THE POSITIONAL DISTRIBUTION OF FATTY ACIDS INCORPORATED INTO TRIACYLGLYCEROLS BY RAT ADIPOSE TISSUE SLICES IN VITRO

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SUMMARY: Rat adipose tissue slices incorporated [1-^{14}C]-palmitic acid, [1-^{14}C]-stearic acid, [1-^{14}C]-oleic acid and [1-^{14}C]-linoleic acid from mixtures similar to those found in plasma into triglycerides at comparable rates in vitro. Although similar relative proportions of oleic acid and linoleic acid entered each of the three positions of the triglycerides as occur naturally, somewhat less palmitic acid entered position sn-1, less stearic acid entered position sn-2 and considerably more oleic acid, formed de novo from stearic acid, entered position sn-2 than are found in these positions naturally. Positional distributions are therefore not controlled solely by the availability of fatty acids and the specificities of the acyl transferases.

The fatty acid components of adipose tissue triglycerides may be synthesised de novo in adipose tissue itself or may originate from the diet or elsewhere in the body of the animal and be transported to the adipose tissue via the plasma. Some modification of preformed fatty acids can also occur in adipose tissue e.g. desaturation of stearic to oleic acid. The relative contributions of fatty acids from each source will vary considerably with species but in the rat, a high proportion of the fatty acids are synthesised de novo in adipose tissue (1); an active desaturase system is also present (2). Fatty acids are utilised for triglyceride biosynthesis via the glycerol-3-phosphate pathway in rat adipose tissue (3) although the 2-monoglyceride pathway is important in some species. The control mechanism by which fatty acid biosynthesis is coupled to triglyceride biosynthesis is poorly understood,

however. Some information on this process has been obtained from structural analyses of triglycerides. In mammary gland, for example, it has been suggested that stearic acid desaturation is coupled to the acylation of position sn-3 and may exert a controlling influence (4). In pig adipose tissue, there were apparently differences in the manner in which fatty acids synthesised de novo were esterified to the three positions of the triglyceride molecules compared to fatty acids from other sources (5). In this study, the proportions in which exogenous [^{14}C]-labelled free fatty acids were esterified to each of the three positions of the triglycerides by rat adipose tissue slices in vitro have been determined for comparison with the natural distribution of the acids in the triglycerides.

MATERIALS AND METHODS

Parametrial adipose tissue slices (approx. 100 mg in total) from rats were incubated in Krebs-Ringer bicarbonate buffer (2.5 ml) containing bovine serum albumin (100 mg), insulin (10 μg) and glucose (2.5 mg) at 37°C together with a [^{14}C]-labelled unesterified fatty acid (2.5 μ moles, 0.2 μCi). The unesterified fatty acid was solubilised by dissolving in 0.1N potassium hydroxide solution (0.1 ml) containing bovine serum albumin (4 mg) and consisted of a mixture of palmitic acid (30 per cent), stearic acid (10 per cent), oleic acid (40 per cent) and linoleic acid (20 per cent); the component carrying the [^{14}C]-label (0.2 μCi in each instance) was varied. These proportions were similar to those found in the free fatty acid fraction of plasma and in the triglyceride fraction of rat adipose tissue. The amounts added were sufficient to swamp the effects of any unesterified fatty acids endogenous to the tissue. Control samples containing [1-^{14}C]-palmitic acid (2.5 μ moles) alone were incubated simultaneously. The amounts and fatty acid compositions of the unesterified

fatty acids in the incubation media were checked independently by gas chromatography in the presence of heptadecanoic acid internal standard after separation by preparative thin layer chromatography (TLC) (6) so that any residual unesterified fatty acid in the albumin could be allowed for. After incubation, the adipose tissue slices were removed from the medium with forceps before the lipids were extracted by homogenisation with chloroform-methanol (2:1, v/v). An unlabelled carrier consisting of a mixture of unesterified fatty acids, diglycerides and phospholipids was added before lipid classes were separated by TLC on layers of silica gel G (0.5 mm thick); hexane-diethyl ether-formic acid (80:20:2 by vol.) was the developing solvent. Fractions were counted in suspension on the silica gel G in Unisolve 1 (Koch-Light Laboratories, Colnbrook, Bucks.) in a Tricarb 2425 liquid scintillation spectrometer (Packard Instruments, Reading, Berks.). The procedure for stereospecific analysis of triglycerides containing isotopically-labelled fatty acids has been described in detail elsewhere (7, 8). [1-^{14}C]-Stearic acid and [1-^{14}C]-oleic acid formed from it were separated by silver nitrate chromatography of their methyl ester derivatives (8, 9).

RESULTS AND DISCUSSION

The results for the distribution of [1-^{14}C]-palmitic acid esterified to the various lipid products with respect to time in the control samples are illustrated in Fig. 1. Triglycerides were the main product and were formed rapidly in increasing proportions. Diglycerides and phospholipids accumulated much more slowly and reached maximum levels after about 4 hours. [1-^{14}C]-Palmitic acid added to the medium in the unesterified fatty acid mixture was esterified at essentially the same rate. Similarly, the rates of esterification of [1-^{14}C]-stearic acid (without allowing for desaturation), [1-^{14}C]-oleic acid and [1-^{14}C]-

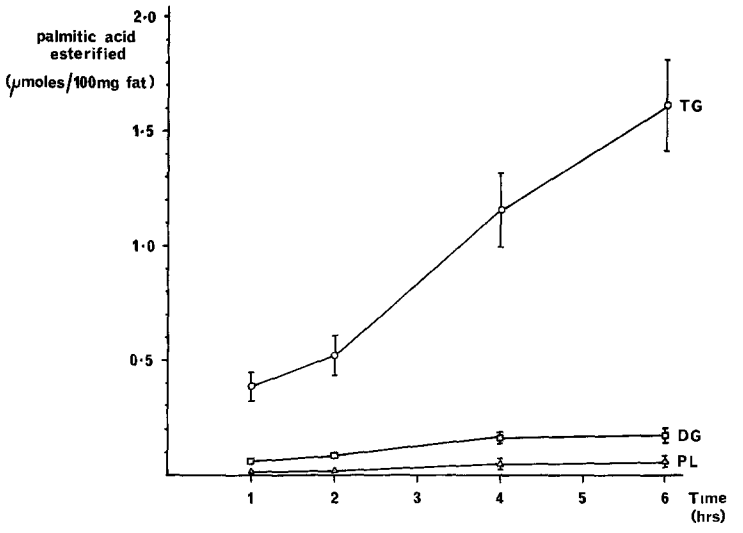

Fig. 1. The amounts of [1-^{14}C]-palmitic acid (control samples) entering each lipid class with respect to time (means and standard deviations of four experiments. O, Triglycerides (TG); □, diglycerides (DG); △, phospholipids (PL).

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linoleic acid and the pattern of products did not differ significantly from that of the control sample when the results were corrected for the differing initial concentration of each substrate. Stearic acid was desaturated uniformly to oleic acid; the mean value for the proportion of desaturation occurring with subsequent esterification to triglycerides at 1, 2, 4 and 6 hour intervals was 26.4 ± 6.2 per cent of the labelled fatty acid esterified.

Duplicate samples from incubations for 2, 4 and 6 hours for each labelled fatty acid in the mixture were subjected to stereospecific analysis with tri-[10,11-^{3}H]-heptadecanoin as internal standard. As very similar results were obtained for the relative proportion of each esterified fatty acid formed in each position at these time intervals, the results were meaned and are compared with the normal distributions of the same fatty acids in rat adipose tissue triglycerides in table 1. [1-^{14}C]-Oleic acid (added to the medium in this form) and [1-^{14}C]-

Table 1. Relative incorporation of [^{14}C]-labelled fatty acids into the three position of sn-triacylglycerols by rat adipose tissue slices.

Fatty Acid	Proportional (%) distribution in each position					
	Normal distribution[a]			Incorporation in vitro[b]		
	1	2	3	1	2	3
16:0	54.2 ± 1.5	11.8 ± 0.3	34.0 ± 1.5	45.7 ± 0.3	14.3 ± 0.4	40.0 ± 0.6
18:0	56.9 ± 4.3	21.3 ± 1.6	21.8 ± 2.9	51.6 ± 1.6	5.8 ± 1.1	42.6 ± 2.2
18:1	24.3 ± 0.8	40.0 ± 0.8	35.7 ± 1.4	22.9 ± 1.0	38.6 ± 0.5	38.5 ± 0.7[c]
				18.2 ± 6.2	63.3 ± 4.0	18.5 ± 4.1[d]
18:2	16.0 ± 0.8	56.4 ± 0.9	27.6 ± 0.4	16.2 ± 0.6	52.8 ± 1.0	31.0 ± 1.4

a Means and standard deviations of three analyses of rat adipose tissue triglycerides
b Means and standard deviations of duplicate samples from 2, 4 and 6 hour incubations.
c Oleic acid itself added.
d Oleic acid formed from 18:0 in the medium.

linoleic acid entered the three positions of the triglyceride molecule in very similar relative proportions to the natural distribution of each in adipose tissue triglycerides. A somewhat higher proportion of [1-^{14}C]-palmitic acid entered positions sn-2 and sn-3 and correspondingly less entered position sn-1 than is found normally; fat-free homogenates of rat adipose tissue esterified [1-^{14}C]-palmitic acid to each position of the triglycerides in relative proportions virtually the same as those occurring naturally on the other hand (7). Much less [1-^{14}C]-stearic acid entered position sn-2 with a corresponding increase in position sn-3 than was found naturally. A considerably higher proportion of oleic acid formed in the medium from [1-^{14}C]-stearic acid was esterified to position sn-2

and correspondingly less was found in positions sn-1 and sn-3 than was expected from the natural distribution of oleic acid in adipose tissue or from incubation with [1-^{14}C]-oleic acid itself.

Samples incubated with [1-^{14}C]-linoleic acid and [1-^{14}C]-stearic acid for one hour gave very similar results to those in table 1. Samples incubated with [1-^{14}C]-palmitic acid and [1-^{14}C]-oleic acid for one hour gave much lower relative proportions of each substrate in position sn-1 (29.4 per cent and 8.2 per cent respectively) than was obtained with longer incubations presumably because the adipose tissue slices contained some endogenous unlabelled sn-1-acyl precursors of triglycerides, that were soon used up by the reaction.

Apparently then, the supply of exogenous fatty acids controlled the relative proportions of the fatty acids entering the triglycerides but other factors controlled the positional distribution. These other factors which probably include the fatty acid desaturase enzyme system and the enzymes of fatty acid synthesis may also contribute to the control of the rate of triglyceride biosynthesis in adipose tissue. This aspect of the problem is currently being investigated. In contrast to this finding, subcellular homogenates of adipose tissue were recently used in vitro (3) to demonstrate that the relative proportions of palmitic and oleic acid entering position sn-2 of triglycerides were apparently controlled entirely by the transacylases of the glycerol-3-phosphate pathway. However, it would appear from the study reported here that a wider range of fatty acids should be utilised and full stereospecific analyses of the triglycerides should be carried out before definitive conclusions are drawn about the nature of the control mechanism.

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