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Glycerolipid Biosynthesis in Rat Adipose Tissue

INFLUENCE OF ADIPOSE-CELL SIZE AND SITE OF ADIPOSE TISSUE ON TRIACYLGLYCEROL FORMATION IN LEAN AND OBESE RATS*

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The rates of lipid formation were compared in different fat-depots from lean and obese rats by using [^{14}C]glycerol 3-phosphate, [^{14}C]glucose or [^{14}C]acetate as substrates. In lean animals, subcutaneous adipose tissue showed significantly lower rates of lipid synthesis than did perirenal and gonadal fat-tissue. In obese animals, the rates of lipid synthesis were significantly higher and did not vary from one fat-depot to another. Differences in the rates of lipid formation between lean and obese rats disappeared during dietary restriction of obese animals. The isolated adipocyte preparation did not reflect the true metabolic activity of the adipose organ, since this preparation was mainly derived from smaller adipocytes that were metabolically less active than larger adipocytes. The present study suggests that it is better to use whole tissue preparations to measure lipogenesis and esterification reactions, because these measurements represent the contribution of both larger and smaller adipocytes towards lipid formation.

The epididymal adipose tissue of the rat is the most widely studied adipose tissue, and results from this tissue have been applied to adipose tissue from other body sites. However, studies on the comparison of esterification and lipolytic rates and the measurements of glycerol kinase activities from various adipose-tissue sources suggest that epididymal adipose tissue is not always representative of other types of adipose tissue (Durham *et al.*, 1971; Zucker, 1972; Persico *et al.*, 1975). In the studies reported by Durham *et al.* (1971), adipose-tissue fragments from perirenal, epididymal and omental fat-pads were incubated with [^{14}C]palmitate and the rates of palmitate esterification into triacylglycerols were compared in different fat-depots. Esterification by the epididymal and omental adipose tissue did not differ significantly, but the values from both of these were higher than for perirenal tissue. However, in these earlier studies, the rates of esterification were expressed on the basis of tissue weight, and such an expression of the metabolic function of adipose tissue has been questioned by several workers (Zinder *et al.*, 1967; Salans *et al.*, 1968; Hubbard & Matthew, 1971; Holm *et al.*, 1975). It is now generally accepted that the metabolic functions of adipose tissue should be expressed on the basis of either number or size of adipocytes. Since these parameters were not measured by Durham *et al.* (1971), it is not clear whether the observed differences in esterification rates are related to physiological differences in fat-tissue from various areas, changes in the number or size of adipocytes, or are artifacts arising from differences in the permeability to [^{14}C]palmitate because of morphological differences between various fat-pads.

Therefore the question of differential rates of esterification in various fat-pads was re-examined. The tissue fragments, the isolated adipocytes and the fat-free homogenates derived from gonadal, perirenal and subcutaneous fat-pads were used to measure the rates of triacylglycerol formation from [^{14}C]glucose, [^{14}C]acetate or *sn*-[^{14}C]glycerol 3-phosphate.

Materials and Methods

sn-[1,3-^{14}C]Glycerol 3-phosphate (sp. radioactivity 30 mCi/mmol) was purchased from ICN Chemicals and Radioisotope Division, Irvine, CA, U.S.A. [1,2-^{14}C]Acetate (sp. radioactivity 96.8 mCi/mmol) and [U-^{14}C]glucose (sp. radioactivity 240 mCi/mmol) were purchased from New England Nuclear Corp., Boston, MA, U.S.A. Most of the other chemicals were of A.R.-grade quality and were purchased from the sources reported previously (Jamdar & Fallon, 1973*a*,*b*; Jamdar *et al.*, 1976). Male and female obese (*fa*/*fa*) rats and their lean controls (*FA*/–) were either from our animal colony or were purchased from Harriet G. Bird Memorial Laboratory, Stow, MA, U.S.A.

Initial studies were conducted with the adipose tissues from albino rats to determine optimum incubation conditions. Sprague–Dawley rats of the same age as the obese rats were purchased from Flow

* This is paper no. 4 of a series; no. 3 is Jamdar (1977).

Laboratories, Dublin, VA, U.S.A. The incubation conditions developed with adipose tissues from these animals were found to be satisfactory to measure the esterification rates in adipose tissues from both lean and obese rats. At the time of death, lean and obese rats were 67–75 days old unless otherwise stated. Rats were fed on laboratory chow (Ralston Purina Laboratories, St. Louis, MO, U.S.A.).

Conversion of [^{14}C]acetate or [^{14}C]glucose into lipid

Small fragments of gonadal, perirenal and subcutaneous adipose tissue (60–100mg wet wt.) were incubated in the presence of [^{14}C]acetate or [^{14}C]-glucose as described by Christophe *et al.* (1961*a*,*b*). In the final volume of 2ml, the reaction mixture contained 1.5ml of Krebs–Ringer bicarbonate buffer with half the recommended concentration of Ca^{2+} (Cohen, 1959) containing 3% albumin, 0.5mM-acetate, 1μCi of [^{14}C]acetate and 20mM-glucose. In some studies, radioactive acetate was replaced by [^{14}C]glucose (2μCi/μmol). The contents were incubated in a shaking water bath at 37°C in 20ml plastic scintillation vials with O_2/CO_2 (19:1) as the gas phase. Under these conditions, incorporation of radioactive substrates into lipids by adipose tissue of both lean and obese rats was linear with time up to 3h.

In the standard assay the incubation was continued for 1h. At the end of incubation, CO_2 was released by injecting 0.25ml of 0.5M-H_2SO_4. The vials were incubated for an additional 30min at room temperature (23°C) to collect CO_2 in plastic wells that contained filter-paper strips soaked with 0.2ml of Hyamine hydroxide. At the end of the incubation, paper strips were removed and transferred into scintillation vials containing 10ml of Aquasol 2 (New England Nuclear) and counted for radioactivity. Tissue lipids along with the medium were extracted with chloroform/methanol (2:1, v/v) and purified as described by Folch *et al.* (1957).

One portion (25μl) of the lipid sample was counted for radioactivity to measure incorporation of label into total lipids. Another portion was subjected to t.l.c. to determine incorporation into triacylglycerol, and a third portion was saponified with 0.5M-NaOH at 90°C for 1h. The samples were neutralized by the addition of 0.5ml of 12M-HCl and diluted with 1.5ml of water. Fatty acids were extracted with 2×4ml of light petroleum (b.p. 30–60°C). The ether extracts were combined, dried under N_2 and processed to determine incorporation into fatty acids. It has been demonstrated by Kovanen *et al.* (1975) that acetate can be utilized for cholesterol formation by the adipose tissue. Therefore a portion of lipid extract (200μl) containing non-saponified lipid was further processed to determine the incorporation of [^{14}C]-acetate into cholesterol fractions (Kovanen *et al.*, 1975).

Conversion of [^{14}C]glycerol 3-phosphate into lipid

Gonadal, perirenal and subcutaneous adipose tissues from individual animals were removed and homogenized separately with 3vol. of buffer containing 0.25M-sucrose, 1mM-Tris/HCl, pH7.5, 1mM-EDTA and 1mM-dithiothreitol (Medium A). The homogenization was performed at speed 5 for 30s in the cold (4°C) with a Tekmar Tissumizer (Tekmar Instruments, Cincinnati, OH, U.S.A.). The homogenate was centrifuged at 600*g* for 15min and separated into upper fat cake, a pellet (containing nuclei, cell debris and other tissue fragments), and an intermediate layer. This intermediate layer, called the fat-free homogenate, was used to measure triacylglycerol formation.

Triacylglycerol formation from *sn*-glycerol 3-phosphate was measured in the presence of fat-free homogenates derived from subcutaneous, gonadal and perirenal fat-pads (Jamdar *et al.*, 1976). In a final volume of 0.75ml, the reaction mixture contained 24mM-Tris/HCl buffer, pH7.5, 50mM-KCl, 0.42mM-*sn*-[^{14}C]glycerol 3-phosphate (0.25μCi/μmol), 0.7mM-dithiothreitol, 1.05mM-ammonium palmitate, 3mM-ATP, 3.6mM-$MgCl_2$, 0.01mM-CoA and 1.25mg of fatty acid-poor albumin. The reaction was initiated with 0.1–0.2ml of homogenate containing 200–300μg of protein. Incubation was under air at 37°C in a shaking water bath. The reaction was linear with time for 20min and was terminated by the addition of 3ml of chloroform/methanol/1M-HCl (1:2:0.025, by vol.), 1ml of chloroform and 1ml of water.

Radioactive lipids formed were extracted as described by van den Bosch & Vagelos (1970) and dried under N_2. The dry lipids were dissolved in 0.5ml of benzene. Samples were applied in a volume of 0.1ml and separated on thin-layer plates coated with silica gel G (E. Merck, Darmstadt, Germany) slurried in 0.1M-sodium borate. The different classes of lipids were separated by t.l.c. and identified as described earlier (Jamdar *et al.*, 1976).

The rates of incorporation of [^{14}C]acetate, [^{14}C]-glucose and *sn*-[^{14}C]glycerol 3-phosphate into different lipids were expressed in relation to fat-cell number. Previously, we have demonstrated that this consideration is particularly important in the studies with adipose tissue from obese rats because obesity in animals is associated with a significant increase in the non-fat-cell population, which causes a rise in the proportion of adipose-tissue protein content derived from non-fat-cells (Jamdar *et al.*, 1976).

Portions of the adipose tissue and of the isolated adipocytes used in different studies were processed for the determination of adipocyte number and size by the method of Hirsch & Gallian (1968). When the rates of triacylglycerol formation from [^{14}C]acetate or [^{14}C]glucose were compared between the isolated

adipocytes and the adipose-tissue fragments, adipose-cell size was determined by measuring the diameter of 200–250 cells (OsO_4-fixed) under an optical microscope as described by Anderson *et al.* (1972). In some experiments, cell size distribution was studied by the method of Sjöstrom *et al.* (1971).

Protein was determined by the method of Lowry *et al.* (1951), with crystalline bovine albumin as standard.

Statistical comparisons were made by Student's *t* test. Differences between means were considered non-significant if $P > 0.05$.

Results and Discussion

If it is assumed that the structural differences between fat-pads affect the entry of a substrate into the adipose cell and thereby its further metabolism, then one could avoid this problem by using isolated adipocytes instead of the tissue fragments to measure the esterification rates. To compare the two systems, triacylglycerol formation from [^{14}C]glucose or [^{14}C]acetate was measured by using isolated adipocytes or adipose-tissue fragments (Table 1). Tissue fragments showed much higher rates of glucose and acetate utilization compared with the isolated adipocytes. This observation was unexpected and could be related to derangement of glucose or acetate metabolism caused by the isolation procedure with collagenase, or to the presence of larger adipocytes in tissue fragments than in the isolated adipocyte preparations.

Observations from several laboratories do not indicate that collagenase treatment causes any derangement in the glucose metabolism of adipocytes (Rodbell, 1964). However, during counting and sizing of fat-cells, it was observed that the samples of isolated adipocytes contained smaller numbers of larger adipocytes than the samples obtained from whole tissue fragments (Fig. 1, Table 2). Therefore the presence of larger adipocytes in the tissue fragments might have been responsible for the increased rates of glucose or acetate utilization in this preparation.

It has been demonstrated by a number of workers that glucose metabolism of adipose tissue increases in direct proportion to cell size (Zinder *et al.*, 1967;

Table 1. *Conversion of* [^{14}C]*glucose and* [^{14}C]*acetate into triacylglycerol*

The adipose-tissue fragments and the isolated adipocytes from gonadal, perirenal and subcutaneous fat-pads from male Zucker rats (lean) were incubated in the presence of (*a*) [^{14}C]glucose or (*b*) [^{14}C]acetate. Adipocytes were isolated by collagenase digestion as described by Rodbell (1964), except that the incubation medium did not contain glucose and the concentration of collagenase was 1 mg/ml. Adipocytes were separated from non-adipocytes by suspending them in warm (collagenase-free) medium and then centrifuging the suspension at 2000 rev./min for 1 min in a GLC2 centrifuge (Sorvall Instruments). The old incubation medium was aspirated out with a 10ml syringe equipped with plastic capillary tubing and replaced by fresh medium (5 ml). The cells were suspended in the fresh medium by drawing in and out gently five times with 1 ml plastic Biotip pipettes (Schwarz/Mann, Orangeburg, NY, U.S.A.). This procedure freed fat-cell clumps. The tubes were again centrifuged to liberate any additional non-fat cells, and this procedure was repeated three times. The samples of isolated adipocytes and tissue fragments were each processed for the determination of adipocyte number. The rates of conversion of glucose and acetate into triacylglycerols were expressed as nmol/h per 10^6 adipocytes. Each value is the mean ± S.D. for three determinations .*, Significantly different from isolated adipocytes ($P < 0.05$); **, significantly different from isolated adipocytes ($P < 0.01$); †, significantly different from other samples (adipocytes of adipose-tissue fragments) of the same experiment ($P < 0.05$).

Triacylglycerol formed from	Tissue source of adipocytes			Tissue fragments from		
	Gonadal	Perirenal	Subcutaneous	Gonadal	Perirenal	Subcutaneous
(*a*) [^{14}C]Acetate	27.03 ±4.52	9.08 ±1.46	5.59† ±1.60	106.21* ±45.03	91.33** ±7.97	10.32† ±4.17
(*b*) [^{14}C]Glucose	25.02 ±9.07	19.06 ±2.71	13.15† ±0.23	90.40* ±30.40	64.10** ±8.98	23.43**,† ±3.10

Table 2. *Recovery of adipocytes during collagenase digestion*

For details see legend of Fig. 1. The recovery of osmium-fixed material derived from the tissue fragments from the right fat-pad was considered as 100% to calculate the recovery of adipocytes during collagenase digestion from the left fat-pads. Each value is the mean ± S.D. for four animals.

Body wt. (g)	Fat-pad wt. (g)		10^{-6} × No. of adipocytes/fat-pad		Recovery of adipocytes by collagenase method (%)
	Right	Left	Right (real)	Left (recovered)	
320 ± 21.6	1.05 ± 0.21	0.97 ± 0.18	5.22 ± 0.78	4.06 ± 0.22	79 ± 14

Salans *et al.*, 1968; Smith *et al.*, 1974; Holm *et al.*, 1975), although no such correlation for acetate utilization has been established. Adipocytes obtained by collagenase digestion and fixed in the presence of OsO_4 also differed in their appearance from the osmium-fixed cells derived from whole tissue fragments (Plate 1). However, these differences may not be related to the variation in the metabolic activities between these two preparations.

It is known that osmium fixation is accompanied by cell swelling, and the method used in our studies is

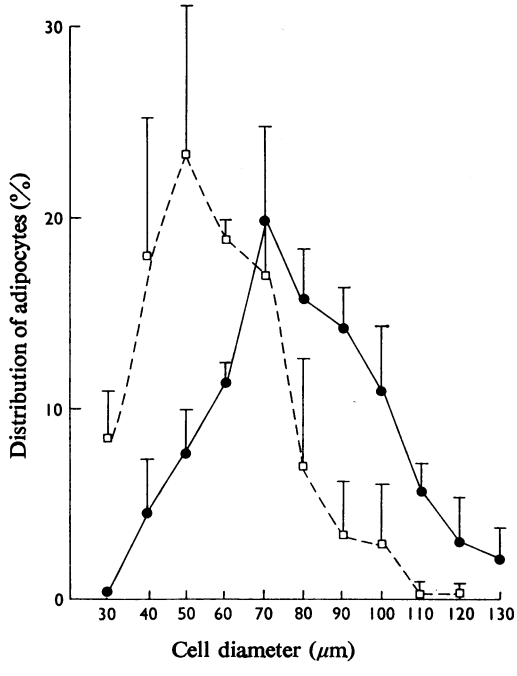


Fig. 1. *Size distribution of osmium-fixed adipocytes*
Four male Sprague–Dawley rats (8–10 weeks old) were used. The tissue fragments from right epididymal fat-pads from individual animals were fixed directly in OsO_4. The left fat-pads were subjected to collagenase digestion (Rodbell, 1964) and the resultant adipocytes were fixed with OsO_4 for 48 h at 37°C. The osmium-fixed cells from these two preparations were counted in a Coulter Counter to determine the total number of adipocytes in each fat-pad. The diameters of 200–250 cells (osmium-fixed) were measured with the help of a micrometer eye-piece under an optical microscope to determine the size distribution of adipocytes between these two preparations. The solid line shows the size distribution of osmium-fixed cells derived from the tissue fragments and the broken line indicates the size distribution of the isolated adipocyte preparation. Each point is the mean ± S.D. of four rats. The photographs of the two preparations are shown in Plate 1 and data on the adipocyte recovery are shown in Table 2.

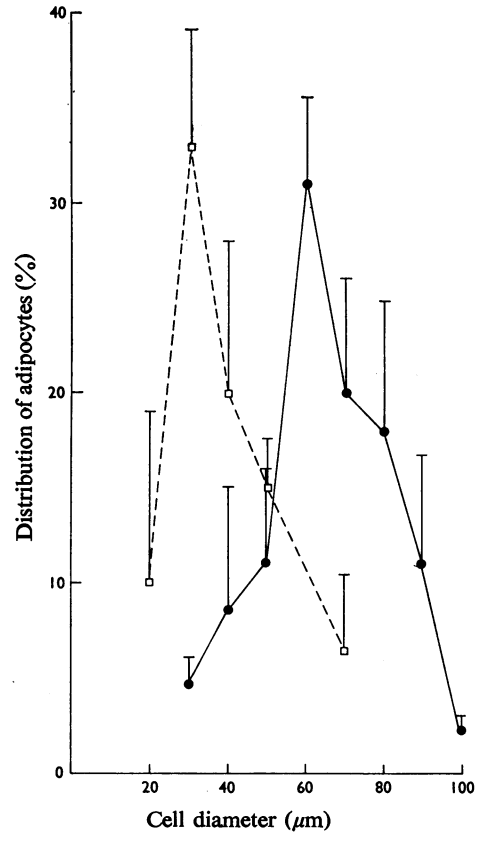
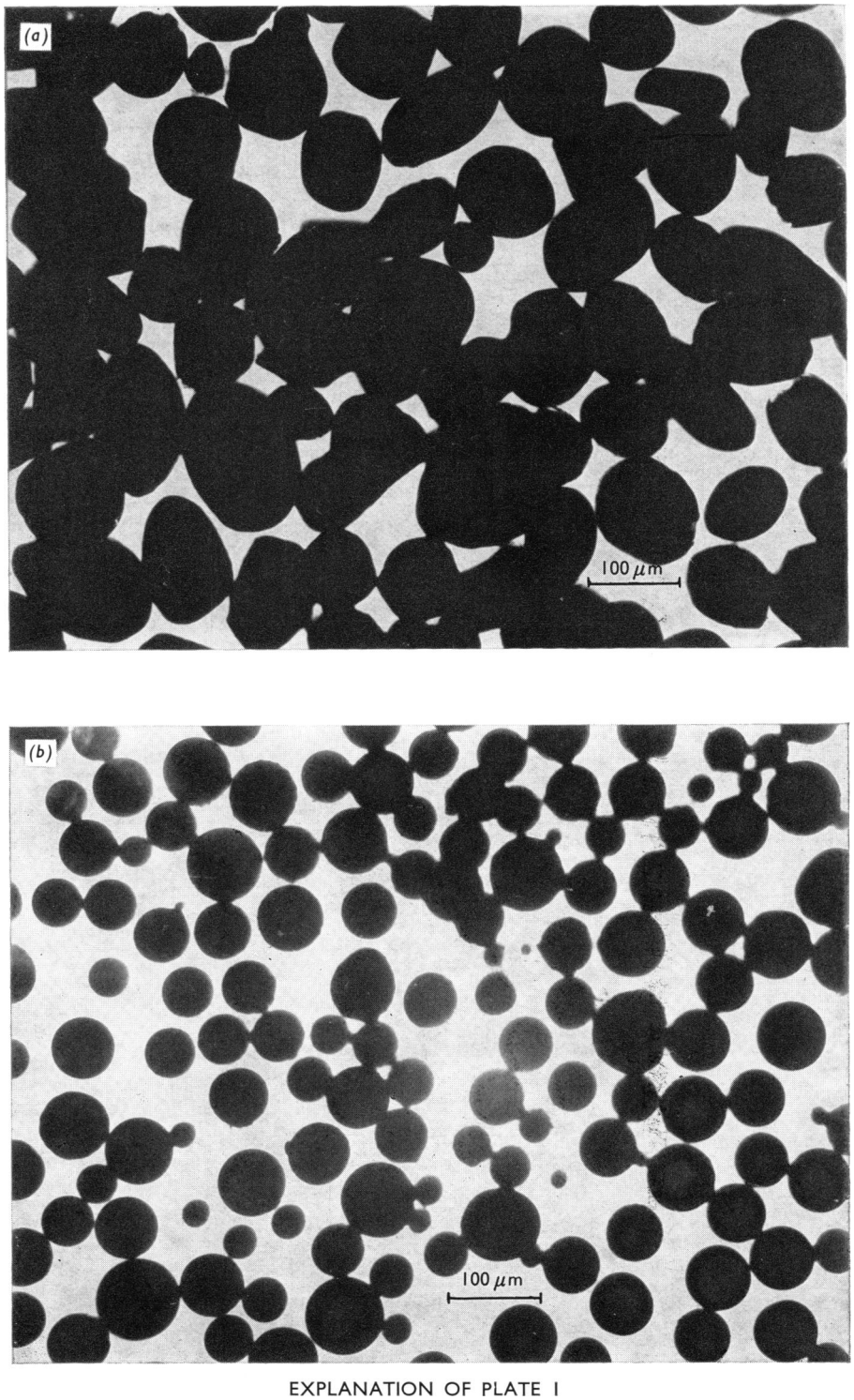
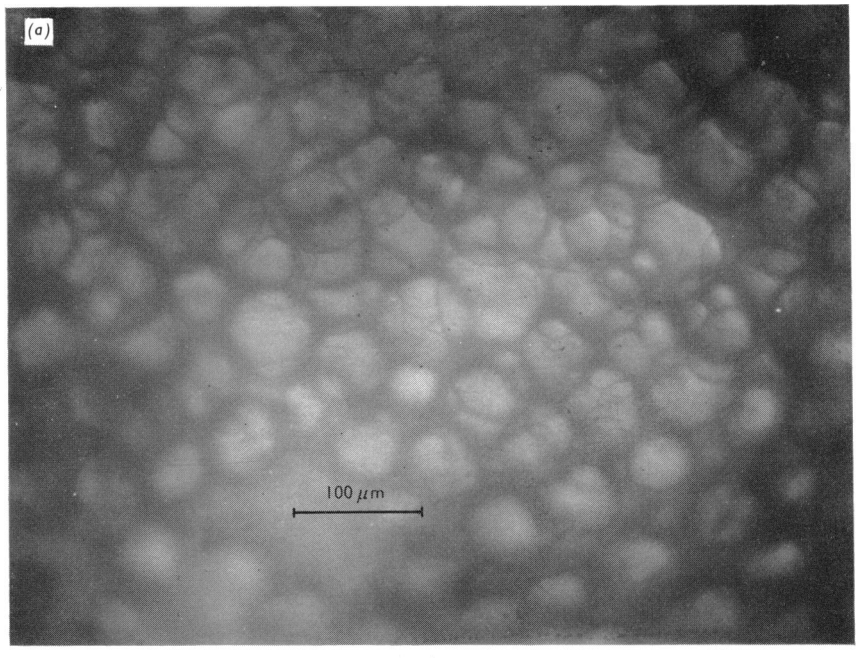


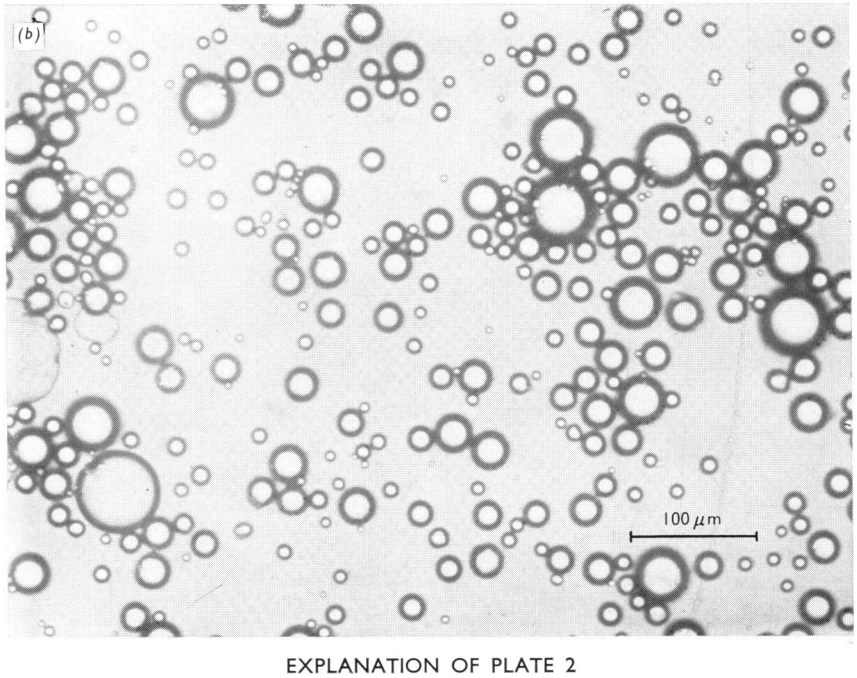
Fig. 2. *Size distribution of adipocytes by the freeze-cutting method*
Five male Sprague–Dawley rats (300 ± 20 g) were used. The small tissue fragments from right epididymal fat-pads were fixed in 35% formaldehyde for 8 min. These sections were then frozen immediately with CO_2 and thick sections (100 μm) were cut with the help of a sliding microtome (American Optical, model 860). These sections were then transferred to a plastic Petri dish (60 mm × 15 mm, Falcon) containing Krebs–Ringer bicarbonate buffer. While the sections were floating, photomicrographs were taken of them with a Nikon 83210 inverted microscope with attached Polaroid camera. The cell size was measured with a micrometer (2 mm division into units of 0.1 mm; American Optical). The left fat-pads were subjected to collagenase digestion to isolate adipocytes and the resultant adipocytes were fixed with 35% formaldehyde for 8 min and then transferred to a Petri dish containing Krebs–Ringer bicarbonate buffer to determine the cell size as described above. The diameters of 100–150 cells were measured. The solid line shows the size distribution of adipocytes from the tissue fragments and the broken line shows the distribution of the isolated adipocyte preparation. Each point is the mean ± S.D. of five animals. The photomicrographs of the two preparations are shown in Plate 2.



EXPLANATION OF PLATE 1

Photomicrographs of osmium-fixed cells derived from (a) the tissue fragments and (b) the isolated adipocyte preparation





EXPLANATION OF PLATE 2

Photomicrographs of adipocytes from (a) tissue fragments and (b) the isolated adipocyte preparation

Table 3. *Characteristics of adipose-tissue depots from lean and obese rats*

Adipose-tissue fragments from the subcutaneous, perirenal and gonadal fat-pads were incubated in the presence of [^{14}C]acetate (in duplicate) to study lipogenesis as described in the Materials and Methods section. A portion of adipose tissue was fixed in OsO_4 to determine adipocyte number and size as described by Hirsch & Gallian (1968). These results were obtained from seven lean and seven obese male rats. Each value is the mean ± S.D. Lean rats weighed 239 ± 58 g and obese rats weighed 298 ± 41 g. *, Significantly different from lean animals ($P < 0.01$); †, significantly different from one fat-depot to another in the same group of animals ($P < 0.05$).

	Lean rats			Obese rats		
Parameters measured	Gonadal	Perirenal	Subcutaneous	Gonadal	Perirenal	Subcutaneous
10^{-6} × No. of adipocytes/g	4.68 ± 1.50	3.70 ± 0.82	6.89 ± 0.84	0.55* ± 0.21	0.57* ± 0.17	0.56* ± 0.22
Cell size (μg of lipid/cell)	0.17 ± 0.04	0.26 ± 0.06	0.08† ± 0.03	1.79* ± 0.73	1.52* ± 0.39	1.61* ± 0.53
[^{14}C]Acetate incorporation (μmol/h per 10^6 adipocytes) into						
(*a*) CO_2	0.07 ± 0.02	0.08 ± 0.03	0.07 ± 0.02	1.06* ± 0.35	0.84* ± 0.12	0.92* ± 0.33
(*b*) Fatty acids	0.14 ± 0.04	0.18 ± 0.07	0.04† ± 0.02	1.61* ± 0.51	0.51* ± 0.31	0.63* ± 0.36
(*c*) Triacylglycerols	0.09 ± 0.03	0.13 ± 0.05	0.024† ± 0.008	0.94* ± 0.51	0.37* ± 0.15	0.60* ± 0.35

Table 4. *Glycerolipid formation in different fat-depots from lean and obese rats*

Glycerolipid formation was measured in the presence of [^{14}C]glycerol 3-phosphate and a palmitoyl-CoA-generating system with fat-free homogenates as the enzyme source. Assays were carried out in duplicate. In these experiments adipose tissues were obtained from lean and obese male Zucker rats. Other details of the animals are given in Table 3. Each value is the mean ± S.D. for the number of animals in parentheses. The rates of glycerolipid formation were expressed as nmol/min per 10^6 adipocytes. Abbreviations: PA, phosphatidate, DG, diacylglycerol; TG, triacylglycerol; GP, [^{14}C]glycerol 3-phosphate. *, Significantly different from other fat-depots ($P < 0.01$); †, significantly different from obese rats ($P < 0.01$); ‡, significantly different from gonadal tissue ($P < 0.01$).

	Lean rats				Obese rats			
		Distribution of GP (%) into				Distribution of GP (%) into		
Source of tissue	Lipid synthesis	PA	DG	TG	Lipid synthesis	PA	DG	TG
Gonadal (7)	10.8† ± 2.9	44 ± 13	11 ± 4	45‡ ± 13	37.8 ± 12.0	34 ± 12	15 ± 13	51 ± 13
Perirenal (5)	13.4† ± 5.5	45 ± 14	13 ± 9	42 ± 10	25.6 ± 9.0	39 ± 13	17 ± 10	44 ± 21
Subcutaneous (7)	4.4*† ± 2.6	84 ± 51	6 ± 6	12 ± 10	23.9 ± 9.8	58‡ ± 22	11 ± 5	31 ± 16

optimized for fixation of tissue fragments rather than for isolated adipocytes (Hirsch & Gallian, 1968). Therefore, in some experiments the freeze-cutting method described by Sjöstrom *et al.* (1971) was used to study the cell-size distribution of adipocytes in tissue fragments. The results of these studies also demonstrate that the adipose-tissue fragments contain larger adipocytes compared with isolated adipocyte preparations (Fig. 2 and Plate 2).

It has been demonstrated previously that the subcellular fractions from isolated adipocytes were less active in lipid synthesis from *sn*-glycerol 3-phosphate by using a palmitoyl-CoA-generating system than were the corresponding fractions derived from whole adipose tissue that contained both adipocytes and non-adipocytes (Jamdar *et al.*, 1976). Since the non-adipocytes did not contribute towards triacylglycerol formation, the lower capacity for lipid synthesis by the isolated fractions was attributed to an inactivation of various triacylglycerol-synthesizing enzymes during the isolation of adipocytes by the collagenase digestion. The present studies suggest that the differences in the capacities for triacylglycerol synthesis between these two preparations may also result from variation in size of adipocytes.

In addition to cell size, other differences in cellular breakage or cellular integrity during the metabolic studies cannot be ruled out as possible causes of variation in the rates of lipid synthesis between these two preparations. The preparation of subcellular fractions from whole adipose tissue usually represents the contribution of all the adipocytes; however, such preparations from isolated adipocytes may be derived mainly from smaller adipocytes, which are

metabolically less active than the larger adipocytes (Zinder *et al.*, 1967; Salans *et al.*, 1968; Smith *et al.*, 1974; Holm *et al.*, 1975). Since the metabolic activity measured in the presence of whole adipose-tissue fragments is derived from the total number of adipocytes in the adipose organ, further metabolic and enzymic studies were conducted with the adipose-tissue fragments instead of isolated adipocytes.

Table 3 shows the characteristics of lean and obese rats. Obese rats were heavy and had enlarged subcutaneous, perirenal and gonadal fat-pads compared with lean animals. Adipocytes were enlarged in all the fat-depots of the obese animals, with no significant variation in the size of the adipocytes from one depot to another. The adipocytes from gonadal and perirenal fat-pads did not differ significantly with respect to cell size in lean animals. However, adipocytes from the subcutaneous fat-depots were smaller than those from the gonadal and perirenal fat-pads. Similar differences in the sizes of adipocytes in the fat-pads from lean and obese rats have been described previously (Johnson *et al.*, 1971; Zucker, 1972).

The boundaries of the gonadal and the perirenal fat-pads in the obese animal were not easily distinguishable from one another. Therefore the absolute weight of the fat-pad was not recorded, and changes in the total number of adipocytes in different fat-depots could not be calculated. However, previous studies suggest that along with the cell enlargement, total adipose-cell number is also increased in the different fat-depots of the obese rat (Johnson *et al.*, 1971).

The rates of [^{14}C]acetate conversion into CO_2 and lipid were significantly higher in subcutaneous, perirenal and gonadal fat-pads from obese rats than in adipose tissue derived from lean animals (Table 3). A major portion of the newly synthesized fatty acid formed from [^{14}C]acetate was found in the triacylglycerol molecule (53±10%, S.D., five animals) with lesser amounts in the phospholipid (10±4.2%) and diacylglycerol (20±6%), and the rest in unesterified fatty acids.

Although it has been demonstrated that acetate can be utilized in the formation of cholesterol (Kovanen *et al.*, 1975), no significant incorporation of [^{14}C]-acetate into cholesterol by the adipose tissues from either lean or obese rats could be demonstrated in the present studies. In lean animals, the gonadal and the perirenal fat-pads showed significantly higher rates of triacylglycerol formation from acetate compared with the subcutaneous adipose tissue. These rates did not differ significantly in the different fat-pads in the obese rats, although they were several times higher than in lean animals (Table 3).

Triacylglycerol synthesis from *sn*-glycerol 3-phosphate and palmitoyl-CoA-generating system was also higher in the obese rats than in lean animals in all the

Table 5. *Effect of food restriction on lipogenesis (fatty acid synthesis) and glycerolipid formation in different fat-depots of lean and obese rats*

The details of the animals used are given in Table 6. Lipogenesis from [^{14}C]acetate was measured with adipose-tissue fragments from gonadal fat-pads. Gonadal, perirenal and subcutaneous fat-free homogenates were used to measure glycerolipid formation from [^{14}C]glycerol 3-phosphate and palmitoyl-CoA-generating system. The rates of lipogenesis were expressed as nmol/h per 10^6 adipocytes and the rates of glycerolipid formation were expressed as nmol of products formed/min per 10^6 adipocytes in parentheses. The relative distribution of glycerophosphate into phosphatidate (PA), diacylglycerol (DG) and triacylglycerol (TG) is given as a percentage. Each value is the mean ± S.D. from four rats. *, Significantly different from obese control ($P > 0.05$); **, significantly different from obese control ($P < 0.01$); †, significantly different from other fat-depots from the same group of animals ($P<0.025$).

	Lipogenesis	Glycerolipid formation											
	Gonadal	Gonadal				Perirenal				Subcutaneous			
Group (treatment)		Lipid synthesis	PA	DG	TG	Lipid synthesis	PA	DG	TG	Lipid synthesis	PA	DG	TG
Lean (control)	27 ±11	(15.1* ±1.9)	47 ±5	11 ±2	42 ±6	(15.1* ±2.9)	39 ±9	12 ±4	49 ±18	(5.4**,† ±1.2)	72 ±23	10 ±2	18† ±5
Obese (control)	220* ±150	(48 ±21)	24 ±10	16 ±10	60 ±35	(24.6 ±6.9)	34 ±8	15 ±4	51 ±31	(45.5 ±21)	30 ±10	11 ±8	53 ±34
Obese (food-restricted)	37 ±12	(19.2 ±12.3)	42 ±23	14 ±7	44 ±33	(10.5 ±6.4)	44 ±27	9 ±3	37 ±19	(8.7 ±4.6)	59 ±33	8 ±4	28 ±21

Table 6. *Effect of dietary restrictions on the characteristics of adipose tissue in lean and obese rats*

Female lean and obese rats (67 days old) were divided into four groups with four animals per group. Lean and obese food-restricted animals received one-third of the amount of chow consumed by their respective controls (freely fed). After 14 days on this diet, all animals were killed. Adipose tissues from different fat-depots were used to determine the adipocyte number and adipocyte size. Adipose tissues from these animals were also used to determine lipid formation from [^{14}C]acetate and [^{14}C]glycerol 3-phosphate. Each value is the mean ± S.D. of four animals. The rates of lipid formation are shown in Table 5. The lean animals subjected to dietary restriction showed no significant adipose tissue. Therefore different parameters were not measured in this group of animals. Abbreviations: G, gonadal; PR, perirenal; SC, subcutaneous. *, Significantly different from obese groups (control and food-restricted) ($P < 0.05$).

Group (treatment)	Body wt (g)		Food consumption	10^{-6} × No. of adipocytes/g			Adipocyte size (μg of lipid/cell)		
	Initial	Final	(g/rat per day)	G	PR	SC	G	PR	SC
Lean (control)	152 ±6.4	188 ±9.0	15 ±0.36	3.39* ±1.15	3.27* ±1.0	4.30* ±2.37	0.20* ±0.03	0.22* ±0.04	0.14* ±0.07
Lean (food-restricted)	163 ±6.0	109 ±4.2	5 ±0.11						
Obese (control)	213 ±9.4	291 ±8.4	26 ±0.75	0.37 ±0.12	0.45 ±0.07	0.30 ±0.13	2.54 ±1.0	1.92 ±0.26	3.06 ±1.24
Obese (food-restricted)	228 ±18.7	196 ±11.0	9 ±0.75	0.85 ±0.27	0.92 ±0.24	0.75 ±0.18	1.14 ±0.44	1.05 ±0.36	1.12 ±0.38

fat-depots examined (Table 4). In addition to triacylglycerols, adipose-tissue homogenates incubated in the presence of *sn*-glycerol 3-phosphate formed phosphatidate and diacylglycerol. In lean animals, the fat-free homogenates from perirenal and gonadal fat-pads usually formed more neutral lipids (sum of di- and tri-acylglycerols) compared with phosphatidate and were more active in lipid formation than was the subcutaneous adipose tissue. In obese rats the rates of lipid formation did not differ significantly from one fat-depot to other fat-depot. However, the subcutaneous-fat-tissue homogenates from these animals, like those of lean animals, also formed more phosphatidate than neutral lipids. In the present studies individual enzyme activities involved in glycerolipid metabolism were not measured. It is possible that these differences in the product formation may be related to variation in intrinsic enzyme activities involved in lipid formation in the different fat-depots, as reported previously for obese and lean mice (Jamdar *et al.*, 1976). These results confirm the earlier observations of Bray and co-workers (Bray, 1968; Bray *et al.*, 1970) and demonstrate that an enhanced capacity for lipogenesis and esterification in the obese animals is not only limited to epididymal fat-pads but is also found in the other fat-depots as well.

An accelerated rate of adipose-tissue lipogenesis in obese rats has been described by Bray *et al.* (1974) at 6 weeks of age. However, at 18 weeks of age, lipogenesis was identical in both lean and obese animals. Martin (1974) and Martin & Lamprey (1975) also demonstrated an enhanced rate of lipogenesis in the epididymal fat-pads from obese animals at 5 weeks of age. However, at later ages when the obese rats were pair-fed along with the lean controls, no significant differences were observed. These studies suggest that an increased capacity of lipogenesis in the adipose tissue from obese animals may be related to increased food intake.

Dietary restriction in the obese rats caused a significant decrease in the rates of lipid synthesis from either [^{14}C]acetate or [^{14}C]glycerol 3-phosphate (Table 5), supporting the results of Martin & Lamprey (1975). Although during dietary restriction of obese animals the differences in the rates of lipid formation observed between lean and obese animals disappeared, adipocytes from food-restricted obese animals were still considerably larger than those of normal lean controls (Table 6). The lean animals subjected to similar dietary restriction showed a complete loss of their adipose tissue from all the sites, and therefore the measurements of different parameters could not be conducted in these animals.

Several conclusions can be drawn from these studies. First, isolated adipocytes do not provide optimum material for measurement of lipogenesis or esterification in fat-tissue. Fragments with proper corrections for non-adipocyte protein, or expression of results on the basis of adipocyte number (Jamdar *et al.*, 1976), are more representative of the metabolic activity of the adipose organ. Secondly, there are considerable differences in the rates of lipogenesis and esterification in lean animals by adipose tissue from various sites. Subcutaneous tissue is much less active than gonadal or perirenal tissue. Thirdly, adipose tissues from obese rats have much greater rates of lipogenesis and esterification at all sites. Finally, a marked decrease in the lipid synthesis in

adipose tissue of obese rats, which occurs after food restriction, is greater than can be accounted for by the decrease in adipocyte size.

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