SELECTIVE DAMPENING OF LIPOGENIC ENZYMES OF LIVER BY EXOGENOUS POLYUNSATURATED FATTY ACIDS*

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SUMMARY: Oral administration of methyl esters of linoleic, linolenic and arachidonic acids to rats maintained on a high-carbohydrate, fat-free diet brought about a progressive dimunition of the activity of liver enzymes which participate in the synthesis of saturated and monounsaturated fatty acids: acetyl CoA carboxylase (CBX), fatty acid synthetase (FAS), citrate cleavage enzyme (CCE), malic enzyme (ME), and glucose-6-phosphate dehydrogenase (G6PDH). In the same three day experimental period the fatty acid composition of liver phospholipid and free fatty acids reflected the exogenous input of polyunsaturated fatty acids.

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It was previously reported by this laboratory that high-carbohydrate, low-fat alimentation by rats and mice was associated with severe deficiency in linoleic and arachidonic acids in total liver lipids in less than two days. Coincident with these compositional changes was a striking adaptive rise in the activity of the key enzymes catalyzing fatty acid synthesis in liver: acetyl CoA carboxylase (CBX) and fatty acid synthetase (FAS) (1,2,3). Addition of methyl linoleate to the fat-deficient diet returned enzyme levels toward normal and restored the fatty acid spectrum seen with balanced diets. Methyl palmitate, methyl oleate, fats containing saturated fatty

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acids, and cholesterol were virtually without effect in halting hyperlipogenesis.

The present study was undertaken to examine this phenomenon under more controlled conditions, in relation to the metabolism of higher polyunsatufated fatty acids in the rat, and to include other 'lipogenic' enzymes for comparison: citrate cleavage enzyme (ATP-citrate lyase) (CCE), malic enzyme (ME) and glucose-6-phosphate dehydrogenase (G6PDH). Rats were adapted to a high-sucrose, fat-free diet for ten days and trained to accept supplementary treatment by stomach tube such that known doses of methyl esters of fatty acids could be delivered during the subsequent experimental period. The relative concentrations of fatty acids in the phospholipid and free fatty acid fractions of liver lipids were examined with the same specimens of liver used for enzyme activity determinations (4).

METHODS: Male Wistar strain rats weighing about 200 grams were kept on a high-sucrose, fat-free diet (General Biochemicals) for ten days following two days of starvation. Animals were then divided into three general groups: (a) animals switched to normal balanced chow diet over three days (FN_3); (b) animals continued on high carbohydrate diet and fed methyl esters of various fatty acids by gastric intubation each morning for three days; (c) animals on high carbohydrate diet and intubated with physiological saline each morning for three days (F). On the fourth morning animals were killed by decapitation. The livers were removed, washed in cold physiological saline, and divided into halves.

One portion of liver was homogenized in two volumes of buffer containing 0.25 M sucrose, 0.1 M potassium phosphate buffer (pH 7.4), 0.07 M $KHCO_3$, 1 mM EDTA, and 1 mM dithiothreitol. The soluble liver supernatant which was obtained by centrifuging the homogenate at 105,000 x g for 60 minutes was used for determining the activity of five enzymes: CBX, FAS, ME, CCE, and G6PDH. Four enzymes were assayed spectrophotometrically: FAS (5), ME (6),

CCE (7), and G6PDH (8). Acetyl CoA carboxylase (CBX) activity was measured by following $^{14}CO_2$ incorporation into malonyl CoA according to Majerus et al. (9).

The second portion of each liver was used for determining the fatty acid composition of several lipid fractions. The liver was extracted in chloroform methanol (2:1) according to Folch et al. (10) with the exception that 2.5 mM EDTA-Na_2K_2 and 1/5 volume of 0.2% H_2SO_4 were added in order to obtain a complete recovery of free fatty acids (FFA) from the lower phase. Phospholipids were isolated on an acid-treated florisil column (95% recovery) (11). Free fatty acids were separated on a florisil column as described by Carroll (12). Further FFA purification was achieved by thin-layer chromatography. Overall recovery of FFA was more than 80%. The lipid fractions obtained by these methods were methylated in redistilled methanol, containing 2% H_2SO_4, under N_2 gas for two hours at 70° C. The esters were separated by gas-liquid chromatography (10% EGSS-X; column temperature, 180° C). Arachidic acid (Applied Science Lab., Inc.) served as an internal standard (30 µg/gr liver tissue). Commercial methyl esters of fatty acids used in these experiments (99% pure) were purchased from the Hormel Institute, except for γ-linolenic acid (ONO-Pharmaceutical Co., Osaka, Japan). Peroxides were not detectable in the methyl esters of fatty acids, nor in the lipid extracts of liver after methyl esters were administered. Peroxide formation was determined by measuring diene conjugation absorption (13).

RESULTS: ENZYMES: In Fig. 1 are shown the induction patterns of the five enzymes during high-carbohydrate, fat-free refeeding following two days of starvation. At the tenth day various methyl esters of fatty acids were introduced by stomach tube. Enzyme activities were measured at the tenth day and three days later. The activity of the five lipogenic enzymes studied fell in response to oral administration of methyl esters of poly-

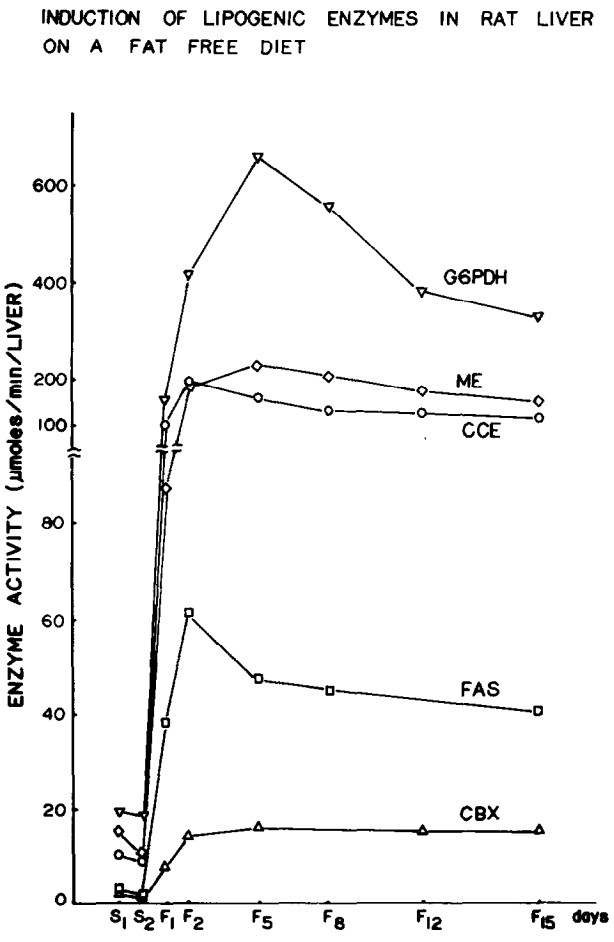

Fig. 1. Adaptive rise in the activity of five enzymes catalyzing fatty acid synthesis in rat liver during high-carbohydrate, fat-free refeeding over fifteen days following two days of starvation.

unsaturated fatty acids. The degree of fall in enzyme activity was a function of the dose of the several esters employed and the length of the experimental period.

In Fig. 2 activities of the five enzymes are presented after administration of 0.25 grams of various methyl esters (per 100 grams body weight) over three days. A suboptimal dose was selected to afford a comparison of the several esters. The dampening effect appeared to be proportional to the degree of cis unsaturation in the fatty acid ester tested, methyl arachidonate being the most effective in this series. Animals receiving a balanced diet

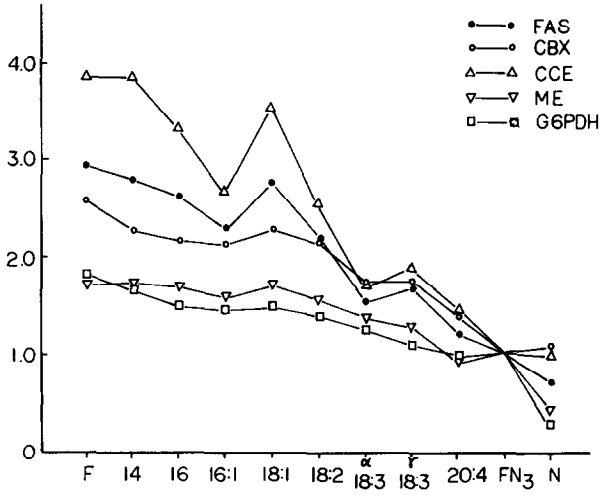

Fig. 2. Effect of oral administration of methyl esters of fatty acids on the activity of the five enzymes in Fig. 1. All activities are compared with the level of FN_3 (refed balanced diet) set equal to one. The baseline level for the five enzymes were: G6PDH, 196 µmoles of NADPH/min./liver; ME, 86 µmoles NADPH/min./liver; FAS, 11.8 µmoles/min./liver; CCE, 29 µmoles/min./liver; and CBX, 5.5 µmoles CO_2/min./liver.

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ad lib over three days returned to a normal pattern. In the latter case, however, there was no control of lipid intake. The stock diet contained 4.3% fat (w/w) with 39% linoleic acid in total fatty acids. The five lipogenic enzymes behaved as a constant proportionality set regardless of the kind of nutritional manipulation.

LIPIDS: As previously observed (1,2,3) rats maintained on a high-carbohydrate, fat-free diet were deficient in linoleic and arachidonic acids in the lipid fractions of liver. Fatty acids of the ω-9 series (eg. oleate) which can be synthesized de novo are elevated. Table I shows the changes in fatty acid composition in the free fatty acid fraction of liver following oral administration of the same methyl esters indicated in Fig. 2. The polyunsaturated content of the free fatty acids reflected the exogenous fatty acid input. Added saturated and monounsaturated esters had virtually no effect on the 'fat-free' pattern. Administration of the members of the ω-6 fatty acid family tended to restore normal fatty acid patterns, viz; the ω-9 series was diminished and the ω-6 series was restored. There were individual

TABLE I

Effect of Oral Administration of Methyl Esters of Fatty Acids on the Percent Composition of the Free Fatty Acids of Rat Liver.

SERIES	RATS	14	16	16:1 (ω7)	18	18:1 (ω9)	18:2 (ω6)	20:3 (ω9)	20:4 (ω6)	22:5 (ω6)	22:6 (ω3)	μg FFA g liver
F	28	1.2	38.4	5.1	25.4	16.3	3.0	2.7	3.4	0.9	3.6	69
14:0	4	3.2	38.9	5.8	24.9	18.3	2.3	1.0	3.0	0.9	1.7	64
16:0	4	1.9	39.0	5.0	23.2	18.3	3.3	1.3	4.2	0.9	2.9	50
16:1 (ω7)	4	2.0	34.8	6.5	22.4	19.3	4.4	1.7	4.7	0.9	3.3	66
18:1 (ω9)	8	1.3	34.0	4.2	27.0	18.7	3.1	3.3	4.5	0.9	3.0	81
18:2 (ω6)	12	1.8	34.1	4.1	25.7	13.2	7.6	1.2	7.5	1.5	3.2	70
18:3 (ω3)	4	2.0	28.2	4.1	23.5	14.1	2.1	1.7	4.3	0.3*	6.9	72
18:3 (ω6)	8	1.7	38.2	4.5	23.7	13.0	2.6	-	9.3	2.1	2.6	65
20:4 (ω6)	4	1.2	45.5	3.3	21.7	11.9	3.8	-	8.8	1.0	2.8	52
FN_3	16	1.6	32.6	3.3	28.0	10.3	9.0	-	8.4	1.0	4.8	50
N	8	1.3	34.3	2.1	25.8	10.6	10.3	-	10.2	1.3	4.1	48

* 20:5 (ω3) rose to 8.8%.

differences in response, however: linoleate (18:2) restored both 18:2 and arachidonate (20:4) levels, whereas γ-linolenate (18:3) restored only arachidonate without affecting the low linoleate level. This relationship is consistent with the known metabolic conversion of linoleate to γ-linolenate to arachidonate (14,15). Administration of α-linolenate led to accumulation of higher members of the ω-3 series. The relative composition of fatty acids in the total lipid extract and the phospholipid fraction exhibited the same changes in pattern.

DISCUSSION: Polyunsaturated fatty acids exert a specific effect *in vivo* in

causing the lowering of activities of five enzymes concerned with the synthesis of saturated and monounsaturated fatty acids in liver. This is coincident with a rapid reciprocal change in the relative amounts of ω-6 and ω-9 series fatty acids in the liver lipids.

Examination of these five enzymes in vitro reveals that free fatty acids inhibit enzyme activity and that the polyunsaturated fatty acids are more potent in this respect than saturated fatty acids. The concentration of arachidonate required for 50% inhibition of CBX is 7×10^{-5} M (16, also see 17,18). This is 3 to 4 fold the maximum concentration of free arachidonic acid achieved in whole liver (Table I). It is also unlikely that free fatty acids directly inhibit these cytoplasmic enzymes in vivo, in view of the fact that 80% or more of FFA are bound to the particulate elements of the liver cell (16). Other studies in our laboratory indicate that there is a net loss of enzyme protein during the adaptive fall in enzyme activity in vivo. This would support the concept that polyunsaturated fatty acids (or a component containing or derived from them) may function as co-repressors in the synthesis of the lipogenic enzyme set (3).

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