The Role of the Cytoplasmic Redox Potential in the Control of Fatty Acid Synthesis from Glucose, Pyruvate and Lactate in White Adipose Tissue

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The metabolism of lactate, pyruvate and glucose was studied in epididymal adipose tissue of starved, normally fed and starved–re-fed rats. Lactate conversion into fatty acid occurred at an appreciable rate only in the adipocyte of starved–re-fed animals. *NNN'*-Tetramethyl-*p*-phenylenediamine, an agent that transports reducing power from the cytoplasm to the mitochondria, caused large increments of fatty acid synthesis from lactate and a smaller one from glucose but a decrease in that from pyruvate. Glucose (1.0mM) increased fatty acid synthesis from lactate 4.3-fold but only 1.67-fold from pyruvate in adipocytes from normally fed animals. 2-Deoxyglucose decreased fatty acid synthesis from lactate to a greater degree (threefold) compared to that from pyruvate in adipocytes from starved–re-fed animals. L-Glycerol 3-phosphate contents were approximately equal in epididymal fat-pads, incubated in the presence of lactate or pyruvate, from normally fed animals, whereas the addition of 1mM-glucose resulted in a tenfold increase in L-glycerol 3-phosphate content only in the presence of lactate. The L-glycerol 3-phosphate content was tenfold higher in adipose tissue from starved–re-fed animals incubated in the presence of lactate than in the presence of pyruvate. 2-Deoxyglucose caused these values to be slightly lowered in the presence of lactate. We suggest that lactate metabolism is limited by the rate of NADH removal from the cytoplasm. In the starved–re-fed state, this occurs by reduction of dihydroxyacetone phosphate formed from glycogen to produce L-glycerol 3-phosphate, thus permitting lactate conversion into fatty acid. When glucose is the substrate, and rates of transport are not limiting, the rate of removal of cytoplasmic NADH limits glucose conversion into fatty acid.

Rat adipose tissue when incubated in a bicarbonate medium is capable of converting glucose, pyruvate and (to a much smaller extent) lactate into triglycerides (Kneer & Ball, 1968). The conversion of glucose in physiological concentrations proceeds readily only in the presence of insulin, presumably owing to the impermeability of the cell membrane to glucose in the absence of insulin (Winegrad & Renold, 1958; Flatt & Ball, 1964). Pyruvate, however, is converted readily into triglyceride in both the presence and absence of insulin (Winegrad & Renold, 1958) and is therefore able to supply the necessary NADPH and L-glycerol 3-phosphate required for triglyceride synthesis. Lactate in the starved or normally fed rat is a very poor substrate relative to pyruvate for triglyceride synthesis, but in the starved–re-fed rat it is as good as pyruvate (Reshef, Hanson & Ballard, 1969; Schmidt & Katz, 1969).

The present studies were undertaken to investigate these differences between lactate and pyruvate. We suggest that in the starved and normally fed rats

lactate uptake and metabolism is limited by the rate of removal of cytoplasmic NADH, as the rate of transfer of reducing power into the mitochondrion of adipose tissue proceeds only at a low rate (Robinson & Halperin, 1970). In the starved–re-fed rats the metabolism of adipose-tissue glycogen resulted in the production of L-glycerol 3-phosphate, thereby re-oxidizing the cytoplasmic NADH; this would then allow lactate to be as potent a precursor for fatty acid synthesis as pyruvate.

MATERIALS

Rats. These were as described in the preceding paper (Robinson & Halperin, 1970).

Chemicals. Enzymes, glycolytic intermediates, adenine nucleotides, sodium pyruvate and triethanolamine were obtained from Boehringer Corp., New York, N.Y., U.S.A. 2-Deoxyglucose, bovine serum albumin (fraction V) and insulin were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. ³H-labelled water was obtained from New England Nuclear Corp., Boston, Mass., U.S.A. Sodium

[2-¹⁴C]pyruvate, L-[U-¹⁴C]lactic acid and D-[U-¹⁴C]-glucose were obtained from Calbiochem, Los Angeles, Calif., U.S.A. AnalaR DL-lactic acid and TMPD* were obtained from British Drug Houses Ltd., Poole, Dorset, U.K.; the DL-lactic acid was neutralized with NaOH and analysed spectrophotometrically with lactate dehydrogenase before use.

Media. Fat-pads were incubated in a Krebs bicarbonate-buffered medium (Krebs & Henseleit, 1932) gassed with O₂ + CO₂ (95:5) in a volume of 2.5 ml/fat-pad. The concentrations of various additions are given in the text or tables.

METHODS

Incubation of epididymal fat-pads. Epididymal fat-pads were prepared and incubated after a 30 min preincubation period as described by Denton & Randle (1967).

Extraction and analysis of fat-pads. At the end of the incubation, pads were blotted lightly and frozen in liquid N₂. A portion of the pads was extracted in 25 ml of chloroform-methanol (2:1, v/v) and assayed for ¹⁴C in glyceride glycerol and fatty acids or ³H in fatty acids as described by Denton & Randle (1967). The remainder of the frozen pad was extracted with ice-cold 5% (w/v) HClO₄ and peroxide-free diethyl ether as described by Denton & Halperin (1968).

Analysis of the incubation media and tissue. Glucose, pyruvate and lactate uptake, pyruvate, lactate and gly-

cerol output, and tissue ATP and L-glycerol 3-phosphate were measured as described by Denton & Halperin (1968) and Halperin & Denton (1969).

Radioactivity assay. ¹⁴C- and ³H-labelled compounds were assayed in a Nuclear-Chicago liquid-scintillation system (Mark I model). For aqueous solutions the scintillator was dioxan containing naphthalene, 2,5-diphenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene (Butler, 1961). For solutions in *n*-heptane the scintillator was toluene containing 2,5-phenyloxazole and 1,4-bis-(5-phenyloxazol-2-yl)benzene (Synder, 1961). Quenching corrections were calculated from the channels-ratio method by using an external standard of ¹³³Ba.

RESULTS

The rates of fatty acid synthesis and glyceride glycerol formation from glucose, lactate or pyruvate as substrate are shown in Table 1. Although fatty acid synthesis did occur with lactate (5 mM) as substrate in normally fed rats the rate was much lower than with pyruvate or glucose. Increasing the lactate concentration to 100 mM did not increase the rate of fatty acid synthesis.

The influence of the nutritional state of the animal on fatty acid synthesis from pyruvate, lactate and glucose was examined (Table 2). With pyruvate as substrate, the rate of fatty acid synthesis in the starved animals was considerably less than in the fed animals, whereas in the starved-re-fed animals

* Abbreviation: TMPD, *NNN'*-tetramethyl-*p*-phenylenediamine.

Table 1. Comparison of substrates for fatty acid and glyceride glycerol synthesis in epididymal fat pads of normally fed rats

Fat-pads were preincubated for 30 min in medium containing the substrates as indicated below plus insulin (10 m-units/ml) and then transferred into similar medium containing in addition either ¹⁴C-labelled substrate or ³H-labelled water (0.2 μCi/ml). The pads were paired directly in Expts. 1-3, and in Expts. 4-6 the pads at both the lowest and highest concentrations of substrates were paired with pads incubated at the intermediate substrate concentration. Results are expressed as means ± S.E.M. for paired observations. * *P* < 0.01.

Expt. no.	No. of observations	Substrate	Concn. (mM)	Isotope	Rate (μg-atoms incorporated/h per g wet wt.)	
					Fatty acid synthesis	Glyceride glycerol synthesis
1	4	Glucose	10	¹⁴ C	14.9 ± 1.3	3.4 ± 0.2
	4	Lactate	5	¹⁴ C	3.2 ± 0.7*	0.2 ± 0.01*
2	4	Glucose	10	¹⁴ C	19.0 ± 3.8	3.5 ± 0.2
	4	Pyruvate	5	¹⁴ C	21.9 ± 4.0	2.0 ± 0.2*
3	4	Pyruvate	5	¹⁴ C	12.0 ± 1.4	1.5 ± 0.2
	4	Lactate	5	¹⁴ C	3.0 ± 0.5*	0.4 ± 0.1*
4	4	Pyruvate	2	³ H	15.4 ± 2.4*	—
	8		5	³ H	23.6 ± 2.8	—
	4		15	³ H	22.0 ± 3.6	—
5	4	Lactate	5	³ H	4.0 ± 0.8	—
	8		10	³ H	3.6 ± 0.7	—
	4		100	³ H	3.8 ± 0.8	—
6	4	Glucose	5	³ H	36.8 ± 4.2	—
	8		10	³ H	34.4 ± 3.3	—
	4		15	³ H	38.8 ± 3.7	—

Table 2. *Effect of nutritional state on fatty acid synthesis in adipose tissue*

Details of incubation were as given in Table 1. Results are the means of four sets of paired observations $\pm$ S.E.M. * $P < 0.01$.

Expt. no.	Substrate	Concn. (mm)	Rate of fatty acid synthesis (μ g-atoms of ^3H incorporated/h per g)		
			Starved	Normally fed	Starved-re-fed
1	Glucose	10	8.2 ± 2.1	19.6 ± 2.8	51.1 ± 7.2
	Pyruvate	10	$2.2 \pm 0.54^*$	23.8 ± 4.0	67.8 ± 10.4
2	Pyruvate	10	4.8 ± 0.62	24.8 ± 2.8	40.6 ± 3.4
	Lactate	10	$0.2 \pm 0.04^*$	$3.8 \pm 0.6^*$	32.0 ± 1.8
3	Glucose	10	6.4 ± 1.0	21.4 ± 3.2	48.2 ± 4.1
	Lactate	10	$0.1 \pm 0.02^*$	$6.2 \pm 0.9^*$	46.8 ± 5.2

Table 3. *Effect of TMPD on the rate of fatty acid synthesis, adipose-tissue ATP content and the outputs of lactate and pyruvate in normally fed rats*

Details of incubation were as given in Table 1. Results are means of four sets of paired observations $\pm$ S.E.M. * $P < 0.01$ for appropriate control.

Expt. no.	Substrate	TMPD (50 μ M)	ATP content (nmol/g wet wt.)	Output (μ g-atoms of C/h per g)		Lactate/pyruvate output ratio	Rate of fatty acid synthesis (μ g-atoms of ^3H incorporated/h per g)
				Lactate	Pyruvate		
1	Glucose (10 mm)	—	100 ± 8.1	1.71 ± 0.2	0.57 ± 0.2	3.13 ± 0.12	30.8 ± 2.1
		+	104 ± 9.2	1.41 ± 0.3	$1.08 \pm 0.3^*$	$1.31 \pm 0.27^*$	$37.0 \pm 2.0^*$
2	Pyruvate (5 mm)	—	102 ± 10.0	—	—	—	24.0 ± 4.8
		+	110 ± 8.1	—	—	—	$19.8 \pm 3.6^*$
3	Lactate (5 mm)	—	108 ± 16.0	—	1.26 ± 0.2	—	4.6 ± 1.4
		+	116 ± 10.3	—	$2.64 \pm 0.1^*$	—	$9.8 \pm 3.2^*$

the rate was approximately doubled compared with that in the fed state. With lactate as substrate there was virtually no fatty acid synthesis in the starved state and, as reported in Table 1, very little in the fed state. In the starved-re-fed animals lactate was almost as effective as pyruvate as a source of carbon for fatty acid synthesis. Glucose was a much better precursor than pyruvate for fatty acid synthesis in the starved state, but not in the fed animals.

To demonstrate that lactate even at very high concentrations (100 mm) did not inhibit fatty acid synthesis, lactate was added to fat-pads incubated in the presence of glucose plus insulin. The addition of 100 mm-lactate to 10 mm-glucose plus 10 m-units of insulin/ml increased fatty acid synthesis from 37.2 ± 2.0 to 48.8 ± 2.4 ($P < 0.01$) μ g-atoms of ^3H incorporated/h per g wet wt.

In the absence of TMPD neither NADH nor NADPH is oxidized by adipose-tissue mitochondria; however, NADH (but not NADPH) is oxidized at a rapid rate in the presence of 50 μ M- and 100 μ M-TMPD (Robinson & Halperin, 1970). In Table 3, results of experiments with epididymal fat-pads in

which TMPD was added to the incubation medium with either pyruvate, lactate or glucose as substrate are reported. To confirm that TMPD acts in the whole tissue in a manner similar to that with isolated mitochondria, the lactate and pyruvate outputs were measured. TMPD caused the lactate/pyruvate output ratio to decrease from 3.13 ± 0.12 to 1.31 ± 0.27 ($P < 0.01$) when glucose was substrate, suggesting that the cytoplasmic $[\text{NADH}]/[\text{NAD}^+]$ ratio decreases. With glucose as substrate TMPD resulted in a small but statistically significant ($P < 0.01$) increase in fatty acid synthesis. With pyruvate as substrate TMPD resulted in a small but statistically significant ($P < 0.01$) decrease in the rate of fatty acid synthesis, and in Expt. 3, with lactate as substrate, TMPD resulted in a twofold increase in the rate of fatty acid synthesis, as well as a significant ($P < 0.01$) increase in pyruvate output. Under all these experimental conditions the tissue content of ATP was maintained at the normal values.

In order to study the mechanism whereby starved-re-fed rats were able to synthesize fatty acid from lactate, the role of the contribution of glycogen in

Table 4. *Effect of 2-deoxyglucose and glucose on the rate of fatty acid synthesis, glycerol output and the tissue content of L-glycerol 3-phosphate in the epididymal adipose tissue of the starved-re-fed rat*

Details of incubation were as given in Table 1. Results are expressed as the means $\pm$ S.E.M. for eight paired observations. * $P < 0.01$; ** $P < 0.05$.

Expt. no.	Substrate	Addition	Nutritional state	Rate of fatty acid synthesis (μ g-atoms of ^3H incorporated/h per g)	Glycerol output (μ mol/h per g)	L-glycerol 3-phosphate content (nmol/g)
1	Pyruvate (15 mM)	—	Starved-re-fed	37.9 $\pm$ 6.4	0.75 $\pm$ 0.09	7.1 $\pm$ 1.01
		2-Deoxyglucose (10 mM)		29.7 $\pm$ 3.5*	0.57 $\pm$ 0.08	8.1 $\pm$ 0.96
2	Lactate (15 mM)	—	Starved-re-fed	34.6 $\pm$ 3.2	0.59 $\pm$ 0.05	129.0 $\pm$ 12.0
		2-Deoxyglucose (10 mM)		16.4 $\pm$ 1.0*	0.46 $\pm$ 0.04**	97.0 $\pm$ 7.0**
3	Pyruvate (5 mM)	Insulin (10 m-units/ml)	Normally fed	25.1 $\pm$ 2.4	0.34 $\pm$ 0.06	10.4 $\pm$ 1.8
		Insulin (10 m-units/ml) + glucose (1 mM)		41.5 $\pm$ 4.8	0.36 $\pm$ 0.05	9.7 $\pm$ 2.8
4	Lactate (5 mM)	Insulin (10 m-units/ml)	Normally fed	5.8 $\pm$ 1.6	0.45 $\pm$ 0.03	14.8 $\pm$ 4.0
		Insulin (10 m-units/ml) + glucose (1 mM)		22.3 $\pm$ 1.8*	0.74 $\pm$ 0.07*	120 $\pm$ 14.9*

Table 5. *Lactate and pyruvate outputs from epididymal adipose tissue in starved-re-fed, starved and normally fed rats*

Glucose (10 mM) was the substrate in all the experiments. Incubation conditions were as outlined in Table 1. Results are expressed as the means $\pm$ S.E.M. for four paired observations. All preincubations were carried out in the absence of insulin. * $P < 0.01$ for corresponding control value.

Nutritional state	Insulin (10 m-units/ml)	Output (μ g-atoms of carbon/h per g wet wt.)		
		Lactate	Pyruvate	Lactate/pyruvate output ratio
Starved-re-fed	—	6.36 $\pm$ 0.81	0.92 $\pm$ 0.07	8.92 $\pm$ 2.61
	+	25.4 $\pm$ 6.30*	1.89 $\pm$ 0.13*	13.3 $\pm$ 0.94
Normally fed	—	1.68 $\pm$ 0.16	0.42 $\pm$ 0.03	3.99 $\pm$ 0.28
	+	3.54 $\pm$ 0.36*	0.58 $\pm$ 0.04*	6.07 $\pm$ 0.17*
Starved	—	5.22 $\pm$ 0.32	0.79 $\pm$ 0.07	6.72 $\pm$ 0.64
	+	13.4 $\pm$ 1.51*	2.43 $\pm$ 0.45*	5.62 $\pm$ 0.81

these tissues was studied. The addition of 1 mM-glucose to fat-pads of normally fed rats with lactate as substrate resulted in a large increase (4.3-fold) in the rate of fatty acid synthesis from lactate (Expt. 4, Table 4). The total glucose uptake in this experiment was $8.0 \pm 0.4 \mu$ g-atoms of glucose carbon/h per g wet wt. of tissue in both the presence and absence of lactate. If all this glucose were converted into fatty acid it could only account for 33% of the rate of fatty acid synthesis observed. Further, the rate of fatty acid synthesis from 1.0 mM-glucose alone as substrate (not reported in this table) ($6.1 \pm 0.7 \mu$ g-atoms of ^3H /h per g) plus that from lactate alone ($5.8 \pm 1.6 \mu$ g-atoms of ^3H /h per g) was about half that seen when both glucose and lactate were added to the incubation medium ($22.3 \pm 1.8 \mu$ g-atoms of ^3H /h per g). In a similar experiment with pyruvate as substrate (Expt. 3, Table 4) the added glucose resulted in a

smaller increase (1.7-fold) in the rate of fatty acid synthesis.

Glycerol outputs from fat-pads of normally fed animals were $0.41 \pm 0.04 \mu$ mol/h per g wet wt. of tissue with either pyruvate (5 mM) or lactate (5 mM) as substrate. The addition of 1 mM-glucose did not increase glycerol output in the presence of pyruvate whereas in the presence of lactate glycerol output was increased to $0.74 \pm 0.07 \mu$ mol/h per g wet wt. ($n = 4$, $P < 0.01$). In pads from starved-re-fed animals 2-deoxyglucose resulted in a small but significant fall in glycerol output when lactate (15 mM) was present in the medium (0.59 ± 0.05 to 0.46 ± 0.04 ; $P < 0.05$).

If one could, in the starved-re-fed animals, remove the effect of the large tissue storage of glycogen (39 mg/g wet wt.) which might be analogous to the added glucose in the experiments with normally fed

animals described above, one should find a much greater inhibition of the rate of fatty acid synthesis from lactate as compared with pyruvate. This was attempted by adding 10mM-2-deoxyglucose to the incubation medium containing fat-pads of starved-re-fed animals, with either lactate or pyruvate as substrate. The results are presented in Expts. 1 and 2 in Table 4. 2-Deoxyglucose resulted in a 3.0-fold greater fall in the rate of fatty acid synthesis with lactate as substrate as compared with pyruvate.

The addition of insulin to the incubation medium did not result in any significant change in the rate of fatty acid synthesis from pyruvate or lactate in the normally fed or starved-re-fed rats (results not shown).

The outputs of lactate and pyruvate from epididymal adipose tissue were measured in the starved, normally fed and starved-re-fed rats in the presence and absence of insulin (Table 5). Glucose was the substrate in all experiments. The [lactate]/[pyruvate] ratio was significantly lower than 6 only in the normally fed animal in the absence of insulin.

DISCUSSION

The rate of fatty acid synthesis in all the experiments reported in the present paper was measured with paired rat epididymal fat-pads to minimize the differences between animals. Calculation of absolute rates therefore resulted in larger standard errors than would be found if relative rates of fatty acid synthesis were reported (i.e. percentage changes of the control rate).

The results in Table 1 indicate that glucose and pyruvate serve equally well as precursors of fatty acid synthesis in rat epididymal adipose tissue in the normally fed rat, whereas lactate is a poor precursor.

The only pathway for lactate metabolism is the conversion of lactate into pyruvate catalysed by lactate dehydrogenase, with resultant conversion of NAD^+ into NADH. The pyruvate once formed should undergo the same metabolic fate as pyruvate that had been added to the incubation media. Therefore the fact that lactate is a poor precursor for fatty acid synthesis suggests that the limiting factor may be the rate of removal of NADH from the cytoplasm or possibly the rate of lactate transport into the cell.

Fourteen molecules of NADPH are required for the synthesis of palmitate from acetyl-CoA. Reducing power generated in adipose tissue mitochondria when pyruvate is oxidized could be transported into the cytoplasm via L-malate (Halperin, Robinson, Martin & Denton, 1969), and transferred to NADP^+ by the 'malic' enzyme (EC 1.1.1.38). In addition, reducing equivalents of cytoplasmic NADH could be transferred to NADP^+ by the malate dehydrogenase (EC 1.1.1.37)–'malic' enzyme pathway

(Kornacker & Ball, 1965). When lactate is metabolized, a significant proportion is not converted into fatty acid, but is either released as pyruvate into the medium or oxidized to carbon dioxide: both these pathways would result in NADH accumulation in the cytoplasm and this accumulation might limit further flow through the lactate dehydrogenase reaction.

TMPD was added to the incubation medium when lactate, pyruvate or glucose was the substrate for fatty acid synthesis. When lactate was the substrate TMPD resulted in a twofold increase in the rate of fatty acid synthesis, presumably by catalysing the transfer of cytoplasmic NADH into the mitochondria. When pyruvate was substrate TMPD decreased the rate of fatty acid synthesis and this could be explained by a decrease in the quantity of NADPH generated. When glucose was substrate for fatty acid synthesis, TMPD resulted in a small but significant increase in the rate of fatty acid synthesis, suggesting that the $[\text{NADH}]/[\text{NAD}^+]$ ratio might be important in the conversion of glucose into cytoplasmic acetyl-CoA and fatty acid synthesis. This is in agreement with earlier suggestions by Flatt & Ball (1964, 1966), Rognstad & Katz (1966), and Schmidt & Katz (1969).

Lactate can be as good a source of carbon atoms for fatty acid synthesis in the starved-re-fed animals as pyruvate or glucose. For this to happen the NADH generated in the cytoplasm from lactate must be removed and also there must be a significant synthesis of L-glycerol 3-phosphate, the precursor of glyceride glycerol. This may be rendered possible by the availability of glycogen, which is present in appreciable amounts (39mg/g wet wt.) in the adipose tissue of the starved-re-fed rat (Frerichs & Ball, 1962). Since Jungas & Ball (1964) demonstrated high basal lipolytic rates in the absence of insulin in starved-re-fed adipose tissue and that free fatty acids do not accumulate then the rate of L-glycerol 3-phosphate utilization must be high.

To investigate the role of glycogen, 2-deoxyglucose was added to the incubation medium (Table 4). The accumulation of intracellular 2-deoxyglucose 6-phosphate should inhibit the phosphohexose isomerase reaction competitively with respect to glucose 6-phosphate (Wick, Drury, Nakada & Wolfe, 1957) and thereby limit the conversion of glycogen into dihydroxyacetone phosphate.

2-Deoxyglucose inhibited fatty acid synthesis from lactate far more than that from pyruvate; this result is consistent with the conclusion that, in the starved-re-fed rats, lactate is able to be a significant precursor for fatty acid synthesis because the NADH generated when pyruvate is formed from lactate is reoxidized by dihydroxyacetone phosphate formed from glycogen. In the normally fed rat lactate may be a poor precursor of glyceride glycerol as there is a

very limited quantity of adipose-tissue glycogen (0.25mg/g wet wt.) to supply dihydroxyacetone phosphate (Gutman & Shafir, 1963).

Both in the adipose tissue from starved-re-fed animals incubated in the presence of lactate and in the epididymal fat-pad from normally fed animals incubated in the presence of lactate plus 1mM-glucose the content of L-glycerol 3-phosphate was much higher than in the corresponding condition when pyruvate was present instead of lactate (Table 4). The glycerol release was significantly greater from those pads where the L-glycerol 3-phosphate content was very high ($>0.1 \mu\text{mol/g}$ wet wt.). High L-glycerol 3-phosphate contents may favour fatty acid synthesis from lactate by acting either as an acyl acceptor (triglyceride synthesis) or having reoxidized cytoplasmic NADH. Robinson & Halperin (1970) have demonstrated that L-glycerol 3-phosphate is oxidized (very slowly) by mitochondria from the adipose tissue of starved-re-fed but not of normally fed rats. This would result in NADH transport from the cytoplasm into the mitochondria and would facilitate lactate utilization.

The results reported in the present paper are in close agreement with those recently reported by Schmidt & Katz (1969), i.e. that the rate of removal of cytoplasmic NADH limits lactate utilization in white adipose tissue and, in the adipocyte of normally fed or starved animals, there is very little transfer of hydrogen equivalents from cytoplasm into mitochondria. The conclusions differ somewhat in the adipocyte of the starved-re-fed rat as there is some transfer of reducing power into the mitochondria via the L-glycerol 3-phosphate pathway (Robinson & Halperin, 1969) and there is a glycogen-dependent cytoplasmic NADH oxidation as outlined above.

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