Glycerolipid biosynthesis in rat adipose tissue

Influence of adipocyte size

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(Received 13 June 1980/Accepted 5 September 1980)

A simple one-step filtration method is described to separate larger adipocytes from the smaller ones by using nylon screen (52 μm pore size). Adipocytes retained on the screen were larger (60–90 μm) compared with those that passed through the screen. By using this separation technique, activities of various enzymes involved in triacylglycerol formation from *sn*-glycerol 3-phosphate were measured in the larger and smaller adipocytes isolated from gonadal fat-depots. The homogenates from larger adipocytes were more active in lipid formation compared with those derived from small adipocytes. This was evident from the increased activities of *sn*-glycerol 3-phosphate acyltransferase, Mg^{2+} -dependent phosphatidate phosphohydrolase and diacylglycerol acyltransferase in the larger adipocytes. The activities of these enzymes were also measured in the adipocytes isolated from gonadal, perirenal and subcutaneous fat-depots. Subcutaneous adipocytes were smaller and were less active in lipid formation than gonadal and perirenal adipocytes. These measurements in the activities of individual enzymes provide evidence that the entire pathway of esterification via *sn*-glycerol 3-phosphate is accelerated in the larger adipocytes.

It is now clear that triacylglycerol biosynthesis in adipose tissue is initiated by the stepwise acylation of either *sn*-glycerol 3-phosphate, dihydroxyacetone phosphate or monoacylglycerol (Rose & Shapiro, 1960; Steinberg *et al.*, 1961; Schultz & Johnson 1971; LaBelle & Hajra, 1972). Comparisons of the relative rates of esterification from these various precursors indicated that the *sn*-glycerol 3-phosphate pathway is the major route of triacylglycerol biosynthesis in adipose tissue (Dodds *et al.*, 1976).

Several metabolic functions of adipose tissue have been shown to increase in direct proportion to cell size. These functions include glucose incorporation into lipids (Salans & Cushman, 1978), lipoprotein lipase activity (Guy-Grand & Bigorie, 1974), lipolysis (Zinder & Shapiro, 1971; Holm *et al.*, 1975) and esterification of exogenously added fatty acids into lipids (Zinder *et al.*, 1967; Salans & Cushman, 1978). The last observation could be the result of either increased uptake of fatty acids and thus elevation of cell-associated fatty acids, or increased activities of esterifying enzymes in the larger adipocytes, or both. However, as for the relationship between triacylglycerol-biosynthesizing enzymes and a cell size is concerned, very little information is available. Therefore, in the present investigation, changes in enzyme activities involved in triacylglycerol biosynthesis from *sn*-glycerol

3-phosphate were studied as a function of adipocyte size.

Materials and methods

1,2-Diacyl-*sn*-glycerol and phosphatidate (99% pure) were purchased from Serdary Research Laboratories, London, Ont., Canada. The fatty-acid composition of diacylglycerol and phosphatidate was 36% palmitic acid, 37% oleic acid, 15% stearic acid and 12% linoleic acid (by weight). 1,2-Diacyl-*sn*-glycerol was dispersed in 0.1% (w/v) Tween 20 and phosphatidate was dispersed in deionized water by sonication for 20 s with a Branson model W 185 Sonifier (Ultrasonics, Plainview, NY, U.S.A.) equipped with a micro-tip probe. *sn*-[1,3- ^{14}C]Glycerol 3-phosphate (sp. radioactivity 30 Ci/mol) was purchased from ICN Chemicals and Radioisotope Division, Irvine, CA, U.S.A. [1- ^{14}C]Palmitoyl-CoA (sp. radioactivity 56 Ci/mol) was obtained from New England Nuclear Corporation, Boston, MA, U.S.A. Collagenase (158 units/mg of protein; lot 4196 CLS 49 S 240) was obtained from Worthington Corporation, Freehold, NJ, U.S.A. Most of the other chemicals were of A.R.-grade quality and were purchased from the sources reported by Jamdar (1977).

Sprague–Dawley rats (both sexes) were either

from our colony or purchased from Flow Laboratories, Dublin, VA, U.S.A. Rats were placed in air-conditioned animal facility maintained on a 12h-dark/12h-light schedule. Animals were fed laboratory chow (Ralston Purina Laboratory, St. Louis, MO, U.S.A.).

Isolation of adipocytes

Gonadal, perirenal or subcutaneous fat-pads (2–4g wet wt. of tissue per two to five rats) were used to isolate adipocytes by the method of Rodbell (1964) with slight modification. Tissue fragments were incubated in the presence of Krebs–Ringer bicarbonate buffer containing 4% (w/v) bovine serum albumin and 1 mg of collagenase/ml. For each gram of tissue, 5 ml of buffer was used. After incubation at 37°C for 30 min in a shaking water bath, adipocytes were separated from non-adipocytes by centrifugation at 200 rev./min for 1–2 min. The sedimented non-adipocytes along with buffer were removed by aspiration without disturbing the upper adipocyte layer. Adipocytes were suspended in a warm buffer (32°C) by drawing the mixture gently in and out five times with 1 ml plastic Biotip pipettes (1 mm diameter tip; obtained from Schwarz/Mann, Orangeburg, NY, U.S.A.) to free fat-cell clumps. The suspension was filtered through two layers of gauze to remove remaining undigested material, including small tissue fragments and blood capillaries.

To separate adipocytes of different sizes, a filtration technique was used in which isolated adipocytes were filtered through HC52 (pore size 52 μ m) nylon screen (Tetko Inc., Elmsford, NY, U.S.A.). For preparation of filter screen, the wide mouth (6.5 cm) of the plastic funnel was melted by placing the funnel on the hot plate for a few seconds. The melted edge of the funnel was pressed on the nylon screen, which was spread on a round-bottom flask (250 ml). This created a concave-surfaced screen that allowed sufficient area to filter 3–5 ml of adipocyte preparation. Usually, the smaller adipocytes (<60 μ m diameter) were filtered through and the larger ones remained on the screen. To remove smaller adipocytes completely, the larger adipocytes were washed with several volumes of warm buffer. The larger adipocytes were collected in a plastic centrifuge tube by tilting the funnel and pouring warm buffer on the screen. After this separation, adipocytes were harvested by low-speed (200 rev./min) centrifugation and washed with warm (30–32°C) medium A (0.25 M-sucrose/1 mM-dithiothreitol/1 mM-EDTA/1 mM-Tris, pH 7.5) to remove adherent albumin. This procedure was carried out twice. The washed adipocytes were suspended in a known volume of warm medium A (2 ml).

One portion (50 μ l) of this suspension was taken for the cell counting, which was done with the help

of a 0.2 mm haemocytometer (Carnie *et al.*, 1979). Another portion (50 μ l) was taken for the fixation of cells in OsO₄ (Cushman & Salans, 1978). The third portion (300 μ l) was added to 5 ml of chloroform/methanol (2:1, v/v) for lipid extraction (Folch *et al.*, 1957). To the remaining adipocytes, 1 ml of cold medium A was added before homogenizing with a Teckmar-tissuemizer (10N shaft; Teckmar Company, Cincinnati, OH, U.S.A.) at speed 5 for 30 s at 4°C. The resultant suspension was centrifuged at 2500 rev./min for 15 min in a refrigerated centrifuge (International Equipment Company, model B20-A) to prepare cell-free homogenates as described previously (Jamdar & Fallon, 1973a). Throughout the procedure, adipocytes were handled carefully and plastic-ware was used to minimize the cell breakage. After protein determination (Lowry *et al.*, 1951), adipocyte homogenates were used for various enzyme assays.

Enzyme assays

sn-Glycerol 3-phosphate acyltransferase (glycerophosphate acyltransferase; EC 2.3.1.15), phosphatidate phosphohydrolase (phosphatidate phosphatase; EC 3.1.3.4) and diacylglycerol acyltransferase (EC 2.3.1.20) were assayed as described previously (Jamdar & Fallon, 1973a,b; Jamdar *et al.*, 1976).

The acylation of *sn*-glycerol 3-phosphate was studied in the presence of palmitate, ATP, CoA and Mg²⁺. In a final volume of 0.75 ml, the reaction mixture contained 24 mM-Tris/HCl buffer, pH 7.5, 50 mM-KCl, 0.84 mM-*sn*-glycerol 3-phosphate, 0.1 μ Ci of *sn*-[1,3-¹⁴C]glycerol 3-phosphate, 0.7 mM-dithiothreitol, 1.05 mM-ammonium palmitate, 3 mM-ATP, 3.6 mM-MgCl₂, 0.01 mM-CoA and 1.25 mg of fatty-acid-poor albumin. The reaction was started with 0.1–0.3 ml of enzyme containing 100–120 μ g of homogenate protein. Incubation was under air at 37°C in a shaking water bath. The reaction was linear with time 15 min, when fat-free homogenate was used as the enzyme source.

Phosphatidate phosphohydrolase was assayed in the presence of aqueous-dispersed substrate as previously described (Jamdar & Fallon, 1973b) and the release of P_i was taken as a measure of phosphatidate phosphohydrolase activity. In a final volume of 1 ml, the reaction mixture contained 60 mM-Tris/maleate buffer, pH 7.5, 1.2 mM-phosphatidate, 5 mM-MgCl₂ and 0.8 mg of bovine serum albumin. The reaction was started by the addition of 100–120 μ g of homogenate protein and was linear with time up to 30 min. After incubation at 37°C for 30 min, the reaction was terminated by the addition of 1 ml of 10% (w/v) trichloroacetic acid, and the protein-free filtrate was collected by centrifugation. A portion of this filtrate (1 ml) was used to measure P_i content (Jamdar & Greengard, 1969). The

difference in the phosphate content between experimental reaction and the reaction stopped at zero time was taken as a measure of phosphatidate phosphohydrolase activity.

1,2-Diacyl-*sn*-glycerol acyltransferase was measured in the presence of 1,2-diacyl-*sn*-glycerol dispersed in Tween 20. The standard assay was in a final volume of 1 ml, containing 50 mM-Tris/HCl, pH 7.5, 1.2 mM-1,2-diacyl-*sn*-glycerol, 0.25 mM-dithiothreitol, 1.15 mM-spermine, 0.08 mM-palmitoyl-CoA (containing 0.1 μ Ci of [14 C]palmitoyl-CoA) and 1.25 mg of fatty-acid-poor albumin. The contents were incubated at 37°C for 2 min. The reaction was started by adding 0.1 ml of homogenate (100–120 μ g of protein) and was linear with time up to 10 min. The formation of [14 C]triacylglycerol was taken as a measure of the enzyme activity.

Radiochemical analysis of lipids

The radioactive lipids formed during the reaction were extracted as described by Van den Bosch & Vagelos (1970) and dried under N₂. The dry lipids were dissolved in 0.5 ml of benzene. Samples were applied in a volume of 0.1 ml and separated on thin-layer plates coated with silica gel G (E. Merck, Darmstadt, Germany) slurried in 0.1 M-sodium borate. The lipid classes were identified by appropriate standards in an adjacent lane.

The separation of phosphatidate was performed with the solvent system chloroform/methanol/3 M-NH₃ (65:38:8, by vol.) and chloroform/methanol/acetic acid/water (50:25:8:4, by vol.) (Renkonen, 1968; Blank & Snyder, 1970). Neutral lipids were separated with hexane/diethyl ether/acetic acid (73:25:2, by vol.) (Pieringer & Kunnes, 1965). The lipids were located by exposure of the plates to I₂. After sublimation of I₂ at room temperature, appropriate areas from the plates were scraped directly into scintillation vials containing 10 ml of Liquifluor in toluene. The radioactivity was measured in a Beckman LS 250 scintillation counter.

Since adipocytes isolated by Rodbell's procedure are usually contaminated with collagenase and also with small amounts of bovine serum albumin (Salans & Dougherty, 1971), the calculations of the enzyme reaction rates were based on the adipocyte number rather than on the adipocyte protein content.

Results and discussion

It is now known that several metabolic functions of adipose tissue are subject to change with adipocyte size. To study adipose metabolism as a function of cell size, two approaches are commonly used. In the first approach, various metabolic parameters of smaller adipocytes from young animals are measured and compared with larger adipocytes from older animals (Rudman &

DiGirolamo, 1967). In the second approach, adipocytes from the same donor are separated into different sizes before measuring various metabolic parameters (Björntorp & Karlsson, 1970; Holm *et al.*, 1975). The second approach is preferable over the first because, the source of adipocytes being the same, such measurements clearly reflect changes as a function of cell size rather than the secondary changes caused by the differences in the hormonal, dietary and age-related conditions of the donors. Therefore, in the present investigation, the second approach was used to study glycerolipid metabolism as a function of cell size.

Although techniques are available to separate fat-cells of different sizes from the same donors (Björntorp & Karlsson, 1970; Hansen *et al.*, 1974; Etherton *et al.*, 1977), these were found unsuitable for the present study. In the techniques developed by Hansen *et al.* (1974) and Etherton *et al.* (1977), adipocytes of different sizes were separated after the fixation with OsO₄. The flotation technique (Björntorp & Karlsson, 1970) was developed mainly for the separation of fewer numbers of adipocytes derived from the biopsy samples of patients. When large numbers of adipocytes (8×10^6 cells) were used, the separation by this method was not satisfactory. Therefore, a new method was developed in which isolated adipocytes were filtered through a nylon screen. This single-step filtration method separated smaller adipocytes from the larger ones. The sample of adipocytes that was retained on the screen contained mainly (85% or more) large adipocytes measuring 60–90 μ m diameter. The sample that passed through the nylon screen consisted of smaller adipocytes from 20 to 70 μ m in diameter. The typical separation of adipocytes by this method is shown in Fig. 1. This method was found to be reproducible, less time consuming, inexpensive and handled large numbers of adipocytes with good yields. The separation of larger adipocytes from smaller adipocytes by this method was also evident from the determination of mean cell size, which was expressed as μ g of lipid/cell (Table 1). Adipocytes retained on the filter screen were two times larger than the adipocytes that were filtered through the nylon screen.

Table 1 shows the changes in *sn*-glycerol 3-phosphate acyltransferase, phosphatidate phosphohydrolase and diacylglycerol acyltransferase activities as a function of cell size. To measure various enzyme activities, adipocyte homogenates prepared from large and small adipocytes were used as the enzyme source.

sn-Glycerol 3-phosphate acyltransferase activity measured in the presence of [14 C]glycerol 3-phosphate and palmitoyl-CoA-generating system was three times more active in the larger adipocytes compared with smaller ones. The major radioactive

Table 1. Influence of cell size on *sn*-glycerol 3-phosphate acyltransferases (GPAT), phosphatidate phosphohydrolase (PPH) and diacylglycerol acyltransferase (DGAT)

Adipocytes isolated from gonadal fat-pads from male rats (215 ± 6 g) ($n = 12$) were sized by the filtration technique described in the Materials and methods section. Adipocyte homogenates prepared from smaller and larger adipocytes were used to assay various enzyme activities, which were expressed as nmol/min per 10^6 adipocytes. The separation profile of adipocytes by the filtration method is shown in Fig. 1. Mean adipocyte size is expressed as μg of lipid/cell. Each value is the mean $\pm$ S.E.M. from six different experiments. For each experiment, gonadal fat-pads from two rats were pooled for the preparation of adipocytes. Values for large cells were significantly different from small cells at: ** $P < 0.01$, * $P < 0.05$.

Adipocytes	Mean cell size (μg of lipid/cell)	Enzyme activity (nmol/min per 10^6 adipocytes)		
		GPAT	PPH	DGAT
Small ($<60 \mu\text{m}$)(A)	0.023 ± 0.003	0.73 ± 0.24	3.37 ± 1.14	2.3 ± 0.61
Large ($>60 \mu\text{m}$)(B)	$0.052 \pm 0.01^{**}$	$2.58 \pm 0.87^*$	$6.26 \pm 1.8^*$	$8.13 \pm 1.90^{**}$
Ratio of B/A	2.21	3.53	1.85	3.51

Fig. 1. Separation of adipocytes of different sizes. In each experiment, gonadal fat-pads from two male rats were pooled for the preparation of adipocytes as described in the Materials and methods section. Isolated adipocytes were filtered through the HC52 nylon screen. A sample of adipocytes ($50 \mu\text{l}$) retained on the nylon screen and sample of adipocytes passed through the screen were fixed in 4 ml of 2% OsO_4 by the method of Cushman & Salans (1978). After fixation, adipocytes were processed as described by Hirsch & Gallian (1968), except that instead of $25 \mu\text{m}$ nylon screen, $10 \mu\text{m}$ screen was used to collect OsO_4 -fixed adipocytes. Adipocytes were washed with several volumes of water and finally with 0.9% NaCl and suspended in 0.9% NaCl. The diameters of 200–250 adipocytes (OsO_4 -fixed) were measured with the help of a micrometer eye piece (American Optical) to determine the adipocyte distribution in these two samples. The solid line shows the size distribution of adipocytes passed through the nylon screen. The broken line indicates the size distribution of adipocytes retained on the nylon screen. Each point is the mean $\pm$ S.E.M. of six different experiments with 12 male rats (215 ± 6.6 g body wt.).

Table 2. Incorporation of [^{14}C]glycerol 3-phosphate into lipids as a function of adipocyte size

sn-Glycerol 3-phosphate acyltransferase was assayed in the presence of [^{14}C]glycerol 3-phosphate, palmitate, ATP, CoA and Mg^{2+} by using adipocyte homogenates as the enzyme source. The rate of glycerolipid formation and other details of the experiments are given in Table 1. The relative distribution of [^{14}C]glycerol 3-phosphate into phosphatidate, diacylglycerol and triacylglycerol is given as a percentage. Each value is a mean $\pm$ S.E.M. from six different experiments with assays carried out in duplicate.

Adipocytes	Distribution of glycerophosphate (%) into:		
	Phosphatidate	Diacylglycerol	Triacylglycerol
Small	50 ± 9	11 ± 2	39 ± 6
Large	67 ± 3	9 ± 1	24 ± 2

products of the *sn*-glycerol 3-phosphate acyltransferase reaction were identified as phosphatidate, diacylglycerol and triacylglycerol with less than 5% of the lipid recovered as lysophosphatidate (Table 2). The butanol extraction procedure (Dae & Bremer, 1970) also gave similar quantitative recoveries of the acylated products. The proportions of the phosphatidate and neutral lipids (sum of di- and tri-acylglycerols) formed did not differ significantly as a function of cell size.

Previously, we have reported the presence of both Mg^{2+} -dependent and Mg^{2+} -independent phosphatidate phosphohydrolase activities in adipose tissue and demonstrated that mainly the Mg^{2+} -dependent enzyme is involved in acylglycerol synthesis (Jamdar & Fallon, 1973b). Due to lack of sufficient material, such distinction was not made in the present studies and the total phosphatidate phosphohydrolase activity in the presence

of Mg^{2+} was measured. Adipocyte homogenates prepared from larger adipocytes were twice as active in the utilization of phosphatidate compared with homogenates derived from the smaller adipocytes (Table 1).

There was also a significant increase (3.5 times) in the diacylglycerol acyltransferase activity in the larger adipocytes compared with the smaller ones. Thus, these measurements of the individual enzyme activities provide evidence that the entire pathway of esterification via *sn*-glycerol 3-phosphate is accelerated in the larger adipocytes. These enzymic changes may at least be partly responsible for the increased rates of esterification of exogenously added fatty acids noted in the larger adipocytes (Zinder *et al.*, 1967; Salans & Cushman, 1978).

There are known differences in the sizes of adipocytes in various fat-depots in the rat and in several studies an advantage has been taken of this property to study adipose metabolism as a function of cell size (Hartman, 1977). Adipocytes from the subcutaneous fat-pads are usually smaller compared with those derived from the gonadal and perirenal fat-depots (Jamdar, 1978). Comparison of the esterification rates from different fat-depots indicated that glycerolipid formation by the subcutaneous adipose tissue was significantly low compared with the gonadal or the perirenal adipose tissues (Jamdar, 1978). However, in our previous studies, instead of isolated adipocyte preparations, whole-tissue preparations were used to study glycerolipid formation. Since adipose tissue is comprised of as much as 45% non-adipocytes (Rodbell, 1964), the question of the differences in the rates of esterification in the different fat-depots was re-examined by using isolated adipocyte preparations. As shown from the mean-cell-size determination (μg of lipid/cell) and adipocyte distribution studies (Table 3 and Fig. 2), the subcutaneous adipocytes were significantly smaller than the adipocytes derived from the gonadal and perirenal fat-depots.

The activities of *sn*-glycerol 3-phosphate acyltransferase, phosphatidate phosphohydrolase and diacylglycerol acyltransferase were also significantly lower in the subcutaneous adipocytes compared with the gonadal and perirenal adipocytes. The gonadal and perirenal adipocytes did not differ from each other with respect to cell size or enzyme activities (Table 3). These results were similar to those reported previously for whole-tissue preparations (Jamdar, 1978).

Fig. 2. Size distribution of adipocytes in different fat depots

The samples of adipocytes prepared separately from gonadal, perirenal and subcutaneous fat-pads were fixed in 2% OsO_4 . After fixation, the diameters of 200–250 cells were measured as described in the legend to Fig. 1. In each experiment, fat-pads from four to five female rats were pooled to isolate adipocytes. Each point is the mean $\pm$ S.E.M. from four different experiments with 23 female rats. The details of the animals are given in Table 3. Symbols: $\blacktriangle$, gonadal; $\bigcirc$, perirenal; $\bullet$, subcutaneous.

Table 3. Differences in *sn*-glycerol 3-phosphate acyltransferase (GPAT), phosphatidate phosphohydrolase (PPH) and diacylglycerol acyltransferase (DGAT) activities in different fat-depots

Adipocyte homogenates prepared from gonadal, perirenal and subcutaneous fat-pads from female rats [body wt. 255 ± 49 g ($n = 23$)] were used to assay various enzyme activities. For each experiment, fat-pads from four to five rats were pooled for adipocyte preparation. Each value is a mean $\pm$ S.D. for four different experiments. Enzyme activities were expressed as nmol/min per 10^6 adipocytes. Mean cell size is expressed as μg of lipid per cell. The adipocyte size profile is shown in Fig. 2.

Source of fat	Cell size (μg of lipid/cell)	Enzyme activity (nmol/min per 10^6 adipocytes)		
		GPAT	PPH	DGAT
Gonadal	0.068 ± 0.028	1.39 ± 0.36	6.07 ± 2.0	4.91 ± 0.24
Perirenal	0.076 ± 0.032	1.23 ± 0.56	7.61 ± 3.32	4.36 ± 0.87
Subcutaneous	$0.017 \pm 0.005^*$	$0.29 \pm 0.31^*$	$0.67 \pm 0.39^*$	$0.38 \pm 0.18^*$

* Result significantly different from gonadal and perirenal fat-depots at $P < 0.02$.

Besides an increase in the activities of various enzymes involved in triacylglycerol formation, there was also a significant increase in the total protein content in the larger adipocytes ($P < 0.05$). For 10^6 cells, the larger adipocytes contained 0.39 ± 0.08 mg of protein, whereas the protein concentration in the smaller adipocytes was 0.24 ± 0.05 mg (mean $\pm$ s.e.m. from six different experiments). However, previous studies by Salans & Dougherty (1971) demonstrated that adipocytes isolated by Rodbell's procedure are usually contaminated with collagenase and bovine serum albumin. In their studies, the collagenase contamination with adipocytes was 33%, regardless of the cell size, whereas the contamination with albumin was less than 1%, but was increased proportionately with the adipocyte size. Therefore it is not presently clear whether the observed rise in the protein content in the larger adipocytes reported here is related to selective increase in the enzyme protein or is a reflection of the contamination of adipocytes by collagenase and bovine serum albumin, as noted previously by Salans & Dougherty (1971).

This investigation was supported by Grant R01 AM 26181 from the National Institute of Health. This is paper no. 5 in a series. Paper no. 4 is Jamdar (1978).

References

- Björntorp, P. & Karlsson, M. (1970) *Eur. J. Clin. Invest.* **1**, 112–117
- Blank, M. L. & Snyder, F. (1970) *Biochemistry* **9**, 5034–5036
- Carnie, J. A., Smith, D. G. & Mavris-Vavayannis, M. (1979) *Biochem. J.* **184**, 107–112
- Cushman, S. W. & Salans, L. B. (1978) *J. Lipid Res.* **19**, 269–273
- Daae, L. N. W. & Bremer, J. (1970) *Biochim. Biophys. Acta* **210**, 92–104
- Dodds, P. F., Gurr, M. I. & Brindley, D. N. (1976) *Biochem. J.* **160**, 693–700
- Etherton, T. D., Thomson, E. H. & Allen, C. E. (1977) *J. Lipid Res.* **18**, 552–557
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Guy-Grand, B. & Bigorie, B. (1974) in *The Regulation of the Adipose Tissue Mass* (Vague, J. & Boyer, J., eds.), pp. 52–57, American Elsevier Publishing Company, New York
- Hansen, F. J., Neilson, H. J. & Gliemann, J. (1974) *Eur. J. Clin. Invest.* **4**, 411–418
- Hartman, A. D. (1977) *Am. J. Physiol.* **232**, 316–323
- Hirsch, J. & Gallian, E. (1968) *J. Lipid Res.* **9**, 110–119
- Holm, B., Jacobson, B., Björntorp, P. & Smith, U. (1975) *J. Lipid Res.* **12**, 286–293
- Jamdar, S. C. (1977) *Arch. Biochem. Biophys.* **182**, 723–731
- Jamdar, S. C. (1978) *Biochem. J.* **170**, 153–160
- Jamdar, S. C. & Fallon, H. J. (1973a) *J. Lipid Res.* **14**, 507–516
- Jamdar, S. C. & Fallon, H. J. (1973b) *J. Lipid Res.* **14**, 517–525
- Jamdar, S. C. & Greengard, O. (1969) *Arch. Biochem. Biophys.* **134**, 228–238
- Jamdar, S. C., Shapiro, D. & Fallon, H. J. (1976) *Biochem. J.* **158**, 327–334
- LaBelle, E. F. & Hajra, A. K. (1972) *J. Biol. Chem.* **247**, 5825–5834
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Pieringer, R. A. & Kunnes, R. S. (1965) *J. Biol. Chem.* **240**, 2833–2838
- Renkonen, O. (1968) *Biochim. Biophys. Acta* **152**, 114–135
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Rose, G. & Shapiro, B. (1960) *Bull. Res. Council. Isr. Sect. A* **9**, 15–22
- Rudman, D. & DiGirolamo, M. (1967) *Adv. Lipid Res.* **5**, 35–117
- Salans, L. B. & Cushman, S. W. (1978) *Adv. Mod. Nutr.* **2**, 267–301
- Salans, L. B. & Dougherty, J. W. (1971) *J. Clin. Invest.* **50**, 1399–1410
- Schultz, F. M. & Johnston, J. M. (1971) *J. Lipid Res.* **12**, 132–138
- Steinberg, D., Vaughan, M. & Margolis, S. (1961) *J. Biol. Chem.* **236**, 1631–1637
- Van den Bosch, H. & Vagelos, P. R. (1970) *Biochim. Biophys. Acta* **249**, 318–330
- Zinder, O. & Shapiro, B. (1971) *J. Lipid Res.* **12**, 91–95
- Zinder, O., Arad, R. & Shapiro, B. (1967) *Isr. J. Med. Sci.* **3**, 787–791