The Regulation of Glyceride Synthesis in Isolated White-Fat Cells

THE EFFECTS OF PALMITATE AND LIPOLYTIC AGENTS

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1. 0.5 mM-Palmitate stimulated incorporation of [U-¹⁴C]glucose into glyceride glycerol and fatty acids in normal fat cells in a manner dependent upon the glucose concentration. 2. In the presence of insulin the incorporation of 5 mM-glucose into glyceride fatty acids was increased by concentrations of palmitate, adrenaline and 6-*N*-2'-*O*-dibutyryl-adenosine 3':5'-cyclic monophosphate up to 0.5 mM, 0.5 μ M and 0.5 mM respectively. Higher concentrations of these agents produced progressive decreases in the rate of glucose incorporation into fatty acids. 3. The effects of palmitate and lipolytic agents upon the measured parameters of glucose utilization were similar, suggesting that the effects of lipolytic agents are mediated through increased concentrations of free fatty acids. 4. In fat cells from 24 h-starved rats, maximal stimulation of glucose incorporation into fatty acids was achieved with 0.25 mM-palmitate. Higher concentrations of palmitate were inhibitory. In fat cells from 72 h-starved rats, palmitate only stimulated glucose incorporation into fatty acids at high concentrations of palmitate (1 mM and above). 5. The ability of fat cells to incorporate glucose into glyceride glycerol in the presence of palmitate decreased with increasing periods of starvation. 6. It is suggested that low concentrations of free fatty acids stimulate fatty acid synthesis from glucose by increasing the utilization of ATP and cytoplasmic NADH for esterification of these free fatty acids. When esterification of free fatty acids does not keep pace with their provision, inhibition of fatty acid synthesis occurs. Provision of free fatty acids far in excess of the esterification capacity of the cells leads to uncoupling of oxidative phosphorylation and a secondary stimulation of fatty acid synthesis from glucose.

There is evidence that the conversion of glucose into glyceride fatty acids in white adipose tissue *in vitro* is limited by the ability to supply acetyl-CoA for fatty acid synthesis and not by the ability to convert acetyl-CoA into fatty acids. Disproportionate changes in fatty acid synthesis from glucose compared with changes in lactate+pyruvate production by the tissue, achieved by alteration of dietary status or by addition of hormones to the incubation medium, suggested that control of the oxidation of pyruvate is important in the regulation of carbohydrate conversion into fatty acids (Denton & Halperin, 1968; Halperin & Robinson, 1970; Saggerson & Greenbaum, 1970*a,b*). Adipose tissue pyruvate dehydrogenase has now been shown to be activated and inhibited in tissues treated with insulin and adrenaline respectively. The manner of regulation appears to be through a phosphorylation-dephosphorylation cycle (Jungas, 1970; Coore *et al.*, 1971). It is also significant that a considerable proportion of pyruvate derived from glucose in incubated adipose tissues is reduced to lactate, which is released from the tissue. In incubated adipose tissues there appears to be a balance between the production of cytoplasmic reducing equivalents and their utilization in that

compartment (Katz *et al.*, 1966; Rognstad & Katz, 1966, 1969; Saggerson & Greenbaum, 1970*a*). It has been proposed that under normal conditions there is no transfer of reducing equivalents from cytoplasm to mitochondria in the tissue (Rognstad & Katz, 1969; Schmidt & Katz, 1969; Robinson & Halperin, 1970). Limitation of the reoxidation of cytoplasmic NADH may therefore impose a restraint upon the rate of conversion of glucose into pyruvate. This is supported by the finding that increased utilization of cytoplasmic NADH in the presence of artificial electron acceptors increases fatty acid synthesis from glucose (Halperin & Robinson, 1970; Saggerson, 1972). In addition Flatt (1970) has proposed that the accepted pathway of glucose conversion into fatty acids is limited by the ability of the tissue to utilize ATP produced in excess by this process. Theoretically fatty acid synthesis from glucose should be facilitated by an increase in ATP utilization or by a decrease in the efficiency of ADP phosphorylation by the tissue. In fact, glucose conversion into fatty acids is increased in the presence of acetate, presumably through increased utilization of ATP for acetate activation (Flatt & Ball, 1966; Del Boca & Flatt, 1969; Saggerson & Greenbaum, 1970*a,b*). Fatty acid synthesis

from glucose is also increased in the presence of uncoupling agents and oligomycin (Saggerson, 1972). The enzymic details of control by limitation of ATP utilization have not been specified, though Flatt (1970) has suggested that ATP production through oxidation of endogenous fatty acids would restrain glucose conversion into fatty acids. Linn *et al.* (1969) have proposed that mitochondrial oxidation of fatty acids should generate ATP, giving rise to inhibition of pyruvate dehydrogenase. Pyruvate conversion into glyceride fatty acids in fat cells is decreased in the presence of palmitate (Saggerson & Tomassi, 1971); however, glucose conversion into fatty acids has been found to be increased in the presence of exogenous free fatty acids under certain conditions (Felber *et al.*, 1966; Cushman, 1970). Felber *et al.* (1966) suggested that this effect was due to increased utilization of ATP and cytoplasmic NADH for fatty acid activation and esterification by glycerol 1-phosphate. Similarly, low concentrations of lipolytic hormones stimulate glucose conversion into fatty acids (Flatt & Ball, 1964; Bray, 1967) though higher concentrations inhibit fatty acid synthesis (Denton & Randle, 1967; Saggerson & Greenbaum, 1970a). Free fatty acids, lipolytic hormones and 6-*N*-2'-*O*-dibutyryladenine 3':5'-cyclic monophosphate have been found to increase glucose uptake, oxidation and incorporation into total lipids in fat-pads and in fat cells *in vitro* (Leboeuf *et al.*, 1959; Hagen & Ball, 1960; Lynn *et al.*, 1960; Cahill *et al.*, 1960; Leboeuf & Cahill, 1961; Blecher, 1967) and it has been suggested that many of the effects of lipolytic agents upon adipose tissue metabolism are secondary to increased production of free fatty acids (Leboeuf & Cahill, 1961; Ho & Jeanrenaud, 1967; Vassalli & Jeanrenaud, 1970; Bihler & Jeanrenaud, 1970; Angel *et al.*, 1971b). Alternatively it has been suggested that lipolytic agents have a cyclic nucleotide-mediated effect upon glucose utilization that is independent of lipolysis (Blecher, 1967; Bray, 1967; Bray & Goodman, 1968; Blecher *et al.*, 1969; Schimmel & Goodman, 1971).

The present study uses the isolated cell preparation of Rodbell (1964), which permits the examination of a large number of experimental conditions with a single batch of cells, to examine the role that various concentrations of external free fatty acids may play in the control of lipogenesis in fat cells and to compare these effects with the effects upon glucose utilization of a wide concentration range of lipolytic agents.

Materials and Methods

Chemicals

Nucleotides, collagenase (from *Clostridium histolyticum*) and other enzymes, phosphoenolpyruvate (potassium salt) and triethanolamine hydrochloride

were obtained from Boehringer Corp. (London) Ltd. (London W.5, U.K.). Insulin (6×recrystallized) was from Boots Pure Drug Co. Ltd. (Nottingham, U.K.), anti-insulin serum from the Wellcome Research laboratories (Beckenham, Kent, U.K.) and adrenaline from Sigma (London) Chemical Co. Ltd. (London S.W.6, U.K.). Adrenaline and insulin were freshly dissolved at a concentration of 1 mg/ml in 10 mM-HCl and 3 mM-HCl respectively and diluted appropriately in incubation media. Bovine serum albumin (fraction V) from the Armour Pharmaceutical Co. Ltd. (Eastbourne, Sussex, U.K.) was treated with charcoal to remove fatty acids (Chen, 1967), dialysed against distilled water and freeze-dried. Palmitic acid (Sigma) was converted into the sodium salt and associated with defatted bovine serum albumin (Evans & Mueller, 1963). Sodium pyruvate was obtained from the Merck Corporation (Darmstadt, W. Germany), all radiochemicals from The Radiochemical Centre (Amersham, Bucks., U.K.) and 2,5-bis-(5-*t*-butylbenzoxazol-2-yl)thiophen from CIBA (A.R.L.) Ltd. (Duxford, Cambs., U.K.). All other chemicals were of A.R. grade and were used without further purification.

Animals

Male Wistar rats of 130–140 g body wt. were purchased from A. Tuck and Son Ltd. (Rayleigh, Essex, U.K.). The animals were maintained on cube diet 41 B (Bruce & Parkes, 1949) with water supplied *ad libitum* until initiation of the experiments. Animals designated as normally fed weighed 150–180 g at death. Periods of starvation of 24 or 72 h before death were started with animals of this weight range.

Techniques with fat cells

Preparation. The technique of Rodbell (1964) for preparation of fat cells was used as previously described (Saggerson & Tomassi, 1971).

Incubation of fat cells and measurement of metabolic products. Portions of fat cells equivalent to one-third of a fat-pad were incubated in 4 ml volumes with the required additions (Saggerson & Tomassi, 1971). The final concentrations of albumin present in the incubations varied in different experiments and are recorded in the individual result tables.

The measurement of incorporation of ^{14}C into glyceride fatty acids and glyceride glycerol was as described by Saggerson & Greenbaum (1970a) and Saggerson & Tomassi (1971). In experiments in which the incorporation of tritium from $^3\text{H}_2\text{O}$ into fatty acids was measured, tritium was present at a specific radioactivity of $0.02 \mu\text{Ci}/\mu\text{mol}$ in incubation media. The measurement of incorporation of tritium was identical with that for ^{14}C except that hexane solutions of tritiated extracted lipids were washed twice with 1 mM- H_2SO_4 .

Free fatty acids in incubated cell suspensions were determined as described previously (Saggerson & Tomassi, 1971), by using the method of Itaya & Ui (1965).

Incubated cell suspensions were deproteinized, neutralized and assayed for lactate, pyruvate and glycerol (Saggerson & Greenbaum, 1970a; Saggerson & Tomassi, 1971). Normally glycerol was assayed by the method of Garland & Randle (1962). However, when pyruvate was included in incubation media as a substrate the method of Wieland (1957) was used.

Determination of fat cell DNA. Portions (1 ml) of non-incubated fat cells (equivalent to one fat pad) were extracted as described by Denton *et al.* (1966) and DNA was determined with calf thymus DNA used as a standard (Burton, 1956). A mean value of 123 μg of DNA/g dry wt. of cells was obtained for cells prepared from normally fed animals.

Expression of results

Rates of incorporation of substrates are expressed throughout as μg -atoms of substrate carbon/h per 100 μg of fat cell DNA and are the means $\pm$ S.E.M. of the number of determinations (usually 3–5). In a single determination one measurement was made of each parameter presented in a particular table. A different fat cell preparation was used for each determination.

Results

Figs. 1 and 2 show the dependence of fatty acid synthesis and glyceride glycerol formation from [^{14}C]glucose upon the initial glucose concentration in the medium. In the absence of palmitate, 5 mM-glucose gave maximal rates of lipogenesis in the presence of insulin. In the presence of 0.5 mM-palmitate considerable increases in the rate of fatty acid synthesis, compared with those in the absence of palmitate, were observed at higher glucose concentrations in the presence and absence of insulin. Even greater percentage increases in glyceride glycerol synthesis were observed in the presence of palmitate. The results of Table 1 demonstrate that the concentration of insulin used in the experiment shown in Figs. 1 and 2 was saturating as far as fatty acid synthesis from [^{14}C]glucose is concerned. It can be inferred therefore that the rate of [^{14}C]glucose incorporation into fatty acids reaches a limiting value with 5 mM-glucose + 20 munits of insulin/ml unless other agents such as palmitate are added to the incubation medium.

In Table 2 it may be seen that the production of lactate and pyruvate, though influenced by the presence of insulin and dependent upon the glucose concentration, is less affected by variation in these parameters than is glucose incorporation into fatty

Fig. 1. Effect of glucose concentration upon [^{14}C]glucose incorporation into glyceride fatty acids by normal fat cells in the presence and absence of insulin and sodium palmitate

Fat cells from normal rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 1.0% (w/v) albumin together with the required concentration of [^{14}C]glucose and anti-insulin serum (10 munits/ml), insulin (20 munits/ml) and sodium palmitate (0.5 mM) where indicated. The results are the means of four determinations. ●, Anti-insulin serum; ▲, anti-insulin serum + palmitate; ○, insulin; Δ, insulin + palmitate.

Fig. 2. Effect of glucose concentration upon [^{14}C]glucose incorporation into glyceride glycerol by normal fat cells in the presence and absence of insulin and sodium palmitate

The conditions of incubation were as described in the legend to Fig. 1. The results were obtained in the same experiment as that summarized in Fig. 1. ●, Anti-insulin serum; ▲, anti-insulin serum + palmitate; ○, insulin; Δ, insulin + palmitate.

acids. These findings provide additional supporting evidence for the involvement of insulin in the regulation of pyruvate oxidation in the fat cell as discussed by Coore *et al.* (1971). It may also be seen in Table 2 that lactate/pyruvate ratios in the fat cell suspensions after incubation for 1 h were consistently lower in the presence than in the absence of insulin.

Table 3 shows the effects of different concentrations of palmitate upon lipogenesis from 5 mM-glucose + 20 munits of insulin/ml (maximal conditions) and from 1 mM-glucose (an arbitrarily chosen concentration of glucose, which does not give maximal rates of lipogenesis). Fatty acid synthesis from 5 mM-glucose was stimulated by palmitate, but fatty acid synthesis from 1 mM-glucose was not significantly

affected. Maximal stimulation was obtained with 0.5 mM-palmitate (the concentration used in the experiment shown in Figs. 1 and 2). Glyceride glycerol formation from glucose at either concentration was progressively increased with increasing palmitate concentration. The increases in glyceride glycerol formation were accompanied by decreases in the lactate/pyruvate ratios in the cell suspensions. This may reflect increased utilization of cytoplasmic NADH for glycerol 1-phosphate synthesis.

Tables 4 and 5 demonstrate that similar effects may be produced if free fatty acids are generated in the cell suspensions through the actions of lipolytic agents. Fatty acid synthesis from 5 mM-glucose + insulin was stimulated in the presence of adrenaline or 6-*N*-2'-*O*-dibutyryl adenosine 3':5'-cyclic monophosphate, the effects being optimum at approx. 0.5 μ M-adrenaline and 0.5 mM-6-*N*-2'-*O*-dibutyryl adenosine 3':5'-cyclic monophosphate respectively. Significant stimulations of fatty acid synthesis from 1 mM-glucose were not observed. The effects of adrenaline and 6-*N*-2'-*O*-dibutyryl adenosine 3':5'-cyclic monophosphate upon glyceride glycerol formation and upon the lactate/pyruvate ratio were similar to those observed with palmitate.

Stimulation of fatty acid synthesis in the presence of palmitate is not apparent when pyruvate or lactate is used as substrate (Saggerson & Tomassi, 1971). Palmitate in fact inhibits fatty acid synthesis from these substrates. Similarly it may be seen in Table 6 that fatty acid synthesis from pyruvate is decreased in the presence of adrenaline in both the presence and absence of insulin. The antilipolytic action of insulin may be seen in the higher concentrations of adrenaline necessary both to increase glycerol production and to decrease fatty acid synthesis in the presence of insulin.

In Table 7 it may be seen that total fatty acid synthesis, as measured by tritium incorporation into

Table 1. *Effect of insulin upon incorporation of [U-¹⁴C]-glucose into glyceride fatty acids*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin, 5 mM-[U-¹⁴C]glucose and the required concentration of anti-insulin serum or insulin. The results are means $\pm$ S.E.M. of three determinations.

Anti-insulin serum (munits/ml)	Insulin (munits/ml)	Incorporation of [U- ¹⁴ C]glucose into glyceride fatty acids (μ g-atoms/h per 100 μ g of DNA)
0	0	5.20 $\pm$ 0.16
10	0	5.99 $\pm$ 0.13
0	0.50	17.71 $\pm$ 0.46
0	1.25	18.30 $\pm$ 0.09
0	5.00	18.95 $\pm$ 0.17
0	50	18.93 $\pm$ 0.04

Table 2. *Dependence of glucose utilization upon glucose concentration in the presence and absence of insulin*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 1.0% (w/v) albumin, the required concentration of [U-¹⁴C]glucose and either 10 munits of anti-insulin serum/ml or 20 munits of insulin/ml. The results are means $\pm$ S.E.M. of four determinations.

Concn. of glucose (mM)	Incorporation of [U- ¹⁴ C]glucose into glyceride fatty acids (μ g-atoms/h per 100 μ g of DNA)		Production of lactate + pyruvate (μ g-atoms/h per 100 μ g of DNA)		Lactate/pyruvate ratio	
	With anti-insulin serum	With insulin	With anti-insulin serum	With insulin	With anti-insulin serum	With insulin
0.3	0.78 $\pm$ 0.03	3.75 $\pm$ 0.81	5.71 $\pm$ 0.46	9.13 $\pm$ 0.65	16.41	8.98
0.6	1.50 $\pm$ 0.20	6.26 $\pm$ 1.65	6.91 $\pm$ 0.82	11.96 $\pm$ 0.94	18.51	8.13
1.0	1.98 $\pm$ 0.25	8.44 $\pm$ 2.28	7.14 $\pm$ 0.91	13.67 $\pm$ 1.18	13.41	7.48
2.0	2.85 $\pm$ 0.37	9.77 $\pm$ 3.00	8.19 $\pm$ 0.93	14.66 $\pm$ 1.48	11.04	7.75
3.0	3.09 $\pm$ 0.39	10.49 $\pm$ 2.86	8.93 $\pm$ 0.98	15.11 $\pm$ 1.60	11.95	8.32
5.0	3.52 $\pm$ 0.44	10.63 $\pm$ 2.90	9.15 $\pm$ 0.61	14.81 $\pm$ 1.47	10.15	8.01

Table 3. *Effect of sodium palmitate upon glucose utilization by normal fat cells*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 5.0% (w/v) albumin together with the required concentrations of sodium palmitate, [U-¹⁴C]glucose, insulin and anti-insulin serum. The results are means $\pm$ S.E.M. of five determinations and are expressed as μ g-atoms/h per 100 μ g of DNA.

Concn. of sodium palmitate (mM)	1 mM-Glucose + anti-insulin serum (10 munits/ml)				5 mM-Glucose + insulin (20 munits/ml)			
	Incorporation of [U- ¹⁴ C]glucose into		Production of lactate + pyruvate	Lactate/pyruvate ratio	Incorporation of [U- ¹⁴ C]glucose into		Production of lactate + pyruvate	Lactate/pyruvate ratio
	Glyceride fatty acids	Glyceride glycerol			Glyceride fatty acids	Glyceride glycerol		
0	4.28 $\pm$ 1.40	1.54 $\pm$ 0.26	4.74 $\pm$ 0.61	6.88	22.03 $\pm$ 2.15	3.82 $\pm$ 0.58	15.99 $\pm$ 2.47	5.12
0.5	3.51 $\pm$ 1.46	8.20 $\pm$ 1.24	4.68 $\pm$ 0.68	2.67	32.61 $\pm$ 3.54	11.47 $\pm$ 0.99	19.18 $\pm$ 3.44	3.41
1.0	3.54 $\pm$ 1.53	9.46 $\pm$ 1.44	4.77 $\pm$ 0.64	2.70	30.20 $\pm$ 3.28	18.22 $\pm$ 1.77	22.18 $\pm$ 4.30	2.75
2.0	3.74 $\pm$ 1.56	11.11 $\pm$ 1.54	5.08 $\pm$ 0.61	2.37	23.98 $\pm$ 3.39	26.12 $\pm$ 2.58	26.04 $\pm$ 5.39	2.40
4.0	4.48 $\pm$ 1.87	13.28 $\pm$ 1.95	5.53 $\pm$ 0.92	2.27	20.99 $\pm$ 2.64	29.54 $\pm$ 2.98	27.73 $\pm$ 5.57	2.38

fatty acids from ³H₂O (Jungas, 1968), was affected by palmitate in a manner similar to the incorporation of [¹⁴C]glucose. Similarly when pyruvate was the only external substrate supplied to fat cells palmitate decreased equally [¹⁴C]pyruvate incorporation into fatty acids and total fatty acid synthesis.

Fig. 3 demonstrates the effects of starvation upon the response of glyceride synthesis from 5mM-glucose + insulin to increasing palmitate concentrations. Palmitate gave a smaller percentage stimulation of fatty acid synthesis in fat cells from 24h-starved animals and the optimum concentration of palmitate was lower (0.25mM or below) than in cells from normally fed animals. In fat cells from 72h-starved animals the profile of fatty acid synthesis versus palmitate concentration was entirely different. Concentrations of palmitate up to 0.75mM had no effect on fatty acid synthesis. Higher concentrations of palmitate increased [¹⁴C]glucose incorporation into fatty acids in a manner that was virtually linear with palmitate concentration. This effect is also demonstrated in Table 8, which shows an experiment in which the stimulation of [¹⁴C]glucose incorporation into fatty acids was linearly related to the palmitate concentration between 1 and 4mM-palmitate. As is discussed below, this effect of palmitate in 72h-starved fat cells could reflect an uncoupling effect of free fatty acids upon oxidative phosphorylation. In Fig. 3 it may also be seen that glyceride glycerol formation increased with increasing palmitate concentration in all three dietary conditions though the esterifying ability of the cells was decreased with increasing time of starvation.

Table 9 demonstrates that, though starvation considerably decreases the ability of fat cells to incorporate [¹⁴C]glucose into fatty acids (Fig. 3), corresponding decreases in lactate + pyruvate formation are not observed. Similar results are obtained with fat-pad segments (Saggerson & Greenbaum, 1970b). It

may also be seen that the lactate/pyruvate ratio is decreased as glyceride glycerol formation is increased with increasing palmitate concentration in all three dietary states.

In the experiment shown in Table 10, measurements were made of the amounts of free fatty acids and glycerol in incubated cell suspensions at the beginning and end of 1 h of incubation. Glycerol contents were found to be negligible at the start of the incubations. Fat-cell suspensions from 24h-starved rats differed from normal cells in having higher rates of lipolysis (as indicated by glycerol production), in having higher free fatty acid concentrations at the start of the incubation, and in utilizing (presumably esterifying) more endogenous fatty acid during the course of the incubation. Fat cells from 72h-starved rats had higher rates of lipolysis than normal cells and showed a net output of fatty acids during the course of the incubation. These results are relevant in explaining the different effects of palmitate on lipogenesis in these three types of fat cells as discussed below.

Discussion

The results presented in this study indicate that free fatty acids, adrenaline and 6-*N*-2'-*O*-dibutyryl-adenosine 3':5'-cyclic monophosphate have very similar effects upon the parameters of glucose utilization measured. These results therefore support suggestions that effects of lipolytic agents upon glucose metabolism are mediated through the resulting increased concentrations of free fatty acids. In general the data are consistent with the proposal that increased free fatty acid production by lipolysis (as measured by glycerol production) gives rise to increased esterification of fatty acids (as measured by glyceride glycerol formation) with resultant utilization of ATP and cytoplasmic NADH, thereby relieving restraints upon fatty acid synthesis. In the

Table 4. *Effect of adrenaline upon glucose utilization and glycerol release by normal fat cells*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 5.0% (w/v) albumin together with the required concentrations of adrenaline, [U-¹⁴C]glucose, insulin and anti-insulin serum. The results are means $\pm$ S.E.M. of four determinations and are expressed as μ g-atoms/h per 100 μ g of DNA, with the exception of glycerol release, which is expressed as μ mol/h per 100 μ g of DNA.

Concn. of adrenaline (μ M)	1 mM-Glucose + anti-insulin serum (10 munits/ml)				5 mM-Glucose + insulin (20 munits/ml)			
	Incorporation of [U- ¹⁴ C]- glucose into		Production of lactate + pyruvate	Lactate/ pyruvate ratio	Incorporation of [U- ¹⁴ C]- glucose into		Production of lactate + pyruvate	Lactate/ pyruvate ratio
	Glyceride fatty acids	Glycerol			Glyceride fatty acids	Glycerol		
0	2.90 $\pm$ 0.36	1.09 $\pm$ 0.05	5.18 $\pm$ 0.79	15.20	23.38 $\pm$ 2.16	4.20 $\pm$ 0.41	17.45 $\pm$ 1.64	10.28
0.05	3.14 $\pm$ 0.39	2.08 $\pm$ 0.27	4.53 $\pm$ 0.97	8.59	25.52 $\pm$ 2.42	3.77 $\pm$ 0.32	17.98 $\pm$ 1.74	9.23
0.10	2.89 $\pm$ 0.43	3.23 $\pm$ 0.42	4.22 $\pm$ 1.03	5.56	26.89 $\pm$ 2.85	3.95 $\pm$ 0.43	17.86 $\pm$ 1.94	9.21
0.25	2.45 $\pm$ 0.31	4.63 $\pm$ 0.25	4.26 $\pm$ 1.02	4.80	31.42 $\pm$ 3.60	5.91 $\pm$ 0.81	18.76 $\pm$ 1.96	6.86
0.50	2.42 $\pm$ 0.26	5.20 $\pm$ 0.38	3.97 $\pm$ 0.89	6.27	36.70 $\pm$ 4.55	9.83 $\pm$ 1.40	19.22 $\pm$ 2.27	4.75
0.75	2.32 $\pm$ 0.21	5.22 $\pm$ 0.36	3.69 $\pm$ 0.80	6.27	36.64 $\pm$ 4.53	12.76 $\pm$ 1.52	20.66 $\pm$ 2.15	4.46
1.00	2.34 $\pm$ 0.19	5.21 $\pm$ 0.34	3.65 $\pm$ 0.60	6.20	35.11 $\pm$ 4.28	15.86 $\pm$ 2.50	22.59 $\pm$ 2.24	3.80
2.00	2.84 $\pm$ 0.35	5.42 $\pm$ 0.34	3.62 $\pm$ 0.64	5.39	32.86 $\pm$ 3.14	18.92 $\pm$ 2.15	20.00 $\pm$ 1.61	3.79
5.00	3.31 $\pm$ 0.50	5.69 $\pm$ 0.46	3.77 $\pm$ 0.59	5.79	28.54 $\pm$ 2.28	20.17 $\pm$ 2.07	18.36 $\pm$ 1.35	3.70
								11.44 $\pm$ 0.65

Table 5. *Effect of dibutyladenosine 3':5'-cyclic monophosphate upon glucose utilization and glycerol release by normal fat cells*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 2.0% (w/v) albumin, together with the required concentrations of 6-N-2'-O-dibutyladenosine 3':5'-cyclic monophosphate, [U-¹⁴C]glucose, insulin and anti-insulin serum. The results are means $\pm$ S.E.M. of five determinations and are expressed as μ g-atoms/h per 100 μ g of DNA, with the exception of glycerol release, which is expressed as μ mol/h per 100 μ g of DNA.

Concn. of dibutyl- adenosine 3':5'-cyclic mono- phosphate (mM)	1 mM-Glucose + anti-insulin serum (10 munits/ml)				5 mM-Glucose + insulin (20 munits/ml)			
	Incorporation of [U- ¹⁴ C]- glucose into		Production of lactate + pyruvate	Lactate/ pyruvate ratio	Incorporation of [U- ¹⁴ C]- glucose into		Production of lactate + pyruvate	Lactate/ pyruvate ratio
	Glyceride fatty acids	Glycerol			Glyceride fatty acids	Glycerol		
0	5.83 $\pm$ 0.95	1.82 $\pm$ 0.43	10.53 $\pm$ 2.77	7.54	15.15 $\pm$ 1.46	3.88 $\pm$ 0.78	20.89 $\pm$ 4.86	5.77
0.03	6.47 $\pm$ 1.01	2.41 $\pm$ 0.52	10.90 $\pm$ 3.07	8.60	17.35 $\pm$ 2.03	4.15 $\pm$ 0.77	22.39 $\pm$ 4.61	5.66
0.06	6.49 $\pm$ 1.05	2.67 $\pm$ 0.50	10.28 $\pm$ 2.37	7.97	17.27 $\pm$ 1.88	4.63 $\pm$ 0.91	22.51 $\pm$ 4.35	5.19
0.15	6.29 $\pm$ 1.12	3.30 $\pm$ 0.69	11.30 $\pm$ 2.82	7.31	18.59 $\pm$ 1.67	5.44 $\pm$ 0.91	23.34 $\pm$ 4.15	4.69
0.30	5.63 $\pm$ 0.88	3.74 $\pm$ 0.55	10.31 $\pm$ 3.31	5.58	19.44 $\pm$ 0.75	6.95 $\pm$ 1.18	23.76 $\pm$ 4.46	4.02
0.50	5.63 $\pm$ 1.00	4.69 $\pm$ 0.78	9.93 $\pm$ 2.91	4.83	19.78 $\pm$ 1.01	9.27 $\pm$ 1.40	25.70 $\pm$ 4.74	3.63
0.75	5.13 $\pm$ 0.56	6.25 $\pm$ 0.82	11.62 $\pm$ 2.65	3.92	18.10 $\pm$ 1.29	13.01 $\pm$ 1.90	26.93 $\pm$ 5.07	3.17
1.00	4.23 $\pm$ 0.63	5.94 $\pm$ 1.17	9.19 $\pm$ 2.52	4.05	16.17 $\pm$ 1.23	16.10 $\pm$ 2.83	27.58 $\pm$ 5.78	2.88
2.00	2.52 $\pm$ 0.59	5.00 $\pm$ 1.32	7.95 $\pm$ 2.60	4.85	12.26 $\pm$ 1.32	18.38 $\pm$ 2.89	31.99 $\pm$ 5.66	2.74
								17.16 $\pm$ 1.47

Table 6. *Effect of adrenaline upon pyruvate utilization and glycerol release by normal fat cells*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 5.0% (w/v) albumin together with 5 mM-[U-¹⁴C]sodium pyruvate and the required concentrations of adrenaline, insulin and anti-insulin serum. The results are means $\pm$ S.E.M. of four determinations and are expressed as μ g-atoms/h per 100 μ g of DNA, with the exception of glycerol release, which is expressed as μ mol/h per 100 μ g of DNA.

Concn. of adrenaline (μ M)	5 mM-Pyruvate + anti-insulin serum (10 munits/ml)				5 mM-Pyruvate + insulin (20 munits/ml)			
	Incorporation of [U- ¹⁴ C]pyruvate into		Production of lactate	Production of glycerol	Incorporation of [U- ¹⁴ C]pyruvate into		Production of lactate	Production of glycerol
	Glyceride fatty acids	Glyceride glycerol			Glyceride fatty acids	Glyceride glycerol		
0	19.12 $\pm$ 1.10	1.75 $\pm$ 0.22	14.67 $\pm$ 1.65	0.58 $\pm$ 0.21	20.15 $\pm$ 1.48	1.87 $\pm$ 0.30	10.90 $\pm$ 0.94	0.72 $\pm$ 0.28
0.05	17.48 $\pm$ 1.40	2.11 $\pm$ 0.26	15.40 $\pm$ 1.22	0.69 $\pm$ 0.23	20.20 $\pm$ 1.48	1.91 $\pm$ 0.27	11.72 $\pm$ 1.53	0.53 $\pm$ 0.17
0.10	14.11 $\pm$ 0.79	2.39 $\pm$ 0.34	16.61 $\pm$ 1.22	1.20 $\pm$ 0.49	19.21 $\pm$ 1.47	1.93 $\pm$ 0.26	12.23 $\pm$ 1.15	0.51 $\pm$ 0.18
0.25	13.05 $\pm$ 0.85	2.55 $\pm$ 0.46	17.38 $\pm$ 0.76	3.48 $\pm$ 1.20	16.27 $\pm$ 1.20	2.42 $\pm$ 0.34	13.67 $\pm$ 2.03	0.72 $\pm$ 0.25
0.50	14.18 $\pm$ 0.73	2.49 $\pm$ 0.46	16.61 $\pm$ 1.24	6.98 $\pm$ 2.29	13.38 $\pm$ 0.42	2.89 $\pm$ 0.41	13.99 $\pm$ 1.83	1.47 $\pm$ 0.44
0.75	14.28 $\pm$ 0.54	2.33 $\pm$ 0.43	15.61 $\pm$ 1.56	8.00 $\pm$ 2.59	12.93 $\pm$ 0.83	3.04 $\pm$ 0.51	14.76 $\pm$ 2.49	2.45 $\pm$ 0.55

Table 7. *Comparison of the effects of sodium palmitate upon ³H₂O and [U-¹⁴C]glucose or [U-¹⁴C]pyruvate incorporation into fatty acids*

Fat cells from normally fed rats were incubated for 1 h in Krebs-Ringer bicarbonate containing either (A) 4.5% (w/v) albumin together with 5 mM-[U-¹⁴C]glucose, 20 munits of insulin/ml and ³H₂O, or (B) 1.5% (w/v) albumin together with 5 mM-sodium [U-¹⁴C]pyruvate and ³H₂O. Sodium palmitate was added as indicated. The results are means $\pm$ S.E.M. of four determinations and are expressed as μ g-atoms/h per 100 μ g of DNA. The figures in parentheses refer to incorporations in the presence of palmitate expressed as percentages of the same measurement made in the absence of palmitate.

A				B			
Concn. of palmitate (mM)	Incorporation of [U- ¹⁴ C]-glucose into glyceride fatty acids		Ratio ¹⁴ C/ ³ H	Concn. of palmitate (mM)	Incorporation of [U- ¹⁴ C]-pyruvate into glyceride fatty acids		Ratio ¹⁴ C/ ³ H
	Incorporation of ³ H ₂ O into glyceride fatty acids				Incorporation of ³ H ₂ O into glyceride fatty acids		
0	25.04 $\pm$ 1.60 (100)	18.51 $\pm$ 1.30 (100)	1.36 $\pm$ 0.02	0	13.31 $\pm$ 0.90 (100)	10.59 $\pm$ 0.57 (100)	1.26 $\pm$ 0.04
0.25	34.78 $\pm$ 2.25 (139)	23.78 $\pm$ 1.49 (128)	1.46 $\pm$ 0.03	0.10	10.51 $\pm$ 0.76 (79)	8.17 $\pm$ 0.49 (77)	1.28 $\pm$ 0.03
0.50	35.97 $\pm$ 2.45 (144)	25.79 $\pm$ 1.85 (139)	1.40 $\pm$ 0.03	0.25	8.57 $\pm$ 0.55 (64)	6.53 $\pm$ 0.39 (62)	1.31 $\pm$ 0.01
1.00	34.34 $\pm$ 3.23 (137)	23.31 $\pm$ 2.55 (126)	1.48 $\pm$ 0.05	0.50	7.61 $\pm$ 0.63 (57)	5.87 $\pm$ 0.45 (55)	1.30 $\pm$ 0.01
2.00	28.25 $\pm$ 3.07 (113)	20.99 $\pm$ 2.09 (113)	1.34 $\pm$ 0.03	1.00	7.83 $\pm$ 0.48 (59)	6.04 $\pm$ 0.34 (57)	1.29 $\pm$ 0.02
3.00	24.86 $\pm$ 2.86 (99)	18.77 $\pm$ 1.88 (101)	1.32 $\pm$ 0.04				

presence of albumin, glycerol production appears to be a reasonable indication of free fatty acid generation (Angel *et al.*, 1971a). However, it is apparent in Tables 4 and 5 that at low concentrations of adrenaline or 6-N-2'-O-dibutyryl adenosine 3':5'-cyclic monophosphate, sufficient to stimulate fatty acid synthesis from 5 mM-glucose in the presence of insulin, no appreciable stimulation of glycerol production or

glyceride glycerol formation is apparent. It may, however, be that only partial lipolysis of triglycerides is proceeding under these conditions. Angel *et al.* (1971a) have observed partial hydrolysis of triglycerides under certain conditions and Winaud *et al.* (1971) have observed that lipolytic agents increase the amounts of diglycerides in fat cells. Resynthesis of triglyceride from lower glycerides would increase

the ATP utilization of the cell and should therefore increase the synthesis of fatty acids from glucose as observed without any change in glyceride glycerol formation being apparent.

An antilipolytic effect of insulin is apparent when adrenaline is the lipolytic agent (Tables 4 and 6), but not when 6-*N*-2'-*O*-dibutyryladenine 3':5'-cyclic monophosphate is used to increase lipolysis. This is in accord with earlier observations (Blecher *et al.*, 1968; Hall & Ball, 1970) and may reflect an inability of cyclic nucleotide phosphodiesterases in the tissue to degrade this cyclic nucleotide analogue as discussed by Blecher *et al.* (1970).

In normal fat cells, rates of triglyceride synthesis were potentially very high indeed and virtually linear

with palmitate concentration; starvation appeared to decrease the esterifying capacity of the cells (Shapiro *et al.*, 1957; Bally *et al.*, 1960; Rubenstein *et al.*, 1965; Angel & Roncari, 1967). Decreases in lactate/pyruvate ratios closely paralleled increased rates of triglyceride synthesis, indicating that this process may be important in governing the cytoplasmic redox state in adipose tissue (Saggerson & Greenbaum, 1970a).

The varying response of the rate of fatty acid synthesis to changes in concentration of free fatty acid or to changes in lipolytic rate is interesting. Presumably fatty acid synthesis from 5 mM-glucose in the presence of insulin has reached a 'self-limited' value, as described by Flatt (1970). Increased triglyceride synthesis allows this process to be increased until a maximum is reached. Increased provision of fatty acids beyond this level then leads to a gradual decrease in fatty acid synthesis. The maximal stimulation of glucose-derived fatty acid synthesis achieved through provision of free fatty acids (0.5 mM-palmitate, 0.5 μ M-adrenaline or 0.5 mM-6-*N*-2'-*O*-dibutyryladenine 3':5'-cyclic monophosphate for normal cells incubated with 5 mM-glucose+insulin) cannot correspond to a maximal utilization of ATP or cytoplasmic NADH since esterification rates and changes in the lactate/pyruvate ratio are not maximized at this point. It can also be shown that the capacity of the cells to synthesize fatty acids is not maximal under these conditions (Saggerson, 1972). Two competing effects, one stimulatory and one inhibitory, are therefore apparent in regulating the rate of fatty acid synthesis from glucose. It is suggested that the fatty acid-esterifying system(s) become increasingly saturated as the provision of free fatty acids is increased, thus allowing free fatty

Table 8. *Effect of sodium palmitate upon glucose incorporation into glycerides by fat cells from animals starved for 72 h*

Fat cells from 72h-starved rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 6.0% (w/v) albumin, 5 mM-[U-¹⁴C]glucose, 20 munits of insulin/ml and the required concentration of sodium palmitate. The results are means $\pm$ S.E.M. of four determinations and are expressed as μ g-atoms/h per 100 μ g of DNA.

Concn. of palmitate (mM)	Incorporation of [U- ¹⁴ C]glucose into	
	Glyceride fatty acids	Glyceride glycerol
0	0.396 $\pm$ 0.057	2.59 $\pm$ 0.15
1.0	0.308 $\pm$ 0.024	7.13 $\pm$ 0.25
2.0	0.382 $\pm$ 0.017	7.75 $\pm$ 0.25
3.0	0.515 $\pm$ 0.020	8.60 $\pm$ 0.32
4.0	0.627 $\pm$ 0.032	8.60 $\pm$ 0.46

Table 9. *Effect of sodium palmitate upon lactate and pyruvate production by fat cells from normal, 24h-starved and 72h-starved animals incubated with glucose and insulin*

Fat cells from normal, 24h-starved or 72h-starved rats were incubated for 1 h in Krebs-Ringer bicarbonate containing 2.75% (w/v) albumin, 5 mM-[U-¹⁴C]glucose and 20 munits of insulin/ml together with the required concentration of sodium palmitate. The results are means $\pm$ S.E.M. of five determinations and are expressed as μ g-atoms/h per 100 μ g of DNA. The results were obtained in the same experiment as the data presented in Fig. 3.

Concn. of palmitate (mM)	Normally fed rats		24h-starved rats		72h-starved rats	
	Production of lactate + pyruvate	Lactate/pyruvate ratio	Production of lactate + pyruvate	Lactate/pyruvate ratio	Production of lactate + pyruvate	Lactate/pyruvate ratio
0	19.63 $\pm$ 2.20	6.57	22.18 $\pm$ 1.86	11.46	26.16 $\pm$ 1.68	8.87
0.25	20.16 $\pm$ 1.63	4.22	26.46 $\pm$ 1.73	9.94	31.07 $\pm$ 1.76	6.10
0.50	22.22 $\pm$ 1.43	3.54	29.78 $\pm$ 1.82	7.97	31.18 $\pm$ 1.69	5.18
0.75	23.50 $\pm$ 1.86	2.95	31.38 $\pm$ 2.60	6.76	30.10 $\pm$ 1.31	5.06
1.00	25.26 $\pm$ 1.93	2.73	31.78 $\pm$ 2.81	5.52	30.66 $\pm$ 1.95	4.46
2.00	29.25 $\pm$ 2.88	2.08	34.79 $\pm$ 2.10	4.51	27.43 $\pm$ 2.07	3.97

Fig. 3. Effect of sodium palmitate upon [U-¹⁴C]glucose incorporation into glyceride fatty acids and glyceride glycerol by fat cells obtained from normal, 24h-starved and 72h-starved animals

Fat cells were incubated for 1 h in Krebs–Ringer bicarbonate containing 2.75% (w/v) albumin together with 5mM-[U-¹⁴C]glucose and insulin (20munits/ml), and the required concentration of sodium palmitate. The results are the means of five determinations. ●, ○, Normal fat cells; ▲, △, 24h-starved cells; ■, □, 72h-starved cells. ●, ▲, ■, Incorporation into fatty acids; ○, △, □, incorporation into glyceride glycerol.

Table 10. Changes in free fatty acid content and glycerol release by fat cells from normal, 24h-starved and 72h-starved animals incubated with glucose and insulin

Fat cells from normal, 24h-starved or 72h-starved rats were incubated for 1 h in Krebs–Ringer bicarbonate containing 2.75% (w/v) albumin, 5mM-glucose and 20munits of insulin/ml. The results are means ± S.E.M. of four determinations.

Dietary status	Free fatty acids (nequiv./ml of incubated cell suspension)		Change in free fatty acid content (nequiv./h per 100 μg of DNA)	Glycerol release (nmol/h per 100 μg of DNA)
	At 0 min	At 60 min		
Normal	48.9 ± 4.1	43.7 ± 6.3	−63.3	244 ± 26
24h-starved	77.1 ± 14.1	56.9 ± 10.7	−249.4	470 ± 17
72h-starved	19.2 ± 5.9	25.7 ± 3.0	+86.1	410 ± 33

acid concentrations inside the cells to rise with resultant inhibitory effects. In support of this argument it may be seen that fatty acid synthesis from pyruvate is inhibited at far lower concentrations of palmitate or adrenaline to induce lipolysis (Tables 6 and 7) than those required to inhibit fatty acid synthesis from glucose. The ability to esterify palmitate

is severely limited when pyruvate is the sole substrate supplied to fat cells (Saggerson & Tomassi, 1971). In Fig. 3 it may be seen that fatty acid synthesis in 24h-starved cells is decreased by palmitate concentrations above 0.25mM, whereas in normal cells the effect is only apparent above 0.5mM-palmitate. This presumably reflects a lower esterifying capacity of the

24h-starved cells together with an increased production and initial amount of endogenous free fatty acids in cells incubated without palmitate (Table 10). Presumably the profile of the response to palmitate is the same in 24h-starved cells as in normal cells but has been moved towards the left of the graph in Fig. 3. In the absence of insulin, palmitate and lipolytic agents did not produce appreciable stimulations of 1mm-glucose incorporation into fatty acids (Tables 3–5). However, when the glucose concentration was 5mm (Figs. 1 and 2) and glyceride glycerol formation was raised to an amount comparable with that found in the presence of insulin, fatty acid synthesis was then stimulated by 0.5mm-palmitate in the absence of insulin.

A third effect upon fatty acid synthesis from glucose is apparent when palmitate is added to 72h-starved cells. In these cells the fatty acid-esterifying capacity is severely curtailed and the provision of endogenous free fatty acids by lipolysis in cells incubated without palmitate is greater than in normal cells (Table 10). Under these conditions the profile of the response to palmitate is presumably moved even further to the left of the graph shown in Fig. 3. Higher concentrations of palmitate caused a secondary stimulation of fatty acid synthesis from glucose in 72h-starved cells, which was proportional to the concentration of palmitate and became apparent when the esterifying capacity of the cells was virtually saturated (Fig. 3 and Table 8). Free fatty acids are known to uncouple oxidative phosphorylation in isolated mitochondria (Pressman & Lardy, 1956) and Angel *et al.* (1971b) have suggested that ATP concentrations fall in fat cells incubated with lipolytic agents in the absence of glucose, as a result of free fatty acid-mediated uncoupling of oxidative phosphorylation. In the fat cells from 72h-starved animals, used in this study, glucose will have no protecting effect against uncoupling once a palmitate concentration has been reached that saturates the esterifying capacity of the cells. Stimulation of glucose conversion into fatty acids through uncoupling of oxidative phosphorylation is in accord with the predictions of Flatt (1970) and with reported effects of other uncoupling agents upon fatty acid synthesis from glucose in fat cells (Saggerson, 1972). In brown fat cells, controlled uncoupling through free fatty acids has been proposed to play a physiological role (Prusiner *et al.*, 1968). The results presented in this study suggest that extreme unphysiological conditions have to be employed to produce such an effect in white fat cells.

From the results presented in Table 7, since the profiles of $^3\text{H}_2\text{O}$ incorporation in the presence of $[\text{U}-^{14}\text{C}]\text{glucose}$ or $[\text{U}-^{14}\text{C}]\text{pyruvate}$ were the same as the profiles of incorporation of these substrates with varying palmitate concentration, it would seem likely that there is little formation of fatty acids from

acetyl-CoA, which is derived by oxidation of endogenous fatty acids. Alternatively if there is dilution of the acetyl-CoA pool that is the precursor of fatty acid synthesis, free fatty acid accumulation must affect some step(s) after acetyl-CoA formation in the lipogenic pathway. It would seem most likely, however, since lactate+pyruvate output increases as fatty acid synthesis decreases when provision of free fatty acids is high (cells incubated with 5mm-glucose+insulin; Tables 3–5), that pyruvate oxidation is decreased under these conditions. If pyruvate oxidation decreases as a result of increased fatty acid oxidation, which in turn increases with increasing concentration of free fatty acid (Bally *et al.*, 1960), some dilution of the acetyl-CoA pool should be observed with resultant alterations in the $^{14}\text{C}/^3\text{H}$ ratios presented in Table 7. Significant alterations in these ratios are not observed. However, if there is normally a tight gearing between ATP production and utilization in the cells, the observed changes in fatty acid synthesis may result from very small changes in fatty acid oxidation, which would not cause significant changes in the specific activity of acetyl-CoA. Alternatively acetyl-CoA derived from fatty acid oxidation may not enter the acetyl-CoA pool that is the precursor for fatty acid synthesis.

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