Biochem. J. (1988) 250, 325–333 (Printed in Great Britain)

Comparison of triacylglycerol synthesis in rat brown and white adipocytes

Effects of hypothyroidism and streptozotocin-diabetes on enzyme activities and metabolic fluxes

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1. Adipocytes were isolated from the interscapular brown fat and the epididymal white fat of normal, streptozotocin-diabetic and hypothyroid rats. 2. Measurements were made of the maximum rate of triacylglycerol synthesis by monitoring the incorporation of [U-^{14}C]glucose into acylglycerol glycerol in the presence of palmitate (1 mM) and insulin (4 nM) and of the activities of the following triacylglycerol-synthesizing enzymes: fatty acyl-CoA synthetase (FAS), mitochondrial and microsomal forms of glycerolphosphate acyltransferase (GPAT), dihydroxyacetonephosphate acyltransferase (DHAPAT), monoacylglycerol phosphate acyltransferase (MGPAT), Mg^{2+}-dependent phosphatidate phosphohydrolase (PPH) and diacylglycerol acyltransferase (DGAT). 3. FAS activity in brown adipocytes was predominantly localized in the mitochondrial fraction, whereas a microsomal localization of this enzyme predominated in white adipocytes. Subcellular distributions of the other enzyme activities in brown adipocytes were similar to those shown previously with white adipocytes [Saggerson, Carpenter, Cheng & Sooranna (1980) Biochem. J. **190**, 183–189]. 4. Relative to cell DNA, brown adipocytes had lower activities of triacylglycerol-synthesizing enzymes and showed lower rates of metabolic flux into acylglycerols than did white adipocytes isolated from the same animals. 5. Diabetes decreased both metabolic flux into acylglycerols and the activities of triacylglycerol-synthesizing enzymes in white adipocytes. By contrast, although diabetes decreased metabolic flux into brown-adipocyte acylglycerols by 80%, there were no decreases in the activities of triacylglycerol-synthesizing enzymes, and the activity of PPH was significantly increased. 6. Hypothyroidism increased metabolic flux into acylglycerols in both cell types, and increased activities of all triacylglycerol-synthesizing enzymes in brown adipocytes. By contrast, in white adipocytes, although hypothyroidism increased the activities of FAS, microsomal GPAT and DGAT, this condition decreased the activities of mitochondrial GPAT and PPH. 7. It was calculated that the maximum capabilities for fatty acid oxidation and esterification are approximately equal in brown adipocytes. In white adipocytes esterification is predominant by approx. 100-fold. 8. Diabetes almost abolished incorporation of [U-^{14}C]glucose into fatty acids in both adipocyte types. Hypothyroidism increased fatty acid synthesis in white and brown adipocytes by 50% and 1000% respectively.

INTRODUCTION

Brown adipose tissue is a highly specialized organ involved both in non-shivering thermogenesis, which is important during early postnatal life and during cold exposure, and in diet-induced thermogenesis. Heat is derived from a high rate of uncoupled respiration of fatty acids, involving a unique proton-conductance pathway that is dissociated from oxidative phosphorylation. It is well established that these catabolic events are supported by the lipolysis of triacylglycerols stored in the multiple droplets found within the brown adipocytes (see reviews by Nedergaard & Lindberg, 1982; Nicholls & Locke, 1983, 1984; Cannon & Nedergaard, 1985*a*,*b*) and facilitated by a high activity of carnitine palmitoyltransferase (Saggerson & Carpenter, 1982). Clearly the brown adipocyte must possess the capability to replenish these stores by synthesizing triacylglycerol by using fatty acids of endogenous or exogenous origin. It is established that brown adipose tissue *in vivo* is capable of high rates of fatty acid synthesis (McCormack & Denton, 1977; Agius & Williamson, 1980), particularly in cold-adapted animals (Trayhurn, 1981). Glucose appears to be the predominant substrate for fatty acid synthesis in this tissue, and a stimulatory role for insulin is established (McCormack & Denton, 1977; Paetzke-Brunner *et al.*, 1979; Sugden *et al.*, 1982; McCormack, 1982). Plasma lipoprotein triacylglycerols metabolized by lipoprotein lipase can also provide an alternative source of fatty acids to the tissue (Bertin *et al.*, 1978; Guerrier & Pellet, 1979). By contrast, our knowledge of the triacylglycerol-synthesis pathway and its regulation in the brown adipocyte is extremely limited. [^{14}C]Glucose incorporation into the glycerol moiety of acylglycerols and esterification of [^{3}H]oleate have been demonstrated with brown-adipose-tissue preparations *in vitro* (Joel, 1965;

Abbreviations used: DGAT, diacylglycerol acyltransferase (EC 2.3.1.20); DHAPAT, dihydroxyacetonephosphate acyltransferase (EC 2.3.1.42); FAS, ATP-dependent long-chain fatty acyl-CoA synthetase (EC 6.2.1.3); GPAT, glycerolphosphate acyltransferase (EC 2.3.1.15); MGPAT, monoacylglycerolphosphate acyltransferase (EC 2.3.1.51); NEM, *N*-ethylmaleimide; PPH, phosphatidate phosphohydrolase (EC 3.1.3.4).

Himms-Hagen, 1965; Fain *et al.*, 1967; Prusiner *et al.*, 1968; Angel, 1969; Knight & Myant, 1971). It has been stated that fatty acid esterification in a microsomal preparation from hamster brown adipose tissue occurs via both the monoacylglycerol and the glycerolphosphate pathways (Schultz & Johnston, 1971), whereas it has been reported that only the glycerolphosphate pathway is operative in rabbit cells (Schenk *et al.*, 1975). The only enzyme from the triacylglycerol-synthesis pathway to be investigated is FAS, which is involved in both esterification and oxidation processes. It is reported that this enzyme has a high specific activity (Pederson *et al.*, 1975; Norman & Flatmark, 1980), and it is claimed to be localized only on the mitochondrial outer membrane, none being found in the endoplasmic reticulum. Surprisingly, this short resumé represents more or less our total knowledge of this process in brown adipose tissue.

The purpose of this study was to make an assessment of the capabilities of the triacylglycerol-synthesis pathway in brown adipose tissue. By using brown and white adipocytes from the same animals as source material, it is possible to make direct comparison with the more extensively characterized triacylglycerol-synthesis system in white adipose tissue. We have made comparisons of the maximum activities of several enzymes, together with their subcellular distributions, and have measured flux rates into triacylglycerol in incubated cells. In addition, we have investigated the effects of streptozotocin-diabetes and hypothyroidism on enzyme activities and flux rates. These abnormal hormonal conditions were chosen because of the growing awareness that brown adipose tissue may be an important site of insulin action (McCormack, 1982; Cooney *et al.*, 1985; Ferré *et al.*, 1986) and because both diabetes (Seydoux *et al.*, 1983, 1984; Jamal & Saggerson, 1987, 1988) and hypothyroidism (Mory *et al.*, 1981; Seydoux *et al.*, 1982; Sundin *et al.*, 1984; Woodward & Saggerson, 1986) cause considerable changes in brown-adipose-tissue metabolism.

MATERIALS AND METHODS

Chemicals

These were obtained and treated as described by Bates & Saggerson (1979), Saggerson *et al.* (1980); Hall & Saggerson (1985), Taylor & Saggerson (1986) and Woodward & Saggerson (1986). In addition diacyl-*sn*-[^{3}H]glycerol 3-phosphate was a gift from Professor D. N. Brindley (University of Nottingham, U.K.).

Animals

These were male Sprague–Dawley rats bred in the animal colony at University College London and, unless indicated otherwise, were selected for experimentation at 160–180 g body wt. The animals were kept at approx. 21 °C on a 13 h-light/11 h-dark cycle, with light from 06:00 to 19:00 h, with Rat and Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.) supplied *ad libitum*. Streptozotocin-diabetes was induced over 2 days as described by Saggerson & Carpenter (1987), and hypothyroidism over 4 weeks by feeding rats with a low-iodine diet and 6-n-propyl-2-thiouracil (Chohan *et al.*, 1984; Woodward & Saggerson, 1986).

Isolation of adipocytes

White and brown adipocytes were invariably obtained from the same animals. Brown adipocytes were isolated from the pooled interscapular brown fat of four rats as described by Woodward & Saggerson (1986), based on the original method of Fain *et al.* (1967) and elaborated by Nedergaard & Lindberg (1982). White adipocytes were isolated from the pooled epididymal fat-pads of four rats by the method of Rodbell (1964).

Preparation of defatted homogenates

Freshly isolated preparations of brown and white adipocytes were washed twice in albumin-free Krebs–Ringer bicarbonate medium (Krebs & Henseleit, 1932) and then transferred to ice-cold sucrose medium [0.25 M-sucrose/10 mM-Tris/HCl buffer (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol]; 5–10 ml of this medium was used for brown and 8–16 ml for white adipocyte preparations respectively. The cells were then homogenized in an ice-cooled Potter–Elvehjem homogenizer with six up-and-down strokes of a rotating Teflon pestle (450 rev./min) with a radial clearance of 0.2 mm. Floating fat was removed by brief centrifugation (10 s at approx. 200 $g_{av.}$), and the resulting infranatant was removed by aspiration. These defatted homogenates either were used for subsequent subcellular fractionation or were immediately frozen at −20 °C in small batches.

Subcellular fractionation

This was as described by Saggerson *et al.* (1980) to isolate nuclear (N), mitochondrial (M), microsomal (E) and 105000 *g*-supernatant (S) fractions.

Measurements of flux rates in incubated adipocytes

Freshly isolated brown and white adipocytes were washed twice in Krebs–Ringer bicarbonate containing fatty-acid-poor albumin (10 mg/ml) and were then dispensed at approx. 2×10^6 cells/ml into 25 ml silicone-treated Erlenmeyer flasks containing 4 ml of Krebs–Ringer bicarbonate medium, 5 mM-[U-^{14}C]glucose (2 μCi/flask), 1 mM-sodium palmitate, insulin (4 nM) and fatty-acid-poor albumin (40 mg/ml). The flasks were incubated at 37 °C with shaking for 40 min under an atmosphere of O_2/CO_2 (19:1) before extraction of cell lipids and measurement of [^{14}C]glucose incorporation into glycerol and fatty acids of acylglycerols (Saggerson, 1972; Saggerson & Greenbaum, 1970).

Analytical methods

FAS was assayed fluorimetrically at 25 °C (Hall & Saggerson, 1985) by measurement of the incorporation of (1,N^6-etheno)-CoASH into the acid-precipitable reaction product, palmitoyl-(etheno-CoA). GPAT was assayed radiochemically at 30 °C (Saggerson *et al.*, 1980; Rider & Saggerson, 1983) by measuring the incorporation of 1 mM-[U-^{14}C]glycerol 3-phosphate into butanol-soluble products. The proportion of GPAT which is insensitive to NEM was assayed in the presence of 10 mM-NEM with 40 μM-palmitoyl-CoA to optimize conditions. The remaining proportion of GPAT was either assayed as NEM-sensitive activity with 120 μM-palmitoyl-CoA as substrate, or with 120 μM-oleoyl-CoA in the absence of NEM (see the Results and discussion section). MGPAT was assayed spectrophotometrically at 25 °C and 412 nm (Saggerson *et al.*, 1980) by observing

the transfer of CoA from oleoyl-CoA to 5,5′-dithiobis-(2-nitrobenzoic acid) (Okuyama *et al.*, 1971). Mg^{2+}-dependent PPH was assayed at 37 °C by measurement of phosphate release from an aqueous dispersion of phosphatidate. The procedure was essentially as described by Taylor & Saggerson (1986), except that the assay (0.5 ml final volume) was modified to contain 100 mM-Tris/maleate buffer (pH 7.4), 1 mM-dithiothreitol, 2.5 mM-$MgCl_2$ and 0.75 mM-sodium phosphatidate. In some preliminary experiments Mg^{2+}-dependent PPH was also assayed by measuring the conversion of diacyl-*sn*-[^{3}H]glycerol 3-phosphate into [^{3}H]diacylglycerol (Sturton & Brindley, 1978). DHAPAT was assayed radiochemically at 30 °C by using dihydroxy-[^{14}C]acetone phosphate generated *in situ* from [U-^{14}C]fructose 1,6-bisphosphate as described by Bates & Saggerson (1979). DGAT was assayed radiochemically at 30 °C (Coleman & Bell, 1976) by measurement of the dioleoylglycerol-dependent incorporation of [^{14}C]palmitoyl-CoA into hexane-soluble products. Lactate dehydrogenase (EC 1.1.1.27), citrate synthase (EC 4.1.3.7) and $NADP^+$–cytochrome *c* reductase (EC 1.6.2.4) were assayed spectrophotometrically at 25 °C by the methods of Saggerson (1974), Shepherd & Garland (1969) and Phillips & Langdon (1962) respectively. Protein was measured by the method of Lowry *et al.* (1951), with fatty-acid-poor bovine albumin as a standard. DNA was measured in defatted homogenates by the method of Switzer & Summer (1971).

Statistical methods

Throughout, *n* values refer to the numbers of separate adipocyte preparations. Statistical significance was assessed by Student's *t* test for unpaired samples.

RESULTS AND DISCUSSION

Enzyme assays: general aspects

GPAT activity in liver or white-adipocyte mitochondrial fractions differs substantially from that in microsomal fractions. The mitochondrial form has a higher affinity for acyl-CoA substrates (Yamada & Okuyama, 1978; Bates & Saggerson, 1979; Saggerson *et al.*, 1979; Rider & Saggerson, 1983), has a higher preference for acylation at the 1-position of glycerol phosphate (Daae, 1972; Monroy *et al.*, 1972) and preferentially uses saturated fatty acyl-CoA substrates (Monroy *et al.*, 1972, 1973; Saggerson *et al.*, 1979). In addition, the microsomal GPAT from liver or white adipocytes is almost completely inhibited by thiol-group reagents such as NEM, whereas the mitochondrial activity is barely affected (Lands & Hart, 1965; Monroy *et al.*, 1972; Haldar & Pullman, 1975; Bates & Saggerson, 1979; Saggerson *et al.*, 1979; Nimmo, 1979; Rider & Saggerson, 1983). In liver and white adipocytes, NEM-insensitive activity (mitochondrial) is optimal at 40–60 μM-palmitoyl-CoA (Bates & Saggerson, 1979; Rider & Saggerson, 1983) under the assay conditions used here ([albumin] = 27 μM). In preliminary experiments (not shown) the same was found to hold for this activity in brown-adipocyte mitochondrial fractions. Because mitochondrial GPAT has very low activity with unsaturated acyl-CoA substrates, it has been found convenient to assay for microsomal GPAT in liver extracts with oleoyl-CoA as substrate, as well as assaying for NEM-sensitive activity with palmitoyl-CoA (Saggerson & Topping, 1981). The same was found to be true for brown adipocytes, in that 10 mM-NEM inhibited more than 95% of GPAT activity measured with oleoyl-CoA in brown-adipocyte homogenates or in any subcellular fraction (results not shown). Furthermore, GPAT activities assayed with palmitoyl-CoA + NEM or with oleoyl-CoA alone showed the same subcellular distribution (results not shown). As found in liver and white adipocytes (Bates & Saggerson, 1979; Rider & Saggerson, 1983), GPAT activity in brown-adipocyte microsomal fraction also had a lower affinity for acyl-CoA substrates than did the mitochondrial activity (results not shown), and 120 μM-oleoyl-CoA or 120 μM-palmitoyl-CoA (+NEM) was needed to give near-maximum activities. Brown-adipose-tissue mitochondria contain an active glycerolphosphate oxidase. It was therefore considered possible that this might interfere with GPAT assays in mitochondrial fractions. This possibility was discounted, because addition of antimycin A (1 μg/ml) to such assays had no effect (preliminary experiments; results not shown).

Sturton & Brindley (1978, 1980) have shown that rat liver contains phospholipase activity that deacylates phosphatidate. Subsequent dephosphorylation of the resulting glycerol phosphate then leads to overestimation of liver PPH activity if this enzyme is assayed as P_i release from phosphatidate, particularly in liver microsomal fractions. This necessitates the use of an alternative assay for PPH, namely conversion of [^{3}H]phosphatidate into [^{3}H]diacylglycerol, if this problem is apparent. Lawson *et al.* (1981), however, showed that this does not appear to apply to white adipose tissue, since they observed no formation of lysophosphatidate or glycerol phosphate when rat epididymal-fat-pad soluble or microsomal fractions were incubated with [^{3}H]phosphatidate. In preliminary experiments (results not shown) we have established that measurements of PPH activity are essentially identical when either P_i formation or [^{3}H]diacylglycerol formation is monitored with 1,2-diacyl[^{3}H]glycerol 3-phosphate as substrate, with mitochondrial, microsomal or 105000 *g*-supernatant fractions from rat brown adipocytes. In addition, the percentage distribution of PPH activity between these three fractions was the same for both assays. This finding provided reassurance that the simpler phosphate-release assay of PPH could also be used with brown adipocytes.

In further preliminary experiments, the kinetic properties of Mg^{2+}-dependent PPH in S fractions were found to be similar for brown and white adipocytes. All PPH activity in brown-adipocyte S fraction was Mg^{2+}-dependent and showed similar dependence on [Mg^{2+}] and [phosphatidate] to that found previously in this laboratory for the white-adipocyte enzyme (Saggerson, 1988). In addition Mg^{2+}-dependent PPH in S fraction from both adipocyte types had a pH optimum of approx. 7.6.

Subcellular distributions of enzyme activities

Preparations of white adipocytes were fractionated at the same time as the brown adipocytes, so that comparisons between the two cell types could be made. Relative specific activities and percentage distributions are shown in Fig. 1.

There were differences between the two cell types in

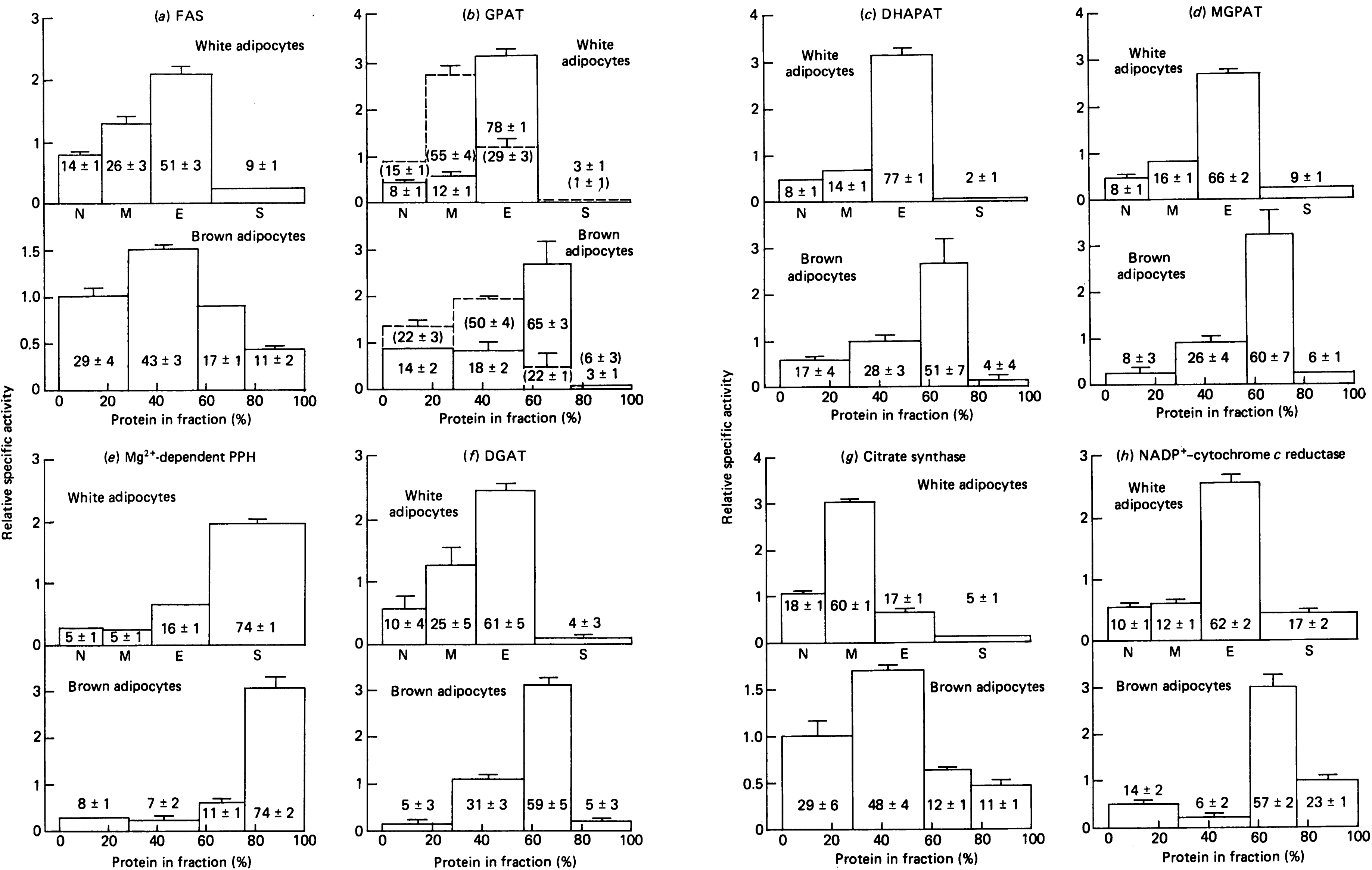

Fig. 1. Activities of enzymes in subcellular fractions

Adipocytes were obtained from 6-week-old normal animals. The values are means ± S.E.M. for three experiments. The histograms indicate the relative specific activity, which is defined as % of total activity in a fraction/% of total protein in that fraction, the value for the defatted homogenate being 1. Broken lines in (*b*) indicate NEM-insensitive GPAT, and continuous lines indicate GPAT assayed with oleoyl-CoA. The numbers on the histograms show the percentage distributions of enzyme activities in subcellular fractions, where the sum of each activity in fractions N + M + E + S is set to a value of 100. In (*b*), values in parentheses indicate NEM-insensitive GPAT and the others indicate GPAT assayed with oleoyl-CoA.

Table 1. Rat and adipose-tissue wet weights: effects of age, diabetes and hypothyroidism

Rats	Body wt. (g)	Wt. of eight pooled epididymal fat-pads (g)	Wt. of four pooled interscapular brown-fat pads (g)
6-week-old control	190 ± 1 ($n = 28$)	2.92 ± 0.12 ($n = 7$)	1.64 ± 0.12 ($n = 7$)
6-week-old diabetic	156 ± 1 ($n = 24$)	1.92 ± 0.20 ($n = 6$)	1.48 ± 0.08 ($n = 6$)
9-week-old control	280 ± 3 ($n = 20$)	6.84 ± 0.20 ($n = 5$)	2.52 ± 0.12 ($n = 5$)
9-week-old hypothyroid	145 ± 2 ($n = 20$)	3.60 ± 0.12 ($n = 5$)	2.00 ± 0.08 ($n = 5$)

the distribution of protein: 56% was found in the N+M fractions in brown adipocytes, compared with 38% in white adipocytes. Both cell types had roughly similar proportions in the E fraction (19% and 24% for brown and white adipocytes respectively), whereas white adipocytes had a substantially larger proportion of soluble protein (48%, compared with 25% in brown adipocytes). Within the N+M fractions, the percentage distributions of both citrate synthase and NEM-insensitive GPAT were slightly greater in the N fraction and smaller in the M fraction in brown adipocytes.

Subcellular distributions of both forms of GPAT, MGPAT, Mg^{2+}-dependent PPH and DGAT from brown adipocytes were similar to those for white adipocytes (Fig. 1; see also Saggerson *et al.*, 1980). DHAPAT in brown adipocytes showed a higher percentage distribution in the N+M fractions, possibly reflecting the presence of some mitochondrial or peroxisomal DHAPAT, as in liver (Declercq *et al.*, 1984).

There were clear differences between the cell types in the distribution of FAS. In brown adipocytes N+M fractions contained the majority of this activity, which was significantly enriched only in the M fraction. By contrast, FAS distribution in white adipocytes suggested a mainly microsomal localization. Assuming that FAS has only a mitochondrial and microsomal localization, the 'true' percentage distribution of FAS activity was calculated by using the citrate synthase and $NADP^+$–cytochrome *c* reductase distribution to correct for cross-contamination. On this basis, the 'true' percentage distribution for brown-adipocyte FAS was found to be mitochondrial 87 ± 1%, microsomal 13 ± 1%. For white adipocytes the distribution was mitochondrial 29 ± 7%, microsomal 71 ± 7% (means ± S.E.M. for three determinations). This clear difference between the two cell types reflects the dual purpose of FAS, namely to form acyl-CoA thioesters both for β-oxidation and for use in triacylglycerol synthesis. The former process is of considerable importance in the brown adipocyte, but is relatively minor in white adipocytes (Harper & Saggerson, 1976).

Enzyme activities: comparisons between brown and white adipocytes from normal animals

Details of animal and tissue weights in this series of experiments are shown in Table 1. Epididymal-fat-pad weight relative to body weight declined in diabetes, whereas brown-fat weight increased slightly in diabetes and quite substantially in hypothyroidism relative to body weight. Expression of enzyme activities relative to DNA content permitted direct comparisons between the two adipocyte types on a cellular basis. Cells were isolated from normal animals aged 6 or 9 weeks, and in both cases the protein contents relative to DNA were similar for brown and white adipocytes (Table 2). In general, at either age in normal animals activities of enzymes from the triacylglycerol-synthesis pathway were higher in white than in brown adipocytes. Brown/white adipocyte differences were also apparent for the activities of the marker enzymes. $NADP^+$–cytochrome *c* reductase activity was 3–3.5-fold higher in white adipocytes, whereas citrate synthase activity was 3–4-fold higher in brown adipocytes. A 2-fold higher activity of lactate dehydrogenase in white adipocytes was only evident at 9 weeks. Combining data for the calculated 'true' subcellular distribution of FAS and the absolute activities of this enzyme in the cells from the 6-week-old normal rats, it may be calculated that in brown cells FAS activity in mitochondria and microsomal fractions is 13.1 and 2.0 nmol/min per 100 μg of cell DNA respectively. By contrast, the corresponding calculated values for white adipocytes are 8.9 and 21.9. Thus FAS activity is higher in mitochondria of brown adipocytes, but substantially higher in the endoplasmic reticulum of white adipocytes.

Enzyme activities: effects of age in normal animals

Jamdar & Osborne (1982) reported that rat epididymal white-adipocyte total GPAT activity was increased by 65% between ages 30 and 60 days. On the other hand, Jamdar *et al.* (1984) showed that white-adipose-tissue Mg^{2+}-dependent PPH activity is not changed over this period. In accord with these findings, both GPAT activities in white adipocytes were significantly increased between 6 and 9 weeks of age, whereas there was no significant change in PPH activity in this cell type (Table 2). The age-dependent increase was more pronounced for the mitochondrial GPAT (2.6-fold) than for the microsomal form (1.4-fold). Age also increased MGPAT activity (1.6-fold), but had no significant effect on DGAT and decreased FAS activity by 25%. In brown adipocytes there were significant, but different, changes in enzyme activities with age. The mitochondrial GPAT showed the largest increase (2.7-fold), compared with 1.8-fold for microsomal GPAT and 1.6-fold for PPH and DGAT. MGPAT and FAS activities were unaffected by age in brown adipocytes.

Enzyme activities: effects of diabetes

The diabetic rats were age-matched against the 6-week-old normal animals. Diabetes had completely different effects in white adipocytes compared with brown adipocytes. Relative to whole epididymal-fat-pad protein, Taylor & Saggerson (1986) and Saggerson & Carpenter (1987) reported significant decreases in FAS, both forms of GPAT, MGPAT and Mg^{2+}-dependent PPH. Table 2 shows similar changes in the diabetic state

Table 2. **Enzyme activities in brown and white adipocytes: effects of age, diabetes and hypothyroidism**

Assays were performed at the substrate concentrations and temperatures indicated in the Materials and methods section. Values are means ± S.E.M. and are expressed as nmol/min per 100 μg of adipocyte DNA. [a]$P < 0.05$, [b]$P < 0.02$, [c]$P < 0.01$, [d]$P < 0.001$ for comparison of 6- and 9-week-old normal animals; [e]$P < 0.05$, [f]$P < 0.02$, [g]$P < 0.01$, [h]$P < 0.001$ for comparison of the diabetic or the hypothyroid states with the appropriate age-matched controls.

	6-week-old controls (n = 7)		6-week-old diabetics (n = 6)		9-week-old controls (n = 5)		9-week-old hypothyroids (n = 5)	
Adipocytes ...	Brown	White	Brown	White	Brown	White	Brown	White
Cell protein (mg/100 μg of DNA)	5.2 ± 0.4	5.3 ± 0.3	5.3 ± 0.3	4.0 ± 0.3[g]	8.3 ± 0.4[d]	9.1 ± 0.9[c]	13.9 ± 0.7[h]	9.8 ± 0.4
FAS	1500 ± 80	3080 ± 210	1580 ± 170	1910 ± 150[h]	1530 ± 60	2310 ± 260[a]	2800 ± 80[h]	3860 ± 230[g]
Mitochondrial GPAT (A)	2.5 ± 0.4	10 ± 1	2.2 ± 0.3	3.6 ± 0.3[h]	6.8 ± 0.4[d]	26 ± 2[d]	32 ± 3[h]	11 ± 1[h]
Microsomal GPAT (B)	14 ± 1	73 ± 4	17 ± 2	52 ± 4[g]	25 ± 1[d]	103 ± 9[b]	44 ± 4[g]	160 ± 6[h]
Ratio B/A	5.6	7.3	7.7	14.4	3.7	4.0	1.4	14.5
DHAPAT	–	–	–	–	1.9 ± 0.1	12 ± 1	4.7 ± 0.5[h]	14 ± 1
MGPAT	326 ± 27	695 ± 65	430 ± 58	461 ± 38[g]	361 ± 22	1120 ± 100[c]	777 ± 33[h]	891 ± 66
Mg^{2+}-dependent PPH	131 ± 8	343 ± 30	207 ± 26[f]	305 ± 30	210 ± 15[d]	457 ± 43	520 ± 24[h]	338 ± 20[e]
DGAT	3.9 ± 0.9	7.7 ± 1.2	3.3 ± 0.6	2.3 ± 0.4[g]	6.3 ± 0.5[a]	5.5 ± 1.1	8.2 ± 0.8	9.4 ± 0.4[g]
Lactate dehydrogenase	1500 ± 240	1160 ± 170	1570 ± 190	1030 ± 100	1980 ± 80	3610 ± 310[d]	4440 ± 290	1880 ± 90[h]
Citrate synthase	8390 ± 490	2700 ± 250	8390 ± 950	1500 ± 190[g]	14200 ± 600[d]	3770 ± 280[b]	16600 ± 700[e]	3420 ± 230
$NADP^+$–cytochrome c reductase	50 ± 5	164 ± 19	62 ± 8	132 ± 19	83 ± 7[c]	283 ± 23[c]	218 ± 14[h]	237 ± 15

expressed relative to white-adipocyte DNA, for FAS, GPAT (both forms) and MGPAT, but in this particular instance the decrease in PPH activity was not statistically significant and a significant decrease in DGAT activity was now also observed. By contrast, and most surprisingly, there was no significant effect of diabetes on any of the measured activities in brown adipocytes, except for Mg^{2+}-dependent PPH, which was increased by 58%. In this respect the brown adipocyte more closely resembles the liver cell in its response to diabetes, since diabetic states either increase or do not change FAS, total GPAT, MGPAT, Mg^{2+}-dependent PPH and DGAT activities in liver (Brandes *et al.*, 1973; Whiting *et al.*, 1977; Bates & Saggerson, 1977; Dang *et al.*, 1984; Murthy & Shipp, 1979; Woods *et al.*, 1982; Young & Lynen, 1969).

Enzyme activities: effects of hypothyroidism

The hypothyroid state was induced over a period of 4 weeks (Chohan *et al.*, 1984; Saggerson & Carpenter, 1986), and for this reason these animals were matched against 9-week-old controls. In brown adipocytes hypothyroidism significantly increased cell protein and all measured enzyme activities relative to cell DNA, except for DGAT and citrate synthase. For enzymes of the triacylglycerol-synthesis pathway, these increases were 1.8–2.5-fold, except for mitochondrial GPAT, which was increased 4.7-fold, such that this value approached that of the microsomal activity. It is also noteworthy that hypothyroidism increased brown-adipocyte $NADP^+$–cytochrome c reductase activity by 2.6-fold, possibly suggesting a general increase in the endoplasmic reticulum in this state. In white adipocytes the response to hypothyroidism was more complex. There was no significant change in cell protein content or in DHAPAT and MGPAT activities. Mitochondrial GPAT and Mg^{2+}-dependent PPH activities were significantly decreased, whereas FAS, microsomal GPAT and DGAT activities relative to adipocyte DNA were significantly increased by 1.6–1.7-fold. These findings may be compared with the previous observation that, relative to whole epididymal-fat-pad protein, hypothyroidism decreased PPH activity and also, by contrast, decreased total GPAT activity by 22% (Taylor & Saggerson, 1986).

Measurements of metabolic fluxes into triacylglycerol: comparison with changes in enzyme activities

In preliminary experiments (results not shown) it was found that, as with white adipocytes (Saggerson, 1972), addition of palmitate to incubations of brown adipocytes considerably increased incorporation of [^{14}C]glucose into the glycerol moiety of cell acylglycerols. It was also established that maximal rates of this incorporation were achieved with either cell type with 5 mM-[U-^{14}C]glucose, 1 mM-sodium palmitate (with 40 mg of albumin/ml) and with 4 nM-insulin added. Incorporations were linear from zero time for at least 1 h. Accordingly, ^{14}C incorporation into acylglycerol glycerol was taken as a reasonable measure of the maximum capability of the intact cells to synthesize triacylglycerol *de novo*. There are two possible criticisms of this approach. Firstly, triacylglycerol synthesis might be underestimated if glycerol mobilized via lipolysis were to be recycled back via glycerokinase. This is unlikely to be appreciable, since no lipolytic agents were present and glycerol accumulation was negligible, rates of glucose incorporation were

Table 3. Incorporation of glucose into acylglycerol glycerol and fatty acid moieties by incubated brown and white adipocytes

Incubation conditions are described in the Materials and methods section. The values are means ± S.E.M. ($n = 6$). [a]$P < 0.02$, [b]$P < 0.01$ for comparison of 6- and 9-week-old normal animals; [c]$P < 0.05$, [d]$P < 0.01$, [e]$P < 0.001$ for comparison of the diabetic or the hypothyroid states with the appropriate age-matched controls.

	[U-^{14}C]Glucose incorporation (μg-atoms/h per 100 μg of cell DNA)			
	Into acylglycerol glycerol		Into acylglycerol fatty acids	
Rats Adipocytes ...	Brown	White	Brown	White
6-week-old control	3.05 ± 0.32	7.16 ± 0.27	0.81 ± 0.23	4.18 ± 0.70
6-week-old diabetic	0.68 ± 0.10[e]	3.17 ± 0.42[e]	0.011 ± 0.004[d]	0.10 ± 0.03[e]
9-week-old control	3.73 ± 0.21	4.91 ± 0.70[a]	0.93 ± 0.19	1.15 ± 0.21[b]
9-week-old hypothyroid	6.82 ± 1.19[c]	12.0 ± 1.1[e]	10.2 ± 2.1[d]	1.74 ± 0.27

linear with time (see above), which would not be expected if accumulating glycerol began to dilute the glycerol phosphate precursor pools, and lastly, incorporation of radiolabelled glycerol into acylglycerol glycerol was low (T. McAllister & E. D. Saggerson, unpublished work). Secondly, the triose phosphate precursor pool might be diluted by other endogenous metabolites. Again, this is unlikely to be appreciable, since relative rates of incorporation of ^3H_2O and [^{14}C]glucose into brown-adipocyte fatty acids are similar under a variety of conditions (E. D. Saggerson, unpublished work). In addition, formation of acylglycerol glycerol from C_3 metabolites such as lactate is negligible in brown adipocytes (T. McAllister & E. D. Saggerson, unpublished work).

In accord with the lower activities of triacylglycerol-synthesizing enzymes in brown adipocytes from normal rats (Table 2), rates of acylglycerol glycerol formation by brown adipocytes were less than in white adipocytes, although this disparity decreased with age (Table 3).

For white adipocytes, the 31% decrease in metabolic flux with age correlated well with 25% and 29% decreases in FAS and DGAT activities, but did not correlate with the increases with age in GPAT, MGPAT and PPH activities. Diabetes caused a 56% decrease in metabolic flux in white adipocytes, and this generally correlated with the decreases in enzyme activities seen in this state (Table 2; Taylor & Saggerson, 1986; Saggerson & Carpenter, 1987). By contrast, in brown adipocytes, although diabetes did not decrease the activities of any of the triacylglycerol-synthesizing enzymes (Table 2), metabolic flux was decreased by 78%. The implication is that other factors, such as glucose transport, glycolytic flux or glycerol phosphate concentration, may be particularly important in constraining triacylglycerol synthesis in brown adipocytes from diabetic rats.

Hypothyroidism increased rates of acylglycerol synthesis by 1.8-fold and 2.4-fold in brown and white adipocytes respectively (Table 3). Although other factors, such as glucose transport and control of glycolytic flux, should also be considered, this increase is clearly quite well correlated with observed increases in enzyme activities in brown adipocytes (Table 2). It is less easy to make correlations for white adipocytes in hypothyroidism, where the increase in metabolic flux reasonably matches increases in FAS, microsomal GPAT and DGAT only. Thus, overall, in the white adipocytes it is only changes in FAS and DGAT activities which correlate with alterations in metabolic flux through the changes of age, diabetes and hypothyroidism.

Fatty acid oxidation and esterification in adipocytes

Maximum rates of noradrenaline-stimulated O_2 consumption in brown adipocytes from 6-week-old normal rats are 96 μmol/h per 100 μg of DNA (Jamal & Saggerson, 1988). If fatty acid were the only substrate supporting this respiration, this would represent the consumption of approx 4.2 μmol of 16–18-chain-length acyl units/h per 100 μg of DNA. Table 3 indicates that the maximum rate of esterification in these cells is 3.1 μmol of acyl units/h per 100 μg of DNA. Assuming a doubling of reaction rate for every 10 °C increase in temperature, it may be calculated that the maximum activity at 37 °C of overt carnitine palmitoyltransferase is 3.5 μmol/h per 100 μg of DNA (from Saggerson & Carpenter, 1982) and that total GPAT activity at 37 °C could initiate the conversion into triacylglycerol of 4.8 μmol of acyl units/h per 100 μg of DNA (from Table 2). It therefore appears that the maximum capabilities for fatty acid oxidation and esterification are approximately equal in the brown adipocyte. This allows the possibility that regulation of either pathway could influence the other. This almost certainly is not the case in white adipocytes, where maximum rates of fatty-acyl-unit oxidation are only approx 0.1 μmol/h per 100 μg of DNA (Harper & Saggerson, 1976), compared with esterification rates of 7.2 μmol/h per 100 μg of DNA (Table 3). In addition, maximum activities in white adipocytes of overt carnitine palmitoyltransferase and of total GPAT-initiated triacylglycerol formation at 37 °C may be calculated as approx. 0.2 (Saggerson & Carpenter, 1983) and 24 μmol/h per 100 μg of DNA (from Table 2) respectively. In white adipocytes, therefore, it is difficult to envisage regulation of esterification being initiated by changes in the rate of fatty acid oxidation.

Fatty acid synthesis

Although not a primary objective of this study, we also extracted the acylglycerol fatty acids in the course of obtaining the glyceroyl moiety, and measurements of fatty acid synthesis from [^{14}C]glucose are shown in Table 3. In general, the same trends were seen as for acylglycerol glycerol formation, except that in some instances they were exaggerated. Diabetes essentially abolished fatty

acid synthesis in both cell types. Most surprisingly, hypothyroidism increased fatty acid synthesis by 11-fold in brown adipocytes, but only increased this rate by 50% in the white cells (this was not statistically significant). Thus brown adipocytes from hypothyroid rats show fatty acid synthesis rates which are 6-fold higher than in white adipocytes. McCormack (1982) has questioned the competence of brown-adipose-tissue preparations to perform fatty acid synthesis *in vitro*. In our rat population maintained at approx. 21 °C we have observed approximately equal rates of fatty acid synthesis (expressed relative to tissue DNA) from injected ^3H_2O in epididymal white fat and interscapular brown fat after preinjection with a glucose load (E. D. Saggerson, unpublished work), and it is therefore noteworthy that brown and white adipocytes from 9-week-old normal animals exhibited similar rates of fatty acid synthesis. By contrast, rates of fatty acid synthesis in brown adipocytes from the younger rats were low compared with the rates in white adipocytes. It is possible that the cell isolation procedure recovers from the younger rats only a subpopulation of brown adipocytes that is not particularly proficient in fatty acid synthesis. It should, however, be stressed that the incubation conditions do not optimize fatty acid synthesis, particularly in brown adipocytes. In preliminary experiments, it was found that the necessary addition of palmitate to optimize acylglycerol synthesis considerably decreased fatty acid synthesis in brown adipocytes (H. Baht & E. D. Saggerson, unpublished work), but had no effect on, or even slightly increased, fatty acid synthesis in white cells, as previously observed by Saggerson (1972).

Conclusions

This study provides for the first time some characterization of the triacylglycerol-synthesis pathway in brown adipose tissue. Some differences from white adipose tissue are observed in absolute activities of enzymes and in their adaptation to diabetes and hypothyroidism. Some correlations can be made between changes in enzyme activity and in metabolic flux in both adipocyte types. The striking effects of diabetes on acylglycerol synthesis and of hypothyroidism on fatty acid synthesis observed in brown adipocytes merit further investigation.

We thank the Medical Research Council for financial support.

REFERENCES

Agius, L. & Williamson, D. H. (1980) Biochem. J. **190**, 477–480
Angel, A. (1969) Science **163**, 288–290
Bates, E. J. & Saggerson, E. D. (1977) FEBS Lett. **84**, 229–232
Bates, E. J. & Saggerson, E. D. (1979) Biochem. J. **182**, 751–762
Bertin, R., Gaubern, M. & Portet, R. (1978) Experientia Suppl. **32**, 185–189
Brandes, R., Zohar, Y., Arad, R. & Shapiro, B. (1973) Eur. J. Biochem. **34**, 329–332
Cannon, B. & Nedergaard, J. (1985*a*) Essays Biochem. **20**, 110–164
Cannon, B. & Nedergaard, J. (1985*b*) in Circulation, Respiration and Metabolism (Gilles, R., ed.), pp. 502–518, Springer-Verlag, Berlin and Heidelberg
Chohan, P., Carpenter, C. & Saggerson, E. D. (1984) Biochem. J. **223**, 53–59
Coleman, R. & Bell, R. M. (1976) J. Biol. Chem. **251**, 4537–4543
Cooney, G. J., Caterson, I. D. & Newsholme, E. A. (1985) FEBS Lett. **188**, 257–261
Daae, L. N. W. (1972) Biochim. Biophys. Acta **270**, 23–31
Dang, A. Q., Faas, F. H. & Carter, W. J. (1984) Lipids **19**, 738–748
Declercq, P. E., Haagsman, H. P., Van Veldhoven, P., Debeer, L. J., Van Golde, L. M. G. & Mannaerts, G. P. (1984) J. Biol. Chem. **259**, 9064–9075
Fain, J. N., Reed, N. & Saperstein, R. (1967) J. Biol. Chem. **242**, 1887–1894
Ferré, P., Burnol, A.-F., Leturque, A., Terretaz, J., Pericaud, L., Jeanrenaud, B. & Girard, J. (1986) Biochem. J. **233**, 249–252
Guerrier, D. & Pellet, H. (1979) FEBS Lett. **106**, 115–120
Haldar, D. & Pullman, M. E. (1975) Fed. Proc. Fed. Am. Soc. Exp. Biol. **34**, 632
Hall, M. & Saggerson, E. D. (1985) Biochem. J. **226**, 275–282
Harper, R. D. & Saggerson, E. D. (1976) J. Lipid Res. **17**, 516–526
Himms-Hagen, J. (1965) Can. J. Physiol. Pharmacol. **43**, 379–403
Jamal, Z. & Saggerson, E. D. (1987) Biochem. J. **245**, 881–886
Jamal, Z. & Saggerson, E. D. (1988) Biochem. J. **249**, 415–421
Jamdar, S. C. & Osborne, L. J. (1982) Biochim. Biophys. Acta **713**, 647–656
Jamdar, S. C., Osborne, L. J. & Wells, G. N. (1984) Arch. Biochem. Biophys. **233**, 370–377
Joel, C. D. (1965) Handb. Physiol. Sect. 5; Adipose Tissue, pp. 59–85
Knight, B. L. & Myant, N. B. (1971) Biochem. J. **125**, 1–8
Krebs, H. A. & Henseleit, H. (1932) Hoppe-Seyler's Z. Physiol. Chem. **210**, 33–66
Lands, W. E. M. & Hart, P. (1965) J. Biol. Chem. **240**, 1905–1911
Lawson, N., Pollard, A. D., Jennings, R. J., Gurr, M. I. & Brindley, D. N. (1981) Biochem. J. **200**, 285–294
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
McCormack, J. C. (1982) Prog. Lipid Res. **21**, 195–223
McCormack, J. C. & Denton, R. M. (1977) Biochem. J. **166**, 627–630
Monroy, G., Rola, F. H. & Pullman, M. E. (1972) J. Biol. Chem. **247**, 6884–6894
Monroy, G., Keller, H. C. & Pullman, M. E. (1973) J. Biol. Chem. **248**, 2845–2852
Mory, G., Ricquier, D., Pesquies, P. & Hemon, P. (1981) J. Endocrinol. **91**, 515–524
Murthy, V. K. & Shipp, J. C. (1979) Diabetes **28**, 472–478
Nedergaard, J. & Lindberg, O. (1982) Int. Rev. Cytol. **74**, 187–286
Nicholls, D. G. & Locke, R. M. (1983) in Mammalian Therogenesis (Girardier, L. & Stock, M. J., eds.), pp. 8–49, Chapman and Hall, London
Nicholls, D. G. & Locke, R. M. (1984) Physiol. Rev. **64**, 1–64
Nimmo, H. G. (1979) Biochem. J. **177**, 283–288
Norman, P. T. & Flatmark, T. (1980) Biochim. Biophys. Acta **619**, 1–10
Okuyama, H., Eibi, H. & Lands, W. E. M. (1971) Biochim. Biophys. Acta **248**, 263–273
Paetzke-Brunner, I., Loffler, G. & Wieland, O. H. (1979) Horm. Metab. Res. **11**, 285–288
Pederson, J. I., Slinde, E., Grynne, B. & Aas, M. (1975) Biochim. Biophys. Acta **398**, 191–203
Phillips, A. H. & Langdon, R. G. (1962) J. Biol. Chem. **237**, 2652–2660
Prusiner, S. B., Cannon, B., Ching, T. M. & Lindberg, O. (1968) Eur. J. Biochem. **7**, 51–57

Rider, M. H. & Saggerson, E. D. (1983) Biochem. J. **214**, 235–246
Rodbell, M. (1964) J. Biol. Chem. **239**, 275–280
Saggerson, E. D. (1972) Biochem. J. **128**, 1057–1067
Saggerson, E. D. (1974) Biochem. J. **140**, 211–224
Saggerson, E. D. (1988) in CRC Monograph in Enzyme Biology: Phosphatidate Phosphohydrolase (Brindley, D. N., ed.), CRC Press, Boca Raton, FL, in the press
Saggerson, E. D. & Carpenter, C. A. (1982) Biochem. J. **204**, 373–375
Saggerson, E. D. & Carpenter, C. A. (1983) Biochem. J. **210**, 591–597
Saggerson, E. D. & Carpenter, C. A. (1986) Biochem. J. **236**, 137–141
Saggerson, E. D. & Carpenter, C. A. (1987) Biochem. J. **243**, 289–292
Saggerson, E. D. & Greenbaum, A. L. (1970) Biochem. J. **119**, 193–219
Saggerson, E. D. & Topping, D. L. (1981) FEBS Lett. **132**, 124–126
Saggerson, E. D., Sooranna, S. R. & Cheng, C. H. K. (1979) INSERM Colloq. **87**, 223–238
Saggerson, E. D., Carpenter, C. A., Cheng, C. H. K. & Sooranna, S. R. (1980) Biochem. J. **190**, 183–189
Schenk, H., Heim, T., Mende, T., Varga, F. & Goetze, E. (1975) Eur. J. Biochem. **58**, 15–22
Schultz, F. M. & Johnston, J. M. (1971) J. Lipid Res. **12**, 132–138
Seydoux, J., Giacobino, J. P. & Girardier, L. (1982) Mol. Cell Endocrinol. **25**, 213–226
Seydoux, J., Chinet, A., Schneider-Picard, G., Bas, S., Imesch, E., Assimacopoulos-Jeannet, F., Giacobino, J. P. & Girardier, L. (1983) Endocrinology (Baltimore) **113**, 604–620
Seydoux, J., Trimble, E. R., Bouillaud, F., Assimacopoulos-Jeannet, F., Bas, S., Ricquier, D., Giacobino, J. P. & Girardier, L. (1984) FEBS Lett. **166**, 141–145
Shepherd, D. & Garland, P. B. (1969) Biochem. J. **114**, 597–610
Sturton, R. G. & Brindley, D. N. (1978) Biochem. J. **171**, 263–266
Sturton, R. G. & Brindley, D. N. (1980) Biochim. Biophys. Acta **619**, 494–505
Sugden, M. C., Watts, D. I., Marshall, C. E. & McCormack, J. G. (1982) Biosci. Rep. **2**, 289–297
Sundin, U., Mills, I. & Fain, J. N. (1984) Metab. Clin. Exp. **33**, 1028–1033
Switzer, B. R. & Summer, G. K. (1971) Clin. Chim. Acta **32**, 203–206
Taylor, S. J. & Saggerson, E. D. (1986) Biochem. J. **239**, 275–284
Trayhurn, P. (1981) Biochem. Biophys. Acta **664**, 549–560
Whiting, P. H., Bowley, M., Sturton, R. G., Pitchard, P. H., Brindley, D. N. & Hawthorne, J. N. (1977) Biochem. J. **168**, 147–153
Woods, J. A., Knauer, T. E. & Lamb, R. G. (1982) Biochim. Biophys. Acta **666**, 482–492
Woodward, J. A. & Saggerson, E. D. (1986) Biochem. J. **238**, 395–403
Yamada, K. & Okuyama, H. (1978) Arch. Biochem. Biophys. **190**, 409–420
Young, D. L. & Lynen, F. (1969) J. Biol. Chem. **244**, 377–383

Received 11 June 1987/1 October 1987; accepted 9 October 1987