Regulation of Glycolysis and L-Glycerol 3-Phosphate Concentration in Rat Epididymal Adipose Tissue *in vitro*

ROLE OF PHOSPHOFRUCTOKINASE

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1. Attempts were made to define the role of phosphofructokinase in glycolytic control and the factors regulating the concentration of L-glycerol 3-phosphate in rat epididymal fat pads incubated *in vitro*. 2. Glycolysis rates were altered by anoxia or by additions of insulin, adrenaline or both to the incubation medium, and the changes in rate were related to changes in the steady-state concentrations of hexose phosphates, adenine nucleotides, L-glycerol 3-phosphate and citrate in the whole tissue. Measurements were also made of the lactate/pyruvate concentration ratio in the medium after incubation. 3. The mass-action ratios of phosphofructokinase, calculated from the whole-tissue concentrations of products and substrates, were less than 0.1% of the value of the ratio at pH 7.4 at equilibrium. 4. Only in the presence of adrenaline could the observed stimulation of glycolytic flux be related to a possible activation of phosphofructokinase since, in this situation, the concentration of one substrate, fructose 6-phosphate, was not altered and the concentration of the other, ATP, was decreased. Increased glycolytic flux in the presence of insulin may be explained by an observed increase in the concentration of the substrate, fructose 6-phosphate. Under anaerobic conditions, glycolytic flux was decreased but this did not appear to be the result of inhibition of phosphofructokinase, since the concentrations of both substrates, fructose 6-phosphate and ATP, were decreased. The changes in glycolytic flux with insulin and anoxia may be secondary to changes in the rate of glucose uptake. 5. Changes in L-glycerol 3-phosphate concentration appear to be related both to changes in the concentration of dihydroxyacetone phosphate and to changes in the NADH/NAD⁺ concentration ratio in the cytoplasm. They do not seem to be related directly to alterations in glycolytic rate.

The main object of this study was to define the role of PFK* in the control of glycolysis in adipose tissue. It had been established that the properties of rat adipose-tissue PFK are qualitatively similar to those of the enzyme in heart and brain with respect to inhibition by ATP and citrate and activation by ADP, AMP, 3',5'-(cyclic)-AMP and phosphate. Quantitatively, adipose-tissue PFK appears to be more sensitive to activation by 3',5'-(cyclic)-AMP (Denton & Randle, 1966). In the present study, adipose tissue was incubated *in vitro* and changes in glycolytic rate were effected by the addition of insulin or adrenaline, or both, to the incubation medium, or by incubation under anoxic conditions. Such changes were correlated with changes in the steady-state whole-tissue concentrations of intermediates likely to be involved in the control of glycolysis by PFK (hexose phosphates, adenine nucleotides and citrate).

The provision of L-glycerol 3-phosphate for glyceride synthesis may be the major role of glycolysis in adipose tissue under certain conditions (e.g. on incubation in the presence of adrenaline; Denton & Halperin, 1968). In confirmation of an earlier suggestion (Denton, Yorke & Randle, 1966) it is shown below that the tissue concentration of L-glycerol 3-phosphate is related both to the tissue concentration of dihydroxyacetone phosphate and to the NADH/NAD⁺ concentration ratio in the cytoplasm, but not directly to the rate of glycolysis.

EXPERIMENTAL

Materials

Rats. Epididymal fat pads were obtained from male albino Wistar rats (150–200 g.) fed on stock laboratory diet 41B (Short & Parkes, 1949). In all experiments the animals were allowed free access to food before the time of killing (approx. 10 a.m.). In any one experiment the rats were of the same age and similar weight (± 15 g.).

* Abbreviation: PFK, phosphofructokinase (EC 2.7.1.11).

Chemicals. These were as given in Denton & Halperin (1968). Also, dihydroxyacetone phosphate dimethyl ketal (cyclohexylammonium salt), obtained from Boehringer Corp. (London) Ltd. (London W.5), was hydrolysed with Dowex 50 (H⁺ form) for 6 hr. at 40° and neutralized with NaHCO₃ immediately before use.

Media. Fat pads were incubated in Krebs bicarbonate-buffered medium (Krebs & Henseleit, 1932), gassed with O₂ + CO₂ (95:5), except in anaerobic experiments where the gas mixture was N₂ + CO₂ (95:5). The concentrations of the various additions are given in the text or tables. Media containing albumin were dialysed for 18 hr. at 4° against 20 vol. of the buffer for the medium (Denton & Halperin, 1968).

Procedure and methods

Incubation of epididymal fat pads. Rats were killed by decapitation and the fat pads removed as described by Winegrad & Renold (1958). The pads were rinsed and preincubated in paired groups of four pads (except where stated) for 30 min. at 37° in medium (5–10 ml.) containing glucose (1.5 mg./ml.). After preincubation each group of pads (1.5–3.0 g. wet wt. of tissue) was lightly blotted and transferred to flasks containing gassed medium (5–10 ml.) with additions as indicated in the tables. Incubation was then continued for a further 60 min. When the time-course of glucose uptake was measured the flasks were fitted with rubber serum caps through which samples of media were withdrawn at intervals with a 25 μ l. Hamilton syringe. The flasks were not regassed.

Extraction and analysis of fat pads. At the end of each incubation the pads were lightly blotted, rapidly frozen in liquid N₂ and reduced to a fine powder by grinding under liquid N₂. The powder was weighed and extracted in ice-cold 5% (w/v) HClO₄ (5 ml.) and diethyl ether (10 ml.) in an all-glass tissue grinder. After centrifugation the ether layer was removed by suction. The aqueous layer was then carefully decanted, neutralized to pH 6.5 (measured by a glass electrode) with KOH in the presence of triethanolamine hydrochloride and freeze-dried.

Analysis of incubation media. Glucose uptake, and pyruvate, lactate and glycerol outputs were measured as described by Denton & Randle (1967).

Assay of glucose, lactate, pyruvate, glycerol and intermediates. These were assayed spectrophotometrically or fluorimetrically as described by Denton & Halperin (1968). In addition, fructose 6-phosphate and fructose 1,6-diphosphate were measured fluorimetrically by techniques based on the methods of Hohorst (1963) and Bücher & Hohorst (1963) respectively. The recovery of fructose 1,6-diphosphate added at the time of extraction of the tissue powder with HClO₄-diethyl ether was 89 $\pm$ 4% (mean $\pm$ S.E.M. of three observations).

Extraction and assay of L-glycerol 3-phosphate dehydrogenase. Fat pads were rinsed in Krebs bicarbonate-buffered medium, blotted and extracted in an all-glass tissue grinder in ice-cold 0.15 M-KCl adjusted to pH 7.0 with K₂HPO₄ (5 ml./g. wet wt. of tissue). The homogenate was centrifuged at 1000 g for 10 min. at 0°.

The total activity of L-glycerol 3-phosphate dehydrogenase (E.C. 1.1.1.8) in samples of the supernatant was assayed spectrophotometrically at 25° by following NADH oxidation at 340 nm. in 0.1 M-triethanolamine hydrochloride buffer, pH 7.4, containing NADH (0.1 mM), MgSO₄ (6 mM),

KCl (2 mM) and either fructose 1,6-diphosphate (25 mM) plus aldolase (50 μ g./ml.) or dihydroxyacetone phosphate (1 mM). Activities were similar under both conditions and were not increased at higher substrate concentrations. The *K_m* for dihydroxyacetone phosphate was measured by observation of the initial rates of NADH oxidation with different additions of dihydroxyacetone phosphate under the above conditions.

RESULTS

Effect of time of incubation on the rate of glucose uptake by rat epididymal fat pads. Fig. 1 demonstrates that the rates of glucose uptake by fat pads incubated aerobically or anaerobically, in both the presence and the absence of insulin, were linear during the first 1 hr. of incubation, after 30 min. preincubation. This suggests, in agreement with earlier studies (Denton & Halperin, 1968), that there is a steady state of glucose metabolism during the experimental period used in this study.

Effects of insulin, adrenaline and anoxia on flow rates and intermediate concentrations in rat epididymal fat pads in vitro. The effects of insulin, adrenaline and anoxia on the rates of uptake of glucose and of output of glycerol, lactate and pyruvate are shown in Table 1, together with the effects on the lactate/pyruvate concentration ratio in the medium at the end of the incubation period. In Table 2 are shown the effects of insulin, adrenaline and anoxia on the whole-tissue concentrations of

Fig. 1. Uptake of glucose by fat pads as a function of time. Pads were preincubated aerobically in medium containing glucose (1.5 mg./ml.) for 30 min. and then transferred to fresh medium containing glucose (1.5 mg./ml.) with (Δ , $\blacktriangle$) or without ($\circ$, $\bullet$) insulin and incubated either aerobically ($\circ$, Δ) or anaerobically ($\bullet$, $\blacktriangle$). Each point represents the glucose uptake of a single group of four pads. Other details are given in the Experimental section.

Table 1. *Effects of insulin, adrenaline and anoxia on the rates of glucose uptake and the outputs of pyruvate, lactate and glycerol in rat epididymal adipose tissue incubated in vitro*

Fat pads were preincubated for 30 min. in medium containing glucose (1.5 mg./ml.) and then incubated under either aerobic or anaerobic conditions in fresh medium containing glucose (1.5 mg./ml.) and, where added, albumin (20 mg./ml.), insulin (10 milliunits/ml.) and adrenaline (5 μ g./ml.). Results are given as mean values $\pm$ S.E.M. of four observations on groups of four pads. The pads were paired between control and experimental flasks except in Expt. 5, where pads were distributed regularly between three flasks. ** $P < 0.01$; * $P < 0.05$ (versus appropriate control).

Expt. no.	Addition(s) to incubation medium (and incubation conditions)	Carbon atom flow (μ g.atoms of carbon/g. wet wt. of tissue/hr.)				Lactate/ pyruvate concentration ratio in the medium
		Glucose uptake	Lactate output	Pyruvate output	Glycerol output	
1	None (aerobic)	21.8 $\pm$ 1.7	0.84 $\pm$ 0.06	0.28 $\pm$ 0.02	1.78 $\pm$ 0.10	3.0 $\pm$ 0.1
	Insulin (aerobic)	92.4 $\pm$ 1.3**	2.36 $\pm$ 0.11**	0.43 $\pm$ 0.02**	1.27 $\pm$ 0.08**	5.6 $\pm$ 0.2**
2	None (aerobic)	21.2 $\pm$ 2.6	0.85 $\pm$ 0.22	0.27 $\pm$ 0.04	1.38 $\pm$ 0.10	2.7 $\pm$ 0.6
	None (anaerobic)	7.1 $\pm$ 1.4**	3.60 $\pm$ 0.34**	0.31 $\pm$ 0.06	1.18 $\pm$ 0.06	11.9 $\pm$ 3.7*
3	None (aerobic)	16.6 $\pm$ 2.2	1.14 $\pm$ 0.13	0.28 $\pm$ 0.02	2.16 $\pm$ 0.29	4.0 $\pm$ 0.2
	Adrenaline (aerobic)	38.2 $\pm$ 2.9**	3.93 $\pm$ 0.17**	0.96 $\pm$ 0.04**	21.8 $\pm$ 0.56**	4.2 $\pm$ 0.2
4	Albumin (aerobic)	19.4 $\pm$ 3.6	1.23 $\pm$ 0.21	0.47 $\pm$ 0.07	2.91 $\pm$ 0.29	2.9 $\pm$ 0.8
	Albumin + adrenaline (aerobic)	70.2 $\pm$ 3.2**	4.68 $\pm$ 0.24**	0.98 $\pm$ 0.07**	32.1 $\pm$ 1.26**	4.8 $\pm$ 0.2
5	Insulin (aerobic)	63.6 $\pm$ 2.4	2.84 $\pm$ 0.10	0.44 $\pm$ 0.04	1.88 $\pm$ 0.07	6.1 $\pm$ 1.0
	Insulin + adrenaline (aerobic)	74.8 $\pm$ 2.7*	6.96 $\pm$ 0.66**	2.06 $\pm$ 0.08**	28.2 $\pm$ 2.3**	3.35 $\pm$ 0.2*
	Insulin (anaerobic)	51.1 $\pm$ 2.6*	26.0 $\pm$ 3.8**	0.80 $\pm$ 0.06**	1.96 $\pm$ 0.18	32.3 $\pm$ 3.7**

hexose monophosphates, fructose 1,6-diphosphate, citrate, L-glycerol 3-phosphate and adenine nucleotides. With one exception, these intermediates were measured in the same tissue used for the experiments reported in Table 1. Some of the changes in the rates of glucose uptake and concentrations of adenine nucleotides, L-glycerol 3-phosphate, glucose 6-phosphate and citrate observed under aerobic conditions on addition of insulin or adrenaline to the incubation medium have been reported previously (Denton *et al.* 1966; Denton & Randle, 1967; Denton & Halperin, 1968). The changes observed in this study are in good agreement with these earlier reports except that the concentration of ATP found was rather greater this time.

The fructose 6-phosphate concentration was measured in four of the experiments and varied from 19% to 33% of the glucose 6-phosphate concentration. The mean value for the fructose 6-phosphate/glucose 6-phosphate concentration ratio was 0.24 ± 0.02 (for 32 observations), which corresponds closely to the predicted value (0.28) if phosphoglucose isomerase (EC 5.3.1.9) were in equilibrium (Hess, 1963). The low concentration of fructose 6-phosphate made its measurement less reliable than that of glucose 6-phosphate. For this reason, we used values for the latter in the calculation of the tissue mass-action ratio of PFK shown in Table 2.

Insulin increased glucose uptake (4.2-fold) and

the outputs of lactate (2.8-fold) and pyruvate (1.5-fold), and slightly decreased the rate of glycerol output (Table 1, Expt. 1). The lactate/pyruvate concentration ratio in the medium was nearly doubled. There were increases in the concentrations of glucose 6-phosphate (2.1-fold), fructose 6-phosphate (2.1-fold), fructose 1,6-diphosphate (2.2-fold) and L-glycerol 3-phosphate (3.7-fold), but no change was seen in the concentrations of citrate or of adenine nucleotides. The mass-action ratio of PFK in the tissue was not significantly altered.

In the absence of insulin and albumin (Expt. 3) adrenaline increased glucose uptake (2.3-fold) and the outputs of lactate (3.3-fold), pyruvate (3.4-fold) and glycerol (tenfold). There was no significant change in the lactate/pyruvate concentration ratio in the medium. The concentrations of hexose monophosphates did not alter significantly, but there were increases in those of fructose 1,6-diphosphate (2.9-fold), L-glycerol 3-phosphate (2.7-fold) and AMP (3.8-fold), and that of ATP was halved. The mass-action ratio of PFK was increased fivefold. In the presence of albumin (Expt. 4), adrenaline had very similar effects except that the rise in L-glycerol 3-phosphate concentration was not statistically significant and that in citrate concentration was considerably greater. In the presence of insulin (Expt. 5) adrenaline caused a small but significant increase in glucose uptake (1.2-fold) and substantial increases in the outputs of lactate (2.5-fold), pyruvate (4.7-fold) and glycerol (15-fold).

Table 2. *Effects of insulin, adrenaline and anoxia on the whole-tissue concentrations of glucose 6-phosphate, fructose 6-phosphate, fructose 1,6-diphosphate, L-glycerol 3-phosphate, citrate and the adenine nucleotides in rat epididymal adipose tissue incubated in vitro*

Details of incubation conditions were as given in Table 1 and the results were obtained with the same tissues, except in Expt. 1. The PFK mass-action ratio was calculated from the whole-tissue concentrations of fructose 1,6-diphosphate, ATP and ADP, together with the whole-tissue concentration of fructose 6-phosphate derived from the whole-tissue concentration of glucose 6-phosphate by assuming phosphoglucose isomerase to be in equilibrium, i.e. that the fructose 6-phosphate/glucose 6-phosphate concentration ratio is 0.28 (Hess, 1963). ** $P < 0.01$; * $P < 0.02$ (versus appropriate control).												
Expt. no.	Additions to incubation medium (and incubation conditions)	Concentration (nmoles/g. wet wt. of tissue)								PFK mass-action ratio		
		Glucose 6-phosphate	Fructose 6-phosphate	Fructose 1,6-diphosphate	L-Glycerol 3-phosphate	Citrate	ATP	ADP	AMP			
1	None (aerobic)	4.69 $\pm$ 0.39	1.15 $\pm$ 0.13	0.81 $\pm$ 0.05	3.55 $\pm$ 0.34	7.08 $\pm$ 0.30	130 $\pm$ 4.6	58 $\pm$ 2.8	7.8 $\pm$ 0.7	0.28 $\pm$ 0.03		
	Insulin (aerobic)	9.79 $\pm$ 0.48**	2.37 $\pm$ 0.08**	1.74 $\pm$ 0.11**	13.27 $\pm$ 0.96**	6.81 $\pm$ 0.22	140 $\pm$ 1.5	56 $\pm$ 3.2	7.6 $\pm$ 0.6	0.26 $\pm$ 0.02		
2	None (aerobic)	4.60 $\pm$ 0.49	1.50 $\pm$ 0.16	0.77 $\pm$ 0.04	3.4 $\pm$ 0.5	8.39 $\pm$ 0.30	135 $\pm$ 1.2	45 $\pm$ 4.0	8.3 $\pm$ 1.0	0.19 $\pm$ 0.02		
	None (anaerobic)	3.30 $\pm$ 0.19*	1.05 $\pm$ 0.11	3.08 $\pm$ 0.34**	60.0 $\pm$ 3.6**	5.11 $\pm$ 0.28**	81 $\pm$ 9.1**	40 $\pm$ 4.0	61.0 $\pm$ 3.2**	1.84 $\pm$ 0.46**		
3	None (aerobic)	3.98 $\pm$ 0.50	0.76 $\pm$ 0.08	0.63 $\pm$ 0.07	3.44 $\pm$ 0.47	8.06 $\pm$ 0.88	144 $\pm$ 5.6	40 $\pm$ 3.9	3.3 $\pm$ 0.7	0.16 $\pm$ 0.02		
	Adrenaline (aerobic)	4.81 $\pm$ 0.33	0.89 $\pm$ 0.15	1.82 $\pm$ 0.07**	9.16 $\pm$ 1.03**	11.6 $\pm$ 1.08*	79 $\pm$ 6.0**	47 $\pm$ 2.9	12.4 $\pm$ 1.5**	0.83 $\pm$ 0.12**		
4	Albumin (aerobic)	3.80 $\pm$ 0.43	0.93 $\pm$ 0.20	0.85 $\pm$ 0.11	4.81 $\pm$ 0.61	10.6 $\pm$ 0.34	127 $\pm$ 5.5	40 $\pm$ 1.0	7.8 $\pm$ 1.7	0.26 $\pm$ 0.02*		
	Albumin + adrenaline (aerobic)	5.32 $\pm$ 0.82	1.02 $\pm$ 0.11	1.78 $\pm$ 0.11**	5.48 $\pm$ 0.49	23.4 $\pm$ 2.07**	87 $\pm$ 2.2**	41 $\pm$ 2.7	11.5 $\pm$ 1.5	0.61 $\pm$ 0.10		
5	Insulin (aerobic)	10.6 $\pm$ 0.80	—	2.20 $\pm$ 0.15	12.1 $\pm$ 1.4	7.85 $\pm$ 0.43	131 $\pm$ 4.0	42 $\pm$ 1.7	6.9 $\pm$ 0.5	0.24 $\pm$ 0.02		
	Insulin + adrenaline (aerobic)	7.7 $\pm$ 0.46*	—	2.00 $\pm$ 0.09	4.6 $\pm$ 0.9**	24.9 $\pm$ 2.3**	103 $\pm$ 5.0**	48 $\pm$ 1.5*	16.7 $\pm$ 1.0**	0.46 $\pm$ 0.08*		
	Insulin (anaerobic)	8.4 $\pm$ 0.47*	—	3.73 $\pm$ 0.31**	160 $\pm$ 13**	3.66 $\pm$ 0.75**	68 $\pm$ 3.7**	42 $\pm$ 1.4	33.3 $\pm$ 3.4**	1.00 $\pm$ 0.10**		

Details of incubation conditions were as given in Table 1 and the results were obtained with the same tissues, except in Expt. 1. The PFK mass-action ratio was calculated from the whole-tissue concentrations of fructose 1,6-diphosphate, ATP and ADP, together with the whole-tissue concentration of fructose 6-phosphate derived from the whole-tissue concentration of glucose 6-phosphate by assuming phosphoglucose isomerase to be in equilibrium, i.e. that the fructose 6-phosphate/glucose 6-phosphate concentration ratio is 0.28 (Hess, 1963). ** $P < 0.01$; * $P < 0.02$ (versus appropriate control).

The lactate/pyruvate concentration ratio in the medium was approximately halved. The concentrations of glucose 6-phosphate (0.7-fold) and L-glycerol 3-phosphate (0.4-fold) were diminished, but no change in the concentration of fructose 1,6-diphosphate was observed. The ATP concentration was slightly diminished, whereas that of AMP rose. The mass-action ratio of PFK was increased (1.9-fold).

In the absence of insulin the rate of glucose uptake was decreased to one-third under anaerobic conditions (Expt. 2), whereas the output of lactate was increased (4.2-fold). No change in glycerol or pyruvate output was observed. The lactate/pyruvate concentration ratio in the medium was increased fourfold. In addition, the concentrations of fructose 1,6-diphosphate (4.0-fold), L-glycerol 3-phosphate (18-fold) and AMP (7.3-fold) were greatly elevated and those of the hexose monophosphates (0.72-fold), citrate (0.61-fold) and ATP (0.60-fold) were diminished. The mass-action ratio of PFK was increased (9.7-fold). In the presence of insulin anoxia caused qualitatively similar changes to those observed in the absence of insulin (Expt. 5). The decrease in glucose uptake (0.80-fold) was smaller and the increases in lactate and pyruvate outputs were considerably greater, but the change in the lactate/pyruvate concentration ratio (13-fold) was similar. In addition, in the presence of insulin the concentrations of glucose 6-phosphate (0.8-fold) and citrate (0.5-fold) were again lowered by anoxia and those of fructose 1,6-diphosphate (1.7-fold) and especially L-glycerol 3-phosphate (13-fold) and AMP (4.8-fold) were increased. Assuming the intracellular water space in adipose tissue to be 14 μ l./g. wet wt. of tissue (Denton *et al.* 1966), the concentration of L-glycerol 3-phosphate was nearly 12 mM in tissues incubated anaerobically in the presence of insulin.

Activity of L-glycerol 3-phosphate dehydrogenase in rat epididymal adipose tissue. The total activity of L-glycerol 3-phosphate dehydrogenase was assayed at 25° in low-speed supernatants of adipose-tissue homogenates. The activity was 14.9 ± 1.4 units/g. wet wt. of tissue (mean $\pm$ S.E.M. for seven observations). The K_m of the enzyme for dihydroxyacetone phosphate was calculated by using the programme described by Cleland (1967). Observations of the rate of NADH oxidation made at eight different concentrations of dihydroxyacetone phosphate (ranging from 16×10^{-6} to 228×10^{-6} M) gave a K_m value of $86 (\pm 9) \times 10^{-6}$ M.

DISCUSSION

In the following discussion the assumption is made that a change in the whole-tissue concentration of a metabolite reflects a change in the

Table 3. Relationships between the rate of glycolysis and the concentrations of substrates and effectors of PFK

The rates of glucose uptake were taken from Table 1 and the concentrations of intermediates from Table 2. The rates of glycolysis were calculated from the rates of glucose uptake, corrected for the contribution of the pentose phosphate pathway as reported by Katz *et al.* (1966) and Flatt & Ball (1964). ** $P < 0.01$, * $P < 0.05$ when compared with control values (i.e. 100%).

Expt. no.	Addition(s) to medium (and incubation conditions)			Experimental rate (% of control)		Experimental concentration (% of control concentration)					
	Control	Experimental		Glucose uptake	Calculated glycolysis flow	Hexose mono-phosphates	Fructose 1,6-diphosphate	Citrate	ATP	ADP	AMP
1	None (aerobic)	Insulin (aerobic)		424**	403	209**	215**	96	108	97	97
2	None (aerobic)	None (anaerobic)		33**	41	72*	400**	61**	60**	89	735**
3	None (aerobic)	Adrenaline (aerobic)		230**	264	121	289**	144**	55**	118	376**
4	Albumin (aerobic)	Albumin + adrenaline (aerobic)		362**	415	140	209**	221**	69**	103	147
5	Insulin (aerobic)	Insulin + adrenaline (aerobic)		118*	125	73*	91	317**	79**	114*	242**
	Insulin (aerobic)	Insulin (anaerobic)		80*	104	79*	170**	47**	52**	100	483**

concentration of the metabolite throughout the extramitochondrial compartment of the cell. However, the possibility of compartmentation, in particular of citrate and of the adenine nucleotides, must be borne in mind.

Role of PFK in glycolytic regulation

In Table 3, the percentage changes caused by insulin, adrenaline and anoxia in the rates of glucose uptake and of glycolysis by adipose tissue are compared with the percentage changes in the tissue concentrations of the hexose monophosphates, fructose 1,6-diphosphate, citrate and the adenine nucleotides. The rate of glycolysis was calculated from the glucose uptake, corrected under aerobic conditions for the contribution of the pentose phosphate pathway (from the data of Flatt & Ball, 1964, and Katz, Landau & Bartsch, 1966). It was assumed that there is no appreciable flow through the pentose phosphate pathway under anaerobic conditions. No allowance for the flow of carbon atoms through the glycogen pathway was made because this is so small relative to the flow through glycolysis. The changes in hexose monophosphate concentration were calculated from the measured concentrations of glucose 6-phosphate on the assumption that the phosphoglucose isomerase step is in equilibrium (see the legend to Table 2).

Since a proton is produced by the PFK reaction, the mass-action ratio of PFK at equilibrium will depend on the pH. If the intracellular pH of the adipose-tissue cell were 7.4, the value of the mass-action ratio at equilibrium would be about 3000 (Hess, 1963). The measured value was, in fact, never greater than 1.84. Thus, as found in many other tissues, the enzyme in adipose tissue appears to be considerably displaced from equilibrium under all the conditions studied.

Effects of insulin. The stimulation of glucose uptake and of glycolysis observed with insulin can be explained in terms of activation of membrane transport (Crofford & Renold, 1965*a,b*) leading to increases, in turn, in the intracellular concentration of glucose, the rate of glucose phosphorylation and the concentration of hexose monophosphates, and hence to an increased flow rate through PFK as a result of the increased substrate (fructose 6-phosphate) concentration. There is no evidence for a regulatory role of PFK under these conditions.

Effects of anoxia. It has been reported that anoxia increases lactate output (Ball & Jungas, 1963) and decreases the flow of carbon from [^{14}C]glucose into glyceride glycerol, fatty acids and carbon dioxide (Cahill, Leboeuf & Renold, 1959) in epididymal fat pads incubated *in vitro*. Direct observations of the rate of glucose uptake do not appear to have been published. In the present

studies adipose tissue did not exhibit a Pasteur effect and, in fact, in the presence, and especially in the absence, of insulin, anoxia depressed the rate of glucose uptake. This could be the result of inhibition either of membrane transport or of glucose phosphorylation. Without measurements of the intracellular glucose concentration, clear distinction between these two possibilities cannot be made. However, since anoxia decreased the concentrations of glucose 6-phosphate, an inhibitor of hexokinase from adipose tissue (Hernandez & Sols, 1963), it seems likely that membrane transport was inhibited. Such a situation would be in marked contrast with that found in muscle, where it has been suggested that the changes in adenine nucleotide concentration under anaerobic conditions lead to the activation of glucose transport (see Randle & Morgan, 1962). The decreased flow through glycolysis does not appear to be the result of any inhibition of PFK since the concentrations of both substrates, fructose 6-phosphate and ATP, are decreased.

Effects of adrenaline. Like insulin, adrenaline accelerated glucose uptake and glycolysis. It seems reasonable to assume that accelerated glucose uptake was the result of stimulated membrane transport, but the mechanism of this stimulation remains obscure. It may be accounted for by the changes in adenine nucleotides, but in anoxia there are similar changes and glucose uptake is decreased. The stimulation of glycolysis by adrenaline appears to be the result of activation of PFK (the concentration of one substrate, fructose 6-phosphate, is not significantly altered and the concentration of the other, ATP, is decreased in both the presence and the absence of albumin). PFK activation, despite the rise in the concentration of the inhibitor, citrate, could be the result of the changes in the adenine nucleotides, or an increase in 3',5'-(cyclic)-AMP concentration, or both (Butcher, Ho, Meng & Sutherland, 1965).

In the presence of insulin, adrenaline produced small rises in the rates of glucose uptake and glycolysis. The increases are explicable in terms of a stimulation of hexokinase (which may be limiting in the presence of insulin) due to the observed fall in glucose 6-phosphate concentration resulting from the activation of PFK. As in the absence of insulin, the activation of PFK could be the result of the small changes in adenine nucleotides together with an increase in the concentration of 3',5'-(cyclic)-AMP.

Regulation of L-glycerol 3-phosphate concentration

It has been suggested that changes in the rate of glycolysis in adipose tissue may lead to parallel

Table 4. Relationships between the tissue concentration of L-glycerol 3-phosphate and the rate of glycolysis, the lactate/pyruvate concentration ratio in the medium and the tissue concentration of fructose 1,6-diphosphate

Expt. no.	Addition(s) to medium (and incubation conditions)		Experimental calculated glycolysis rate (% of control)	Experimental lactate/pyruvate concentration ratio in the medium (% of control)	Experimental concentration (% of control concentration)			
	Control	Experimental			Fructose 1,6-diphosphate	Observed		Calculated L-glycerol 3-phosphate
						L-glycerol	3-phosphate	
1	None (aerobic)	Insulin (aerobic)	403	187**	215**	374**	274	
2	None (aerobic)	None (anaerobic)	41	441*	400**	1765**	882	
3	None (aerobic)	Adrenaline (aerobic)	264	105	289**	266**	170	
4	Albumin (aerobic)	Albumin + adrenaline (aerobic)	415	166	209**	114	145	
5	Insulin (aerobic)	Insulin + adrenaline (aerobic)	125	55*	91	38**	52	
	Insulin (aerobic)	Insulin (anaerobic)	104	530**	170**	1322**	690	

The rates of glycolysis were taken from Table 3, the lactate/pyruvate concentration ratios from Table 1 and the concentrations of fructose 1,6-diphosphate and L-glycerol 3-phosphate from Table 2. The calculated changes in L-glycerol 3-phosphate concentration are the products of the change in the lactate/pyruvate concentration ratio in the medium and the square root of the change in the fructose 1,6-diphosphate concentration (see the Discussion section). ** $P < 0.01$; * $P < 0.05$ when compared with control values (i.e. 100%).

changes in the concentration of L-glycerol 3-phosphate and that, in this way, the glycolytic rate could be a major factor controlling the rate of triglyceride synthesis (Fritz, 1961; Vaughan, 1961; Leboeuf, 1965). However, the total activity of L-glycerol 3-phosphate dehydrogenase in adipose tissue in these studies (15 units/g. wet wt. of tissue at 25°) is sufficient to account for 100 times the maximum flow through this step in tissue incubated at 37° (Denton & Halperin, 1968). Thus it seems probable that equilibrium is approached at this step and that the concentration of L-glycerol 3-phosphate is determined by the NADH/NAD⁺ concentration ratio in the cytoplasm and by the concentration of dihydroxyacetone phosphate. Neither of these two quantities is necessarily directly related to the rate of glycolysis. The NADH/NAD⁺ concentration ratio is likely to depend on the flux through dehydrogenases additional to those involved in glycolysis. The concentration of dihydroxyacetone phosphate [assuming aldolase (EC 4.1.2.13) is at equilibrium] will be affected by the state of activation of PFK, which apparently does not always parallel the rate of glycolysis in this tissue.

In Table 4, the changes in L-glycerol 3-phosphate concentration observed in the present studies are compared with changes in the lactate/pyruvate concentration ratio in the medium (taken as a measure of the change in the NADH/NAD⁺ concentration ratio in the cytoplasm) and with changes in the fructose 1,6-diphosphate concentration. Instability during freeze-drying made direct assay of dihydroxyacetone phosphate unreliable, but, if aldolase and triose phosphate isomerase (EC 5.3.1.1) are near equilibrium, then changes in the dihydroxyacetone phosphate concentration should be the square root of changes in the fructose 1,6-diphosphate concentration.

As expected, there is no clear correlation between the concentration of L-glycerol 3-phosphate and the rate of glycolysis. In only two (Expts. 1 and 3) of the six experiments shown in Table 4 were there parallel changes in glycolytic flux and L-glycerol 3-phosphate concentration. By using changes in the lactate/pyruvate concentration ratio in the medium as an indicator of changes in the cytoplasmic NADH/NAD⁺ concentration ratio and changes in the fructose 1,6-diphosphate concentration as an indicator of the square of changes in the dihydroxyacetone phosphate concentration, the L-glycerol 3-phosphate changes expected if L-glycerol 3-phosphate dehydrogenase were in equilibrium have been calculated. These are shown in Table 4. In all experiments (except perhaps Expt. 4) there is good correlation between the observed and the calculated changes in L-glycerol 3-phosphate concentration.

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