THE CONTROL OF STEAROYL-CoA DESATURASE BY DIETARY LINOLEIC ACID

R. JEFFCOAT and A. T. JAMES

Biosciences Division, Unilever Research Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, England

1. Introduction

The biosynthesis of fatty acids by rat liver is controlled, at least in part, by the levels of dietary carbohydrate and polyunsaturated fats [1]. In this connection, much attention has been focused upon two cytoplasmic proteins: acetyl-CoA carboxylase and fatty acid synthetase. Although it has been known for many years that feeding carbohydrate-rich diets results in elevated levels of these enzymes, only recently has it been demonstrated with fatty acid synthetase that dietary linoleic acid decreases the level of the enzyme protein [2]. Dietary carbohydrate also elevates the level of stearoyl-CoA desaturase [3]. However little documented data are available on the control of the levels of this enzyme by linoleic acid although it is well established that dietary poly-unsaturated fatty acids suppress its activity [4]. We now present further evidence for the close control of fatty acid synthetase and stearoyl-CoA desaturase and demonstrate the rapid response of the desaturase to dietary linoleic acid. The activity of the enzyme is diminished by approx. 60% in the first 18 h of feeding a polyunsaturated fatty acid diet, which is in marked contrast to a $t_{1/2}$ of 2 days for fatty acid synthetase. The significance of these results is discussed in terms of the control of lipogenesis.

2. Materials and methods

2.1. *Animals and diets*

Male litter weanling rats of the Colworth-Wistar

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strain were used for all the experiments described. Rats were allocated to dietary groups of six and allowed food and water ad libitum. The diets used for these experiments were all based on a purified diet of the following composition: starch (containing 0.35% (w/w) linoleic acid) 44.7%, casein 25%, cellulose powder 6%, sucrose 20%, salts 4%, vitamins 0.3%. When corn oil (60% (w/w) linoleic acid) was added to this diet to the required concentration, the calorie content was kept at 410–415 cal/100 g diet by replacing the starch and adding cellulose powder to maintain the weight. Rats fed a fat-free diet for 14 days had specific activities for fatty acid synthetase and stearoyl-CoA desaturase of 1.5 μmol NADPH oxidised/min/g liver and 1.25 nmol oleate produced/min/mg microsomal protein, respectively.

2.2. *Enzyme assays*

Microsomal fractions and 100 000 × *g* supernatant fractions were prepared as described [5]. Stearoyl-CoA desaturase and fatty acid synthetase were assayed as in [5] and [6], respectively. The individual specific activities shown on all the figures represent the average value from six rats and all enzyme assays were carried out in triplicate.

Protein was determined as in [7] using defatted bovine serum albumin as a standard.

3. Results and discussion

3.1. *Effect of dietary lipid on stearoyl-CoA desaturase activity*

The activity of fatty acid synthetase and stearoyl-CoA desaturase can be modulated by dietary fat [4]. Comparisons of enzyme activities are made on liver

preparations from rats fed either high carbohydrate/ low fat or high fat/low carbohydrate diets [1]. We have investigated the relative amounts of carbohydrate and fat such that the source of dietary linoleic acid is just sufficient to override the inductive effect of the carbohydrate.

Rat were fed ad libitum for two weeks on iso-caloric diets containing either 20% (w/w) sucrose or the same diet supplemented with between 0.5% and 10% (w/w) corn oil. As shown in fig.1 both fatty acid

Fig.1. The effect of dietary corn oil on the specific activities of hepatic fatty acid synthetase and stearoyl-CoA desaturase. All diets contained 20% (w/w) sucrose with increasing amounts of corn oil added at the expense of starch and the bulk made up with cellulose powder to maintain all diets iso-caloric.
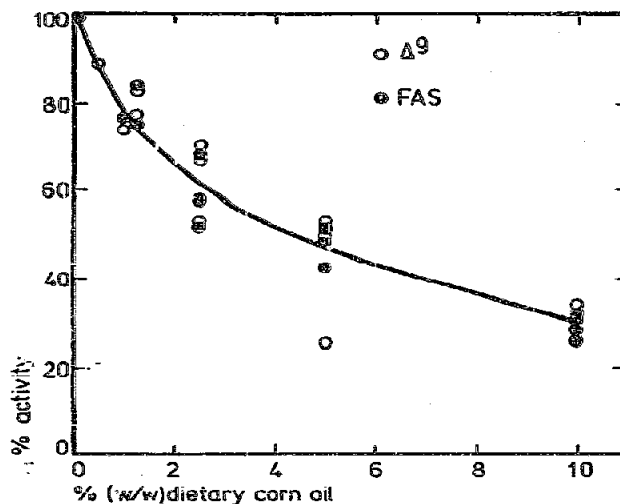
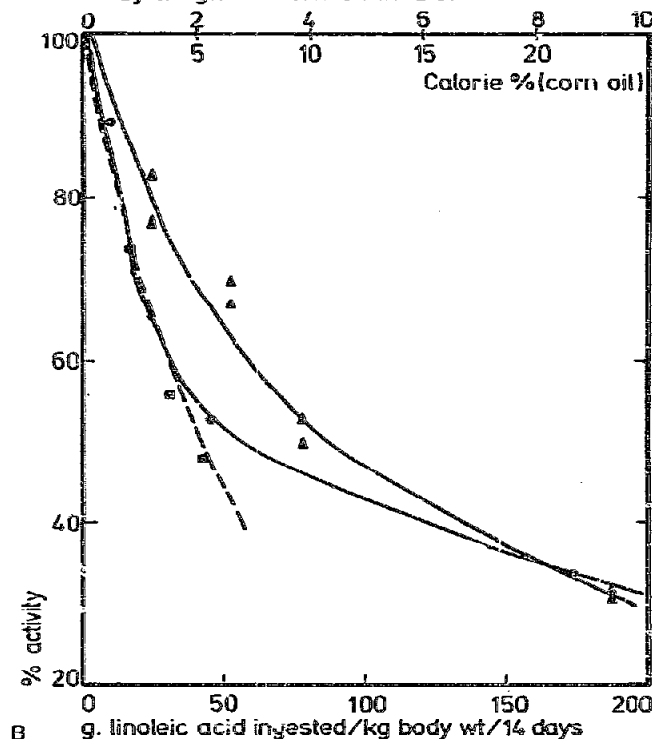
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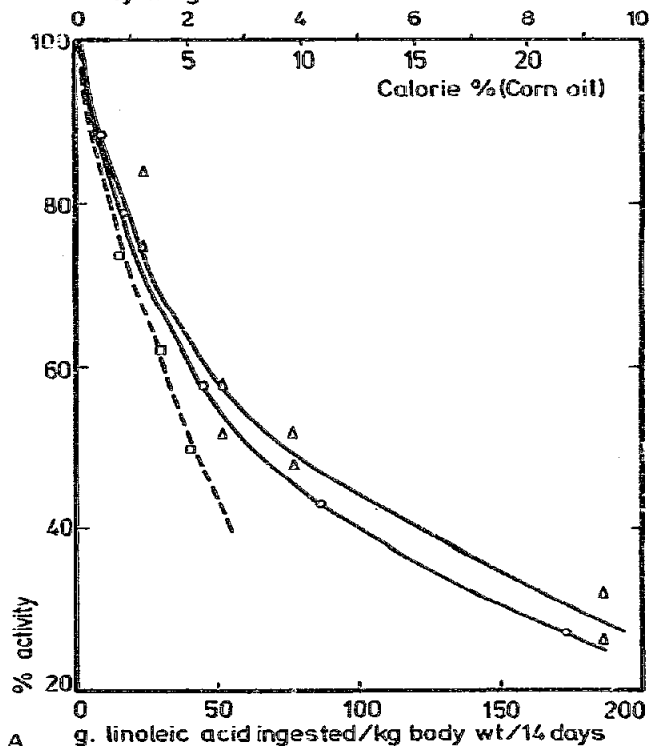
Stearoyl—CoA Desaturase

Fig.2. A comparison of the effects of corn oil and ethyl linoleate on the specific activities of (A) fatty acid synthetase and (B) stearoyl-CoA desaturase. (□■) Specific activities determined when rats were fed sucrose plus ethyl linoleate; (○●) sucrose plus corn oil; (△▲) glucose plus corn oil.

synthetase and stearoyl-CoA desaturase decreased to approx. 30% of their activities on a fat-free diet. A comparison of these data with those reported [4] on the effect of dietary ethyl linoleate, fig.2, shows that the main contributory component of the corn oil is linoleic acid.

3.2. Time course of the linoleic acid effect

The data given above reflect the long term effect of linoleic acid on the levels of two lipogenic enzymes but give no indication of possible short-term effects which might exist as a direct result of the diurnal feeding pattern of the animal in question. To investigate possible acute effects seven dietary groups of rats were fed a fat-free diet containing 20% (w/w) sucrose for 2 weeks. At the end of this feeding period 1 group (6 rats) was sacrificed to determine the level of fatty acid synthetase and stearoyl-CoA desaturase, shown as 100 in fig.3. A second group was maintained on the same diet for a further two weeks. The remaining 5 groups were transferred to a diet containing 5% (w/w) corn oil and 20% (w/w) sucrose and at intervals of approx. 1, 2, 4, 8 and 14 days, groups of rats were sacrificed to determine the level of the two enzymes. A second experiment was also carried out in which the diets were changed to 10% (w/w) corn oil and 20% (w/w) sucrose for the last 14 days of the feeding study. The results of these studies are shown in fig.3 from which three main observations can be made:

(i)   Rats fed 20% (w/w) sucrose for four weeks maintain high levels of fatty acid synthetase and stearoyl-CoA desaturase.
(ii)   Sucrose/corn oil diets reduce the activity of both enzymes.
(iii) The time taken to reduce the activity of fatty acid synthetase and stearoyl-CoA desaturase by 50% is 2 days and less than 12 h, respectively.

These values bear a close correlation with the values reported for the $t_{1/2}$ for fatty acid synthetase [2] and stearoyl-CoA desaturase [3]. Dietary linoleic acid in the form of safflower oil reduces the level of fatty acid synthetase in rat liver not only by decreasing its rate of synthesis but also by increasing its rate of degradation from a $t_{1/2}$ of 3.8 days to 1.9 days [2]. Our data on the half-life of the activity of fatty acid synthetase not only provides a useful control agreeing well with the data [2] but suggests that dietary
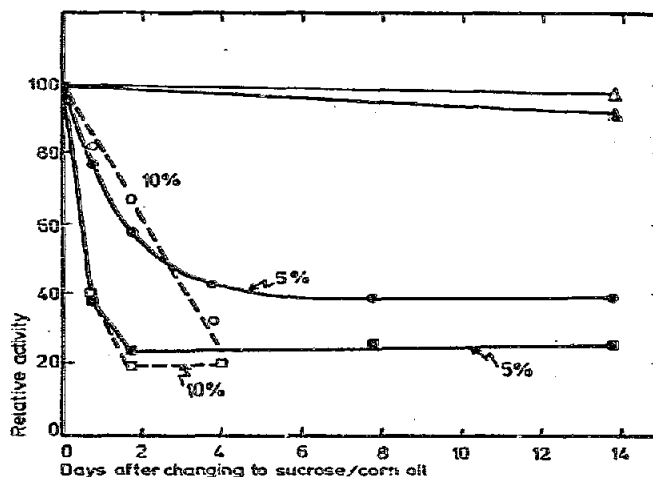

Fig.3. Time course for the effect of dietary corn oil on the specific activities of fatty acid synthetase and stearoyl-CoA desaturase. Rats were fed fat-free diets for 14 days and then switched to 5% or 10% (w/w) corn oil-supplemented diets. Fatty acid synthetase (o●△) and stearoyl-CoA desaturase (□■▲) were measured after 17 h, 41 h, 89 h, 185 h and 329 h.

linoleic acid might also control the level of the desaturase enzyme. The other possibility is that linoleic acid simply inhibits the desaturase either directly or indirectly by modifying the composition of the membrane but this would seem unlikely since stearoyl-CoA desaturase activity is independent of the membrane lipid composition [8].

In order to study the control of the desaturase, rats were starved for 24 h and then re-fed either 20% (w/w) sucrose diets or 5% (w/w) corn oil and 20% (w/w) sucrose diets. Rats were sacrificed at 3 h intervals after re-feeding and the activity of stearoyl-CoA desaturase determined in the liver microsomes. Figure 4 shows the results of these experiments and clearly demonstrates a 6–9 h lag prior to the induction of the enzyme which occurs at a similar rate both in the presence and absence of dietary linoleic acid. These data are very similar to those reported for fatty acid synthetase [2], which differ only in that the induction of the synthetase does not show a lag period. However studies with another microsomal enzyme, palmitoyl-CoA elongase [9] have revealed a 6 h lag period before induction of the enzyme after a period of starvation. Therefore it appears that under certain
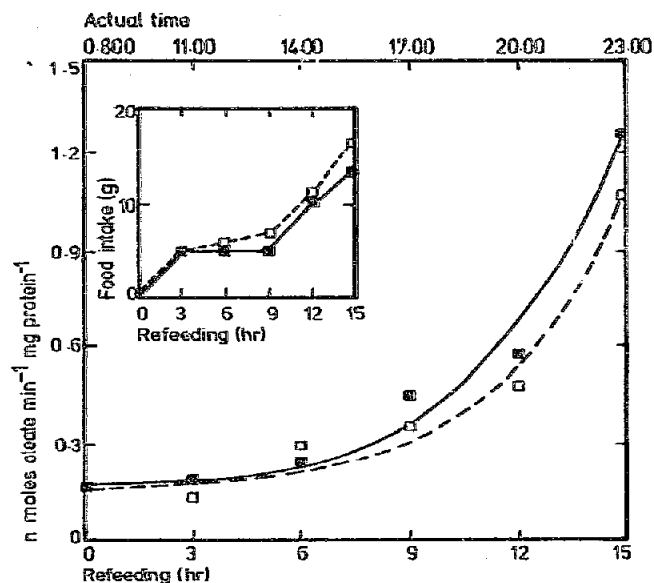
Fig.4. Time course for the induction of stearoyl-CoA desaturase. Rats were starved for 24 h and then fed either a sucrose (■) or a corn oil supplemented diet (□) ad libitum from 08.00 h (time 0). Food intakes were measured and are shown in the inset as g ingested/rat as a function of time after re-feeding.

extreme physiological conditions, the controlling effect of linoleic acid can be suppressed until the normal physiological balance is restored.

3.3. Effect of indomethacin on the activity of stearoyl-CoA desaturase

The high sensitivity and specificity of the linoleic acid effect [4] would suggest that it is a result of a specific property of this fatty acid and not a general fatty acid effect. The effect can be overcome by administering an acetylenic analogue of arachidonic acid which seems to inhibit the further metabolism of linoleic acid [10]. This suppression of the linoleic acid effect can be overcome by feeding arachidonic acid which suggests that one possible mode of action of linoleic acid may be via the prostaglandins. Inhibitions of prostaglandin biosynthesis should therefore reverse the inhibition of lipogenesis by linoleic acid.

Five groups of rats (A–E) were fed a fat-free diet of 20% (w/w) sucrose for 14 days. On days 11, 12, 13 and 14 at 09.00 h, four groups of rats (B–E) were injected intraperitoneally with either indomethacin (0.5 mg/ml polyethylene glycol 400) or polyethylene glycol 400 at a dose level 2 mg indomethacin/kg body wt. On day 14 groups A–C were fed ad libitum for 24 h the same sucrose diet while groups D and E were fed a sucrose diet supplemented with 5% corn oil. The rats were sacrificed and the liver microsomes used to determine the specific activity of the stearoyl-CoA desaturase (table 1).

It is apparent from these results that indomethacin at the dose level used is unable to reverse the effect of dietary linoleic acid. However at this level indomethacin has been shown to cause approx. 70% inhibition of prostaglandin synthesis as determined by the level of urinary metabolites of prostaglandins (Hartop, P. personal communication). It is possible, therefore, that this is insufficient inhibition to reverse the linoleic acid effect on hepatic lipogenesis. Similar results, however, have been obtained [2] suggesting that prostaglandin synthesis is not required for the linoleate-induced decrease in the specific activity of hepatic fatty acid synthetase. However, polyunsatu-

Table 1
The effect of indomethacin injections on the specific activity of stearoyl-CoA
desaturase (nmol/min/mg microsomal protein ± SD)

Treatment	Diet	
	Sucrose (20%)	Sucrose (20%)/corn oil (5%)
—	0.9 ± 0.15	—
Indomethacin	1.05 ± 0.22	0.34 ± 0.1
Polyethylene glycol 400	0.94 ± 0.19	0.43 ± 0.09

rated fatty acids [11—15], in particular α-linolenic acid [13] which can also act as a prostaglandin precursor [16], are also capable of repressing the activities of hepatic fatty acid synthetase and glucose-6-phosphate dehydrogenase. Although the molecular mechanism whereby polyunsaturated fatty acids regulate lipogenesis is still not clear, it does appear that animals on mixed diets have evolved a mechanism whereby these fatty acids, possibly acting as markers of total fat intake, can switch off de novo fat synthesis which operates maximally in animals fed high carbohydrates/low fat diets. It is possible that the dietary polyunsaturated fatty acids act in the short-term (hours) by regulating the level of the desaturase resulting in an accumulation of saturated fatty acids which inhibit acetyl-CoA carboxylase [17]. Longer-term control of lipogenesis by regulation of acetyl-CoA carboxylase with a $t_{1/2}$ 48 h [2] may then be effected by the polyunsaturated fatty acids controlling the levels of these enzymes.

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