Interrelationship and Control of Glucose Metabolism and Lipogenesis in Isolated Fat-Cells

EFFECT OF THE AMOUNT OF GLUCOSE UPTAKE ON THE RATES OF THE PENTOSE PHOSPHATE CYCLE AND OF FATTY ACID SYNTHESIS

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In order to study the quantitative relationship between fatty acid synthesis and pentose phosphate-cycle activity under different hormonal and dietary conditions affecting the extent of glucose uptake, cells isolated from rat epididymal adipose tissue were incubated in bicarbonate buffer containing [U- ^{14}C]-, [1- ^{14}C]- or [6- ^{14}C]-glucose. From the amount of glucose taken up, the production of lactate and pyruvate, and the incorporation of ^{14}C from differently labelled [^{14}C]glucose into CO_2 , fatty acids and glyceride glycerol, the rates of glucose metabolism via different pathways and the extent of lipogenesis under various experimental conditions were determined. The contribution of the pentose phosphate-cycle to glucose metabolism under normal conditions was calculated to be 8%. Starvation and re-feeding, and the presence of insulin, caused an enhancement of glucose uptake, pentose phosphate-cycle activity and fatty acid synthesis. Plots of both pentose phosphate-cycle activity and fatty acid synthesis versus glucose uptake revealed that the extent of glucose uptake, over a wide range, determines the rates of fatty acid synthesis and glucose metabolism via the pentose phosphate cycle. A balance of formation and production of nicotinamide nucleotides in the cytoplasm was established. The total amount of cytoplasmic NADH and NADPH formed was only in slight excess over the hydrogen equivalents required for the synthesis of fatty acids, glyceride glycerol and lactate. Except in cells from starved animals, the pentose phosphate cycle was found to provide only about 60% of the NADPH required for fatty acid synthesis. The results are discussed with respect to an overall control of the different metabolic and biosynthetic reactions in the fat-cells by the amount of glucose transported into the cell.

Adipose tissue has been shown to be a suitable tissue in which to investigate the interrelationship between the pentose phosphate cycle, glycolysis and fatty acid synthesis (Winegrad & Renold, 1958; Flatt & Ball, 1964; Katz *et al.*, 1966; Katz & Rognstad, 1966, 1969; Flatt, 1970; Saggerson & Greenbaum, 1970). A balance of production and utilization of cytoplasmic reducing equivalents revealed that the pentose phosphate cycle in this tissue is not able to provide all the NADPH required for stimulated fatty acid synthesis (Flatt & Ball, 1964; Katz *et al.*, 1966).

The total amount of reduced nicotinamide nucleotides produced in the cytoplasm, however, was found to be sufficient for all the reactions in this compartment depending on reduced pyridine nucleotides (Rognstad & Katz, 1966).

Since Rodbell (1964) introduced a method for the isolation of fat-cells from rat epididymal adipose tissue a considerable amount of information has been accumulated (for reviews see Levine & Pfeiffer,

1970) about glucose and lipid metabolism in these cells. No extensive studies, however, have been reported about the quantitative interrelationship between glucose uptake, pentose phosphate-cycle activity and fatty acid synthesis. To study further the relationship between these metabolic and biosynthetic parameters experiments were carried out with isolated fat-cells. Special attention has been paid to the correlation of pentose phosphate-cycle activity and lipogenesis and their dependence on glucose uptake over a wide range of metabolic activity in these cells.

Glucose metabolism via the glycolytic and the pentose phosphate pathways and the extent of fatty acid synthesis were evaluated by incubating the cells with D-glucose labelled with ^{14}C at different positions and measuring the labelling pattern of various products. The ratios of radioactivity incorporated into CO_2 , fatty acids and glyceride glycerol from [1- ^{14}C]-, [6- ^{14}C]- and [U- ^{14}C]-glucose were used for the estimation of the fluxes and rates of synthesis.

Materials and Methods

Treatment of animals

Male albino rats of a Sprague-Dawley strain (Süd-deutsche Versuchstierfarm, Tuttlingen, Germany), 150–200 g in weight, were used. Control animals were maintained on a standard pellet chow (Altromin). Starved animals were starved for 48 h; starved re-fed rats were re-fed for another 48 h after the same starvation time with bread, and 15% (w/v) glucose in the drinking water.

Cell preparation

Rats were killed by decapitation. The fat-cells were isolated essentially as described by Rodbell (1964), except that glucose was omitted from the medium during the collagenase treatment and washing procedures. The Krebs-Henseleit bicarbonate buffer, pH 7.4 (Krebs & Henseleit, 1932), containing 2% bovine serum albumin, was gassed during handling with $O_2 + CO_2$ (95:5). The pH was controlled, and readjusted if necessary, before the cells were suspended to a final concentration of 15–40 μ mol of triglyceride content/ml. Plastic equipment was used throughout the cell preparation procedure.

Incubation

The cells were incubated at 37°C in stoppered plastic scintillation vials under $O_2 + CO_2$ (95:5) for 120 min. The incubation was started by addition of [$1-^{14}C$]-, [$6-^{14}C$]- or [$U-^{14}C$]-glucose (5 μ mol, 2 μ Ci). When present, 1 unit of insulin was added. The final volume was 2.5 ml. The incubation was stopped by injecting 0.3 ml of 4 M-HClO₄ into the medium. The $^{14}CO_2$ was trapped by 0.2 ml of phenethylamine injected into a small plastic cup hanging from the rubber stopper.

Analytical procedures

The acidified incubation mixtures were transferred to Millipore filters (pore size 0.45 μ m) and filtered by suction. The insoluble residues were washed several times with 0.4 M-HClO₄. The acid filtrates plus washings were neutralized with 2 M-KOH and assayed enzymically for glucose, lactate, pyruvate and free glycerol by using hexokinase and glucose 6-phosphate dehydrogenase, lactate dehydrogenase and glycerol kinase and glycerol 7-phosphate dehydrogenase. The insoluble residues were extracted with chloroform-methanol (2:1, v/v) and the crude lipid extracts were purified as described by Folch *et al.* (1957).

Samples were evaporated to dryness, dissolved in the same volume of ethanol and counted for radioactivity. Portions (5 ml) of the ethanolic lipid extract

were saponified with 0.5 M-KOH in ethanol for 90 min at 80°C. Samples were used for isolation of fatty acids and glyceride glycerol as described by Zoellner & Eberhagen (1965), and for enzymic determination of triglyceride content by the method of Eggstein & Kreutz (1966). DNA content was determined by the method of Burton (1956). Glycogen was assayed enzymically by the method of Keppler & Decker (1970). To measure ^{14}C incorporation, glycogen was extracted with hot KOH, precipitated with 66% (v/v) ethanol and freed from lipids by extraction with hot ethanol. After dialysis overnight glycogen was hydrolysed with 2 M-H₂SO₄ and counted for radioactivity. The ^{14}C radioactivity was measured in a Tracerlab liquid-scintillation counter model LSC-40 in Bray's (1960) solvent. Quenching corrections were made with an external standard. Values obtained from non-incubated fat-cells in the same medium were used as background for all measurements.

Chemicals

Enzymes and coenzymes were purchased from C.F. Boehringer und Soehne G.m.b.H., Mannheim, Germany; highly purified bovine insulin (25.5 units/mg) was a gift of Farbwerke Hoechst A.G., Frankfurt, Germany. Bovine serum albumin was obtained from Behring-Werke, Marburg, Germany. Collagenase was from Worthington Biochemical Corp., Freehold, N.J., U.S.A. [$1-^{14}C$]-, [$6-^{14}C$]- and [$U-^{14}C$]-glucose were from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals were of analytical reagent grade.

Calculations

The contribution of the pentose phosphate cycle to glucose metabolism was estimated by using four different methods described by Wood *et al.* (1963) and by Katz *et al.* (1966) (methods A, B, C and D).

Net synthesis of fatty acids, glyceride glycerol and CO_2 was calculated from the incorporation of ^{14}C from D-[$U-^{14}C$]glucose and the known specific radioactivity. No correction was made for dilution of the label by endogenous pools. All relative metabolic and biosynthetic rates given in this paper are based on the amount of glucose recovered in the products CO_2 , lactate, pyruvate, glyceride glycerol and fatty acids (Table 1). The balance of production and utilization of cytoplasmic reducing equivalents was constructed by using the calculations given by Rognstad & Katz (1966).

Results

The effects of diet and insulin on glucose utilization, lactate and pyruvate production, and on the incorporation of ^{14}C from glucose (labelled in various

Table 1. *Effect of diet and insulin on glucose uptake and utilization in isolated fat-cells*

The amount of [¹⁴C]glucose incorporated into fatty acids, glyceride glycerol and CO₂ was calculated from the specific radioactivities used. The values for [1-¹⁴C]- and [6-¹⁴C]-glucose are expressed in terms of nmol of glucose, as if the amount of radioactivity measured was derived from [U-¹⁴C]glucose. The recovery was calculated from lactate and pyruvate production as determined enzymically, and from the incorporation of [U-¹⁴C]glucose into fatty acids, glyceride glycerol and CO₂. Experimental and assay conditions are described in the Materials and Methods section.

Diet and hormone	No. of expts.	Metabolism (nmol/2h per 100 μmol of triglyceride)			Glucose label	[¹⁴ C]Glucose incorporated (nmol/2h per 100 μmol of triglyceride)				[¹⁴ C]Glucose recovered (%)		
		Glucose utilization	Lactate production	Pyruvate production		Fatty acids	Glyceride glycerol		CO ₂			
Control	6	1200 (1140–1290)	640 (560–730)	125 (123–127)	1 6 U	157 211 152	(121–191) (142–292) (90–182)	221 216 243	(189–263) (168–214) (219–257)	273 121 281	(245–324) (96–141) (191–324)	88 (80–94)
Insulin (1 unit)	4	3360 (3230–3480)	2230 (2180–2290)	480 (440–510)	1 6 U	737 1147 753	(626–848) (1014–1280) (752–755)	362 380 452	(340–380) (350–410) (390–510)	853 118 678	(839–868) (103–132) (650–706)	96 (93–98)
48 h starved	8	1070 (840–1420)	925 (630–1120)	200 (170–230)	1 6 U	10 13 11	(5–20) (8–22) (6–21)	227 175 216	(190–320) (143–245) (166–292)	78 49 79	(43–126) (33–73) (29–147)	81 (76–90)
48 h starved + 48 h re-fed	8	1480 (1040–1880)	648 (225–1050)	82 (48–116)	1 6 U	301 463 286	(151–427) (251–698) (172–375)	144 150 151	(83–198) (91–214) (82–201)	385 89 348	(248–575) (35–131) (247–497)	78 (77–82)
Starved-re-fed + insulin (1 unit)	4	3810 (3460–4170)	1490 (840–2130)	197 (160–230)	1 6 U	1066 1918 1107	(1036–1097) (1823–2012) (1107–1111)	245 263 281	(224–266) (234–291) (281–311)	1190 95 871	(1127–1272) (79–111) (871–911)	80

Table 2. *Percentage contribution of pentose phosphate cycle to glucose metabolism in isolated fat-cells as affected by diet and insulin*

Values were calculated by the four methods described by Katz *et al.* (1966). A, From specific yield in $^{14}\text{CO}_2$ from $[1-^{14}\text{C}]$ glucose and $[6-^{14}\text{C}]$ glucose; B, from the ratio in fatty acids from $[1-^{14}\text{C}]$ - and $[6-^{14}\text{C}]$ -glucose (γ); C, from the ratio in fatty acids (γ) and lipid glycerol (δ) from $[1-^{14}\text{C}]$ - and $[6-^{14}\text{C}]$ -glucose; D, from the $^{14}\text{CO}_2$ specific yields and γ . $\bar{X}$ is the mean value.

Conditions	A	B	C	D	$\bar{X}$
Control	6.1	10.3	7.9	6.5	7.7
Insulin (1 unit)	9.5	15.6	14.1	9.6	12.2
48 h starved	1.3	—	—	1.6	1.5
48 h starved+	11.4	16.2	13.2	11.7	13.1
48 h re-fed					
Starved-re-fed+	16.6	21	19.3	16.7	18.4
insulin (1 unit)

Fig. 1. *Relative interrelationship between the synthesis of fatty acids (a) and production of lactate plus glyceride glycerol (b) and the activity of the pentose phosphate cycle in isolated fat-cells*

Mean values from four to eight experiments are plotted. Values are expressed in % of glucose recovered in lactate, pyruvate, triglyceride and CO_2 . ■, Starved; □, control; △, insulin (1 unit); ○, 48 h starved and re-fed; ▲, starved-re-fed and insulin (1 unit). For experimental details see the text.

positions) into fatty acids, glyceride glycerol and CO_2 , are shown in Table 1. Both starvation re-feeding and insulin stimulated glucose utilization and the incorporation of ^{14}C into fatty acids but not into glyceride glycerol. Similar changes in $^{14}\text{CO}_2$ release from $[1-^{14}\text{C}]$ glucose were observed, whereas $^{14}\text{CO}_2$ release from $[6-^{14}\text{C}]$ glucose remained almost constant under all experimental conditions, indicating a pronounced increase in pentose phosphate-cycle activity without any influence on tricarboxylic acid-cycle activity. As well as increasing pentose phosphate-cycle activity, insulin markedly stimulated ^{14}C incorporation from ^{14}C -labelled glucose into fatty acids. The actual net synthesis of fatty acids is represented by ^{14}C incorporation from $[\text{U}-^{14}\text{C}]$ -glucose. In contrast, starvation resulted in a decrease of both fatty acid synthesis and $^{14}\text{CO}_2$ production from differently labelled glucose. Compared with the control, lactate formation was increased in the presence of insulin and to a lesser extent also in cells from starved animals. Pyruvate production was stimulated by insulin and decreased in cells from starved-re-fed animals.

The recovery of utilized glucose in products listed in Table 1, representing the percentage of glucose catabolized via glycolysis and the pentose phosphate cycle, is given in the last column. Since in starvation and also starvation and re-feeding, the recovery was only about 80%, control experiments were performed to determine the amount of glucose converted into glycogen. Glycogen content was extremely low and showed no pronounced changes during the incubation with glucose, and incorporation of $[^{14}\text{C}]$ glucose was less than 1% of utilized glucose.

Table 2 shows the calculation of the percentage contribution of the pentose phosphate cycle to glucose metabolism, by four methods as described by Katz *et al.* (1966). The calculations are based on the amount of glucose recovered in products. The values obtained by the different methods are in reasonably good agreement. As indicated by the mean values in

the last column, insulin, as well as starvation and re-feeding, stimulates glucose breakdown via the pentose phosphate cycle, whereas starvation causes a drastic decrease.

In Fig. 1 the percentage of glucose converted into fatty acids (a) and into lactate and glyceride glycerol (b) is plotted versus the percentage contribution of pentose phosphate cycle to glucose metabolism. There is a linear increase of fatty acid synthesis with increasing pentose phosphate-cycle activity, accompanied by an almost linear decrease of lactate and glyceride glycerol production regardless of the dietary and hormonal state.

In Fig. 2 NADPH production in the pentose phosphate cycle (a) and NADPH utilization for fatty

acid synthesis (b) under the various experimental conditions are plotted versus glucose uptake. Fig. 2 illustrates the following points: (1) more NADPH is required in isolated fat-cells for fatty acid synthesis than is produced by the pentose phosphate cycle (see also Table 4); (2) the rates of both production and utilization of NADPH are largely dependent on glucose uptake over a wide range of glucose uptake in the experimental cases studied.

Discussion

The purpose of this work was to study the quantitative relationship of pentose phosphate-cycle activity and fatty acid synthesis under different

Fig. 2. NADPH production through the pentose phosphate cycle (a) and NADPH utilization for fatty acid synthesis (b) related to glucose uptake in isolated fat-cells

Individual experimental values are given in μmol/2h per 100 μmol of triglyceride. □, Control; △, insulin (1 unit); o, 48h starved and re-fed; ▲, starved-re-fed and insulin (1 unit). For experimental conditions and calculations see the text and the legend to Table 4.

Table 3. Effect of diet and insulin on the distribution of glucose carbon in products formed by isolated fat-cells as compared with rat adipose tissue

The results for adipose tissue were taken from Katz *et al.* (1966).

Conditions	Source	Relative amount of glucose carbon utilized	Glucose carbon converted (%) into			
			Total carbon dioxide	Fatty acids	Glyceride glycerol	Lactate + pyruvate
Fed <i>ad libitum</i> , no hormone	Adipose tissue	100	29	21	20	30
	Fat-cells	100	27	14	23	36
Starved-re-fed + insulin	Adipose tissue	770	38	45	5	12
	Fat-cells	290	28	36	9	27

hormonal and dietary conditions affecting the extent of glucose uptake in isolated fat-cells.

Since similar experimental conditions and the same calculations for pentose phosphate-cycle activity were used as described by Katz *et al.* (1966), our results obtained in isolated fat-cells are comparable with their findings in adipose tissue.

Table 3 shows a comparison between the distributions of glucose carbon under two different conditions in products formed by isolated fat-cells from rat epididymal tissue and by rat adipose tissue. The relative amount of lactate and pyruvate production is higher in isolated cells than in the tissue, whereas the relative rates of fatty acid synthesis and total CO₂ production are lower. Moreover, glucose uptake in fat-cells from starved re-fed animals and the influence of insulin were less pronounced than in the experiments of Katz *et al.* (1966).

With respect to incorporation of ¹⁴C from [1-¹⁴C]- and [6-¹⁴C]-glucose into CO₂, fatty acids and glyceride glycerol, our results are in good agreement with those of Ho & Jeanrenaud (1967), who studied hormone action on glucose and lipid metabolism in isolated fat-cells. Values for the pentose phosphate-cycle activity given by these authors are about twice as high as those calculated from our results, obtained under comparable experimental conditions.

This discrepancy is due to the different reference values used to calculate specific yields. Whereas our calculations are based on the amount of glucose recovered in the products, CO₂, triglycerides, lactate and pyruvate, Ho & Jeanrenaud (1967) consider only the sum of CO₂ and lipids. When our results are calculated on their basis, values in the same range (about 13% in the controls) are obtained. Since the amount of glucose taken up is not given, net synthesis of fatty acids from glucose cannot be calculated from their results.

Fig. 1 shows that over a wide range of metabolic states the rate of fatty acid synthesis and the formation of lactate and glyceride glycerol are inversely related if plotted versus pentose phosphate-cycle activity. From this it follows that a high rate of fatty acid synthesis is coupled with a high pentose phosphate-cycle activity on one hand, and a low rate of lactate and glyceride glycerol production on the other hand, or vice versa. Because of this mutual and strong correlation presented in Fig. 1 the metabolic situation of the cells can be predicted rather accurately if one of the three parameters is known.

Table 4 shows that production and utilization of cytoplasmic reducing equivalents is balanced under all experimental conditions. The cytoplasmic hydrogen equivalents (NADPH and NADH) exceed only slightly the hydrogen required for reduction, indicating that all hydrogen equivalents are produced and utilized in the same compartment. Therefore net transfer of reducing equivalents from the cytosol into

Table 4. *Balance of non-mitochondrial formation and utilization of reduced nicotinamide nucleotides in isolated fat-cells*

Values are expressed in nmol/2h per 100 μ mol of triglyceride. The balance is based on the following considerations. Utilization of reducing equivalents: for the formation of 1 μ mol of glyceride glycerol or lactate 1 μ mol of NADH is required. For the reduction of 1 μ mol of acetyl-CoA to an ethylene group, 2 μ mol of NADPH are required. On the basis of determinations by g.l.c. an average chain length of fatty acids of 17 carbon atoms was used for calculations. Therefore 0.875 μ mol of NADPH is required for 1 μ g-atom of carbon of newly synthesized fatty acids. Production of reducing equivalents: 1 μ mol of non-mitochondrial NADH is formed for every μ mol of glyceraldehyde 3-phosphate oxidized to pyruvate. This equals twice the amount of glucose recovered in products minus half the amount of glucose catabolized via the pentose phosphate cycle minus glyceride glycerol production. For each μ mol of glucose metabolized via the pentose phosphate cycle 3 μ mol of CO₂ and concomitantly 6 μ mol of NADPH are formed.

Conditions	NADH production (glyceraldehyde 3-phosphate dehydrogenase)		NADPH production (pentose phosphate cycle)		NADH utilization		Total reducing equivalents utilized		Utilization %
					Lactate formation	Glyceride glycerol formation	equivalents produced	equivalents utilized	
Control	1544		487		642	486	2031	1926	95
Insulin (1 unit)	5153		2361		2235	904	7514	7110	95
48 h starved	1280		111		925	398	1391	1381	99
48 h starved + 48 h re-fed	1834		907		648	302	2741	2451	90
Starved-re-fed + insulin (1 unit)	4978		3346		1490	562	8324	7863	95

mitochondria, or vice versa, is not necessary. This result is consistent with that reported by Katz *et al.* (1966) and Rognstad & Katz (1966).

The contribution of the pentose phosphate cycle to glucose metabolism in isolated fat-cells is correlated with fatty acid synthesis via NADPH. Although enough reduced nicotinamide nucleotides for all metabolic and synthetic reactions were formed in the cytoplasm (Table 4), the pentose phosphate cycle in our experiments also under normal conditions provided only about 60% of the NADPH required for fatty acid synthesis (Table 4, Fig. 2). For stimulated lipogenesis our results are consistent with the findings of Flatt & Ball (1964) and Katz *et al.* (1966) in adipose tissue. Only when lipogenesis was extremely low (cells from starved rats) did the NADPH yield from this pathway apparently cover the requirement. From our findings one has to assume that other reactions contribute to the formation of NADPH, e.g. the malate-pyruvate cycle as proposed by Lowenstein (1961). Evidence for the existence of an operating citrate shuttle supplying acetyl-CoA for fatty acid synthesis and oxaloacetate for this pathway has been obtained by Martin & Denton (1971) in studies with fat-cell mitochondria.

Fig. 2 shows a linear relationship between the rates of NADPH production via the pentose phosphate cycle or NADPH utilization for fatty acid synthesis and the rate of glucose uptake over a wide range. Since dietary treatment can alter the triglyceride content of the cells the values in Fig. 2 were also related to the DNA content. In this case a similar linear relationship between glucose uptake and pentose phosphate-cycle activity or fatty acid synthesis was obtained. Because of this strong correlation it can be assumed that glucose transport rather than intracellular control mechanisms of the different metabolic pathways determines the overall metabolism and the biosynthetic rates in fat-cells. This concept is supported by the observations of Rodbell (1966), Lavis & Williams (1970) and Crofford *et al.* (1970) that a number of agents and treatments (trypsin, phospholipase, thiol reagents, ouabain, glucose concentration in the medium) that enhance glucose transport show insulin-like effects on glucose and lipid metabolism in fat-cells. It should be emphasized, however, that changes of fatty acid synthesis rate in the presence of dibutyl cyclic 3':5'-AMP (Schimmel & Goodman, 1971) or of lipolytic hormones (Cahill *et al.*, 1960; Leboeuf & Cahill, 1961; Denton & Randle, 1967) do not correspond with changes in glucose uptake.

The relationship between pentose phosphate-cycle activity or fatty acid synthesis and glucose uptake also does not hold for cells from starved rats. In this case lower values for pentose phosphate-cycle activity and fatty acid synthesis were obtained than should be expected from Fig. 2 regardless of

whether these values were related to triglyceride or DNA content. The results reported by Wieland *et al.* (1971) about the effect of starvation on pyruvate dehydrogenase interconversion might provide an explanation for this deviation. These authors observed a marked decrease of the active form of rat heart pyruvate dehydrogenase in starved animals, leading to a diminished production of acetyl-CoA, thus limiting fatty acid synthesis. Nakanishi & Numa (1970) have shown that the concentration of acetyl-CoA carboxylase was markedly decreased in liver extracts from rats starved for 48 h. Since this enzyme plays a key role in the control of fatty acid synthesis (Numa *et al.*, 1970) the lower rate of fatty acid synthesis might also be due to the decrease of acetyl-CoA carboxylase activity. Because the pentose phosphate-cycle activity in cells from starved animals was also decreased at the same time, one might assume that the rate of glucose conversion via this pathway is determined by the requirement for NADPH in these cells for lipid synthesis.

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