

Alternative substrates for triacylglycerol synthesis in isolated adipocytes of different size from the rat

Albert A. FRANZENDESE and Mario DIGIROLAMO

Division of Endocrinology, Department of Medicine, Emory University School of Medicine,
Atlanta, GA 30303, U.S.A.

(Received 9 April 1980/Accepted 20 June 1980)

The metabolic utilization of ^{14}C -labelled acetate, pyruvate, lactate and glucose by isolated epididymal fat-cells was compared in two groups of rats fed *ad libitum*, one group young and lean (150–200 g body wt.), the other older and spontaneously obese (500–650 g body wt.). The influence of unlabelled glucose (6 mM) and insulin on substrate utilization by adipocytes was also studied. (1) Pyruvate and lactate were found to be good precursors for fatty-acid synthesis in small fat-cells, but not in larger fat-cells. On the other hand, lactate conversion into CO_2 and the glycerol moiety of acylglycerols proceeded actively in both types of cells, and in some cases, it even exceeded the rates of glucose utilization. (2) The addition of glucose or glucose plus insulin, but not insulin alone, enhanced the metabolism of acetate, pyruvate and lactate in both types of fat-cells. (3) Fatty-acid synthesis *de novo* in large fat-cells was markedly decreased regardless of the substrate utilized. These findings point to lactate as a significant precursor for triacylglycerol synthesis in adipocytes. Furthermore, decreased fatty-acid synthesis *de novo* appears to be an acquired metabolic deficiency of enlarging adipocytes, independent of precursor substrate availability.

Recent studies in rats fed *ad libitum* have shown that changes in adipocyte glucose metabolism and insulin response take place concomitantly with fat-cell enlargement. The most important changes observed are a decrease in lipogenesis (fatty-acid synthesis), an increase in the conversion of glucose into the glycerol moiety of acylglycerols and a marked decrease in responsiveness of the cells to the effect of insulin on the acceleration of glucose metabolism (Gliemann, 1965; Salans & Dougherty, 1971; DiGirolamo *et al.*, 1974; Olefsky, 1977). Adipocytes from spontaneously obese animals also exhibit a greater rate of basal lipolysis (DiGirolamo & Owens, 1976). From studies *in vitro* on the metabolic behaviour of adipocytes, it is hard to reconcile these two observations (increased basal lipolysis and decreased lipogenesis from glucose) with a preserved or even increasing adipocyte size and lipid content in animals that continue to become more obese with age and feeding *ad libitum* (Hirsch & Han, 1969; DiGirolamo & Mendlinger, 1971). An obvious explanation for these findings is that the conditions *in vitro* do not fully reflect the contribution to lipid storage by circulating lipids in the form of unesterified fatty acids and lipoprotein-bound

triacylglycerols. Another possible explanation may reside in a shift in metabolic substrate preference from glucose to some other substrate(s), which would be used by the enlarging adipocytes for fatty-acid and triacylglycerol synthesis. Such shifts are not unknown in other species (Ballard *et al.*, 1969).

The present study explores the possibility that substrates other than glucose may be important to the overall triacylglycerol synthesis and storage activities of the maturing adipocyte.

Materials and methods

Animals, hormones and reagents

Male Wistar rats (from Royallhart Laboratory Animals, New Hampton, NY, U.S.A.) were allowed access *ad libitum* to Purina rat chow and water from weaning until the time of death. They were housed in individual cages in a temperature-controlled room (22°C) and exposed to light–dark intervals of 12 h (07:00 h to 19:00 h). Rats of 6–8 weeks of age (150–200 g body wt.) and 8–12 months (500–650 g) were killed in the fed state by cervical dislocation between 10:00 h and 11:30 h on the day of the experiment.

Crystalline pork insulin (monocomponent; 25.4 units/mg) was a gift of Eli Lilly and Co., Indianapolis, IN, U.S.A. D-Glucose (anhydrous) was from Mallinckrodt Chemical Works (St. Louis, MO, U.S.A.). Bovine serum albumin (lot no. 107C-0307), DL-lactic acid (sodium salt, 60% syrup), pyruvic acid (sodium salt, crystalline type II) and sodium acetate (crystalline trihydrate) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). [U-¹⁴C]Glucose (sp. radioactivity 5 Ci/mol), [2-¹⁴C]pyruvic acid (sodium salt, sp. radioactivity 40 Ci/mol) and [2-¹⁴C]acetate (sodium salt, sp. radioactivity 2 Ci/mol) were purchased from New England Nuclear Corp. (Boston, MA, U.S.A.); DL-[2-¹⁴C]lactic acid (sodium salt, sp. radioactivity 2 Ci/mol) was from American Radiochemical Corp. (Sanford, FL, U.S.A.). Collagenase type I (132 units/mg) was from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.).

Cell preparation, incubation procedures and analytical techniques

For each experiment, epididymal adipose tissue from nine small rats or one large rat was excised, cut into small pieces (20–50 mg) and incubated (Rodbell, 1964) for 1 h at 37°C with 30 mg of collagenase dissolved in 15 ml of Krebs–Ringer bicarbonate (KRB) buffer containing 4% albumin at pH 7.4. After a passage through a 149 μm nylon mesh screen to remove undigested tissue, the isolated cells were washed four times with fresh medium without collagenase, re-suspended and transferred in 1 ml portions to polyethylene liquid-scintillation counting vials. Each incubation vial contained (per ml): 0.5–1.25 μCi of [U-¹⁴C]glucose or 0.50 μCi of sodium [2-¹⁴C]lactate, [2-¹⁴C]pyruvate or sodium [2-¹⁴C]acetate; 40 mg of albumin; unlabelled analogous substrate to reach a concentration of 6 mM; and (where indicated) additions of glucose (6 mM) and/or insulin (10 munits/ml). All substrates were prepared immediately before use in KRB buffer and the medium pH was adjusted to 7.4. Measurements of pH performed before and after 1 h or incubation under the described conditions showed no significant change from pH 7.4.

The incubation vials were individually gassed with CO₂/O₂ (1:19), capped with a rubber stopper fitted with a plastic hanging well (Kontes Glass Co., Vineland, NJ, U.S.A.), and incubated at 37°C in a Dubnoff metabolic shaker at 80 strokes/min. Incubations with substrates other than glucose were carried out in triplicate, with glucose in quadruplicate.

The methodology described by Rodbell (1964) was followed to terminate the incubations. Recovery of the radioactivity in CO₂ and the glycerol and fatty-acid moieties of acylglycerols was done as previously described (DiGirolamo *et al.*, 1974). A

portion of the cell suspension was stained with Methylene Blue and the diameter of 300 fat-cells was measured by a microscopic technique to provide a measure of the mean cell diameter and volume. The number of fat-cells in the original fat-cell suspension and in the individual incubation flasks was calculated by dividing the lipid content by the mean fat-cell lipid (mean fat-cell lipid = (mean fat-cell volume) × lipid density) (DiGirolamo *et al.*, 1971).

For each experimental condition tested (in four to five experiments), results were expressed as μg-atoms of carbon of a given substrate converted into specified product in 1 h per 10⁷ fat-cells.

Statistical evaluation of the data

Mean, s.d. and s.e.m. values were calculated in the usual way (Snedecor & Cochran, 1969). Where appropriate, the groups of observations were analysed by using the method of repeated-measures analysis of variance (Winer, 1971). The 'honestly significant difference' (Winer, 1971) procedure for multiple comparisons was used to test the significance (at the *P* = 0.05 level) of specific contrasts between various experimental conditions. These analyses were performed on the logarithms of the raw data to stabilize variances. The program BMDP2V was used to perform the statistical calculations (Dixon & Brown, 1977).

Results

Glucose metabolism in small and large fat-cells

The results of basal and insulin-stimulated glucose metabolism in both small and large fat-cells (Tables 1 and 2), carried out for comparison with the other substrates, agree with those consistently observed in this laboratory (DiGirolamo *et al.*, 1974; DiGirolamo & Owens, 1976). In the small fat-cells, insulin produced a 6-fold increase in [U-¹⁴C]glucose conversion into CO₂, a 2-fold increase in conversion into the glycerol moiety of acylglycerols and a 10-fold increase in conversion into the fatty-acid moiety of acylglycerols. Compared with small fat-cells, large fat-cells presented a much decreased synthesis of the fatty-acid moiety of acylglycerols and production of CO₂ from glucose under both basal and insulin-stimulated conditions (*P* < 0.05). Synthesis of the glycerol moiety of acylglycerols was, however, increased in the large cells, both basally and in the presence of insulin (*P* < 0.01). Overall stimulation of total glucose metabolism by insulin was characteristically decreased in large cells.

Acetate metabolism in small and large fat-cells

In both the small and large fat-cells of the rat (Tables 1 and 2), we found acetate in the absence or presence of insulin to be converted into CO₂ and the

Table 1. *Metabolism of [U - ^{14}C]glucose, [2 - ^{14}C]acetate, [2 - ^{14}C]pyruvate and [2 - ^{14}C]lactate in small fat-cells*

Values shown are means (\pm S.E.M.) for triplicate determinations averaged from five experiments with small fat-cells (mean cell volume = 56 ± 3 pl) from 6–8-week-old rats. All radiolabelled substrates concentrations were 6 mM, unlabelled glucose concentration was 6 mM, and insulin concentration (when present) was 10 munits/ml. The 'Total' value represents the sum of the mean of the three products (CO_2 + glycerol and fatty-acid moieties of acylglycerols), with the relative conversion of each radiolabelled substrate into the three products in each group respectively as a percentage in parentheses.

Incubation condition	Substrate	Substrate carbon converted into specified products (μ g-atoms/ 10^7 fat-cells per h)			
		CO_2	Glycerol	Fatty acid	Total
Basal	[U - ^{14}C]Glucose	4.56 ± 0.42	7.32 ± 0.24	2.82 ± 0.36	14.70 (31, 50, 19)
	[2 - ^{14}C]Acetate	0.38 ± 0.06	0.68 ± 0.08	0.61 ± 0.08	1.67 (23, 40, 37)
	[2 - ^{14}C]Pyruvate	7.44 ± 0.36	7.11 ± 0.57	11.01 ± 1.50	25.56 (29, 28, 43)
	[2 - ^{14}C]Lactate	15.84 ± 1.05	34.41 ± 6.15	6.06 ± 0.75	56.31 (28, 61, 11)
+ Insulin	[U - ^{14}C]Glucose	24.90 ± 0.96	15.78 ± 0.54	28.20 ± 1.56	68.88 (36, 23, 41)
	[2 - ^{14}C]Acetate	0.44 ± 0.04	0.66 ± 0.04	0.82 ± 0.08	1.92 (23, 34, 43)
	[2 - ^{14}C]Pyruvate	6.69 ± 0.42	6.42 ± 0.48	9.69 ± 1.11	22.80 (29, 28, 43)
	[2 - ^{14}C]Lactate	17.61 ± 1.32	34.11 ± 3.99	7.68 ± 0.90	59.40 (30, 57, 13)
+ Glucose	[2 - ^{14}C]Acetate	0.32 ± 0.04	1.18 ± 0.18	1.98 ± 0.22	3.48 (9, 34, 57)
	[2 - ^{14}C]Pyruvate	8.13 ± 0.39	9.33 ± 1.62	26.22 ± 2.46	43.68 (19, 21, 60)
	[2 - ^{14}C]Lactate	17.46 ± 1.14	22.08 ± 2.82	50.10 ± 4.26	89.64 (19, 25, 56)
+ Glucose + insulin	[2 - ^{14}C]Acetate	0.12 ± 0.02	1.84 ± 0.32	6.18 ± 0.76	8.14 (1, 23, 76)
	[2 - ^{14}C]Pyruvate	6.75 ± 0.51	19.14 ± 3.48	50.22 ± 5.64	76.11 (9, 25, 66)
	[2 - ^{14}C]Lactate	9.96 ± 1.08	26.94 ± 7.59	65.97 ± 6.51	102.87 (10, 26, 64)

Table 2. *Metabolism of [U - ^{14}C]glucose, [2 - ^{14}C]acetate, [2 - ^{14}C]pyruvate and [2 - ^{14}C]lactate in large fat-cells*

Values shown are means (\pm S.E.M.) for triplicate determinations averaged from five experiments with large fat-cells (mean cell volume = 446 ± 26 pl) from 8–12 months old rats. All radiolabelled substrates concentrations were 6 mM, unlabelled glucose concentrations 6 mM and insulin concentration (when present) was 10 m-units/ml. The 'Total' value represents the sum of the mean of the three products (CO_2 + glycerol and fatty-acid moieties of acylglycerols), with the relative conversion of each radiolabelled substrate into the three products in each group respectively as a percentage in parentheses.

Incubation condition	Substrate	Substrate carbon converted into specified products (μ g-atoms/ 10^7 fat-cells per h)			
		CO_2	Glycerol	Fatty acid	Total
Basal	[U - ^{14}C]Glucose	3.54 ± 0.30	18.90 ± 1.20	0.17 ± 0.04	22.61 (15, 84, 1)
	[2 - ^{14}C]Acetate	0.66 ± 0.10	0.16 ± 0.02	0.02 ± 0.01	0.84 (79, 19, 2)
	[2 - ^{14}C]Pyruvate	7.56 ± 0.45	7.32 ± 0.57	0.54 ± 0.09	15.42 (49, 47, 4)
	[2 - ^{14}C]Lactate	27.36 ± 2.91	34.08 ± 4.77	0.21 ± 0.03	61.65 (44, 55, 1)
+ Insulin	[U - ^{14}C]Glucose	4.68 ± 0.36	25.92 ± 1.68	0.23 ± 0.04	30.83 (15, 84, 1)
	[2 - ^{14}C]Acetate	0.66 ± 0.10	0.22 ± 0.04	0.04 ± 0.01	0.92 (72, 24, 4)
	[2 - ^{14}C]Pyruvate	7.59 ± 0.63	7.08 ± 0.57	0.57 ± 0.03	15.24 (50, 46, 4)
	[2 - ^{14}C]Lactate	27.09 ± 3.09	26.82 ± 2.40	0.21 ± 0.03	54.12 (50, 50, 1)
+ Glucose	[2 - ^{14}C]Acetate	0.94 ± 0.14	0.38 ± 0.10	0.10 ± 0.04	1.42 (66, 27, 7)
	[2 - ^{14}C]Pyruvate	9.81 ± 0.78	3.36 ± 0.24	1.20 ± 0.24	14.37 (68, 23, 9)
	[2 - ^{14}C]Lactate	51.69 ± 4.59	15.42 ± 2.37	0.78 ± 0.24	67.89 (76, 23, 1)
+ Glucose + insulin	[2 - ^{14}C]Acetate	1.04 ± 0.16	0.42 ± 0.14	0.18 ± 0.06	1.64 (63, 26, 11)
	[2 - ^{14}C]Pyruvate	10.77 ± 0.81	3.15 ± 0.24	1.59 ± 0.33	15.51 (69, 21, 10)
	[2 - ^{14}C]Lactate	49.35 ± 3.60	14.07 ± 1.92	0.93 ± 0.27	64.35 (77, 22, 1)

glycerol and fatty-acid moieties of acylglycerols at a rate considerably lower than that of glucose. Total basal utilization of acetate in small fat-cells was

about 11% that of glucose with nearly 80% of the acetate-derived carbon being converted into the glycerol and fatty-acid moieties of acylglycerols.

Under insulin-stimulated conditions, total utilization of acetate or its pattern of utilization did not appreciably change. Large fat-cells also exhibited low rates of total acetate utilization in comparison with glucose, both basally (4%) and in the presence of insulin (3%). Compared with small fat-cells, the acetate utilization for fatty acid synthesis in the large fat-cells nearly disappeared ($P < 0.05$), and significant ($P < 0.05$) decreases in production of the glycerol moiety of acylglycerols were evident; CO_2 accounted for 70–80% of all acetate carbon metabolized to products by the large cells in either the absence or presence of insulin.

Pyruvate and lactate metabolism in small and large fat-cells

In the absence of insulin, total basal pyruvate utilization was 75% greater than that of glucose in the small fat-cells (Table 1). Fatty-acid synthesis accounted for approx. 40% of the pyruvate carbon metabolism and pyruvate conversion into fatty acids was four times greater than that for glucose. Insulin exerted no significant influence on pyruvate metabolism in small fat-cells, and the relative proportions of pyruvate carbon that appeared in the three products remained unchanged. Thus, when insulin was present, pyruvate was a poorer substrate than glucose and provided 33% of the total product carbon that glucose could provide.

In the large fat-cells (Table 2), the pyruvate contribution to total adipocyte metabolism, both in the absence and presence of insulin, was less than that of glucose, but fatty-acid synthesis from pyruvate was still two to three times greater than that produced by glucose. Of the total pyruvate carbon utilized by large cells, 96% was evenly divided between CO_2 and the glycerol moiety of acylglycerols; in contrast, glucose was mostly (84%) utilized for synthesis of the glycerol moiety of acylglycerols.

Lactate, which favours production of NADH as it enters into the pyruvate pool, produced surprisingly different results. In the absence of insulin, total utilization of lactate carbon by the small fat-cells was four times greater overall than that of glucose (Table 1). Compared with basal glucose, greater contributions of lactate carbon to CO_2 (3.5 times greater) and the glycerol (5 times) and fatty-acid (2 times) moieties of acylglycerols were observed. In the presence of insulin, lactate utilization was nearly equal to that of insulin-stimulated glucose utilization.

The superiority of lactate over glucose as a substrate for adipocyte metabolism was also exhibited by the large fat-cells where, in the presence of insulin, lactate utilization was greater than, or equal to, the carbon utilization from glucose (Table 2).

Effects of glucose on the metabolism of ^{14}C -labelled acetate, pyruvate and lactate in small and large fat-cells

Since glucose exerts certain potentiating effects on fat-cell metabolism (Winegrad & Renold, 1958; Kneer & Ball, 1968; DelBoca & Flatt, 1969; Katz & Wals, 1974), we proceeded to determine the effects of the addition of glucose, or glucose plus insulin, on the metabolism of labelled acetate, pyruvate or lactate in both small and large fat-cells. The results show that, in small fat-cells (Table 1), addition of 6 mM-glucose produced an approximate doubling in the total conversion of acetate, pyruvate and lactate into the sum of the three products. Glucose and insulin produced an even greater increase in substrate conversion, and this was more evident for acetate and pyruvate than lactate.

In contrast, in the large fat-cells, the addition of glucose alone or glucose and insulin produced a minor, if any, enhancement in total substrate utilization.

The results of these experiments also revealed several findings of interest. (a) Insulin in the absence of glucose had a limited capacity to stimulate metabolism of acetate, pyruvate or lactate. (b) In the small fat-cells, the effects of glucose were greatest in the markedly enhanced conversion of all three substrates into fatty acids. (c) Large fat-cells showed a marked decrease in fatty-acid synthesis from any of the substrates tested. Furthermore, the ability of the cells to respond to glucose, insulin or both with an enhanced fatty-acid synthesis was largely lost in these larger cells. Only lactate conversion into fatty acids, although diminished, retained a partial response to these additions. (d) In small fat-cells, conversion of acetate and lactate into the glycerol moiety of acylglycerols was unaffected by glucose addition. Curiously, pyruvate conversion into this product was significantly increased ($P < 0.05$) with addition of glucose and insulin in the small fat-cells and decreased ($P < 0.05$) in the large ones. (e) Lactate conversion into CO_2 by small fat-cells declined significantly ($P < 0.05$) in the presence of glucose and insulin, but increased ($P < 0.05$) in incubations with large fat-cells. The preponderance of the effects shown when both glucose and insulin were present was related more to the presence of glucose than that of insulin.

Evaluation of the relative contributions of glucose, acetate, lactate and pyruvate to the metabolism of small and large fat-cells

In assessing the ability of other substrates to contribute to adipocyte metabolism, we found it useful to express the data relative to the metabolism of basal glucose (Table 3).

Insulin, glucose, or glucose plus insulin did not appreciably affect the relative oxidation of acetate,

Table 3. *Relative contribution in small and large fat-cells, of radiolabelled glucose versus other substrates to production of CO₂ and glycerol and fatty-acid moieties of acylglycerols*

Effects of unlabelled glucose (6 mM) and insulin (10 m-units/ml), singly or in combination, are shown. These data were derived from values reported in Table 1 (small fat-cells) and Table 2 (large fat-cells) respectively. The mean basal [U-¹⁴C]glucose conversion into specified product was set at 100%. All values for the other radiolabelled substrates were expressed relative to that of glucose.

		Relative contribution (% of basal glucose metabolism)							
		Small fat-cells				Large fat-cells			
Product	Substrate	Basal	+Insulin	+Glucose	+Glucose +insulin	Basal	+Insulin	+Glucose	+Glucose +insulin
CO ₂	[U- ¹⁴ C]Glucose	100	546	—	—	100	132	—	—
	[2- ¹⁴ C]Acetate	8.3	9.6	7.0	2.6	19	19	27	29
	[2- ¹⁴ C]Pyruvate	163	147	178	148	214	214	277	304
	[2- ¹⁴ C]Lactate	347	386	383	218	773	765	1460	1394
Glycerol	[U- ¹⁴ C]Glucose	100	216	—	—	100	137	—	—
	[2- ¹⁴ C]Acetate	9	9	16	25	1	1	2	2
	[2- ¹⁴ C]Pyruvate	97	88	127	261	39	37	18	17
	[2- ¹⁴ C]Lactate	470	466	302	368	180	142	82	74
Fatty acids	[U- ¹⁴ C]Glucose	100	1000	—	—	100	135	—	—
	[2- ¹⁴ C]Acetate	22	29	70	219	124	24	59	106
	[2- ¹⁴ C]Pyruvate	390	344	930	1781	318	335	706	935
	[2- ¹⁴ C]Lactate	215	272	1777	2339	124	124	459	547
Total	[U- ¹⁴ C]Glucose	100	469	—	—	100	134	—	—
	[2- ¹⁴ C]Acetate	11	13	26	55	4	4	6	7
	[2- ¹⁴ C]Pyruvate	174	155	297	518	68	67	64	69
	[2- ¹⁴ C]Lactate	383	404	610	700	273	239	300	285

pyruvate or lactate to CO₂ in the small cells; in contrast, glucose or glucose plus insulin doubled the already vigorous oxidation of lactate in the large cells. Acetate was poorly oxidized in either cell type under all conditions, but pyruvate and lactate compared favourably with glucose as a substrate for CO₂ production.

In the small cells, pyruvate and particularly lactate were as good as, or better than, basal glucose for the production of the glycerol moiety of acylglycerols. Surprisingly, this relationship also held true in the presence of glucose plus insulin. In the large cells, pyruvate declined as a substrate of importance for synthesis of the glycerol moiety of acylglycerols, whereas lactate remained relatively better than glucose basally and contributed carbon readily to the total production of the glycerol moiety of acylglycerols in the presence of glucose alone or glucose plus insulin.

Under basal conditions, pyruvate and lactate showed a relatively high contribution to the fatty-acid moiety of acylglycerols compared with glucose. The addition of glucose or glucose plus insulin increased the relative contribution of each substrate to fatty-acid synthesis, and was particularly marked in the case of pyruvate and lactate; nearly twice as much acylglycerol fatty acid could be produced

from these substrates as from glucose (even in the presence of insulin). The absolute amounts of fatty acids produced in the large fat-cells from any substrate were very low (Table 2), but the pattern of relative increases due to the presence of glucose and glucose plus insulin seen in the small cells was nevertheless present.

Basally, both pyruvate and lactate contributed more towards the total metabolism of small fat-cells than glucose (Table 3). In the presence of glucose and glucose plus insulin, the relative contributions of pyruvate and lactate became quite impressive and matched or exceeded that of glucose in the presence of insulin. These patterns changed in the large fat-cells; whereas lactate still contributed more than twice the carbon that glucose could under any condition, pyruvate seemed to be utilized less (relative to glucose) and the stimulatory effects of glucose or glucose plus insulin were not evident on either substrate.

The results reported in Tables 1–3 compare the metabolism of the various substrates at equimolar (6 mM) concentration. In subsequent experiments (results not shown), lactate metabolism was studied at 2 mM concentration, which falls in the upper range of normal plasma lactate concentration (Reiman, 1978). We found that at the 2 mM concentration,

lactate metabolism was qualitatively similar for both types of fat-cells to the values observed at 6 mM concentration; the conversion of lactate into the three products measured was, however, approximately one-half as high.

Discussion

In the present study, significant and novel contributions to adipocyte metabolism by substrates other than glucose have been uncovered in both small and large fat-cells. In particular, pyruvate and lactate have been shown to be potentially important contributors to triacylglycerol synthesis and deposition in adipocytes. The utilization of these alternative substrates to glucose does not appear to be under the influence of insulin, but is markedly influenced by the presence of glucose.

Shifts in metabolic substrate preferences for lipogenesis in developing adipocytes are not unknown. Studies on ruminants, which utilize glucose in the foetal state (Ballard *et al.*, 1969), have shown that the adult ruminant shifts to acetate and butyrate as its primary lipogenic substrates (Hanson & Ballard, 1967). Similar observations have been made in the rabbit (Smith, 1975) and guinea pig (Saggerson, 1974). One of the original aims of the present work was to determine whether enlarged adipocytes (from older fatter rats) that have lost the ability to convert glucose into fatty acids could have shifted substrate-precursor preference for fatty-acid synthesis. Our data show that vicarious substrate utilization for fatty acid synthesis in larger fat-cells does not replace the decreased fatty-acid synthesis associated with these cells. However, in comparing production of CO₂ and the glycerol moiety of acylglycerols from pyruvate or lactate in small versus large fat-cells, the absolute amount of labelled substrate appearing in those two products was similar. This suggests that the cellular capacity for the metabolism of either pyruvate or lactate remains relatively intact in the progression from the small lean fat-cell to the large obese fat-cell with the exception of the loss of the ability to synthesize fatty acids. Thus, it may be concluded that with cellular enlargement, the synthesis *de novo* of fatty acids undergoes a metabolic involution that appears to be related to the activity of the enzymic pathways involved (Czech, 1977) rather than substrate availability.

In contrast with acetate, which contributes little to rat adipocyte metabolism, pyruvate and, in particular, lactate show a remarkable capacity for utilization by adipocytes of different cell size. Pyruvate has been recognized as a potential lipogenic (Winegrad & Renold, 1958; Kneer & Ball, 1968) and glyceroneogenic (Reshef *et al.*, 1970) substrate, but agreement about the extent to which

lactate can be utilized by adipose tissue has not been reached. In some reports, lactate was found to be a good precursor for fatty-acid synthesis (Katz & Wals, 1974; Rath *et al.*, 1975). Other reports have suggested it to be a poor substrate for fatty-acid synthesis (Kneer & Ball, 1968; Schmidt & Katz, 1969; Halperin, 1971; Saggerson, 1974).

With regard to the role of lactate as precursor for glyceroneogenesis, some authors have found it to be significant (Saggerson, 1972), others have found it to be negligible (Schmidt & Katz, 1969; Katz & Wals, 1974; Rath *et al.*, 1975). Our results are in agreement with those authors who have recognized the significant capacity of adipocytes to use lactate as a metabolic precursor. The reasons for the described discrepancies are not clear. Several previous studies have been directed to the elucidation of glyceroneogenesis from glucose and other precursors. The conversion of pyruvate into α -glycerophosphate via glyceroneogenesis has been observed (Reshef *et al.*, 1969). Although subject to possible dietary and hormonal regulation, adequate adipose tissue activities of pyruvate carboxylase (Ballard & Hanson, 1967) and phosphoenolpyruvate carboxykinase (Ballard *et al.*, 1967) are available to support glyceroneogenesis. Formation of the glycerol moiety of acylglycerols is favoured by increased NADH concentrations (Schmidt & Katz, 1969) and lactate produces NADH when it is converted into pyruvate. In experiments where unlabelled lactate is added to ¹⁴C-labelled pyruvate, formation of the glycerol moiety of acylglycerols is considerably enhanced, presumably due to the increase in cytosolic reducing equivalents (Halperin, 1971). This is compatible with our findings of significant glyceroneogenesis from lactate in both small and large fat-cells. Lactate can not only provide carbon for the generation of α -glycerophosphate via its conversion into pyruvate, but it can also alter the cytoplasmic redox state (Kather & Brand, 1975) to favour both glyceroneogenesis and fatty-acid synthesis.

Our results demonstrate the extent to which acetate, pyruvate and lactate can be used when presented to the fat-cells as single substrates at 6 mM concentration in incubations *in vitro*, but their potential contribution in a more physiological setting remains to be determined. The concentrations used in the present study were physiological for glucose, slightly above the physiological range for lactate and definitely supraphysiological for acetate and pyruvate. Consequently for the latter two substrates, the actual contribution to adipocyte metabolism under physiological conditions would probably be below the values shown in Table 3. For lactate, however, the studies reported here at 6 mM concentration (Tables 1, 2 and 3) and the preliminary studies at 2 mM concentration (see the Results section)

indicated a significant contribution to adipocyte metabolism of a magnitude not previously recognized. Furthermore, in a physiological setting, several substrates may be simultaneously available to the fat-cells. A partial elucidation of the role of these substrates in the presence of physiological concentrations of glucose and adequate insulin stimulation was obtained from the results of co-incubations with glucose and the various radio-labelled substrates. The relative acetate contribution to fat-cell metabolism in either cell size was low and agrees with the previously reported assessment of its minor importance (Winegrad & Renold, 1958; DelBoca & Flatt, 1969). However, the contribution of both pyruvate and lactate to fatty-acid synthesis of small fat-cells was most striking. Even in the presence of physiological concentrations of glucose and maximally stimulating doses of insulin, equimolar concentrations of either pyruvate or lactate appeared to be utilized preferentially to glucose. Similarly, examination of the data reveals that, whereas pyruvate contributed to formation of the glycerol moiety of acylglycerols, lactate exceeded both pyruvate and glucose as a precursor for the glycerol moiety of acylglycerols in the small fat-cells and was nearly comparable with glucose in generating the glycerol moiety of acylglycerols in the large fat-cells.

It has been suggested that direct uptake of fatty acids from the blood or circulating lipoproteins could obviate the need for fatty-acid synthesis *de novo* altogether (Masoro, 1963; DeFreitas & Depocas, 1965; Sharago *et al.*, 1971). Evidence also exists showing that lipoprotein lipase activity, exogenous fatty-acid uptake (Björntorp *et al.*, 1975), as well as the overall rate of esterification (Jamdar, 1978), increase with fat-cell size. The continued deposition of triacylglycerol would then rely on an exogenous source of α -glycerophosphate for esterification, and some investigators have postulated that this is a possible rate-limiting step in adipocyte lipid deposition (Tzur *et al.*, 1964). Since the overall fatty-acid synthesis in mature fat-cells is decreased, yet these cells continue to increase in size and triacylglycerol content (Hirsch & Han, 1969; DiGirolamo & Mendlinger, 1971), the continued deposition of triacylglycerol probably occurs in these cells via a direct uptake of exogenous fatty acid with subsequent esterification utilizing α -glycerophosphate derived from a variety of sources. Lactate would appear to be particularly suited to this role and might assume additional importance in physiological states associated with chronic increased plasma lactate concentrations, such as fructose feeding (Akerblom *et al.*, 1972) or infusion (Bergström *et al.*, 1972), obesity (Vendsborg & Bach-Mortensen, 1977) and lactic acidosis (Relman, 1978).

This work was supported in part by USPHS Grant AM-17403.

References

- Akerblom, H. K., Sitlanen, I. & Kallio, A. K. (1972) *Acta Med. Scand. Suppl.* **542**, 195–202
- Ballard, F. J. & Hanson, R. W. (1967) *J. Lipid Res.* **8**, 73–79
- Ballard, F. J., Hanson, R. W. & Leveille, G. A. (1967) *J. Biol. Chem.* **242**, 2746–2750
- Ballard, F. J., Hanson, R. W. & Kronfeld, D. S. (1969) *Fed. Proc. Am. Soc. Exp. Biol.* **28**, 218–231
- Bergström, J., Furst, P., Gallyas, F., Hultman, E., H:son Nilsson, L., Roch-Norlund, A. E. & Vinnars, E. (1972) *Acta Med. Scand. Suppl.* **542**, 57–64
- Björntorp, P., Enzi, G., Ohlson, R., Persson, B., Sponbergs, P. & Smith, U. (1975) *Horm. Metab. Res.* **7**, 230–237
- Czech, M. P. (1977) *Annu. Rev. Biochem.* **46**, 359–384
- DeFreitas, A. S. W. & Depocas, F. (1965) *Can. J. Biol. Chem.* **43**, 437–450
- DelBoca, J. & Flatt, J. P. (1969) *Eur. J. Biochem.* **11**, 127–134
- DiGirolamo, M. & Mendlinger, S. (1971) *Am. J. Physiol.* **221**, 859–864
- DiGirolamo, M. & Owens, J. L. (1976) *Horm. Metab. Res.* **8**, 445–451
- DiGirolamo, M., Mendlinger, S. & Fertig, J. W. (1971) *Am. J. Physiol.* **221**, 850–858
- DiGirolamo, M., Howe, M. D., Esposito, J., Thurman, L. & Owens, J. L. (1974) *J. Lipid Res.* **15**, 332–338
- Dixon, W. J. & Brown, M. B., eds. (1977) *BMDP-77, Biomedical Computer Programs P-Series*, pp. 540–580, University of California Press, Berkeley, CA
- Gliemann, J. (1965) *Diabetes* **14**, 643–649
- Halperin, M. L. (1971) *Can. J. Biochem.* **49**, 736–741
- Hanson, R. W. & Ballard, F. J. (1967) *Biochem. J.* **105**, 529–536
- Hirsch, J. & Han, P. W. (1969) *J. Lipid Res.* **10**, 77–82
- Jamdar, S. C. (1978) *Biochem. J.* **170**, 153–160
- Kather, H. & Brand, K. (1975) *Arch. Biochem. Biophys.* **170**, 417–426
- Katz, J. & Wals, P. A. (1974) *Biochim. Biophys. Acta* **348**, 344–356
- Kneer, P. & Ball, E. G. (1968) *J. Biol. Chem.* **243**, 2863–2870
- Masoro, E. J. (1963) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **22**, 868–873
- Olefsky, J. (1977) *Endocrinology* **100**, 1169–1177
- Rath, E. A., Beloff-Chain, A. & Hems, D. A. (1975) *Biochem. Soc. Trans.* **3**, 513–515
- Relman, A. S. (1978) *N. Engl. J. Med.* **298**, 564–566
- Reshef, L., Hanson, R. W. & Ballard, F. J. (1969) *J. Biol. Chem.* **244**, 1994–2001
- Reshef, L., Hanson, R. W. & Ballard, F. J. (1970) *J. Biol. Chem.* **245**, 5979–5984
- Rodbell, M. (1964) *J. Biol. Chem.* **239**, 375–380
- Saggerson, E. D. (1972) *Biochem. J.* **128**, 1068–1078
- Saggerson, E. D. (1974) *Biochem. J.* **140**, 211–224
- Salans, L. & Dougherty, J. W. (1971) *J. Clin. Invest.* **50**, 1399–1410

- Schmidt, K. & Katz, J. (1969) *J. Biol. Chem.* **244**, 2125–2131
- Sharago, E. J., Glennon, A. & Gordon, E. S. (1971) *Metabolism* **20**, 54–63
- Smith, S. (1975) *J. Lipid Res.* **16**, 324–331
- Snedecor, G. W. & Cochran, W. G. (1969) *Statistical Methods*, 6th edn., Iowa State University Press, Ames, IA
- Tzur, R., Tal, E. & Shapiro, B. (1964) *Biochim. Biophys. Acta* **84**, 18–23
- Winegrad, A. I. & Renold, A. E. (1958) *J. Biol. Chem.* **233**, 267–273
- Winer, B. J. (1971) *Statistical Principles in Experimental Design*, 2nd edn., McGraw-Hill Company, New York
- Vendsborg, P. B. & Bach-Mortensen, N. (1977) *Scand. J. Clin. Invest.* **37**, 317–320