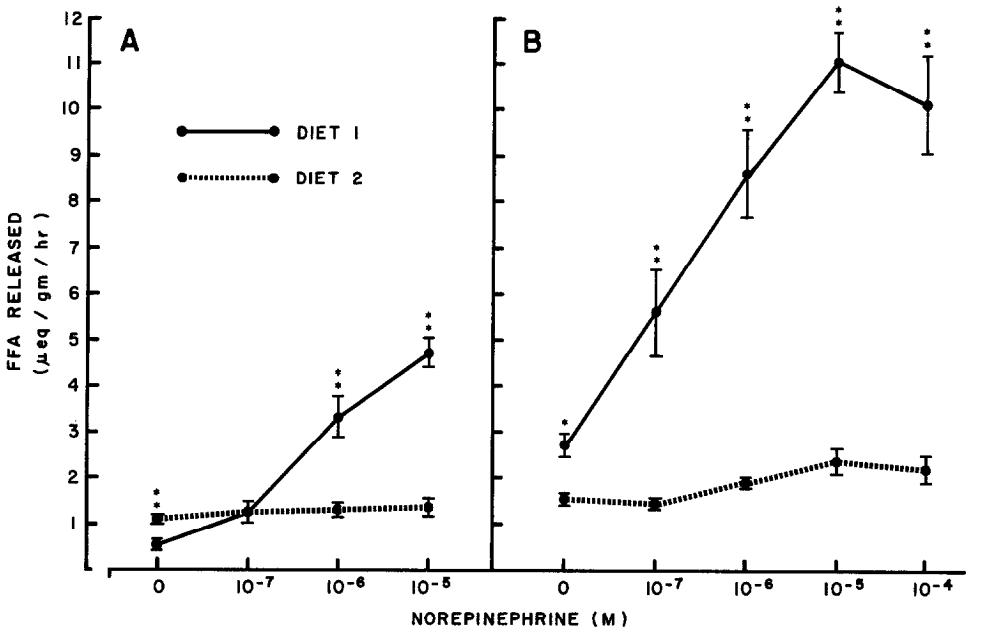

FIGURE 1: The Effect of Dietary Fatty Acid Manipulation on the in vitro Sensitivity of Adipose Tissue to Norepinephrine (NE). Adipose tissue from rats fed either diet 1 (——) or diet 2 (·····) for 4 weeks prior to sacrifice were incubated with varying concentrations (0, 10^{-7}, 10^{-6}, 10^{-5} or 10^{-4}M) of NE. The medium was sampled at the start of the incubation and after one hour and free fatty acid release was determined by the automated method of Lorch and Gey (9). Each point represents the mean ± S.E.M. of at least 4 determinations. Significant differences between sensitivities of adipose tissue from animals fed either diet 1 or diet 2 are indicated by * (P<0.05) or ** (P<0.01).

lipolytic agent. It can be seen also in Figure 1B that, with the addition of a larger concentration of NE (1 X 10^{-4}M) to the incubation medium, a maximum lipolytic response of the adipose tissue to NE occurs at a NE concentration of 1 X 10^{-5}M and that greater amounts of the hormone do not stimulate further the lipolytic rate in fat pads from animals fed either diet 1 or diet 2.

In an attempt to correlate these changes in hormonal responsiveness of the adipose tissue to changes in the lipid composition of this tissue, an analysis of the epididymal fat pad lipids was performed. The results of this analysis are illustrated in Tables 1 and 2. In Table 1, the fatty acid compositions of both the triglyceride and phospholipid components of adipose tissue from animals receiving either diet 1 or diet 2 are reported as a per-

TABLE I

FATTY ACID COMPOSITIONS OF THE TRIGLYCERIDE AND PHOSPHOLIPID FRACTIONS OF EPIDIDYMAL FAT PADS FROM RATS FED DIETS ENRICHED WITH SAFFLOWER OR COCONUT OILS

	Triglycerides		Phospholipids	
Fatty Acid	Diet 1 (Safflower Oil)	Diet 2 (Coconut Oil)	Diet 1 (Safflower Oil)	Diet 2 (Coconut Oil)
		%		
<14:0[a]	Trace[b]	19.8 ± 0.8	---	---
14:0	1.9 ± 0.3	15.0 ± 0.9[d]	1.0 ± 0.4	---
14:1	Trace	0.8 ± 0.1[d]	---	---
16:0	23.1 ± 0.7	27.7 ± 1.1[d]	32.5 ± 1.0	25.9 ± 1.0[e]
16:1	3.4 ± 0.5	6.6 ± 0.3[d]	2.5 ± 0.6	4.5 ± 0.4[f]
18:0	3.8 ± 0.1	2.0 ± 0.1[d]	35.1 ± 1.6	37.5 ± 1.2
18:1	19.7 ± 1.3	27.3 ± 0.6[d]	8.1 ± 0.6	13.0 ± 0.9[d]
18:2	43.6 ± 2.0	Trace	11.4 ± 1.7	1.7 ± 0.3[d]
>18:2	4.3 ± 0.4	Trace	9.6 ± 1.3	7.4 ± 1.1

[a] Chain length : number of double bonds.
[b] <0.5% of the total fatty acids.
[c] Mean ± S.E.M. of 5 observations/group
[d] Significantly different from corresponding diet 1 fatty acid ($P < 0.01$).
[e] Significantly different from corresponding diet 1 fatty acid ($P < 0.02$).
[f] Significantly different from corresponding diet 1 fatty acid ($P < 0.05$).

TABLE II

LIPID CONTENTS OF EPIDIDYMAL FAT PADS IN RATS FED DIETS ENRICHED WITH SAFFLOWER OR COCONUT OILS

	Total Lipids (mg/100 mg wet wt)	Cholesterol (µg/mg wet wt)	Phospholipid (µg/mg wet wt)	Cholesterol:Phospholipid Ratio
Diet 1 (Safflower Oil)	62.4 ± 5.8[a]	0.41 ± 0.02	0.37 ± 0.03	1.15 ± 0.14
Diet 2 (Coconut Oil)	64.5 ± 3.8	0.18 ± 0.01[b]	0.72 ± 0.02[b]	0.25 ± 0.00[b]

[a] Mean ± S.E.M. of 5 observations/group
[b] Significantly different from corresponding diet 1 group ($P < 0.01$)

centage of the total fatty acid content of the various fractions. With regard to the fatty acid composition of the triglyceride fraction, the adipose tissue from animals fed diet 2 had a much higher percentage of fatty acids as smaller saturated fatty acids ($\leq 14:0$) than did the animals fed diet 1. On the

contrary, adipose tissue triglycerides from animals fed diet 1, contained higher percentages of 18:2 and longer fatty acids as compared with diet 2 feeding. Moreover, there were significant differences between the concentrations of major fatty acids due to this dietary manipulation. These differences also exist in the phospholipid fraction with the exception of 18:0 and the polyunsaturated (>18:2) fatty acid concentrations. Unlike the case with the triglyceride fraction, however, the phospholipid fraction from animals fed diet 2 had virtually no measureable fatty acid as 14:0 or smaller. In Table 2, it can be seen that the animals fed diet 2 had a significantly lower adipose tissue cholesterol concentration ($P<0.01$) than did those animals receiving diet 1 for four weeks. Conversely, adipose tissue from animals fed diet 2 had approximately twice as much phospholipid as did adipose tissue from animals fed diet 1. Of special significance to these studies is the marked change in cholesterol : phospholipid ratio seen in adipose tissue from the animals fed the different diets. In this case the ratio was shifted from 1.5 in those animals fed diet 1 to 0.25 in those animals receiving diet 2. It is apparent also, in Table 2, that total lipid content of adipose tissue, which is mainly in the form of triglyceride, did not differ between animals fed either diet 1 or diet 2.

DISCUSSION: Our data clearly show that the responsiveness of rat epididymal adipose tissue to the lipolytic action of NE can be abolished by feeding the animals a diet enriched with saturated fatty acid for four weeks. This decrease in lipolytic response of adipose tissue occured concurrently with alterations in the fatty acid compositions of the triglyceride and phospholipid fractions, as well as alterations in cholesterol : phospholipid ratio, of this tissue. Adipose tissue from animals fed diet enriched with polyunsaturated fatty acids showed a typical lipolytic responsiveness to NE in a clear dose-response fashion.

The alterations in tissue lipid content and composition of adipose tissue by dietary means seen in the present study is in agreement with the reports

of others in adipose tissue (12) as well as in other tissues (2,4,13). Similarly, Smith et al. (14) showed that short-term feeding of diet rich in saturated fat decreased the sensitivity of adipose tissue to insulin. The present studies regarding the altered sensitivity of adipose tissue with differing fatty acid compositions and altered cholesterol : phospholipid ratios to the lipolytic activity of NE, however, do not indicate the cellular site at which these sensitivity changes occur, i.e., whether they are membrane-associated or cytoplasmic in origin. The currently accepted theory concerning NE-stimulated lipolysis is via hormone-receptor interaction on the surface of the membrane resulting in cAMP formation with ultimate stimulation of hormone-sensitive lipase. If, as it is generally assumed, adipocyte phospholipids are associated with the membrane then perhaps the changes noted in the phospholipid content and phospholipid fatty acid composition may, somehow, alter the transduction of the signal produced by hormone-receptor interaction to the adenylate cyclase enzyme. Orly and Schramm (15) demonstrated in vitro an increase in adenylate cyclase activity in turkey erythrocytes associated with a high degree of unsaturation of phospholipid fatty acids in the membrane. Recently, however, Saito et al. (16) have reported stimulation of lipolysis in fat globules within the adipocyte which is independent of cAMP. In this case, alteration of intracellular parameters such as free cholesterol, which is known to be cytoplasmic in origin in adipocytes (17), and/or triglyceride fatty acid composition may somehow result in altered adipocyte sensitivity to lipolytic factors. Further studies are underway to investigate the site(s) of altered adipose tissue sensitivity to NE.

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