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Adipose Tissue Metabolism in Essential Fatty Acid Deficiency

Effects of Prostaglandin E_1, Epinephrine, and ACTH

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Abstract. In an effort to better define some of the metabolic changes that accompany essential fatty acid deficiency (EFAD), we studied glucose metabolism in adipose tissue of EFAD and normal mice under basal conditions and in the presence of prostaglandin E_1 (PGE_1), epinephrine, and $ACTH_{1-18}$. Isolated fat cells were incubated in Krebs-Ringer bicarbonate medium containing glucose 1-^{14}C or 6-^{14}C, and the incorporation of radioactive carbon into CO_2, total fat, fatty acids, and glyceride-glycerol was determined. It was found that EFAD increased glucose uptake over controls which could be attributed to increased oxidation to CO_2 and fatty acid synthesis. The contribution of the pentose cycle to glucose oxidation was 50–80 % higher in EFAD adipocytes as compared to controls. $ACTH_{1-18}$ (0.1 μg/ml) suppressed this by 18 and 30 % in the control and EFAD groups, respectively, while epinephrine decreased pentose cycle activity by 83 and 55 % in the two groups, respectively. PGE_1 alone had no significant effect, but in combination with epinephrine it abolished the inhibitory action of the catecholamine in both groups. It is suggested that, although EFA serve as prostaglandin precursors, the effects of EFAD on the metabolism of fat cells cannot be reversed by PGE_1 *in vitro*. However, these fat cells retain their responsiveness to the action of both lipolytic (ACTH and epinephrine) and antilipolytic (PGE_1) agents.

Introduction

Metabolic alterations in adipose tissue are among the many consequences of essential fatty acid deficiency (EFAD) in growing animals (2). Certain prostaglandins are well known antilipolytic agents (24) and the increased lipolysis, observed in adipose tissue of EFAD rats, has been attributed to diminished

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prostaglandin release. In the present study we investigated some aspects of adipose tissue metabolism in EFAD and intact mice and the effects of prostaglandin E_1 (PGE_1) and two lipolytic hormones: epinephrine and a synthetic analog of $ACTH_{1-18}$.

Materials and Methods

240 3-week-old male albino mice (Carworth CF-1) were divided into two groups of 120 each with 10 mice per cage. The control group received the basal ration consisting of sucrose 61 % purified casein 30 %, corn oil 2 %, balanced salts 5 %, and vitamin mix 2 %. In the EFAD group, reagent grade medium chain triglycerides (Mead Johnson) were substituted for corn oil. Since growth retardation is one of the symptoms of EFAD (1), body weights were recorded at 1- to 2-week intervals. Experiments were carried out after the mice had been on the experimental diet for 6–8 weeks by which time the experimental mice exhibited some of the characteristic signs of EFAD, such as dermatitis and increased liver fat.

Fat cells were isolated by *Rodbell*'s (20) method with some modifications. Epididymal fat pads from 6 to 8 mice per group were incubated for 60 min in 4 ml glucose-free Krebs-Ringer bicarbonate medium (KRB) containing NaCl, 118 m*M*; KCl, 4.7 m*M*; $CaCl_2$, 2.5 m*M*; KH_2PO_4, 1.2 m*M*; and $NaHCO_3$, 25 m*M* as well as 4 mg/ml bacterial collagenase (Worthington). After several washes the cells were dispersed in warm KRB and passed through a 6-ply gauze. Aliquots of this suspension were used for protein determination (17). 0.5 ml cell suspension was pipetted into siliconized Erlenmeyer flasks containing in 0.5 ml KRB; 40 mg BSA (FFA-free; Sigma) and 1 mg glucose. PGE_1 (Ono Pharmaceutical Co.) was diluted freshly with KRB from an ethanolic stock solution (5 mg/ml); epinephrine (*L*-epinephrine bitartrate; Sigma) and $ACTH_{1-18}$ (Ciba-Geigy B72-5) were also dissolved in KRB. Either glucose 1-^{14}C (specific activity 40 mCi/mmol) or glucose 6-^{14}C (specific activity 45 mCi/mmol; Amersham/Searle) was added to each flask (0.25 μCi) before placing them in a Dubnoff metabolic shaker bath (37 °C). After gassing with moist O_2:CO_2 (95 %:5 %) for 5 min, the flasks were stoppered with a rubber stopper equipped with a small plastic cup suspended into the flask and incubated for 2 h. Blank vials without cells but containing the medium and labeled substrates were incubated simultaneously. These flasks were treated identically with flasks containing the cell suspension and the values thus obtained were substracted as 'background'. At the end of the incubation CO_2 was liberated by injecting into the flask 2 *N* H_2SO_4 (0.25 ml) and collected in Hyamine (0.25 ml) which was injected through the rubber stopper into the plastic cup. After shaking for an additional hour to allow for trapping of CO_2, the plastic cups were transferred to scintillation vials containing 10 ml Liquifluor-toluene cocktail and counted in a Packard Tri-carb liquid scintillation spectrometer. Aliquots (0.25 ml) of the media were used to quantitate glucose utilization by an enzymatic method (Glucostat; Worthington). The rest of the contents of the incubation flasks were extracted according to the precedure of *Dole* (7). The heptane layer was washed twice with distilled H_2O before aliquots were transferred to scintillation vials, evaporated to dryness, and counted in 10 ml scintillation cocktail. This represents the incorporation of radioactivity into total lipids. Another portion of the heptane extract was evaporated, the residue taken up in methanolic KOH (2 % w/v), and saponified for 30 min at 90 °C. After acidification, the fatty acids were extracted twice with petroleum ether and the radioactivity determined as before. The difference between the radioactivity of total fat and fatty acids was taken as glucose incorporation into glyceride glycerol. The results are expressed as cpm/mg protein, and the contribution of pentose cycle to glucose metabolism

was calculated according to *Katz and Wood* (14). The limitations of this model require a number of assumptions (e.g., the system is in metabolic and isotopic steady state; there is isotopic equilibrium between hexose phosphates derived from the two major pathways; namely, the Embden-Meyerhof and pentose pathways; CO_2 is liberated solely by the pentose and Krebs cycles, etc.) which have been pointed out and their implications discussed extensively (13–16). However, it has been proven an adequate tool for the estimation of the contribution of the pentose cycle to glucose oxidation in adipose tissue (16).

Livers of ether-anesthetized mice were excised and dropped into liquid nitrogen. Portions (25–50 mg) were homogenized with ice-cold TCA (6 %), centrifuged, and the supernate was used for cyclic AMP determination by radioimmunoassay (23) while protein was determined on the precipitate (18). Liver fat was measured gravimetrically after extraction with chloroform-methanol (19). Data were analyzed for significance by Student's t-test.

Results

By the end of the fourth week, significant ($p < 0.01$) growth retardation was observed on the EFAD diet. By the end of the sixth week, when the weights of the animals began to plateau, the difference between the mean body weight of experimental and control rats was about 20 % ($p < 0.01$) (fig. 1). There was also a moderate hypertrophy of the liver in the EFAD group (2.50 vs. 2.15 g) and a highly significant increase when expressed as percent of body weight ($p < 0.001$) (table I). Consistent with previous reports (3), we found an almost

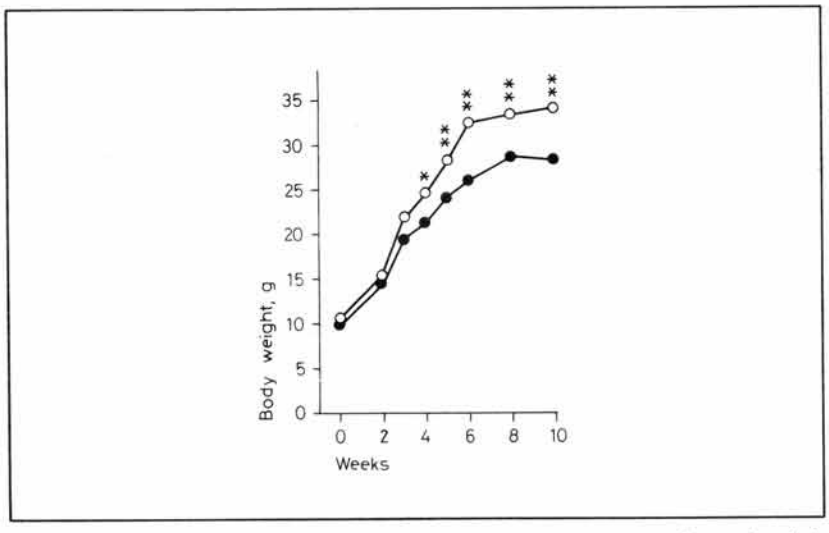

Fig. 1. Growth curve of EFAD (•) and control (○) mice during the experimental period. Asterisks denote the statistical significance between the two groups (* $p < 0.01$; ** $p < 0.005$).

Table I. Effects of EFAD on liver weight, lipid, protein, and cyclic AMP content in mice after 6–8 weeks on the experimental diet

Weight, g		Body weight, %		Lipid, %	
cont. (18)	EFAD (18)	cont.	EFAD	cont. (18)	EFAD (18)
2.15 ± 0.08	2.5 ± 0.07	6.72 ± 0.23	9.25 ± 0.12	5.7 ± 0.42	10.6 ± 1.09
$p < 0.005$		$p < 0.001$		$p < 0.001$	
Protein, %		Cyclic AMP			
		pmole/mg, tissue		pmole/mg, protein	
cont.	EFAD	cont. (9)	EFAD (9)	cont.	EFAD
24.98 ± 3.47	21.12 ±1.59	1.21 ± 0.103	1.64 ± 0.189	5.19 ± 0.53	7.57 ± 0.34
NS		NS		$p < 0.005$	

Numbers in parenthesis indicate the number of livers examined.

doubling of the fat content of liver due to EFAD and a drop in liver protein concentration. Cyclic AMP values (pmol/mg tissue) were higher, though not significantly so, in the experimental group. However, when the cyclic nucleotide levels were related to protein content, the mean difference between the two groups was statistically significant ($p < 0.005$), which is not unexpected in view of the lower protein and higher fat content of EFAD livers.

Evolution of CO_2 from glucose-1-^{14}C was 2–3 times greater ($p < 0.001$) in adipocytes of EFAD mice than in controls, while the oxidation of glucose-6-^{14}C to CO_2 was not affected significantly (fig. 2). However, the incorporation of label into fatty acids was increased more than 5-fold ($p < 0.001$) over controls with both substrates. The incorporation of ^{14}C into glyceride-glycerol was not affected by EFAD when C_1-labeled glucose was used as substrate but it was moderately (10–20 %) increased with glucose-6-^{14}C as substrate. Both $ACTH_{1-18}$ and epinephrine suppressed CO_2 production as well as fatty acid synthesis from glucose-1-^{14}C ($p < 0.0025$ and < 0.01 for EFAD and control, respectively), but only latter was inhibited by epinephrine when glucose-6-^{14}C was used as substrate. PGE_1 alone had no significant effect in either control or EFAD mouse fat cells. However, when added together with epinephrine, it reversed the inhibitory effect of the catecholamine.

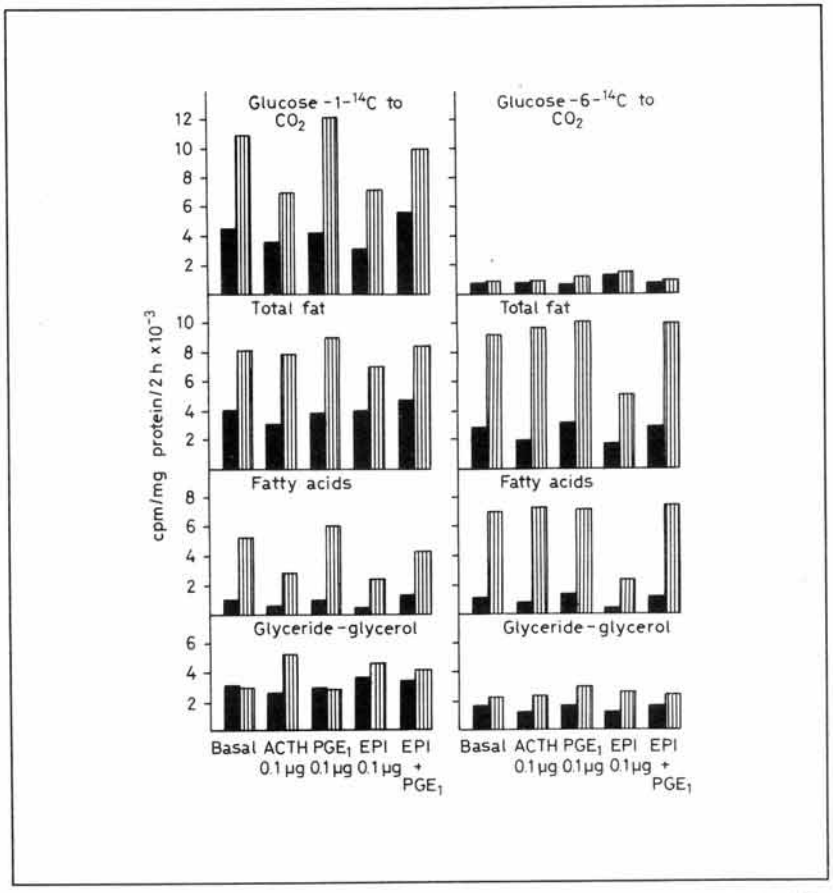

Fig. 2. Effects of PGE_1, $ACTH_{1-18}$, and epinephrine on glucose metabolism in isolated fat cells of EFAD (▣) and control (■) mice. Values were pooled from three experiments in each of which cell suspensions were incubated in triplicates per treatment. Standard errors ranged from 4 to 11 %. Cell protein per vial varied from 0.5 to 0.8 mg in the three experiments.

The percent contribution of the pentose cycle to glucose metabolism was calculated separately for two of the above experiments and is summarized in table II as experiment A and B. Our values for control mice fat cells with and without epinephrine are similar to those found in the literature (16). EFAD caused a 45 and 79 % increase in pentose cycle activity in experiment A and B, respectively. $ACTH_{1-18}$ inhibited pentose shunt by 18 % in control mice and by 30 % in the EFAD groups; while epinephrine brought about a more striking inhibition (83 and 56 %, respectively) which was reversed by PGE_1.

Table II. Percent contribution of pentose cycle to glucose metabolism[1]

Treatment	Control, %	EFAD, %
Experiment A		
None	19.2[2]	27.8
$ACTH_{1-18}$ (0.1 μg)	15.7	19.5
PGE_1 (0.1 μg)	19.6	26.9
Experiment B		
None	13.8	24.7
Epinephrine (0.1 μg)	2.4	11.0
Epinephrine (0.1 μg) + PGE_1 (0.1 μg)	15.0	22.1

[1] Calculated from the equation: $\frac{SY(C_1) - SY(C_6)}{1 - SY(C_6)} = \frac{3PC}{1 + 2PC}$,

where $SY(C_1)$ = specific yield of CO_2 from glucose-1-^{14}C; $SY(C_6)$ = specific yield of CO_2 from glucose-6-^{14}C; PC = percent contribution pentose cycle activity.

[2] Each value was calculated from the mean of triplicate observations.

Discussion

Although increased fatty acid mobilization in EFA deficiency has been repeatedly observed (4, 6), relatively little is known about the metabolism of glucose in adipose tissue of EFAD animals. *Solyom et al.* (22) found that the incorporation of glucose into adipose tissue glycerides was greater in EFAD rats than in controls, but qualitatively both groups responded similarly to fasting, nicotine, and theophylline. They suggested that the increased glyceride synthesis may be due to higher lipolytic activity in EFAD rat fat pads, and in this respect the effect of EFAD is analogous to the action of adipokinetic agents. However, the present experiments indicate that the higher incorporation of glucose into total lipids of EFAD fat cells can be accounted for almost entirely by increased fatty acid synthesis. Two lipolytic hormones, $ACTH_{1-18}$ and particularly epinephrine, inhibited this response in the adipocytes of both EFAD and control mice, whereas addition of PGE_1, an antilipolytic agent, reversed the effect of epinephrine in both groups. Our data are consistent with those of *Du and Kruger* (8) who used uniformly labeled glucose as substrate. By employing specifically labeled substrates, we have also shown that pentose cycle activity is significantly elevated in the fat cells of EFAD mice and that it is affected qualitatively to approximately the same degrée as in normal fat cells by lipolytic agents and PGE_1.

These results are, however, not easy to interpret. On one hand, the increased lipolysis in EFAD fat cells has been linked to decreased prostaglandin synthesis

and release during basal- and hormone-stimulated free fatty acid mobilization (5). On the other hand, however, PGE_1 has been reported (9, 24) to increase glucose uptake and utilization by normal rat fat cells (i.e. exhibit an insulin-like effect). It is conceivable that only the antilipolytic action of PGE_1 like that of insulin, as suggested by the experiments of *Fain* (9) and *Rodbell* (21), is mediated by cyclic AMP, and that the effects on glucose metabolism are not regulated by this nucleotide. It is possible, therefore, that the changes in glucose metabolism of EFAD fat cells are due to the alterations in the physicochemical properties of membranes (11), including mitochondrial (21) membranes, allowing an accelerated uptake and metabolism of glucose.

Although free fatty acid or glycerol release was not investigated in the present study, accelerated lipolysis in EFAD adipose tissue has been observed by a number of investigators (4, 6) who suggested the possibility that this might be due to decreased prostaglandin synthesis from EFA which serve as prostaglandin precursors. It has been proposed on the basis of observations showing increased lipolysis in isolated fat cells in the presence of indomethacin, a potent inhibitor of prostaglandin synthesis, that certain prostaglandins regulate lipolysis by a negative feedback mechanism (12). However, *Fain et al.* (10) failed to confirm the stimulant effect of indomethacin and thus the possible physiological role of prostaglandins in lipolysis remains uncertain. The present studies have also shown that the metabolic effects of EFAD in fat cells is more complex than a simple lack or reduced levels of prostaglandins, since *in vitro* addition of PGE_1 failed to affect EFAD cells.

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