Lipogenesis in human adipose tissue

DAVID J. GALTON

New England Medical Center Hospitals, and the Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111

ABSTRACT The pathways and some critical enzymes involved in lipogenesis in adipose tissue from 82 patients have been studied. Of the glucose-^{14}C metabolized to lipid by isolated adipose cells, approximately 0.6% was recovered in fatty acids and the rest in glyceride-glycerol. Palmitate-1-^{14}C was readily incorporated into neutral lipid.

Homogenates of human adipose tissue contained an active α-glycerophosphate dehydrogenase which was approximately twice as active as malate dehydrogenase. Mitochondria of human adipose tissue contained an NAD-independent α-glycerophosphate dehydrogenase; the reaction product, dihydroxyacetone phosphate, was recovered extramitochondrially.

Homogenates of human adipose tissue also contained an active fatty acyl CoA synthetase which required ATP, CoA, and Mg^{++} for maximal activity. The activity of acyl CoA synthetase varied greatly in a group of 40 patients. By contrast, the range of activity of malate dehydrogenase assayed in the same group of patients was much smaller.

When palmitate or palmitoyl CoA was used as substrate, no difference was found in the rate of incorporation of α-glycerol-1,3-^{14}C phosphate into neutral lipid. If time was allowed for activation of palmitate, the incorporation of α-glycerol-1,3-^{14}C phosphate into lipid was 3.5 times greater than for unactivated palmitate.

Palmitate (200 μM) stimulated lipogenesis in homogenates of human adipose tissue and then caused a severe inhibition at 700 μM. Arachidate over the same concentration range did not depress lipogenesis below initial values.

KEY WORDS adipose tissue · lipogenesis · incorporation · glucose-^{14}C · palmitate-^{14}C · L-α-glycerophosphate dehydrogenase and oxidase · fatty acyl-CoA synthetase · inhibition by fatty acids

ALTHOUGH ADIPOSE TISSUE is an important site of synthesis of lipid (1), tissue from different species varies in its capacity to synthesize fatty acids and glyceride-glycerol from glucose. Jansen, Hutchison, and Zanetti (2) found that in the mouse 90% of the glucose-^{14}C converted into fat by epididymal fat pads is recovered in fatty acids and only 10% in glyceride-glycerol, whereas Goodridge and Ball (3) found that in the pigeon only about 7% of the glucose is metabolized to long-chain fatty acids by adipose tissue. The situation in man is not entirely clear. Reports have variously indicated that human adipose tissue, in vitro, can convert 16% of metabolized glucose to fatty acids and 84% to glyceride-glycerol (4); that the synthesis of fatty acids from glucose is quite variable and, in some cases, undetectable (5); and that human adipose tissue is probably an unimportant site of synthesis of fatty acids since it lacks citrate cleavage enzyme (6). Although part of this variability may be due to nothing more than the different nutritional states of the subjects under study, further investigation seemed indicated. The present studies were designed to examine in more detail the paths of lipogenesis in human adipose tissue and provide information on some of the important enzymatic steps involved.

MATERIALS AND METHODS

Source of Tissue

Human subcutaneous and intra-abdominal adipose tissue was obtained during surgery from a total of 82 patients (33 males, 49 females) whose ages ranged from 17 to 78 yr and body weights from 114 to 216 lbs. Patients with diabetes and jaundice were excluded. Preoperative treatments included starvation for 8 hr, premedication with Nembutal, and general anaesthesia induced by Pentothal and maintained with either cyclopropane and oxygen or halothane and nitrous oxide with oxygen. During the operation approximately 2 liters of lactate, 28 mM, was infused into the patient. The adipose tissue was usually removed at the time of the first incision, although on some occasions the tissue was obtained just before closure of the wound.

Part of this work has been presented in preliminary form (7).

Tissue Preparations

After removal the adipose tissue (1–5 g) was washed and transferred to approximately 3 ml of albumin–bicarbonate buffer, and a mixture of isolated fat cells and cell clumps was prepared by a modification of the procedure of Rodbell (8). Other portions of the tissue were homogenized by hand in a glass mortar in 0.25 M sucrose (1:4). The homogenate was transferred to polyethylene tubes and centrifuged at 700 *g* for 10 min in a Lourdes centrifuge (motor head: 16 RA) at 4°C. The fat cake was then lifted with a spatula and the supernatant fraction decanted into another tube. The residue and fat cake were discarded. The supernate was adjusted to pH 7.6 with 1 M K_2HPO_4 and centrifuged at 17,000 *g* for 20 min in the Lourdes centrifuge or in a refrigerated International centrifuge (motor head: 856). The supernate from this separation (called the soluble cytoplasmic fraction) was kept for assay of enzymes and rates of metabolic pathways. The pellet (called the mitochondrial fraction) was washed twice in 10 ml of 0.25 M sucrose at a pH of 7.6 (adjusted with 1 M K_2HPO_4). After the final wash the pellet was suspended (4:1) in 0.03 M phosphate buffer (pH 7.6) and kept for assay of mitochondrial enzymes.

Assay Procedures with Cells

Isolated adipose cells and cell clumps were incubated in 1 oz polyethylene vials (Packard) stoppered with rubber serum caps supporting a hanging center well of glass. The glass well held a strip of filter paper (Whatman No. 1). The medium contained 1.25 ml of modified Krebs' bicarbonate buffer (9) containing 4% (w/v) fraction V bovine serum albumin (Lot A21908 Armour Pharmaceutical Co., Kankakee, Ill.), glucose 5.6 mM and 0.3 μc/ml of either D-glucose-U-^{14}C or glucose-6-^{14}C (New England Nuclear Corp., Boston, Mass.).

At the end of incubation 0.25 ml of Hyamine [*p*-(diisobutylcresoxy-ethoxyethyl) dimethyl benzylamine] was injected through the serum stopper into the hanging well and 0.2 ml of 1 N H_2SO_4 was injected into the medium. The production of CO_2 was determined by the method of Fain, Scow and Chernick (10) and labeled lipids were determined by the procedure of Rodbell (8). Other incubations were performed with palmitate (1 mM) as substrate added as the neutralized sodium salt and palmitic-1-^{14}C acid (0.3 μc/μmole) added as tracer. At the end of these incubations a modification of the procedure of Dole and Meinertz (11) was used to extract lipids, and the fatty acids were then separated from neutral lipids as described by Borgström (12). The radioactivity in an aliquot of the hexane phase was measured in a liquid scintillation counter (Nuclear-Chicago Corporation) with Bray's scintillation solution (13). Another aliquot of the hexane phase was saponified with 2 ml of ethanolic KOH (1 ml of saturated KOH per 20 ml of 95% ethanol) and the fatty acids were extracted by a modification of the procedure of Dole and Meinertz (11). Radioactivity in the hexane phase was measured in a scintillation counter, and in both cases quenching was corrected for by use of internal standards. Zero-time samples were taken after 5 min equilibration with the medium and treated in a similar manner.

Assay Procedures on the Soluble Cytoplasmic Fraction

The incorporation of palmitic-1-^{14}C acid into triglyceride was measured in the following incubation system, of a total volume of 3 ml: potassium phosphate buffer (pH 7.0) 42 mM: cysteine 7 mM; $MgCl_2$ 2mM; NaF 42mM; DL-glycerolphosphate 6mM; CoA 0.13 mM; ATP 3.5 mM; palmitic-1-^{14}C acid (1 μc/ml) approximately 4 μM; and soluble tissue extract 1.75 ml. At the end of incubation, lipid was extracted by a modification of Dole's procedure. The hexane phase was washed three times with ethanolic NaOH as described by Borgström (12) and radioactivity in the hexane phase was measured as described above. The same method was employed to measure the incorporation of L-α-glycerol-1,3-^{14}C phosphase into neutral lipid, except that the three washes of the hexane phase were omitted.

Fatty acyl-CoA synthetase [acid:CoA ligase (AMP), EC 6.2.1.3] was assayed by the procedure of Kornberg and Pricer (14). Fatty acids were added as their neutralized potassium salts and the incubation system contained in 2 ml: hydroxylamine–HCl buffer (pH 7.4) 500 mM; ATP mM; CoA 0.28 mM; cysteine 15 mM; NaF 12 mM; $MgCl_2$ 7 mM; fatty acid 2 mM; and 0.75 ml of soluble supernatant fraction. Blank tubes were incubated with all the above components except ATP or CoA. A standard curve of acethydroxamate was prepared to convert absorbance units into concentration of hydroxamate (15).

Assays of malate (L-malate:NAD oxidoreductase, EC 1.1.1.37) and L-α-glycerophosphate dehydrogenases (L-glycerol-3-phosphate:NAD oxidoreductase, EC 1.1.-1.8) were performed with an incubation system described elsewhere (16) except that enzyme activity was measured by the change in absorption of nicotinamide nucleotides in a recording spectrophotometer (Bausch & Lomb Incorporated, Spectronic 505) at a wavelength of 340 mμ. The assay was linear with respect to time and quantity of homogenate added to the cuvette. The pH of the system was measured after each assay was 9.4 ± 0.5. The temperature of the cuvette holder was 28 ± 1°C and the specific activities of the dehydrogenases were expressed as mμmoles of NADH formed per min per mg of protein homogenate.

Mitochondrial Assays

The NAD-independent glycerophosphate dehydrogenase

TABLE 1 METABOLISM OF D-GLUCOSE-^{14}C BY ISOLATED HUMAN FAT CELLS

Substrate Added	No. of Expts.	Glucose Converted: Carbon Dioxide	Glucose Converted: Fatty Acids	Glucose Converted: Glyceride-Glycerol
		mμmoles/mmole triglycerides/2 hr		
D-Glucose-U-^{14}C	7	153 ± 34	1.3 ± 0.7	284 ± 34
D-Glucose-6-^{14}C	9	11 ± 4	1.8 ± 0.4	230 ± 30

Fat cells (198 μmoles of triglycerides) were incubated in 1.25 ml of albumin–bicarbonate buffer with glucose 5.6 mM and glucose-^{14}C (0.3 μc/ml) for 2 hr. Results are means ± SEM.

(L-glycerol-3-phosphate:cytochrome *c* oxidoreductase, EC 1.1.2.1) was measured in two ways. The first method was essentially that of Ringler and Singer (17) with methylene blue ($E_0' = +0.011$ v) as the electron acceptor. In the other method mitochondria were incubated in a total volume of 2.9 ml for 45 min at 37°C with: phosphate buffer (pH 7.6) 50mM; DL-α-glycerol phosphate 50mM; and 1 ml of mitochondrial fraction (approximately 1 mg of protein). At the end of the incubation the mitochondria were removed by centrifugation at 17,000 *g* for 20 min at 4°C. The supernatant fraction containing dihydroxyacetone phosphate was transferred to a matched set of test tubes and L-α-glycero-phosphate dehydrogenase, 10 μg, and NADH, approximately 33 μM, were added. The change in optical density at 340 mμ was then measured at room temperature in a spectrophotometer (Bausch and Lomb Spectronic 20) during 15 min. Controls were performed with no added substrate in every case.

Miscellaneous Methods

Supernatant and mitochondrial protein was measured by a modification of the biuret method (17) with crystalline bovine serum albumin as the standard.

Materials

D-Glucose-U-^{14}C, D-glucose-6-^{14}C, palmitic-1-^{14}C acid, and glycerol-1,3-^{14}C were obtained from the New England Nuclear Corp. Coenzyme A, DL-α-glycerol phosphate, and palmitoyl CoA were purchased from the Sigma Chemical Co., St. Louis, Mo. After a series of experiments palmitoyl CoA was assayed by the hydroxamate method (14), and its purity was found to be 99 + %. The purity of glycerides and fatty acids was checked before they were used by thin-layer chromatography; all but the dipalmitin migrated as a single spot. The dipalmitin contained a small amount of material that migrated with the same R_f as monoglyceride. L-α-Glycerol-1,3-^{14}C phosphate was prepared enzymatically by the method described by Tzur, Tal, and Shapiro (18) and its purity was checked by ascending paper chromatography in *n*-butanol–acetic acid–water 4:1:5. L-α-Glycerophosphate dehydrogenase was obtained from C. F. Boehringer and Soehne, Mannheim, Germany.

Calculations

Statistical comparisons were based on means of paired experiments or means by the method of sequential sampling. Values were referred to the total fatty acid content of adipose cells added to each flask or the protein content of homogenates and mitochondrial suspensions.

RESULTS

Utilization of Glucose

Very little D-glucose-6-^{14}C was metabolized by human adipose cells to fatty acids. The major recovery of label was in the glyceride-glycerol fraction (Fig. 1). The extent of incorporation into fatty acids was too small to show on this graph. A similar pattern of recovery was observed when D-glucose-U-^{14}C was used as substrate, except that more radioactivity was now found in the $^{14}CO_2$ fraction (Table 1). Glucose carbons therefore mainly contribute to the glycerol moiety of triglyceride. They probably enter into lipid by the activity of cytoplasmic α-glycerophosphate dehydrogenase, which converts the dihydroxyacetone phosphate of glycolysis into α-glycerol phosphate, the glycerol precursor for the synthesis of triglycerides.

The data in Table 2 show that NAD-dependent α-glycerophosphate dehydrogenase is abundant in human

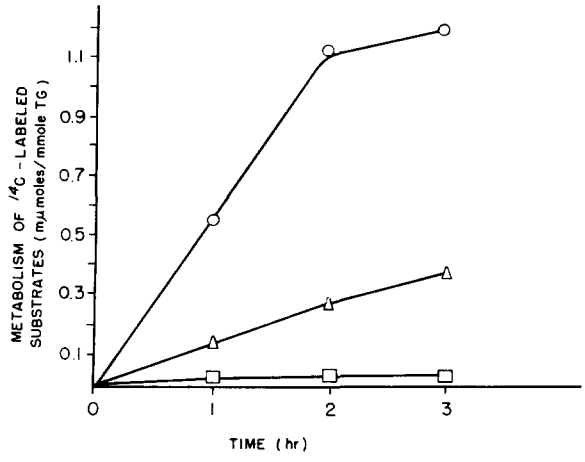

FIG. 1. Metabolism of palmitate-1-^{14}C and glucose-6-^{14}C by isolated human adipose cells. Cells (approximately 200 μmoles of triglycerides) were incubated either in 1.25 ml of albumin–bicarbonate buffer with glucose 5.6 mM and glucose-6-^{14}C 0.2 μc/ml, or in 1.25 ml of albumin–phosphate buffer with palmitate 1 mM, palmitate-1-^{14}C 0.3 μc/ml, and glucose 5.6 mM. The experiments with labeled glucose and palmitate are not paired. Points are means of four experiments. ○, palmitate-1-^{14}C into lipid; △, glucose-6-^{14}C into glyceride-glycerol; □, glucose-6-^{14}C into CO_2. TG, triglyceride.

TABLE 2 ACTIVITIES OF MITOCHONDRIAL AND CYTOPLASMIC ENZYMES IN HUMAN ADIPOSE TISSUE

Activities of:							
Cytoplasmic Fraction				Mitochondrial Fraction			
L-α-Glycerophosphate Dehydrogenase		Malate Dehydrogenase		L-α-Glycerophosphate Dehydrogenase			
mμmoles NADH formed/min per:				*μl O_2/30 min per:*		*mμmoles DHAP*/45 min per:*	
mg protein	*g wet wt*	*mg protein*	*g wet wt*	*mg protein*	*g wet wt*	*mg protein*	*g wet wt*
28 ± 2 (15)	192 ± 16 (15)	15 ± 1 (13)	128 ± 16 (13)	11.1 ± 1.4 (8)	4.5 ± .6 (8)	186 ± 100 (5)	32 ± 8 (5)

Results are given as means ± SEM with the number of experiments in parentheses.
* This assay measured extramitochondrial dihydroxyacetone phosphate (DHAP).

adipose tissue and is approximately twice as active as malate dehydrogenase. The latter enzyme was selected for reference because it has been previously reported as being very active in human adipose tissue (19). Glycerol phosphate produced by the action of the cytoplasmic α-glycerophosphate dehydrogenase can be oxidized by mitochondria of human adipose tissue (Table 2), as well as incorporated into lipid. The data in Table 2 suggest that the α-glycerol phosphate cycle is active in human adipose tissue and that the dihydroxyacetone phosphate produced by the oxidation of α-glycerol phosphate by mitochondria was recovered extramitochondrially. The production of dihydroxyacetone phosphate by mitochondria of human adipose tissue was approximately linear with the quantity of mitochondria added; and better linearity with time was obtained.

Since reports have suggested that monoglycerides can function as acyl acceptors for the synthesis of triglycerides (20), three other acyl acceptors were tested for their capacity to promote the incorporation of palmitate-1-^{14}C into lipid. Table 3 shows that only α-glycerol phosphate stimulated the synthesis of neutral lipid from palmitate-1-^{14}C to any extent. In these experiments dipalmitin and monopalmitin were added as a finely ground powder and time was allowed for the powder to become wet before the experiment was started. The concentrations of dipalmitin and monopalmitin indicated in Table 3 do not therefore represent the actual concentration available for esterification, because of the insolubility of these substrates in an aqueous phase. Detergents were not employed because they are known to inhibit the synthesis of triglycerides (21).

TABLE 3 EFFECTS OF DIFFERENT ACYL ACCEPTORS ON INCORPORATION OF PALMITATE-1-^{14}C INTO NEUTRAL LIPID BY HOMOGENATES OF HUMAN ADIPOSE TISSUE

Acyl Acceptor Added to Medium	Incorporation of Palmitate-^{14}C into Neutral Lipid
	cpm/mg protein/hr
DL-α-Glycerol phosphate 6 mM	3200 ± 670
Glycerol 5 mM	570 ± 130
Monopalmitin 5 mM	360 ± 52
Dipalmitin 5 mM	520 ± 110

Incubations were carried out for 1 hr at 37°C in test tubes containing: phosphate buffer (pH 7.0) 42 mM; cysteine 7 mM; $MgCl_2$ 2 mM; NaF 42 mM; CoA 0.13 mM; ATP 3.5 mM; palmitate-1-^{14}C (1 μc/ml) approximately 4 μM; 1.75 ml of soluble supernatant; and the indicated concentration of acyl acceptor. Results are means of five experiments ± SEM.

TABLE 4 REQUIREMENTS FOR ACTIVITY OF FATTY ACYL-CoA SYNTHETASE IN HOMOGENATES OF HUMAN ADIPOSE TISSUE

Incubation System	Activity of acyl synthetase
	mμmoles hydroxamate/ mg protein/hr
Complete	306
− ATP	0
− Mg^{++}	9
− CoA	28

Incubations were performed for 1 hr in test tubes containing: hydroxylamine buffer (pH 7.4) 500 mM; cysteine 15 mM; NaF 12 mM; palmitate 2 mM; 0.75 ml of soluble supernatant; and, when present, $MgCl_2$ 7 mM; CoA 0.28 mM; and ATP 5 mM. Results are values of one experiment.

Utilization of Fatty Acids

Human adipose cells can readily incorporate palmitate-1-^{14}C into neutral lipid. The time course of this reaction is depicted in Fig. 1 and confirms an earlier report by Hamosh, Hamosh, Bar-Maor, and Cohen (22). The first step in this incorporation requires activation of fatty acids with CoA by acyl-CoA synthetase. Supernatant fractions from human adipose tissue can esterify CoA with fatty acids; the requirements for this reaction in a typical experiment are shown in Table 4. When ATP, CoA, or Mg^{++} was omitted from the incubation medium acylation became negligible. The synthetase activity was also shown to be linearly dependent on the concentration of ATP, CoA, and soluble supernatant material when the other factors were added in excess.

In Fig. 2 the activity of fatty acyl-CoA synthetase is plotted against the chain length of different fatty acids. The concentrations of fatty acids in each assay were 2 mM except for that of oleic acid, which was 0.5 mM. Palmitate was activated to a greater extent than the others and although considerable amounts of oleate are

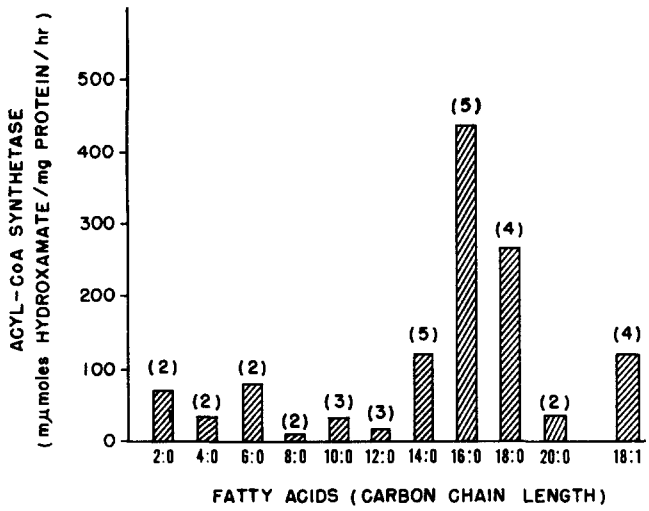

FIG. 2. Effects of chain length of fatty acids on the activity of fatty acyl-CoA synthetase in homogenates of human adipose tissue. Incubation conditions were as described under Methods. Results are means of the number of experiments enclosed in parentheses.

stored in triglycerides in human adipose tissue (23), it was only poorly activated under the experimental conditions of Fig. 2.

The activity of acyl-CoA synthetase for palmitate varied greatly in a group of 40 patients. This variation is presented in Fig. 3A. Although the histogram begins at the origin, this does not necessarily imply that synthetase is absent in adipose tissue from these patients. The assay is carried out under unphysiological conditions of high concentrations of hydroxylamine and in the presence of excess K^+ ions, which were added as KOH to neutralize the hydroxylamine hydrochloride. However, the bars at the origin of Fig. 3A probably represent very low activity of tissue synthetase; when low activity was observed homogenates of rat adipose tissue were measured concurrently to check that the assay was working properly. For contrast, the variation of malate dehydrogenase is presented in Fig. 3B for 20 of the patients in Fig. 3A. The comparison suggests that the variation in fatty acyl-CoA synthetase activity in Fig. 3A is not due simply to nonspecific differences in the daily preparation of adipose tissue, but may be due to differences in the site of origin of the tissue. However, when the activity of acyl synthetase of subcutaneous tissue was compared to that of tissue of intra-abdominal origin the results were, respectively, 250 ± 80 (6) and 250 ± 60 (6) mμmoles of hydroxamate per mg of protein per hr.

The possibility was considered that synthetase activity might be proportional to the over-all rate of incorporation of palmitate-1-^{14}C into lipid by intact cells, i.e., that the enzyme is rate-limiting. A comparison was therefore made between the rate of incorporation of α-glycerol-1,3-^{14}C phosphate into triglyceride when palmitate or palmitoyl CoA was used as substrate. If the activation step is slow, palmitoyl CoA would be expected to stimulate lipogenesis to a much greater extent than palmitate. However, the evidence in Table 5 does not show this. Palmitoyl CoA was added in a concentration range of 5–100 μM and over no part of the curve did stimulation of lipogenesis exceed that seen with palmitate alone.

Since the purity of the palmitoyl CoA had been checked by the hydroxamate method, the possibility that for some reason the thioester was not reaching the site of synthesis of triglyceride was considered. The incorporation of α-glycerol-1,3-^{14}C phosphate into lipid was therefore, compared during 30 min of incubation under two sets of conditions (Table 5). In the first, no time was allowed for activation of fatty acids, i.e., the tubes were incubated for 30 min with palmitate alone, and ATP and CoA were added with the ^{14}C-labeled glycerol phosphate for the final 30 min period of incubation. In the second system, time was allowed for fatty acid activation by incubation of palmitate, CoA, and ATP for 30 min before the addition of ^{14}C-labeled glycerol phosphate. The second system incorporated approximately 3.5 times more glycerol phosphate into neutral lipid during the final 30 min period than the other system (Table 5). This provides indirect evidence that fatty acid activation may be a slow step in the synthesis of neutral lipid.

During the studies on the stimulation of glycerol phosphate incorporation into lipid by palmitate, several additional observations were made to the phenomenon described by Brandes, Olley, and Shapiro (24). They observed that palmitate at 800 μM inhibited glycero-

TABLE 5 STIMULATION OF INCORPORATION OF α-GLYCEROL-1,3-^{14}C PHOSPHATE INTO NEUTRAL LIPID BY PALMITATE AND PALMITOYL COA IN HOMOGENATES OF HUMAN ADIPOSE TISSUE

Incubation Conditions		Incorporation of Glycerol Phosphate into Neutral Lipids
During First 30 Min	During Second 30 Min	
		mμmoles/mg protein/30 min
−ATP, −CoA	−ATP, −CoA, +α-GP	14.5 ± 2.2
−ATP, −CoA	+ATP, +CoA, +α-GP	28.7 ± 5.6
+ATP, +CoA	+ATP, +CoA, +α-GP	101 ± 29
−ATP, −CoA	+ATP, +palmitoyl CoA, +α-GP	15.6 ± 1.1

Incubation were performed for 1 hr at 37°C in test tubes containing: phosphate buffer (pH 7.0) 42 mM; cysteine 7 mM; $MgCl_2$ 2 mM; NaF 42 mM; CoA 0.13 mM; ATP 3.5 mM; palmitate 20 μM or palmitoyl CoA 50 μM; 1.75 ml of soluble supernatant; DL-α-glycerol phosphate 6 mM; and L-α-glycerol-1,3-^{14}C phosphate (approximately 250,000 cpm/tube) in a total volume of 3 ml. Results are means ± SEM of seven experiments. GP, glycerol phosphate.

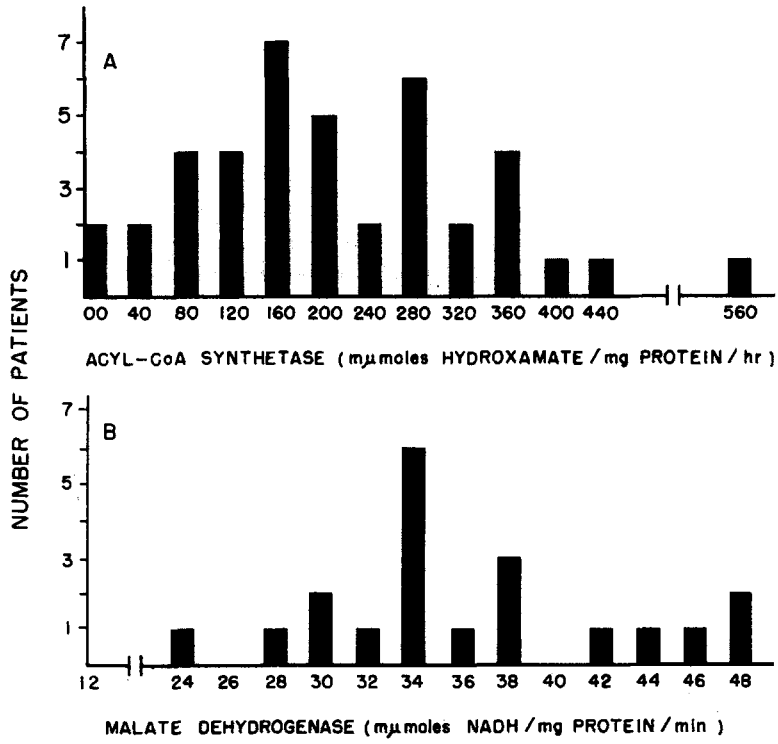

FIG. 3. Variations in activity of fatty acyl-CoA synthetase (A) and malate dehydrogenase (B) in homogenates of human adipose tissue. The fatty acid in A was palmitate 2 mM; other incubation conditions are described under Methods.

phosphate acyl transferase in rat liver but had no effect at 80 μM. Similarly, oleate (800 μM) caused severe inhibition of the enzyme. However, this effect cannot be attributed simply to the toxicity of long-chain fatty acids. Table 6 shows that whereas palmitate (700 μM) and oleate (300 μM) strongly inhibit the incorporation of glycerol phosphate into lipid in homogenates of human adipose tissue, behenate (700 μM) and arachidate (700 μM) have very little effect. Also, the inhibition of lipogenesis by palmitate shows a marked discontinuity at the lower range of concentrations. After an initial stimulation there was a sudden depression of lipogenesis at about 200 μM (Fig. 4). Arachidate over the same concentration range did not depress lipogenesis below initial values (Fig. 4).

TABLE 6 EFFECTS OF FATTY ACIDS OF DIFFERENT CHAIN LENGTH ON INCORPORATION OF α-GLYCEROL-^{14}C PHOSPHATE INTO LIPID BY HOMOGENATES OF HUMAN ADIPOSE TISSUE

Fatty Acid in Medium	No. of Expts.	Recovery of α-Glycerol Phosphate in Lipid		% Inhibition
		Control	+ Fatty Acid	
		mμmoles/mg protein/hr		
Palmitate	10	96.4 ± 7.7	20.7 ± 9.7	79
Oleate	6	76.6 ± 18.3	9.15 ± 1.3	88
Arachidate	6	39 ± 12.6	33 ± 8.8	15
Behenate	5	52 ± 13	35.4 ± 6.4	32

Incubations were performed for 1 hr at 37°C in test tubes under the conditions of Table 5. Results are means ± SEM. Fatty acids were present at a concentration of 700 μM except for oleate which was 300 μM.

DISCUSSION

Under the conditions of the first experiments in this paper glucose is shown to be a poor source of carbon for the synthesis of fatty acids in intact human adipose cells. However, glucose is readily transformed into the glycerol moiety of triglycerides and its incorporation is of sufficient magnitude to account for the tissue's capacity to take up palmitate-^{14}C and convert it to neutral lipid. If the production of glyceride-glycerol greatly exceeded the capacity of adipose tissue to take up palmitate and convert it to triglyceride, then an alternative source of fatty acids would be expected, such as endogenous synthesis.

The entry of glucose into glyceride-glycerol most probably occurs by the activity of a cytoplasmic α-glycerophosphate dehydrogenase that converts dihydroxyacetone phosphate produced during glycolysis into α-glycerol phosphate. The equilibrium constant of this reaction in the direction of oxidation is $K = 5.5 \times 10^{-12}$ M (25) and therefore very much favors the formation of L-α-glycerol phosphate. The enzyme α-glycerophosphate

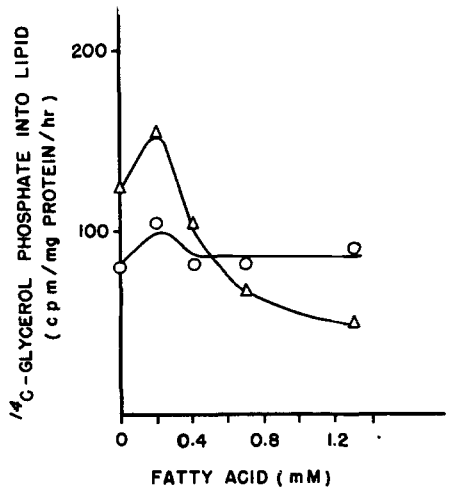

FIG. 4. Effects of fatty acids on the incorporation of α-glycerol-1,3-^{14}C phosphate into lipid by homogenates of human adipose tissue. Points are means of four experiments. Δ, palmitic acid; O, arachidic acid.

dehydrogenase (NAD-dependent) is very abundant in homogenates of human adipose tissue and its activity is greater than malate dehydrogenase (Table 2) which is reportedly very active in human adipose tissue (19). It was also possible to demonstrate the presence of a mitochondrial α-glycerophosphate dehydrogenase in human adipose tissue that oxidizes α-glycerol phosphate by the cytochrome chain and molecular oxygen. The product of this reaction, dihydroxyacetone phosphate, diffuses out of mitochondria and was readily detectable in the buffer after removal of mitochondria by centrifugation (Table 2). It therefore appears that the components of the α-glycerol phosphate cycle are present in human adipose tissue. Since, as is shown in Table 3, α-glycerol phosphate is an obligatory acyl acceptor for the synthesis of triglyceride and is present in very low concentration in human adipose tissue,[1] the balance between the production of α-glycerol phosphate by the cytoplasmic dehydrogenase and its oxidation by the mitochondrial enzyme might affect lipogenesis by varying the supply of α-glycerol phosphate.

Table 1 suggests that glyceride fatty acids are not synthesized from glucose by human adipose tissue in vitro, although this does not exclude the possibility that synthesis occurs from other sources such as pyruvate, which is known to be a better precursor for fatty acids than glucose in rat adipose tissue (26). Human adipose tissue in vitro readily takes up palmitate from the incubation medium and can incorporate it into neutral lipid at a similar rate to the assimilation of glucose into glyceride-glycerol (Fig. 1). Although the experiments in Fig. 1 are not strictly comparable because they were not performed concurrently, they do suggest that glyceride fatty acids of adipose tissue may originate from sources other than adipose tissue. Bortz, Abraham, Chaikoff, and Dozier (27) have shown that homogenates of human liver can actively synthesize fatty acids from acetate and the fatty acids may then be transported in the blood stream to adipose tissue for storage in lipid.

The first step in the incorporation of fatty acids into neutral lipid in adipose tissue is an energy-dependent esterification with CoA. This is performed by a group of enzymes called fatty acyl-CoA synthetase which vary in their specificity for the carbon chain length of the fatty acid in the rat (28). Human adipose tissue contains acyl-CoA synthetase and its activities with fatty acids of different chain length (Fig. 2) correlate well with the proportions of fatty acids stored in human adipose tissue, except for oleic acid. Numerous reports have confirmed that of the total fatty acids stored in human fat approximately 47% consists of oleate and 19% of palmitate (23), so that it was surprising that the acyl synthetase in human adipose was less active for oleate than palmitate in the assay of Fig. 2. This may be due in part to the unphysiological conditions of the assay, in which large amounts of hydroxylamine are present, for this substance at high concentrations has been reported to partially inhibit acyl synthetases (28). Also, the physical form of added fatty acids may not be the same as occurs in vivo, and their structure may be altered during an hour's incubation.

Large variation was observed in the activity of acyl-CoA synthetase in a group of 40 patients. This could not be attributed to nonspecific variations in the daily preparation of the tissue since another enzyme, malate dehydrogenase, was measured concurrently and found to range between much smaller limits. It is also unlikely that the variation in activity of acyl-CoA synthetase was due to different sites of origin of the tissue since similar values were obtained for the enzyme in samples of subcutaneous and intra-abdominal tissue. Also, when the results with human adipose tissue were low, tissue from the rat was assayed concurrently to check that the assay was working properly. It seems possible, then, that the variation in activity of acyl-CoA synthetase may have physiological significance.

It has already been suggested that the activity of acyl-CoA synthetase might control the rate of uptake of fatty acids by adipose tissue of the rat (29), and on thermodynamic grounds the synthetase might be expected to be the rate-controlling enzyme in the sequence from fatty acids to lipid since fatty acid activation is the only energy-dependent step. Glycerol acyl phosphate transferase does not require ATP for maximal activity (24). If the above hypothesis were true then synthesis of

[1] 1 g of human adipose tissue contains approximately 6 mμmoles of L-α-glycerol phosphate (D. Galton, 5 unpublished experiments).

neutral lipid from palmitoyl CoA should proceed at a greater rate than from palmitate, since the slow step has now been circumvented. It was not possible to demonstrate this predicted difference in rate (Table 5) and the possibility was therefore considered that perhaps the exogenous palmitoyl CoA was not reaching the site of synthesis of triglyceride. The situation might be somewhat analogous to the synthesis of lipid in bacteria where the reactions are believed to occur on a carrier protein with a CoA-like prosthetic group (30). When time (30 min) was allowed for activation of fatty acids by supernatant fractions from human adipose tissue and the rates of incorporation of α-glycerol-^{14}C phosphate into lipid were compared to rates achieved by homogenates given no time for activation (Table 5), the average rate of the system that presumably contained activated fatty acids was found to be 3.5 times greater than the system containing unmodified fatty acids. This is indirect evidence to support the hypothesis that synthetase is in fact a limiting step.

Recently, fatty acids, particularly octanoate and oleate, have been postulated to act as regulatory metabolites in the kidney and liver of the rat [(31); see also (32)]. The inhibitory effects of fatty acids on lipogenesis in homogenates of human adipose tissue (Fig. 4 and Table 6) cannot be simply due to the physicochemical properties of long-chain fatty acids, since arachidate and behenate do not behave in the same way as the physiological fatty acids palmitate and oleate. Furthermore, the kinetics of inhibitory effects of palmitate on lipogenesis are unusual (Fig. 4). There is a preliminary stimulation phase followed by a sharp discontinuity in the concentration range of 200–300 μM and then a sharp inhibition of lipogenesis. Since arachidate tested under similar conditions did not show this, it is possible that palmitate and oleate above a certain concentration, which is well within the physiological range, have a specific inhibitory effect on a step in the synthesis of triglyceride beyond the activation reaction (the concentration of palmitate used in the assay for synthetase was 2 mM, with no observable inhibitory effects). Brandes et al. (24), using homogenates of rat liver, found that palmitate and oleate at 800 μM severely inhibited glycerophosphate acyl transferase but had no effect at 80 μM. It is possible that the same enzyme is being inhibited by fatty acids in homogenates of human adipose tissue since the only other reactions are the ones catalyzed by phosphatidic acid phosphatase and by diglyceride acyl transferase.

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