Composition and ^{32}P Turnover of Phospholipids of Rat Adipose Tissue

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Although the composition and metabolism of adipose tissue have been extensively studied during recent years, information on the phospholipids of this tissue remains scant. The only report so far on this subject is that of Spencer and Dempster,[1] who analyzed the composition of mouse brown and white fat. As a first step in study of possible hormonal effects on the phospholipid metabolism of adipose tissue we have assessed the phospholipid composition and the *in vitro* and *in vivo* incorporation of ^{32}P into the individual components of rat epididymal fat.

Methods. White male rats weighing 200–250 g were used after overnight fasting. The excised epididymal fat pads were extracted with chloroform-methanol, 2:1, and the extract washed according to the procedure of Folch *et al.*[2] An aliquot of the washed chloroform phase was taken for P analysis[3] and the rest dried in a stream of nitrogen. Phospholipids were separated from the bulk triglyceride by phase extraction with petroleum ether-87 % ethanol, 2:1.[4] The aqueous ethanol phase was re-extracted twice with the same solvent, the combined extracts taken to dryness, the residue dissolved in a small amount of chloroform-methanol and the phospholipids separated by two-dimensional chromatography on 0.25 mm Silica gel H (Merck). The developing solvents were chloroform-methanol-7 N ammonia (60:35:5) and chloroform-methanol-conc. ammonia (35:60:3).[5] The plates were air-dried and the spots visualized with iodine vapor. The fractions were outlined, scraped off the plate, eluted twice at 50 to 60°C with 1 N HCl in methanol and analyzed for P content.[6]

In the *in vitro* incorporation experiments fat pads were incubated for different periods (30–120 min) in an O_2-CO_2 atmosphere in Krebs-Ringer bicarbonate medium containing 2 mg/ml glucose and 20 μC/ml ^{32}P (Radiochemical Centre, Amersham). After incubation, the tissue was carefully washed with saline and the phospholipids separated and fractionated as described above. To study the labeling of adipose tissue phospholipids *in vivo*, rats were given about 0.2 mC of ^{32}P intraperitoneally and killed 4, 8, 12, 16, and 20 h later. For determination of radioactivity, aliquots of the acid methanol extract of each fraction were dried in counting vials, scintillation solution (0.3 % PPO and 0.03 % dimethyl-POPOP in toluene) was added and counts were made in a Packard Tricarb liquid scintillation counter.

Results. The total concentration of lipid P in the epididymal fat amounted to 60–70 μg/g wet weight. The percentage distribution of P between the individual compounds is given in Table 1. The proportions

Table 1. Proportions and ^{32}P labeling of individual phospholipid components of rat epididymal adipose tissue. The chemical analyses are derived from 10 animals; the *in vitro* incorporation data are means of three incubation experiments; the last column records specific activities in single animals 16 h after ^{32}P injection.

Compound	Per cent of lipid P (mean ± s.d.)	Labeling with ^{32}P, specific activity	
		in vitro cpm/μgP/h	*in vivo* cpm/μgP
Lysophosphatidylcholine	2.3 ± 0.34	460	300
Sphingomyelin	13.6 ± 0.75	21	105
Phosphatidylserine	5.2 ± 0.56	22	85
Phosphatidylinositol	4.2 ± 0.39	1140	540
Phosphatidylcholine	50.1 ± 1.74	490	430
Phosphatidylethanolamine	22.3 ± 1.48	15	145
Phosphatidic acid + polyglycerophosphatides	2.5 ± 0.37	25	190

of the individual phospholipid classes are similar to those given by Spencer and Dempster for white adipose tissue of mouse.[1] Phosphatidylcholine and phosphatidylethanolamine are the major compounds, as in many other tissues.

The incorporation of ^{32}P into the different phospholipid fractions followed a fairly similar pattern *in vitro* and *in vivo* (Table 1). Phosphatidylinositol, phosphatidylcholine and lysophosphatidylcholine showed substantial labeling, while the other compounds were relatively inert. In the *in vivo* experiments incorporation took place mainly between 4 and 8 h after the injection of the label and activity reached a maximum at about 12 h. But the phosphatidylserine and sphingomyelin fractions were still almost cold at 8 h and their activity continued to rise up to 20 h, at least.

It is necessary to emphasize that the present data are valid only for the bulk phospholipid fractions of total adipose tissue and for the metabolic renewal rate of the phosphorus moiety. It has been shown that the different parts of the phospholipid molecule can exhibit markedly different turnover rates,[7] indicating that the labeling represents a net effect of *de novo* synthesis and exchange reactions. A detailed description of the metabolic behavior of phospholipids is not possible without using different labeled precursors and fractionation of cellular subcomponents.

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Synthesis of 2,6,10,14-Tetramethylpentadecanoic Acid (Pristanic Acid)

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Since the discovery of Klenk and Kahlke [1] in 1963 that patients suffering from Refsum's disease accumulate large stores of 3,7,11,15-tetramethylhexadecanoic acid (phytanic acid), considerable effort has been expended in understanding the biochemical defect in this inherited disease. By studying the metabolism of 3,6-dimethyloctanoic acid we have shown [2] that apart from the ordinary β-oxidation and ω-oxidation, an "alternative pathway" exists in mammals for the degradation of branched-chain fatty acids. The demonstration of 2,5-dimethylheptanoic acid as a metabolite [3] shows that an initial α-decarboxylation most likely takes place. In accordance with such an interpretation of the "alternative pathway", F. B. Shorland, New Zealand (personal communication) has demonstrated conversion of phytanic acid into 2,6,10,14-tetramethylpentadecanoic acid (pristanic acid) upon administration of phytanic acid to rats. In view of these results we have undertaken the synthesis of pristanic acid, and studies on its metabolism are in progress.

Pristanic acid, which recently was isolated and identified as a trace constituent in butter-fat [4] was first synthesized in 1948 from phytol in five steps.[5] In the present method pristanic acid is obtained by "stepping down" phytanic acid by one carbon atom, according to the following scheme: