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A Hypothesis Involving Competitive Inhibitions in the Metabolism of Polyunsaturated Fatty Acids*

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The inhibition of metabolism of oleate to eicosatrienoate and of linoleate to higher unsaturated fatty acids by dietary linolenate has been demonstrated. Under some conditions the metabolism of linolenate to docosahexaenoate is inhibited by linoleate or arachidonate. The influence of dietary linolenate upon the several product/precursor ratios for steps in linolenate metabolism has been examined and found to be different for different steps. Competitive inhibition seems to operate between polyunsaturated acids of the oleate, linoleate and linolenate families so that the pattern of polyunsaturated fatty acids is controlled by the concentrations of the precursors of each family.

In recent studies of metabolism of polyunsaturated fatty acids (PUFA), the changes in fatty acid composition of the total lipids of liver[1], heart[2], brain[3], erythrocytes and adipose tissue[4] were recorded as functions of the level of intake of single supplements to a fat-free diet: linoleate, arachidonate and linolenate. These studies revealed that increasing dietary content of linoleate or arachidonate causes an increasing tissue content of 5,8,11,14-eicosatetraenoate (20 : 4) and 4,7,10,13,16-docosapentaenoate (22 : 5ω6)***, both of which have a terminal structure equivalent to linoleate. Likewise, increasing dietary linolenate increases the tissue content of 5,8,11,14,17-eicosapentaenoate (20 : 5), 7,10,13,16,19-docosapentaenoate (22 : 5ω3) and 4,7,10,13,16,19-docosahexaenoate, all of which have terminal structures equivalent to linolenate. These results confirmed in detail the conclusions of Mead and his co-workers[5,6], and demonstrated that there is no metabolic crossover between the families of PUFA.

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*** This shorthand formula indicates 22 carbon atoms and 5 double bonds, the nearest of which lies 6 carbon atoms from the terminal methyl group. This notation is necessary to avoid confusion between isomeric metabolites of oleate, linoleate and linolenate.

In our studies certain unexpected anomalies were observed. When esters of one of these families of PUFA were fed in an otherwise fat-free diet, the concentrations of members of the other family in tissue lipids were depressed. This was particularly consistent and strikingly evident in a study in which linolenate was fed at six different levels together with linoleate at three different dietary intakes[7]. These results suggested metabolic competition between the two families of PUFA.

Such depressions of one PUFA by increasing another have been observed before. The content of hexaenoic acids in liver and brain lipids was less when corn oil was fed than when a fat-free diet was fed to rats[8]. Similarly, when the only dietary fatty acid was linoleate, the hexaenoic acid content of heart, liver, muscle and intestine lipid was less than when a fat-free diet was fed[9]. Hexaenoic acid content of rat liver, kidney, muscle, intestine, skin and adipose tissue was depressed by dietary arachidonate[10]. Privett *et al.*[11] observed a depression in total body arachidonate when linolenate was administered to rats. Klenk and Oette[12] also found that the arachidonate content of liver phospholipids of rats fed linolenate was less than when rats were fed a fat-free diet. These observations were given additional meaning when Marco *et al.*[13] and Machlin[14] reported that when linseed oil (containing linolenate) was fed, the linoleate content of chick liver was higher and the arachidonate content was lower than when linoleate was fed at a level equal to that in the linseed oil. They called attention to the probable inhibition of linoleate metabolism by linolenate. Dhopeshwarkar and Mead[15] have postulated a similar competition between oleic and linoleic acids for enzymes concerned with their metabolism.

The purpose of this communication is to describe the phenomena of competitive inhibition as we have observed them in PUFA metabolism of rats which received only purified fatty acid esters as dietary fat and PUFA precursors.

EXPERIMENTAL

The nutritional and analytical details of the studies of PUFA metabolism appear elsewhere[1,7], and will be repeated here only in general outline. Male weanling rats were fed a fat-free diet, and group-wise were administered orally different amounts of linoleate, arachidonate or linolenate. In one study linoleate was fed at three levels and linolenate at six levels to provide both precursors at different ratios. The food consumption of the rats was measured so that the proportion of calories provided by the supplements could be calculated and controlled. After 100 or 87 days, respectively, the rats were killed and the organs dissected. Lipids were extracted and methyl esters of the total fatty acids were prepared. These were analyzed by gas-liquid chromatography on ethylene glycol succinate columns.

RESULTS AND DISCUSSION

When linoleate was fed alone in increasing levels of the diet[1–3], the only PUFA which was significantly or consistently depressed was the 5,8,11-eicosatrienoic acid, which is elevated in essential fatty acid deficiency and which is synthesized from oleic acid[15]. In these animals (which were fed no linolenate) the levels of linolenate metabolites in the tissue lipids were so low that in many cases they could not be measured. Moreover, the levels of linoleate fed were not sufficiently high to effectively compete with linolenate metabolism. When arachidonate was fed as the sole fatty acid supplement in a fat-free diet, the 22 : 6 content of liver and brain lipids diminished as the arachidonate supplement increased. Thus,

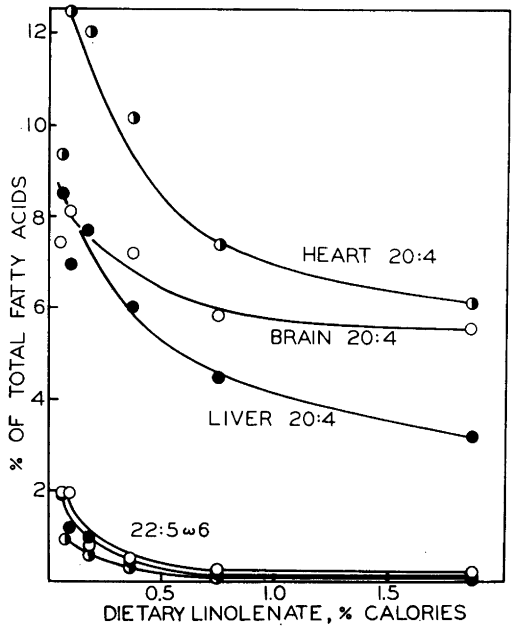

Fig. 1. The effect of increasing levels of dietary linolenate upon the content of two linoleate metabolites in liver and heart lipids of rats fed linoleate at 0.6 % of calories.

arachidonate was able to compete more effectively with linolenate metabolism than could linoleate.

When linolenate was the sole fatty acid supplement to the fat-free diet, several inhibitions of linoleate metabolism were observed. As dietary linolenate increased, 20 : 4 and 20 : 5ω6 contents of liver, heart and brain lipids decreased regularly. The 20 : 4 content of erythrocytes was likewise diminished significantly.

When either linoleate, arachidonate or linolenate was fed, the most striking effect was the depression of 5,8,11-eicosatrienoate, the oleate metabolite prominent in essential fatty acid deficiency. All the dietary supplements depressed this metabolite in all tissues measured, and dietary 20 : 4 was more efficient in depressing it than was dietary linoleate (18 : 2). Linolenate (18 : 3) was similar to arachidonate in this regard.

In the foregoing experiments the contents of tissue PUFA, except those synthesized from the dietary supplement, were low and subject to the animals' dietary history prior to the experiment. In the second group of experiments[7] the animals were fed both linoleate and linolenate to provide at least a minimal constant supply of each precursor in several proportions. In this experimental situation the same competitions and inhibitions were observed more clearly.

At all three levels of linoleate (0.1, 0.3 and 0.6 % of calories) increasing levels of dietary linolenate suppressed the content of 20 : 3ω9 (oleate metabolite), 20 : 3ω6, 20 : 4 and 22 : 5ω6 (linoleate metabolites) in liver and heart lipids. In

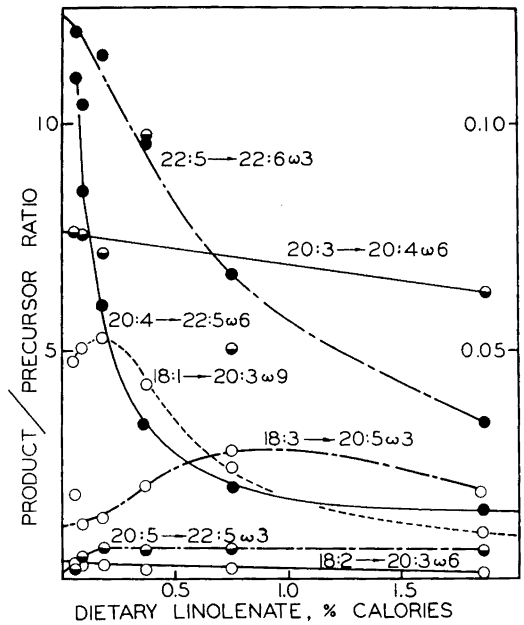

Fig. 2. The effects of increasing level of dietary linolenate upon the product/precursor ratios for several steps of oleate, linoleate and linolenate metabolism. The linoleate content of the diet was held at 0.6 % of calories for all groups. Notice that the right hand scale applies to $20:4 \rightarrow 22:5\omega6$ and $18:1 \rightarrow 20:3\omega9$.

brain lipids $20:4$ and $22:5\omega6$ were likewise depressed. Some typical responses are shown in Fig. 1. The curves indicate that as the precursor to one family of PUFA is increased in the metabolic system, the synthesis of the higher members of the other principal family of PUFA is inhibited.

Conversely, the synthesis of linolenate metabolites was seemingly unaffected by increases in dietary linoleate. However, the three levels of linoleate were quite low and the competitive equilibrium appears to be strongly in favor of the linolenate metabolites. When the ratios of principal product to its precursor are calculated for the conversion $18:1 \rightarrow 20:3$, $18:2 \rightarrow 20:4$, and $18:3 \rightarrow 22:6$ in rats provided a minimal amount of linolenate (0.7 % of calories), these ratios are 0.45, 1.2 and 12.3, respectively. In these rats whose liver lipids contain the respective precursors, more than 30 % 18 : 1, 1.4 to 3.7 % 18 : 2, and 0.4 to 0.8 % 18 : 3, the metabolic pathway of chain lengthening and dehydrogenation obviously favors the substrates in the order linolenate > linoleate > oleate despite the wide differences in precursor concentrations favoring a reverse order.

We consider that in rats which have been fed a constant diet for 87 days the composition of their lipid fatty acids represents a steady state. We assume also that the total fatty acids of liver lipid reflect substrates and products of metabolic conversions of PUFA. If a steady state exists in these lipids, differences in ratio between products and precursors might reflect competitive inhibitions of

specific steps in the metabolic pathway as a competitive metabolite is increased in concentration. Some such relationships are shown in Fig. 2 using data from rats fed a constant level of linoleate of 0.6 % of calories and differing levels of linolenate.

Consider first the product/precursor ratios for the metabolites of linolenate itself, which is the dietary variable. The ratio of 20 : 5/18 : 3, representing the first two steps of its metabolic chain, varies erratically and minimally as the available 18 : 3 is increased. The ratio 22 : 5ω3/20 : 5 rises slightly but consistently through a low narrow range. Thus, these two steps in linolenate metabolism are largely uninhibited by increased linolenate substrate. However, the ratio for the last step of linolenate metabolism 22 : 6/22 : 5ω3 is depressed with increasing dietary 18 : 3, indicating that this step is self-limiting and that 22 : 6 accumulates as the metabolite in principal amount followed by accumulation of 22 : 5ω3.

The conversion of 18 : 1 to 20 : 3 is inhibited distinctly by increasing its competitive inhibitor, linolenate. Under the conditions of this experiment in which linoleate is fed at constant level, the content of 20 : 3 is already depressed by linoleate, but increasing linolenate depresses it even farther.

Within the linoleate family the product/precursor ratio for 18 : 2 $\rightarrow$ 20 : 3ω6 is very low indicating that the latter does not accumulate in high concentration. The low ratios are depressed somewhat by increasing linolenate. The product /precursor ratio for 20 : 3ω6 $\rightarrow$ 20 : 4 is considerably higher but varies erratically as linolenate is increased. The high ratio reflects the accumulation of 20 : 4 which is the metabolite in principal abundance, and its erratic variability through a narrow range indicates that competition from linolenate metabolites does not play a dominant role at this step. However, the last step of the metabolic chain 20 : 4 $\rightarrow$ 22 : 5ω6 is profoundly inhibited by linolenate metabolites. The effect of strong inhibition of this last step is to cause the accumulation of arachidonate as the most abundant metabolite of linoleate. This phenomenon probably operates in natural circumstances, for linolenate metabolites occur widely in foodstuffs.

Let us assume what seems evident, that three metabolic chains of reaction are in competition for a common metabolic system of enzymes:

$$18:1 \rightarrow 18:2 \rightarrow 20:2 \rightarrow 20:3 \qquad \text{(all } \omega 9\text{)}$$

$$18:2 \rightarrow 18:3 \rightarrow 20:3 \rightarrow 20:4 \rightarrow 22:4 \rightarrow 22:5 \qquad \text{(all } \omega 6\text{)}$$

$$18:3 \rightarrow 18:4 \rightarrow 20:4 \rightarrow 20:5 \rightarrow 22:5 \rightarrow 22:6 \qquad \text{(all } \omega 3\text{)}$$

Assume also that enzyme substrate affinities increase with unsaturation. At each chain length stage of metabolism, the metabolites of linolenate would then preferentially occupy the enzyme sites to the inhibition of linoleate and oleate metabolism. Linolenate would be metabolized principally to 22 : 6, and the accumulation of the latter would inhibit the formation of 22 : 5ω6. Arachidonate would accumulate as principal metabolite in its family, all members of which would be depressed by competitive inhibition exerted by linolenate metabolites of comparable chain length. These suppositions in the main agree with the experimental data given in Fig. 2 and other observations mentioned.

The assumption of a common metabolic pathway for the three families of PUFA is not mandatory. Each family could be metabolized *via* a separate path-

way parallel to the others. Competitive inhibition by the other substrates could lead to the same phenomena. Competitive inhibition is known to operate in a similar situation, in the lipoxidase oxidation of linoleate. This reaction is competitively inhibited by oleate, octanoate, conjugated linoleate and *trans,trans,trans*-linolenate, none of which are oxidized by the enzyme[17]. Thus, each separate metabolic pathway of the three fatty acid families could be inhibited competitively by the substrates of the other two families. A third mechanism of inhibition is also possible. If the metabolism of linolenate proceeds much more rapidly than the parallel metabolisms of oleate and linoleate, a common required co-factor, such as coenzyme A, may be preferentially involved in the linolenate metabolic chain, reducing its concentration or availability for the other two metabolic sequences.

It should be pointed out that in considering changes in product/precursor ratios, all metabolites arising from oleate and linoleate are depressed by increasing linolenate, and that we are considering only those increases or decreases which are not proportional to the whole, and are, therefore, specifically involved in competitive inhibition to a disproportionate degree. The observations that individual metabolites are influenced to differing degrees by linolenate precludes the possibility that the inhibitions or depressions observed are mere artifacts of dilution. If, for example, inclusion of linolenate in a diet caused accumulation of it and its metabolites without inhibiting oleate or linoleate metabolism, all of their metabolites would decrease by the same proportion due to dilution. This is clearly not the case, for 5,8,11-eicosatrienoic acid is dramatically reduced, arachidonate is moderately depressed, and saturated and monoenoic acids are essentially unaffected by the same level of linolenate.

CONCLUSION

The changes in composition of tissue PUFA as a consequence of changes in dietary fat have heretofore presented a complex and confused picture. The changes in PUFA involve principally the metabolites of oleate, linoleate and linolenate, although other minor families of unsaturated acids are present. The principal PUFA in fat-deficient animals is an oleate metabolite normally found in low concentrations in tissues. The highly unsaturated fatty acids of normal tissues are metabolites of linoleate and linolenate, and the pattern of these varies with the kind and amounts of these precursors. We offer a unified hypothesis to explain the regulation of all these metabolites and the variations in pattern of PUFA resulting from deprivation or supplementation of essential or polyunsaturated fatty acids. Observations to date indicate that the families of PUFA competitively inhibit each other's metabolism. Competitive inhibition requires that the metabolism of the three families of PUFA have some enzymatic pathway or co-factor in common. Enzyme-substrate affinities of the three families of PUFA are in the order linolenate $>$ linoleate $>$ oleate. When linolenate is present in the substrate pool, its conversion to higher unsaturated acids takes precedence over the metabolism of linoleate by a factor near tenfold. Linoleate metabolism proceeds in preference to oleate metabolism. Oleate metabolism to higher unsaturated acids can take place only when linoleate and linolenate are in very low concentration. Thus, in simplification, the pattern of PUFA in tissue

lipids is controlled by the concentrations of competing substrates in a common metabolic pathway.

The observations reported here and the speculations inspired by them have led to a working hypothesis which fits the experimental observations to date. Undoubtedly, as presented here, it is incomplete and subject to considerable change by future experimentation. The hypothesis is presented in the hope that it will provoke investigations to test its validity. Nutritional experiments and compositional studies perhaps cannot yield more precise data concerning specific enzyme mechanisms, but the insights provided by such experiments can lead to studies of the same phenomena on a cellular or enzymatic level of observation.

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