Regulation of fructose 2,6-bisphosphate concentration in white adipose tissue

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1. Injection of insulin to fed rats diminished the concentration of fructose 2,6-bisphosphate in white adipose tissue. Incubation of epididymal fat-pads or adipocytes with insulin stimulated lactate release and sugar detritiation and also decreased fructose 2,6-bisphosphate concentration. Such a decrease was, however, not observed in fat-pads from starved or alloxan-diabetic rats. 2. Incubation of adipocytes from fed rats with various concentrations of glucose or fructose led to a dose-dependent rise in fructose 2,6-bisphosphate which correlated with lactate output and detritiation of 3-³H-labelled sugar. 3. In adipocytes from fed rats, palmitate stimulated the detritiation of [3-³H]glucose without affecting lactate production and fructose 2,6-bisphosphate concentration. 4. Incubation of epididymal fat-pads from fed rats in the presence of antimycin stimulated lactate output but decreased fructose 2,6-bisphosphate concentration. 5. Changes in lipolytic rates brought about by noradrenaline, insulin, adenosine and corticotropin in adipocytes from fed rats were not related to changes in fructose 2,6-bisphosphate or to rates of lactate output. 6. In fed rats, the activity of 6-phosphofructo-2-kinase was not changed after treatment of adipocytes with insulin, noradrenaline or adenosine. It is suggested that the decrease in fructose 2,6-bisphosphate concentration observed after insulin treatment can be explained by the increase in *sn*-glycerol 3-phosphate, an inhibitor of 6-phosphofructo-2-kinase.

Fru-2,6- P_2 is the most potent stimulator of PFK-1, and its rôle in the regulation of glycolysis and gluconeogenesis in the liver has been extensively studied (for a review, see Hers & Hue, 1983). However, very little is known about its rôle in white adipose tissue. It is present in epididymal fat-pads (Hue *et al.*, 1982) at relatively low concentration (0.1 nmol/g). In rat mammary gland, its concentration rises about 3-fold from late pregnancy to reach 10 μ M at the height of lactation (Sochor *et al.*, 1984).

White-adipose-tissue glycolysis is known to be stimulated by adrenaline (Halperin & Denton, 1969), which induces changes in the kinetic properties of PFK-1, resulting in a small decrease in $V_{\max}$ and a severalfold increase in activity at subsaturating concentration of Fru-6- P (Sooranna

& Saggerson, 1982). This latter effect on the apparent K_m for Fru-6- P was suggested to depend on the association of some hydrophobic ligand with the enzyme. It has been suggested that the increase in fatty acid concentration brought about by noradrenaline-stimulated lipolysis is the explanation for the activation of PFK-1 (Lederer & Hers, 1984).

Insulin also stimulates glycolysis (Halperin & Denton, 1969; Saggerson & Greenbaum, 1970), and a small increase in the $V_{\max}$ of PFK-1 (Sooranna & Saggerson, 1982), as well as an activation of pyruvate kinase (Denton *et al.*, 1979), have been described.

The purpose of the present work was to study whether the changes in glycolytic flux that are observed under different conditions, such as various concentrations of substrates, hormonal treatment, nutritional status of the animal and anoxia, could indeed be explained in terms of changes in the concentration of Fru-2,6- P_2 . The effect of insulin on the kinetic properties of PFK-1 has also been studied.

Abbreviations used: PFK-1, 6-phosphofructo-1-kinase (EC 2.7.1.11); PFK-2, 6-phosphofructo-2-kinase (EC 2.7.1.-); Fru-1,6- P_2 , fructose 1,6-bisphosphate; Fru-2,6- P_2 , fructose 2,6-bisphosphate; Fru-6- P , fructose 6-phosphate; Glc-6- P , glucose 6-phosphate.

Materials and methods

Animals and materials

Fed male Wistar rats weighing 140–180 g were used except where otherwise stated. Pig insulin (Novo Industri, Copenhagen, Denmark), L-noradrenaline bitartrate, adenosine (Sigma, St. Louis, MO, U.S.A.), Dowex AG1-X8 (Bio-Rad, Richmond, CA, U.S.A.), Sephadex G-25 (fine grade) and Blue Sepharose CL-6B (Pharmacia, Uppsala, Sweden), DEAE-cellulose DE-52 (Whatman, Maidstone, Kent, U.K.) and [^3H]-glucose (Amersham, Bucks, U.K.) were obtained as indicated. Bovine albumin (fraction V, from Sigma) was defatted before use (Chen, 1967; Saggerson, 1972). Palmitic acid (from BDH, Poole, Dorset, U.K.) was neutralized with NaOH at 65°C and was then bound to albumin by adding the hot solution to a stirred solution of concentrated albumin, which was then diluted with Krebs–Henseleit (1932) buffer to a final concentration of 4% (w/v). Enzymes and biochemical reagents were either from Boehringer (Mannheim, Germany) or from Sigma. Glucose isomerase immobilized on polystyrene beads (which transformed about 1 μmol of glucose/min per mg of beads) was a gift from Mr. J. Pelgrims, Amylum, Aalst, Belgium. [^3H]Glucose (500 $\mu\text{Ci}/\mu\text{mol}$; 0.5 μmol) was transformed into [^3H]fructose in a total volume of 0.5 ml at pH 8 and 50°C in the presence of 10 mg of beads of immobilized glucose isomerase, which were previously washed in 50 mM-Hepes/0.8 mM-MgSO₄, pH 8. After 3 h of incubation, the radioactive fructose was separated from the radioactive glucose by a two-step procedure. Glucose was first converted enzymically into gluconate, which was removed on Dowex AG1-X8 (Cl⁻ form) (Hue *et al.*, 1978). The radioactive fructose was further purified by chromatography on Dowex AG1-X8 (borate form). The final yield was 20%, and the contamination by glucose in the final preparation was less than 0.4%.

Preparation and incubation of adipocytes

These were obtained (Rodbell, 1964; Saggerson & Tomassi, 1971) and incubated as described previously (Rider & Saggerson, 1983). The incubation flasks contained 0.39 ± 0.04 ($\pm$ S.E.M.; $n = 4$) ml of packed cells in 4 ml of incubation medium. At the end of the incubation the adipocytes were freeze-stopped with liquid N₂ (Rider & Saggerson, 1983) and the incubation media were deproteinized with HClO₄. For the measurement of sugar detritiation, the radioactive sugars (0.2–0.4 $\mu\text{Ci}/\text{ml}$) were added to the cell incubation and $^3\text{H}_2\text{O}$ was separated from the radioactive sugar in neutralized HClO₄ extracts of incubation media as described previously (Bon-

temps *et al.*, 1978). For the measurement of Fru-2,6- P_2 , the cells were deproteinized with NaOH as described previously (Van Schaftingen *et al.*, 1982).

Incubation of epididymal fat-pads

Paired fat-pads were incubated at 37°C, under an atmosphere of O₂/CO₂ (19:1), in 4 ml of Krebs–Henseleit (1932) bicarbonate buffer containing 1% (w/v) of albumin and other additions as indicated. After 30 min of incubation, the pads were frozen in liquid N₂ and the incubation media were deproteinized with HClO₄. Fru-2,6- P_2 was measured in NaOH-deproteinized cell extracts, and lactate and glycerol were measured in HClO₄-deproteinized extracts of the incubation media.

Measurement of enzyme activities

For the measurement of PFK-1, adipocytes were homogenized with an Ultra-Turrax disintegrator in 1 vol. of 50 mM-Hepes/100 mM-KCl/50 mM-KF/5 mM-dithioerythritol/0.1 mM-Fru-6- P /0.3 mM-Glc-6- P /50 μM -Fru-1,6- P_2 , pH 7.4 at 0°C. The extracts were centrifuged for 1 min in an Eppendorf Microfuge to remove fat, and filtered at 4°C through 20 vol. of Sephadex G-25 equilibrated in the same medium. The activity of PFK-1 was measured in the protein fraction. After 6 h in ice, 88% of the V_{max} activity was recovered, whereas only 40% was recovered when Fru-1,6- P_2 was omitted from the homogenization medium. The activity of PFK-1 was measured spectrophotometrically at 340 nm, pH 7.1 and 25°C in the presence of 50 mM-Hepes, 100 mM-KCl, 5 mM-MgCl₂, 1 mM-NH₄Cl, 0.2 mM-NADH, 5 mM-potassium phosphate, 0.1 mM-AMP, 0.5 unit of fructose-bisphosphate aldolase, 5 units of triosephosphate isomerase and 5 units of glycerol phosphate dehydrogenase (desalted by filtration through Sephadex). Other additions are indicated where appropriate. The activity is expressed as nmol of Fru-1,6- P_2 formed per unit of lactate dehydrogenase, which was measured as described by Saggerson (1974).

For the measurement of PFK-2, adipocytes were homogenized with an Ultra-Turrax homogenizer in 2 vol. of 50 mM-Hepes/20 mM-KF/1 mM-dithioerythritol/1 mM-EDTA/1 mM-phenylmethanesulphonyl fluoride, pH 8 at 0°C. The homogenate was centrifuged for 1 min in an Eppendorf Microfuge and the infranant was assayed immediately for PFK-2. The assay was performed at 30°C in a final volume of 0.1 ml containing 50 mM-Hepes, 100 mM-KCl, 20 mM-KF, 5 mM-potassium phosphate, pH 7.1, and 2 mM-Fru-6- P , 6 mM-Glc-6- P and 5 mM-MgATP, except where otherwise stated. After 10 min of incubation, the reaction was stopped by the addition of 0.1 ml of 50 mM-NaOH and the

mixture was kept at 80°C for 10 min. After neutralization with 25 mM-acetic acid/20 mM-Hepes to pH 8, Fru-2,6- P_2 was measured as described below.

Purification of PFK-2 from epididymal adipose tissue

Fat-pads were homogenized in 2 vol. (v/w) of 20 mM-Hepes / 50 mM-KCl / 5 mM-MgCl₂ / 2 mM-EDTA / 1 mM-dithioerythritol / 0.1 mM-Fru-6-P / 0.3 mM-Glc-6-P / 0.1 mM-phenylmethane-sulphonyl fluoride, pH 7.5, containing 10 mg of defatted/albumin/ml, at 4°C in a Waring Blender (4 × 15 s). The infranant obtained after centrifuging the homogenate (40 000 g for 30 min) was fractionated twice with poly(ethylene glycol) (*M*, 6000). The protein precipitated in the 6–15% (w/v) poly(ethylene glycol) fraction was resuspended in 20 mM-Hepes/50 mM-KCl/5 mM-MgCl₂/2 mM-EDTA/1 mM-dithioerythritol/20% (v/v) glycerol/0.1 mM-Fru-6-P/0.3 mM-Glc-6-P/0.1 mM-phenylmethane-sulphonyl fluoride, pH 7.5, and applied to a DEAE-cellulose column (2.5 cm × 15 cm). PFK-2 was eluted (at 0.25 M-KCl) with a linear gradient (100 ml) of Cl⁻ (0.05–0.5 M-KCl in the above buffer) and further purified on a Blue Sepharose column (1 cm × 20 cm). The enzyme was eluted (0.7 M-KCl) with a linear gradient (100 ml) of Cl⁻ (0.1–1 M-KCl in the above buffer) and had a specific activity of 4 nmol of Fru-2,6- P_2 formed/min per mg of protein. The enzyme was purified more than 350-fold with a recovery of 20%.

Analytical methods

Glycerol (Garland & Randle, 1962), lactate (Hohorst, 1963) and Fru-2,6- P_2 (Van Schaftingen *et al.*, 1982) were measured as indicated.

Results and discussion

The experiments reported below were carried out to investigate the relationship between the concentration of Fru-2,6- P_2 and the glycolytic flux. The latter was estimated by two different experimental approaches. The first method involved the measurement of lactate production; the differences in the concentration of glucose were too small to allow an accurate measurement of glucose consumption. Second, the production of ³H₂O from [3-³H]glucose or [3-³H]fructose was used. Since the detritiation of these sugars occurs at the level of triose phosphates and in the pentose phosphate pathway (Katz & Rognstad, 1976; Hue, 1981), the method therefore gives an estimation of the overall flux through glycolysis and the pentose phosphate pathway.

Effects of insulin on lactate production, sugar detritiation and on the concentration of metabolites

The effects of insulin on Fru-2,6- P_2 and the production of lactate were first studied in epididymal fat-pads from rats under various nutritional and hormonal conditions. Table 1 shows that, in agreement with published data (Halperin & Denton, 1969; Saggerson & Green-

Table 1. *Effects of insulin on lactate production and Fru-2,6- P_2 concentration in epididymal fat-pads*

Fat pads from fed, 48 h-starved, 48 h-starved and 12 h-re-fed, fed mature (250 g of body wt) and alloxan-diabetic (45 mg of alloxan intravenously 48 h before the experiment) rats were incubated for 30 min with or without 10 nM-insulin. Antimycin (20 μ M) was added to inhibit the mitochondrial respiratory chain in pads from fed rats. In the experiments with intact rats, anaesthetized (60 mg of Nembutal/kg body wt., intraperitoneally) fed rats were injected intravenously with saline (0.9% NaCl) or insulin (1 unit/kg), and fat-pads were freeze-clamped 15 min after the injection. Values shown are means $\pm$ S.E.M. for the numbers of observations shown in parentheses.

Rats	Addition of insulin	Fru-2,6- P_2 (nmol/g wet wt.)	Lactate production (μ mol/30 min per g wet wt.)
Fed (6)	—	0.30 $\pm$ 0.03	0.64 $\pm$ 0.06
	+	0.17 $\pm$ 0.01 ^a	0.87 $\pm$ 0.03 ^a
Starved (6)	—	0.23 $\pm$ 0.03	0.79 $\pm$ 0.10
	+	0.25 $\pm$ 0.03	1.97 $\pm$ 0.23 ^a
Re-fed (4)	—	0.57 $\pm$ 0.08	2.06 $\pm$ 0.43
	+	0.40 $\pm$ 0.06 ^a	2.62 $\pm$ 0.29
Mature (4)	—	0.18 $\pm$ 0.01	0.52 $\pm$ 0.08
	+	0.13 $\pm$ 0.02 ^a	0.89 $\pm$ 0.10 ^a
Fed + 20 μ M-antimycin (4)	—	0.14 $\pm$ 0.01	2.01 $\pm$ 0.24
	+	0.15 $\pm$ 0.01	3.29 $\pm$ 0.20 ^a
Diabetic (4)	—	0.20 $\pm$ 0.02	0.78 $\pm$ 0.17
	+	0.24 $\pm$ 0.03	1.23 $\pm$ 0.12 ^a
Intact (3)	—	0.46 $\pm$ 0.05	—
	+	0.19 $\pm$ 0.08 ^a	—

^a Statistically significant difference for the effect of insulin ($P < 0.05$).

baum, 1970), insulin stimulates lactate production, but, rather unexpectedly, the hormone decreases the concentration of Fru-2,6- P_2 in fat-pads from fed and starved/re-fed rats. Moreover, the effect of insulin to decrease the concentration of Fru-2,6- P_2 was also observed in fed rats *in vivo*. In fat-pads from starved and alloxan-diabetic rats, Fru-2,6- P_2 tended to increase (four experiments out of six for starved rats, and three out of four for diabetic rats), but the concentrations of Fru-2,6- P_2 after insulin treatment were not significantly different from the control values.

When epididymal fat-pads were incubated with antimycin to inhibit the respiratory-chain activity, lactate production was stimulated 3-fold and the concentration of Fru-2,6- P_2 was decreased by 50%. This effect of antimycin on both parameters is therefore very similar to that of anoxia in isolated hepatocytes (Hue, 1982).

The effects of insulin were further studied with adipocytes isolated from fed rats. As shown in Fig. 1, there was a dose-dependent increase in lactate and $^3\text{H}_2\text{O}$ release in adipocytes incubated with different concentrations of labelled glucose or fructose. Insulin induced a several-fold increase in rates of both lactate production and sugar detritiation, and a decrease in the apparent K_m for glucose, but not for fructose. On the other hand,

the dose-dependent increase in Fru-2,6- P_2 was less marked with insulin than without. In the absence of insulin, therefore, there is a relationship between Fru-2,6- P_2 and the flux; however, the stimulation of flux by insulin cannot be related to an increase in Fru-2,6- P_2 . These results also suggest that the action of insulin cannot be explained merely by its stimulation of glucose transport, since the stimulation of glucose transport by insulin is far greater than that for fructose (Froesch & Ginsberg, 1962; Halperin & Cheema-Dhadli, 1982). As noted above, the increase in sugar detritiation brought about by insulin might result not only from an increased glycolytic flux but also from an increased activity of the pentose phosphate pathway. An increase in the glycolytic flux is evidenced by the enhanced lactate production, and there is ample evidence in the literature for a stimulation of the pentose phosphate pathway by insulin in white adipose tissue (see, e.g., Flatt & Ball, 1964). However, the data presented do not allow the quantification of the relative contributions of these two pathways to the overall detritiation.

The time course of the insulin effect is illustrated in Fig. 2. Palmitate was added to mimic the stimulation of lipoprotein lipase by insulin (Cryer, 1981). The stimulation of the flux by insulin is

Fig. 1. Effect of insulin on Fru-2,6- P_2 concentration, lactate production and sugar detritiation in adipocytes incubated with various concentrations of [$3\text{-}^3\text{H}$]glucose or [$3\text{-}^3\text{H}$]fructose

The concentration of Fru-2,6- P_2 ($\circ$, $\bullet$), the production of lactate ($\triangle$, $\blacktriangle$) and the rate of detritiation of sugar ($\square$, $\blacksquare$) were measured in adipocytes incubated for 30 min with ($\bullet$, $\blacktriangle$, $\blacksquare$) or without ($\circ$, $\triangle$, $\square$) 10 nM-insulin. Values shown are means $\pm$ S.E.M. for three or four separate experiments. Where not shown, the bars lie within the symbols. (*) indicates a statistically significant ($P < 0.05$) effect of insulin compared with the corresponding control.

Fig. 2. Time-course of the effect of insulin on Fru-2,6- P_2 concentration, lactate production and [3- 3 H]glucose detritiation in adipocytes incubated with or without 2mM-palmitate

The concentration of Fru-2,6- P_2 (○, ●), the production of lactate (△, ▲) and the rate of detritiation of 5mM-[3- 3 H]glucose (□, ■) were measured in adipocytes incubated with (●, ▲, ■) or without (○, △, □) 10nM-insulin. Palmitate was bound to albumin. The results are means $\pm$ s.e.m. for three separate experiments. Where not shown, the bars lie within the symbols. (*) indicates a statistically significant ($P < 0.05$) effect of insulin compared with the corresponding control.

clearly a short-term effect, since it is detectable at 10min and lasted for 60min. Palmitate significantly increased the release of $^3\text{H}_2\text{O}$; however, in the absence of insulin, it did not change lactate production. In the presence of insulin, palmitate caused a further stimulation of both lactate and $^3\text{H}_2\text{O}$ release, indicating that the effects of insulin and palmitate were additive. The rise in Fru-2,6- P_2 , which was observed under basal conditions, was prevented by insulin.

The effect of insulin on the concentration of some glycolytic intermediates was also studied. In agreement with published data (Halperin & Denton, 1969; Saggerson & Greenbaum, 1970), insulin increased the concentrations of Glc-6-P, Fru-6-P, 6-phosphogluconate and *sn*-glycerol 3-phosphate 2–3-fold (results not shown). Moreover, in agreement with the results of Claus *et al* (1982) obtained with hepatic PFK-1, *sn*-glycerol 3-phosphate also inhibits PFK-1 activity (K_i about 0.3mM) measured in filtered extracts of epididymal fat-pads or adipocytes (results not shown). We have been unable to confirm the results of Somercorn & Freedland (1982), who showed that 6-phosphogluconate is a positive effector of PFK-1 in the liver. We found that 6-phosphogluconate had no effect on PFK-1 activity in filtered extracts prepared from either liver or fat-pads (results not

shown). Since the concentration of Fru-2,6- P_2 decreases and that of *sn*-glycerol 3-phosphate increases after insulin treatment [15.5 ± 1.5 ($n = 3$) to 28.3 ± 7.7 ($n = 3$) nmol/g after insulin], it is difficult to envisage that the increase in Fru-6-P would be sufficient to account for an actual stimulation of PFK-1 activity in the cell.

Sensitivity of PFK-1 to Fru-2,6- P_2

Fig. 3 shows the sensitivity of adipocyte PFK-1 to Fru-2,6- P_2 under 'physiological' concentrations of substrates and cofactors. Under these conditions, the K_a was $4\mu\text{M}$; when measured in a filtered extract of whole epididymal fat-pads, the K_a was 6-fold lower. Fig. 3 also confirms the results of Sooranna & Saggerson (1982), namely that insulin increases the V_{max} of the enzyme. This effect of insulin may involve a stable change in PFK-1 activity, since the effect persisted for at least 2h after gel filtration. It is not clear whether this increase in V_{max} would be sufficient to compensate for the changes in concentration of regulatory ligands (increase in *sn*-glycerol 3-phosphate and decrease in Fru-2,6- P_2 after insulin), which should lead to an inhibition of PFK-1. This may suggest that some hitherto unknown factor(s) is involved in the activation of PFK-1 by insulin.

Effects of insulin, noradrenaline, adenosine and corticotropin on lactate production, sugar detritiation, lipolysis and the concentration of Fru-2,6-P₂

The effects of noradrenaline and adenosine were compared with those of insulin in adipocytes incubated with either [3-³H]glucose (Table 2) or [3-³H]fructose (Table 3) as substrate. In agreement with the results shown above, insulin stimulated lactate and ³H₂O release, and the absolute rates were about twice as high with glucose as with fructose. In this series of experiments, which were performed in Spring, noradrenaline had no effect on lactate production or ³H₂O release; however, it stimulated lipolysis, as measured by the release of glycerol. A similar result was obtained with 30 nM-corticotropin (results not shown). In another set of experiments, carried out in Autumn, noradrenaline increased the release of ³H₂O from glucose by about 40% (Table 4). However, we do not know whether there is a causal relationship between the time of the year and the results observed. It is also apparent that the various rates of lipolysis brought about by noradrenaline, adenosine, insulin and combinations of these, or by corticotropin, are not related to lactate production or sugar detritiation. This indicates that increased lipolysis and fatty acid production do not necessarily result in a stimulation of lactate production. This finding is in disagreement with the suggestion by Lederer & Hers (1984), namely that the enhanced production of fatty acids brought about by adrenaline stimulates PFK-1 activity and hence glycolysis.

Table 2 and 3 also show that noradrenaline amplifies the insulin effect on ³H₂O release from glucose, but with fructose a decrease in lactate production is observed; however, with either

Fig. 3. Stimulation by Fru-2,6-P₂ of PFK-1 activity from control and insulin-treated adipocytes

Adipocytes were incubated with or without 100 nM-insulin for 30 min. Extracts prepared from freeze-stopped cells were filtered through 20 vol. of Sephadex G-25 and assayed for PFK-1 with 0.1 mM-Fru-6-P, 0.3 mM-Glc-6-P and 5 mM-MgATP.

Table 2. Effect of different agents on the production of lactate and glycerol, detritiation of [3-³H]glucose and the concentration of Fru-2,6-P₂ in adipocytes from fed rats

Adipocytes were incubated for 30 min in the presence of 5 mM-[3-³H]glucose. Values shown are means ± S.E.M. for four separate experiments.

Addition	Glucose detritiation (μmol/30 min per incubation flask)	Lactate production (μmol/30 min per incubation flask)	Glycerol production (μmol/30 min per incubation flask)	Fru-2,6-P ₂ (pmol/incubation flask)
None	0.20 ± 0.03	0.49 ± 0.02	0.21 ± 0.05	12.0 ± 1.5
10 nM-insulin	0.35 ± 0.08 ^a	0.79 ± 0.06 ^a	0.23 ± 0.05	7.8 ± 0.7 ^a
1 μM-noradrenaline	0.22 ± 0.03	0.38 ± 0.04	2.90 ± 0.27 ^a	12.3 ± 1.2
10 μM-adenosine	0.19 ± 0.03	0.42 ± 0.06	0.37 ± 0.05	14.8 ± 0.4
10 nM-insulin + 10 μM-adenosine	0.44 ± 0.12	0.97 ± 0.17 ^d	0.21 ± 0.02	9.6 ± 0.7 ^d
10 nM-insulin + 1 μM-noradrenaline	0.59 ± 0.11 ^{b,c}	0.87 ± 0.05 ^{b,c}	0.87 ± 0.17 ^{b,c}	6.4 ± 0.6 ^c
1 μM-noradrenaline + 10 μM-adenosine	0.28 ± 0.05 ^d	0.44 ± 0.02	2.26 ± 0.30 ^d	13.7 ± 2.6
10 nM-insulin + 10 μM-adenosine + 1 μM-noradrenaline	0.61 ± 0.13 ^{c,f}	0.94 ± 0.11 ^f	0.89 ± 0.20 ^f	6.9 ± 0.4 ^c

^a *P* < 0.05 versus no additions.

^b *P* < 0.05 versus insulin alone.

^c *P* < 0.05 versus noradrenaline alone.

^d *P* < 0.05 versus adenosine alone.

^e *P* < 0.05 versus insulin + adenosine.

^f *P* < 0.05 versus noradrenaline + adenosine.

Table 3. *Effect of different agents on the production of lactate and glycerol, detritiation of [3-³H] fructose and the concentration of Fru-2,6-P₂ in adipocytes from fed rats*

Adipocytes were incubated for 30 min in the presence of 5 mM-[3-³H]fructose. Values shown are means $\pm$ S.E.M. for four separate experiments.

Addition	Fructose detritiation (μ mol/30 min per incubation flask)	Lactate production (μ mol/30 min per incubation flask)	Glycerol production (μ mol/30 min per incubation flask)	Fru-2,6-P ₂ (pmol/incubation flask)
None	0.12 $\pm$ 0.03	0.29 $\pm$ 0.02	0.20 $\pm$ 0.01	7.7 $\pm$ 0.8
10 nM-insulin	0.22 $\pm$ 0.05 ^a	0.37 $\pm$ 0.03 ^a	0.20 $\pm$ 0.04	6.0 $\pm$ 0.5
1 μ M-noradrenaline	0.09 $\pm$ 0.01	0.22 $\pm$ 0.02	2.71 $\pm$ 0.21 ^a	10.0 $\pm$ 0.4 ^a
10 μ M-adenosine	0.10 $\pm$ 0.02	0.28 $\pm$ 0.03	0.40 $\pm$ 0.10	9.7 $\pm$ 0.7
10 nM-insulin + 10 μ M-adenosine	0.20 $\pm$ 0.02 ^d	0.39 $\pm$ 0.04 ^d	0.18 $\pm$ 0.03	8.2 $\pm$ 0.8
10 nM-insulin + 1 μ M-noradrenaline	0.18 $\pm$ 0.03 ^c	0.27 $\pm$ 0.03 ^{b,c}	0.92 $\pm$ 0.26 ^c	7.4 $\pm$ 1.1 ^c
1 μ M-noradrenaline + 10 μ M-adenosine	0.10 $\pm$ 0.01	0.24 $\pm$ 0.03	1.66 $\pm$ 0.21 ^d	9.3 $\pm$ 0.9
10 nM-insulin + 10 μ M-adenosine + 1 μ M-noradrenaline	0.18 $\pm$ 0.03 ^e	0.30 $\pm$ 0.02	1.02 $\pm$ 0.32 ^e	9.0 $\pm$ 0.8

^a $P < 0.05$ versus no additions.

^b $P < 0.05$ versus insulin alone.

^c $P < 0.05$ versus noradrenaline alone.

^d $P < 0.05$ versus adenosine alone.

^e $P < 0.05$ versus noradrenaline + adenosine.

Table 4. *Effect of insulin and noradrenaline on the detritiation of glucose and on the concentration of Fru-2,6-P₂ in adipocytes from fed rats*

Adipocytes were incubated for 30 min in the presence of 5 mM-[3-³H]glucose. Values shown are means $\pm$ S.E.M. for three separate experiments.

Addition	Glucose detritiation (μ mol/30 min per incubation flask)	Fru-2,6-P ₂ (pmol/incubation flask)
None	0.19 $\pm$ 0.01	8.1 $\pm$ 0.8
10 nM-insulin	0.34 $\pm$ 0.04	5.3 $\pm$ 0.8 ^a
1 μ M-noradrenaline	0.27 $\pm$ 0.01 ^a	7.4 $\pm$ 0.2
10 nM-insulin + 1 μ M-noradrenaline	0.47 $\pm$ 0.05	4.5 $\pm$ 0.2 ^b

^a $P < 0.05$ versus no addition.

^b $P < 0.05$ versus noradrenaline.

substrate, insulin could antagonize the stimulation of lipolysis by noradrenaline. One could speculate that noradrenaline acts in synergism with insulin on the transport of glucose, but not of fructose. With glucose as substrate, the ability of insulin to decrease the concentration of Fru-2,6-P₂ was also observed in the presence of adenosine or noradrenaline. Adenosine alone had no effect; however, it amplified the stimulation by insulin and noradrenaline of ³H₂O release from glucose, but not from fructose. It should also be noted that an increase in ³H₂O release was not always in parallel with an increase in lactate production. This may indicate that, as noted above, ³H₂O release is not a good index of the glycolytic flux, or that some glycolytic intermediates are consumed. This might be the case when *sn*-glycerol 3-phosphate is provided for the esterification of fatty acids.

Activity of PFK-2

PFK-2 was measured in homogenates prepared from adipocytes that had been incubated with insulin, noradrenaline or adenosine, and freeze-stopped. These agents had no effect on either the apparent K_m for Fru-2,6-P₂ or the V_{max} .

The kinetic properties of the partially purified enzyme were also measured. At pH 7.1 the apparent K_m values for Fru-6-P and MgATP were 30 and 600 μ M respectively. As described for the liver enzyme (Claus *et al.*, 1982), *sn*-glycerol 3-phosphate inhibited the activity of PFK-2: 1 mM-*sn*-glycerol 3-phosphate increased the K_m for Fru-2,6-P₂ to 60 μ M without changing the K_m for ATP, and decreased the V_{max} by 30%.

Conclusions

The general conclusion that emerges from this study is that the stimulation of glycolysis by insulin

and, when observed, by noradrenaline is not related to Fru-2,6- P_2 concentration. This conclusion should, however, be restricted to fed animals, since the decrease in Fru-2,6- P_2 that is observed after insulin treatment in fed rats is not observed when white adipose tissue from starved or diabetic rats is treated with insulin. Moreover, under basal conditions, i.e. without hormones, the stimulation of glycolysis by substrate concentration might be related to an increase in Fru-2,6- P_2 . If one assumes that the intracellular water space of adipocytes is similar to that of epididymal fat-pads and represents about 3% of the cell volume (Denton *et al.*, 1966; Ballard & Hanson, 1969; Saggerson & Greenbaum, 1970), the concentration of Fru-2,6- P_2 would be 1–2 μM , a concentration range at which PFK-1 is very sensitive to the stimulator.

The mechanism by which insulin exerts a stimulation of glycolysis is multiple: it involves a stimulation of glucose transport (Crofford & Renold, 1965), an increase in V_{max} of PFK-1 (Sooranna & Saggerson, 1982), a stimulation of pyruvate kinase (Denton *et al.*, 1979), and an effect on hexokinase has not been ruled out. The reason for this apparently excessive control of the pathway is not evident. This type of multiple control is also found in the regulation of lipogenesis; it should indeed be remembered that insulin controls the activity of pyruvate dehydrogenase and acetyl-CoA carboxylase.

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