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Lipogenesis in rat brown adipocytes

Effects of insulin and noradrenaline, contributions from glucose and lactate as precursors and comparisons with white adipocytes

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1. Brown adipocytes were isolated from the interscapular depot of male rats maintained at approx. 21 °C. In some experiments parallel studies were made with white adipocytes from the epididymal depot. 2. Insulin increased and noradrenaline decreased [U-^{14}C]glucose incorporation into fatty acids by brown adipocytes. Brown adipocytes differed from white adipocytes in that exogenous fatty acid (palmitate) substantially decreased fatty acid synthesis from glucose. Both noradrenaline and insulin increased lactate + pyruvate formation by brown adipocytes. Brown adipocytes converted a greater proportion of metabolized glucose into lactate + pyruvate and a smaller proportion into fatty acids than did white adipocytes. 3. In brown adipocytes, when fatty acid synthesis from [U-^{14}C]glucose was decreased by noradrenaline or palmitate, incorporation of ^3H_2O into fatty acids was also decreased to an extent which would not support proposals for extensive recycling into fatty acid synthesis of acetyl-CoA derived from fatty acid oxidation. 4. In the absence of glucose, [U-^{14}C]lactate was a poor substrate for lipogenesis in brown adipocytes, but its use was facilitated by glucose. When brown adipocytes were incubated with 1 mM-lactate + 5 mM-glucose, lactate-derived carbon generally provided at least 50% of the precursor for fatty acid synthesis. 5. Both insulin and noradrenaline increased [U-^{14}C]glucose conversion into CO_2 by brown adipocytes (incubated in the presence of lactate) and, in combination, stimulation of glucose oxidation by these two agents showed synergism. Rates of $^{14}CO_2$ formation from glucose by brown adipocytes were relatively small compared with maximum rates of oxygen consumption by these cells, suggesting that glucose is unlikely to be a major substrate for thermogenesis. 6. Brown adipocytes from 6-week-old rats had considerably lower maximum rates of fatty acid synthesis, relative to cell DNA content, than white adipocytes. By contrast, rates of fatty acid synthesis from ^3H_2O *in vivo* were similar in the interscapular and epididymal fat depots. Expressed relative to activities of fatty acid synthase or ATP citrate lyase, however, brown adipocytes synthesized fatty acids as effectively as did white adipocytes. It is suggested that the cells most active in fatty acid synthesis in the brown adipose tissue are not recovered fully in the adipocyte fraction during cell isolation. Differences in rates of fatty acid synthesis between brown and white adipocytes were less apparent at 10 weeks of age.

INTRODUCTION

It is well established that thermogenesis in brown adipose tissue is supported by uncoupled respiration involving a mitochondrial proton-conductance pathway dissociated from oxidative phosphorylation. The major stimulus for thermogenesis appears to be noradrenaline released from the sympathetic nerve supply; and fatty acids released through lipolysis are important substrates for the high rate of respiration (for reviews, see Nedergaard & Lindberg, 1982; Nicholls & Locke, 1983, 1984; Cannon & Nedergaard, 1985*a*,*b*). It is also established that brown adipose tissue in rats or mice is highly active in fatty acid synthesis, particularly in cold-adapted animals (McCormack & Denton, 1977; Trayhurn, 1979, 1981; Agius & Williamson, 1980, 1981; Agius *et al.*, 1981; Gibbins *et al.*, 1985; Buckley & Rath, 1987). Measurements of fatty acid synthesis *in vivo* from injected ^3H_2O show that, in the short term, this process in brown adipose tissue is increased by insulin (McCormack & Denton, 1977; Agius & Williamson, 1980, 1981; Agius *et al.*, 1981; McCormack, 1982) or by electrical stimulation of the ventromedial hypothalamus (Shimazu & Takahashi, 1980) and decreased by noradrenaline (Agius & Williamson, 1980; Gibbins *et al.*, 1985) or by brief exposure to cold (Buckley & Rath, 1987).

Glucose is regarded as a major precursor for newly synthesized fatty acids in white adipose tissue (Hems *et al.*, 1975; McCormack, 1982), although lactate, at physiological concentrations, may also be a relatively important precursor (Saggerson, 1972*b*; Katz & Wals, 1974; Rath *et al.*, 1975). By contrast, the liver makes little use of blood glucose as a direct lipogenic precursor *in vivo* or *in vitro* (Salmon *et al.*, 1974; Clark *et al.*, 1974; Hems *et al.*, 1975), appearing to utilize C_3 precursors preferentially. For brown adipose tissue, glucose (McCormack, 1982), ketone bodies (Agius & Williamson, 1981), acetyl-CoA derived from simultaneously occurring β-oxidation (Buckley & Rath, 1987) or unspecified non-glucose precursors (Trayhurn, 1979) have all been suggested as important under various conditions. In the short term, uptake of glucose or 2-deoxyglucose by brown adipose tissue *in vivo* is stimulated both by insulin and by noradrenaline (Cooney *et al.*, 1985; Ferré *et al.*, 1986; Ma & Foster, 1986). Although the effects of these hormones on the detailed disposition of intracellular glucose metabolism in white adipose tissue have been extensively studied *in vitro* by using incubated fat pieces or adipocytes (for review see Saggerson, 1985), few such

studies have been attempted with brown adipose tissue *in vitro*. As discussed by McCormack (1982), use of incubated brown-adipose-tissue pieces or slices *in vitro* is highly problematical and is probably unsuitable. The alternative, isolated brown adipocytes, does not appear to have been exploited to any extent in the study of glucose metabolism and lipogenesis.

In this study we have attempted to establish the suitability of brown adipocytes in such studies by making direct comparisons with parallel preparations of white adipocytes. We have investigated the relative contributions of glucose and lactate to oxidative metabolism, assessed the extent to which endogenous substrates might dilute glucose carbon flux into fatty acids, and demonstrated effects of insulin, noradrenaline and exogeneous fatty acids. Near the completion of this study, Isler *et al.* (1987) published a study using rat brown adipocytes which has some overlap with the present work.

MATERIALS AND METHODS

Chemicals

These were obtained and treated as described by Saggerson (1972*a*,*b*, 1974) and Woodward & Saggerson (1986).

Animals

For this work, 6-week-old (160–180 g) or 10-week-old (270–300 g) male Sprague–Dawley rats were bred at University College London. Animals had constant access to water and Rat & Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.) and were maintained at approx. 21 °C on a 13 h-light/11 h-dark cycle, with light from 06:00 to 19:00 h.

Isolation of adipocytes

Rats were killed at approx. 10:00–11:00 h. White adipocytes were isolated from the epididymal depot by disaggregation in collagenase (1 mg/ml) essentially as described by Rodbell (1964). Brown adipocytes were isolated from the interscapular depot by the procedure originally described by Fain *et al.* (1967) and elaborated by Nedergaard & Lindberg (1982).

Measurements of flux rates in incubated adipocytes

Freshly isolated cells were dispensed (approx. 2×10^6 cells/ml) into 25 ml silicone-treated Erlenmeyer flasks containing 4 ml of Krebs–Ringer bicarbonate medium (Krebs & Henseleit, 1932) and, unless stated otherwise, fatty acid-poor albumin at 20 mg/ml. Other additions to flasks are indicated in individual Figures and Tables. The flasks were incubated at 37 °C with shaking under O_2/CO_2 (19:1). At the appropriate time, portions of the incubations were taken for extraction of cell lipids and measurement of ^{14}C or ^3H incorporation into acylglycerol fatty acids and glycerol (Saggerson & Greenbaum, 1970*a*; Saggerson, 1972*a*). Other portions were deproteinized, neutralized and assayed for lactate and pyruvate (Saggerson & Greenbaum, 1970*a*). If $^{14}CO_2$ was to be collected, incubations were performed as above, except that these were in 50 ml silicone-treated conical flasks with glass centre wells and the flasks were only gassed vigorously with O_2/CO_2 (19:1) for 2–3 min before being sealed. After 60 min $^{14}CO_2$ was collected and measured as described by Saggerson & Greenbaum (1970*a*). Blanks were performed in parallel with each experiment to correct for the presence of any ^{14}C-labelled volatile substances that may be present in ^{14}C-labelled substrates.

Measurement of lipogenesis *in vivo*

The procedure was similar to that described by Hems *et al.* (1975), Stansbie *et al.* (1976) and McCormack & Denton (1977). At approx. 10:00–11:00 h, rats were injected intraperitoneally with 0.5 ml of 0.15 M-NaCl containing 10 mCi of ^3H_2O and glucose (800 mg/kg body wt.). After 60 min the rats were killed by decapitation. Blood samples were taken and centrifuged to obtain plasma samples, which were used to determine the specific radioactivity of plasma water. Epididymal and interscapular adipose tissues were rapidly removed, weighed, and extracted in 10 ml of propan-2-ol/hexane/1 M-H_2SO_4 (40:10:1, by vol.) (Dole, 1961), by using an Ultra-Turrax tissue disintegrator. After addition of 6 ml of hexane and 7 ml of water and thorough mixing, the resulting hexane layer containing tissue lipids was washed twice with 2×10 ml of 1 mM-H_2SO_4. A 4 ml portion of the washed hexane extract was evaporated to dryness, saponified and treated to measure ^3H in acylglycerol fatty acids as described by Saggerson & Greenbaum (1970*a*).

Measurement of enzyme activities in adipocytes and adipose-tissue pieces

Adipocytes were isolated as described above and washed in albumin-free Krebs–Ringer bicarbonate medium. Tissues were obtained from rats after cervical dislocation. Cells or tissues were transferred to ice-cold sucrose medium [0.25 M-sucrose/10 mM-Tris/HCl buffer (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol] and homogenized in an ice-cold Potter–Elvehjem homogenizer with ten strokes of a rotating (450 rev./min) Teflon pestle with a radial clearance of 0.2 mm. The homogenates were centrifuged briefly in a Sorvall RC5B centrifuge (integrated field-time = 30000 *g*-min) to remove floating fat and some particulate material. The resulting supernatants were then centrifuged (4 °C) for 1 h at 100000 $g_{av.}$ to yield cytosolic fractions, which were stored frozen at −20 °C until assayed for enzyme activities. ATP citrate lyase (EC 4.1.3.8) was assayed spectrophotometrically at 25 °C as described by Martin & Denton (1970). Fatty acid synthase (EC 2.3.1.85) was assayed spectrophotometrically at 25 °C as described by Saggerson & Greenbaum (1970*b*).

Measurement of DNA

The DNA content of adipocyte or tissue homogenates was measured by the method of Switzer & Summer (1971), with calf thymus DNA as standard.

RESULTS AND DISCUSSION

Time courses of glycerolipid synthesis

The time course for glucose carbon flux into fatty acids was similar in brown and white adipocytes (Fig. 1*a*). After short lags, steady rates of synthesis were established in both cell types. With exogenous fatty acid present, both cell types vigorously synthesized the glycerol moiety of cell glycerolipids with a linear time course (Fig. 1*b*). The lower rate of incorporation into acylglycerol glycerol

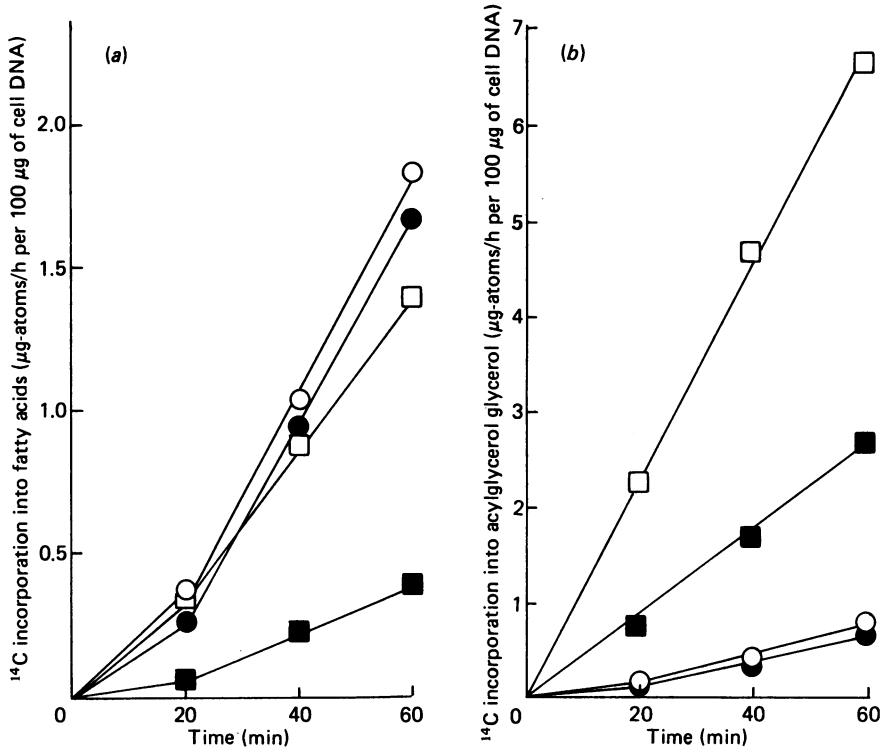

Fig. 1. Time courses of [^{14}C]glucose incorporation into glycerolipids by adipocytes

All incubations contained 5 mM-[U-^{14}C]glucose, fatty acid-poor albumin (40 mg/ml) and 4 nM-insulin. (*a*) Incorporation into acylglycerol fatty acid; (*b*) incorporation into acylglycerol glycerol. ○, □, white adipocytes; ●, ■, brown adipocytes. ○, ●, Without palmitate; □, ■, with 1 mM-palmitate. The results are from a single experiment (10-week-old rats).

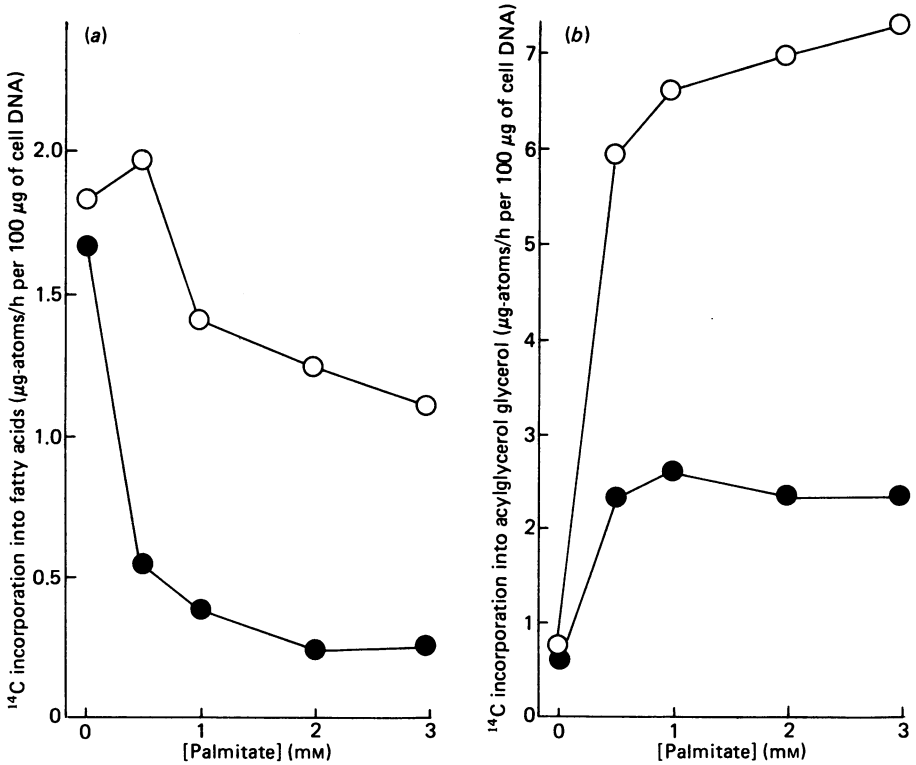

Fig. 2. Effect of palmitate on [^{14}C]glucose incorporation into glycerolipids by adipocytes

All incubations were for 1 h and contained 5 mM-[U-^{14}C]glucose, fatty acid-poor albumin (40 mg/ml) and 4 nM-insulin. (*a*) Incorporation into acylglycerol fatty acids; (*b*) incorporation into acylglycerol glycerol. ○, White adipocytes; ●, brown adipocytes. The results are from a single experiment (10-week-old rats).

Table 1. Glucose metabolism by brown adipocytes in the presence of insulin, noradrenaline or palmitate

Brown adipocytes from 6-week-old rats were incubated for 1 h in the presence of 5 mM-[U-^{14}C]glucose and ^3H_2O. The values are means ± S.E.M. for five separate cell preparations. Statistical significance was determined for paired differences and is indicated by $^aP < 0.02$, $^bP < 0.01$, $^cP < 0.005$ for effects of noradrenaline, and $^dP < 0.05$, $^eP < 0.025$, $^fP < 0.005$ for effects of insulin, and $^gP < 0.025$, $^hP < 0.01$, $^iP < 0.005$ for effects of palmitate.

Additions	Fatty acid synthesis (μg-atoms/h per 100 μg of DNA)			[^{14}C]Glucose conversion into acylglycerol glycerol (μg-atoms/h per 100 μg of DNA)	Lactate + pyruvate production	
	From [^{14}C]-glucose	From ^3H_2O	Ratio $^{14}C/^3H$ in fatty acids		μg-atoms of C/h per 100 μg of DNA	Ratio lactate/pyruvate
None	0.56 ± 0.03	0.62 ± 0.04	0.91 ± 0.05	0.71 ± 0.04	5.59 ± 0.23	2.86 ± 0.15
Noradrenaline (1 μM)	0.31 ± 0.03^c	0.38 ± 0.04^a	0.85 ± 0.02	1.45 ± 0.04^c	10.26 ± 0.23^c	2.57 ± 0.17
Insulin (4 nM)	1.54 ± 0.11^f	1.35 ± 0.10^f	1.14 ± 0.03	0.85 ± 0.02^d	13.08 ± 0.11^f	3.60 ± 0.14^f
Noradrenaline (1 μM) + insulin (4 nM)	0.82 ± 0.09^{be}	0.74 ± 0.08^{bd}	1.11 ± 0.07	2.20 ± 0.07^{cf}	14.03 ± 0.55	2.89 ± 0.14^{ae}
Insulin (4 nM) + palmitate (1.6 mM)	0.74 ± 0.11^h	0.71 ± 0.10^h	1.04 ± 0.03	3.63 ± 0.15^i	14.95 ± 0.46^i	2.87 ± 0.22^g

seen in brown cells may be attributable to their lower content of glycerolipid-synthesizing enzymes (Baht & Saggerson, 1988). The linearity of these time courses, particularly that for the highly energy-dependent glycerolipid synthesis process, gave initial reassurance regarding the integrity of the cells.

Effects of exogenous fatty acid

Fig. 2 shows that acylglycerol glycerol formation by brown adipocytes was maximal at exogenous fatty acid concentrations in excess of 1 mM (in the presence of 4% albumin). In accord with observations by Saggerson (1972*a*), addition of 0.5 mM-palmitate to white adipocytes in the presence of 5 mM-glucose + insulin caused a slight increase in fatty acid synthesis (Fig. 2*a*). Higher concentrations of fatty acid caused some decrease in glucose incorporation. By contrast, all tested concentrations of palmitate substantially decreased fatty acid synthesis in brown adipocytes. In this regard the brown adipocyte is more like the hepatocyte, where, again, fatty acid synthesis is considerably inhibited by physiological concentrations of exogenous fatty acids (Mayes & Topping, 1974; Nilsson *et al.*, 1974).

Effects of hormones on glucose carbon flux

In these experiments 6-week-old rats were used to facilitate comparisons with previous studies with white adipocytes (Saggerson, 1972*a*). Table 1 shows that [^{14}C]-glucose incorporation into fatty acids was increased by insulin and decreased by noradrenaline (in the presence or absence of insulin). These effects are qualitatively similar to those seen in the short term *in vivo* (see the Introduction). Again, brown adipocytes differed in that catecholamines do not appreciably inhibit fatty acid synthesis from glucose in white adipocytes when insulin is present (see Table 4 of Saggerson, 1972*a*). In the presence of noradrenaline or exogenous fatty acids (Prusiner *et al.*, 1968; Fain & Reed, 1970), brown-adipocyte respiration and fatty acid oxidation is substantially increased. It is therefore particularly noteworthy that these agents decreased ^3H_2O incorporation roughly in parallel with the decrease in glucose carbon flux (Table 1). This suggests that extensive recycling of β-oxidation-derived acetyl-CoA back into lipogenesis could not have occurred. Clearly the block in glucose carbon flux into fatty acids seen when noradrenaline (or palmitate) is present must lie distal to pyruvate dehydrogenase (presumably at acetyl-CoA carboxylase). If this were not so, extensive changes in the $^{14}C/^3H$ ratio would have been expected, owing to endogenously derived acetyl-CoA gaining unimpeded access to lipogenesis. It is therefore noteworthy that *in vivo* noradrenaline activates brown-adipose-tissue pyruvate dehydrogenase but inactivates acetyl-CoA carboxylase (Gibbins *et al.*, 1985). Table 1 also shows that acylglycerol glycerol formation and, in particular, lactate + pyruvate output were increased by noradrenaline, suggesting that this agent increased glycolytic flux, in accord with its effect on glucose uptake and lactate + pyruvate output *in vivo* (Cooney *et al.*, 1985; Ma & Foster, 1986). Two more differences from white adipocytes were also apparent. First, lactate + pyruvate formation relative to glucose flux into fatty acids is greater in brown adipocytes, predominating by 8-fold in the presence of insulin and by as mush as 30-fold in the presence of noradrenaline. These findings accord with those of Ma & Foster (1986), who calculated that release of lactate + pyruvate can account for as much as 88% of glucose uptake when brown adipose tissue of cold-acclimated rats is stimulated by noradrenaline *in vivo*. In white adipocytes under similar conditions, glucose fluxes into fatty acid and lactate + pyruvate are generally of equal magnitudes (Saggerson, 1972*a*). It should be stressed that these measurements of lactate + pyruvate release represent flux from exogenous glucose, since freshly isolated brown adipocytes in our hands contain negligible amounts of glycogen (E. D. Saggerson, unpublished work). Second, the ratio of lactate/pyruvate accumulated was considerably lower in brown-adipocytes incubations (Table 1) compared with white adipocyte (Saggerson, 1972*a*).

Table 2. Synthesis of fatty acids from glucose or lactate by brown adipocytes

Brown adipocytes from 6-week-old rats were incubated for 1 h in the presence of ^3H_2O together with [^{14}C]lactate, [^{14}C]glucose or in paired incubations containing either [^{14}C]glucose + [^{12}C]lactate or [^{12}C]glucose + [^{14}C]lactate. The values are means ± S.E.M. for four separate cell preparations. All radiolabels were present at approx. 0.05 μCi/μg-atom of C or H. Statistical significance was determined for paired differences in ^{14}C incorporation and is indicated by $^aP < 0.05$, $^bP < 0.005$ for effects of insulin, $^cP < 0.005$ for effects of glucose on lactate incorporation, or $^dP < 0.05$, $^eP < 0.005$ for effects of lactate on glucose incorporation.

Additions	Fatty acid synthesis (μg-atoms/h per 100 μg of DNA) From ^{14}C-labelled substrate	From ^3H_2O	Ratio $^{14}C/^3H$ in fatty acids
Lactate (1 mM)	0.17±0.01	0.23±0.02	0.75±0.01
Lactate (1 mM)+insulin (4 nM)	0.25±0.02[b]	0.30±0.02	0.83±0.01
Glucose (5 mM)	0.43±0.07	0.41±0.05	1.08±0.15
Glucose (5 mM)+insulin (4 nM)	1.26±0.05[b]	0.96±0.04	1.31±0.01
Glucose (5 mM)+lactate (1 mM)	From glucose: 0.27±0.01[d]		
	From lactate: 0.95±0.09[c]	1.01±0.11	1.22±0.04
	Total: 1.22±0.09		
Glucose (5 mM)+lactate (1 mM)+insulin (4 nM)	From glucose: 0.92±0.04[be]		
	From lactate: 1.24±0.05[ac]	1.36±0.16	1.58±0.10
	Total: 2.16±0.19[b]		

Also, this ratio was significantly increased by insulin in brown cells (Table 1), but decreased by the hormone in white cells (Saggerson, 1972*a*).

Lipogenesis from lactate

When supplied as sole substrate, lactate is a very poor precursor for fatty acid synthesis in rat white adipose tissue (Katz & Wals, 1970; Saggerson & Tomassi, 1971; Saggerson, 1972*b*, 1974), and this is thought to be due to limitation in the disposal of cytosolic NADH in this particular experimental condition. Table 2 shows that the same is true for brown adipocytes. Insulin caused a small but significant stimulation of lactate incorporation. As found in white adipocytes (Saggerson, 1972*b*), addition of glucose facilitated the use of lactate carbon by brown adipocytes. In the absence of insulin this was accompanied by a substantial decrease in the utilization of glucose-derived carbon for lipogenesis. The combination of lactate with glucose at the near-physiological concentrations of 1 mM and 5 mM respectively resulted in substantially higher rates of lipogenesis than were seen with glucose alone.

$^{14}C/^3H$ ratios in fatty acids synthesized *de novo*

Fig. 3 is a compendium of data taken from Tables 1 and 2, showing the relationship between the flux of carbon from exogenous glucose or lactate into fatty acids and the incorporation of ^3H_2O. The latter is taken as being some index of the total flux into fatty acids, irrespective of carbon source. In the presence of glucose + insulin or glucose + lactate + insulin, $^{14}C/^3H$ ratios were comparable with those observed when white adipocytes were incubated with glucose + insulin (see Table 7 of Saggerson, 1972*a*). Lower ^{14}C fluxes were accompanied by lower fluxes of ^3H, but the relationship was not linear. It is presumed that this curvilinearity indicates that there is some dilution of the ^{14}C-labelled precursor pool by endogenous substrates at low rates of flux. However, the possibility that provision of NADPH from the pentose phosphate pathway might be increasing relative to other sources with increasing flux cannot be discounted. This also could cause curvilinearity of the relationship in Fig. 3 (Jungas, 1968).

Lipogenesis and CO_2 production from glucose + lactate

Table 3 shows a study in which brown and white adipocytes isolated in parallel experiments were incubated under conditions approximating to physiological with 5 mM-glucose + 1 mM-lactate. Insulin, as expected, increased fatty acid synthesis from both precursors in the presence or absence of noradrenaline, the percentage effect of insulin being larger with brown cells. By contrast, although noradrenaline decreased fatty acid synthesis from both precursors in the absence of insulin,

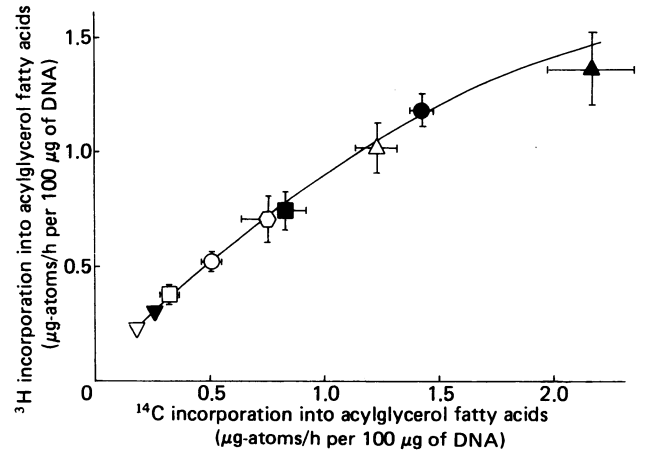

Fig. 3. Correlation between incorporation by brown adipocytes of ^3H_2O or ^{14}C from radiolabelled glucose or lactate into acylglycerol fatty acids

The values are combined values taken from Tables 1 and 2. The bars indicate S.E.M. and, where not shown, lie within the symbol. ○, Glucose, ●, glucose + insulin; □, glucose + noradrenaline; ■, glucose + insulin + noradrenaline; △, glucose + lactate; ▲, glucose + lactate + insulin; ▽, lactate; ▼, lactate + insulin; ⬡, glucose + insulin + palmitate.

Table 3. Effects of insulin and noradrenaline on metabolism of brown and white adipocytes incubated with glucose + lactate

Paired incubation were performed in which brown or white adipocytes from 6-week-old rats were incubated for 1 h either with 5 mM-[U-^{14}C]glucose + 1 mM-[^{12}C]lactate or with 5 mM-[^{12}C]glucose + [U-^{14}C]lactate. The values are means ± S.E.M. for four separate cell preparations in every case. The values in parentheses show the percentage of total incorporation that is attributable to glucose-derived carbon. Statistical significance was determined for paired differences and is indicated by $^{a}P < 0.05$, $^{b}P < 0.025$, $^{c}P < 0.01$, $^{d}P < 0.005$ for effects of noradrenaline, and by $^{e}P < 0.05$, $^{f}P < 0.025$, $^{g}P < 0.01$, $^{h}P < 0.005$ for effects of insulin.

		^{14}C incorporation (μg-atoms/h per 100 μg of cell DNA)				
		Brown adipocytes			White adipocytes	
Additions	^{14}C incorporation from:	Into fatty acids	Into acylglycerol glycerol	Into CO_2	Into fatty acids	Into acylglycerol glycerol
None	Glucose	0.12 ± 0.03	0.28 ± 0.06	0.74 ± 0.16	1.89 ± 0.75	2.15 ± 0.31
	Lactate	0.59 ± 0.15	0.03 ± 0.01	1.51 ± 0.24	3.94 ± 0.84	0.03 ± 0.01
	Both substrates	0.72 ± 0.21	0.31 ± 0.07	2.24 ± 0.39	5.83 ± 1.54	2.18 ± 0.31
		(17)	(90)	(33)	(32)	(99)
Noradrenaline (1 μM)	Glucose	0.04 ± 0.03^{a}	0.93 ± 0.19^{c}	1.38 ± 0.20^{d}	1.07 ± 0.57^{b}	8.67 ± 1.45^{c}
	Lactate	0.18 ± 0.05^{b}	0.01 ± 0.00	2.98 ± 0.40^{d}	1.71 ± 0.59^{d}	0.03 ± 0.01
	Both substrates	0.22 ± 0.08^{a}	0.94 ± 0.19^{c}	4.28 ± 0.60^{b}	2.78 ± 1.16^{a}	8.70 ± 1.44^{c}
		(18)	(99)	(32)	(38)	(99)
Insulin (4 nM)	Glucose	0.76 ± 0.20^{g}	0.57 ± 0.12^{g}	2.33 ± 0.59^{f}	4.67 ± 0.97^{g}	2.54 ± 0.23
	Lactate	1.05 ± 0.21^{f}	0.02 ± 0.00	2.38 ± 0.39^{g}	5.64 ± 0.89^{h}	0.02 ± 0.01
	Both substrates	1.81 ± 0.40^{f}	0.59 ± 0.12^{e}	4.70 ± 1.26^{e}	10.31 ± 1.84^{e}	2.56 ± 0.22
		(42)	(97)	(50)	(45)	(99)
Noradrenaline (1 μM) + insulin (4 nM)	Glucose	0.73 ± 0.25^{f}	1.73 ± 0.31^{ce}	5.36 ± 1.49^{cf}	5.92 ± 1.33^{ag}	5.44 ± 0.35^{de}
	Lactate	0.73 ± 0.25^{e}	0.01 ± 0.01	3.84 ± 0.57^{d}	6.17 ± 1.12^{a}	0.05 ± 0.01
	Both substrates	1.46 ± 0.49^{f}	1.74 ± 0.32^{ce}	9.21 ± 2.06^{ae}	12.09 ± 2.43^{ag}	5.49 ± 0.36^{de}
		(50)	(99)	(58)	(49)	(99)

Table 4. Measurements of fatty acid synthesis *in vivo* and of lipogenic enzyme activities in brown and white whole adipose tissues

^3H_2O incorporation into fatty acids *in vivo* and activities of enzymes in 100000 *g* fractions from whole adipose tissues were measured as described in the Materials and methods section. The values are means ± S.E.M. for the numbers of separate experiments shown in parentheses, and are expressed per g wet wt. tissue. Statistical significance of age differences is indicated by $^aP < 0.05$, $^bP < 0.02$. Statistical significance of differences between white and brown tissues is indicated by $^cP < 0.05$, $^dP < 0.02$, $^eP < 0.001$.

Type of adipose tissue	Age of rats (weeks)	DNA (μg) (*A*)	Fatty acid synthase		ATP citrate lyase		Fatty acid synthesis *in vivo*			
			(nmol/min) (*B*)	$\frac{B}{A}\times 100$	(nmol/min) (*C*)	$\frac{C}{A}\times 100$	(μg-atoms/h) (*D*)	$\frac{D}{A}\times 100$	$\frac{D}{B}\times 100$	$\frac{D}{C}\times 100$
Brown	6	328±22 (11)	458±46 (6)	140	584±75 (6)	178	24.1±3.3 (8)	7.3	5.3	4.1
White	6	210±16[c] (12)	271±39[d] (6)	129	357±45[c] (6)	170	20.3±2.0 (8)	9.7	7.5	5.7
Brown	10	281±19[a] (14)	376±83 (6)	134	397±50 (6)	141	26.9±2.2 (8)	9.6	7.2	6.8
White	10	158±14[ae] (14)	169±15[ac] (6)	107	224±37[ad] (6)	142	13.9±0.9[be] (8)	8.8	8.2	6.2

effects of noradrenaline were less clear-cut in its presence, being insignificant with brown adipcoytes and actually causing a small significant (by paired testing) increase in white adipocytes. In the absence of insulin, white adipocytes derived approx. 60–70% of newly synthesized fatty acid from lactate carbon, whereas more than 80% was lactate-derived in the brown cells. In the presence of insulin both cell types derived 40–50% of the newly synthesized fatty acid from glucose carbon. Noradrenaline did not influence the relative usage of glucose versus lactate. Neither cell type derived any appreciable amount of acylglycerol glycerol from lactate carbon. Acylglycerol glycerol formation from glucose carbon reflects both the provision of glycerol phosphate (which is increased through insulin stimulation of glucose transport) and the provision of non-esterified fatty acid from lipolysis (which is decreased by insulin). It is therefore noteworthy that in the presence of noradrenaline insulin decreased glucose flux into acylglycerol glycerol in white adipocytes, but increased this flux in brown cells. This suggests that the relative effectiveness of insulin in promoting glucose transport compared with inhibiting lipolysis is likely to favour the latter in white cells more than in brown adipocytes. Both insulin or noradrenaline, when added singly, increased the conversion of either substrate into CO_2 by brown adipocytes. When the hormones were combined, their effects on the total CO_2 formation showed synergism. This synergism was solely confined to the utilization of glucose carbon, suggesting an interactive effect of these hormones before pyruvate + lactate formation. It is therefore noteworthy that insulin and noradrenaline have synergistic effects on phosphofructokinase flux in brown adipocytes, as measured by detritiation of [3-^{3}H]glucose (F. Sobrino & E. D. Saggerson, unpublished work).

It has been suggested that glucose may be an important fuel for thermogenesis (Cooney & Newsholme, 1982, 1984; McCormack, 1982). In our hands, brown adipocytes from 6-week-old rats consume 96 μmol of O_2/h per 100 μg of DNA when maximally stimulated with noradrenaline (Jamal & Saggerson, 1988). The 9.2 μmol of CO_2 derived from glucose + lactate in the presence of noradrenaline + insulin (Table 3) could only account for approx. 10% of this maximum rate of respiration, and glucose conversion alone could only account for 5%. It is concluded, in cells from rats maintained at normal temperatures at least, that glucose (and lactate) cannot be a major fuel for brown-adipocyte respiration. Isler *et al.* (1987), also using adipocytes from normal rats, and Ma & Foster (1986), studying cold-acclimated rats *in vivo*, reached the same conclusion.

Comparison between rates of fatty acid synthesis in brown and white adipose cells *in vivo* and *in vitro*

In view of reports that *in vivo* fatty acid synthesis in interscapular brown adipose tissue is substantially higher than in white fat from the same animals (Trayhurn, 1979, 1981; Agius & Williamson, 1980, 1981), it was perplexing to observe from Table 3 that the highest rate of fatty acid synthesis obtainable with brown adipocytes from 6-week-old rats (1.8 μg-atoms/h per 100 μg of DNA in the presence of glucose + lactate + insulin) was only 17% of the rate in white cells under identical conditions. This suggested several possibilities. First, the brown adipocytes might, to a large extent, be non-viable. Second, in our rat population brown-adipose-tissue fatty acid

Table 5. Measurements of fatty acid synthesis *in vitro* and of lipogenic enzyme activities in brown and white adipocytes

For measurements of fatty acid synthesis cells were incubated with 4 nM-insulin in paired incubations containing either 5 mM-[U-^{14}C]glucose + 1 mM-[^{12}C]lactate or 5 mM-[^{12}C]glucose + 1 mM-[U-^{14}C]lactate. Assays of enzymes in 100000 *g* fractions from adipocytes were performed as described in the Materials and methods section. The values are means ± S.E.M. for four separate experiments in every case. Statistical significance of age differences is indicated by $^{a}P < 0.05$, $^{b}P < 0.02$, $^{c}P < 0.01$. Statistical significance of differences between brown and white adipocytes is indicated by $^{d}P < 0.02$, $^{e}P < 0.01$, $^{f}P < 0.001$.

Type of adipocyte	Age of rats (weeks)	Fatty acid synthase activity (nmol/min per 100 μg of cell DNA) (*B*)	ATP citrate lyase activity (nmol/min per 100 μg of cell DNA) (*C*)	^{14}C incorporation into fatty acids: μg-atoms/h per 100 μg of cell DNA (*D*)	$\frac{D}{B} \times 100$	$\frac{D}{C} \times 100$
Brown	6	64 ± 16	62 ± 13	1.8 ± 0.4	2.8	2.9
White	6	273 ± 31^{f}	356 ± 53^{e}	10.3 ± 1.8^{e}	3.8	2.9
Brown	10	107 ± 5^{a}	105 ± 11^{a}	3.9 ± 0.3^{c}	3.6	3.7
White	10	158 ± 16^{bd}	171 ± 26^{a}	5.2 ± 0.1^{ae}	3.3	3.0

synthesis *de novo* might actually be very low. Third, the hormones + substrates presented may not adequately mimic the situation *in vivo*. Fourth, the brown adipocytes obtained in the isolation procedure might not be the cells in the interscapular tissue mass that are most active in lipogenesis. The studies summarized in Tables 4 and 5 were undertaken to gain further insight into this problem.

Rats were administered a glucose load to elicit a high rate of fatty acid synthesis *in vivo*, and these measured rates were then expressed relative to whole-tissue DNA or relative to activities of two 'pathway enzymes' (ATP citrate lyase and fatty acid synthase), which may be considered as reasonable indices of the intrinsic activity of the lipogenic pathway (Table 4). With isolated cells, incorporation of carbon from lactate + glucose (in the presence of insulin) was used as the best estimate of the maximum rate of lipogenesis, and this was expressed on the same basis (Table 5). In brown adipocytes from 6-week-old rats, where there was a large discrepancy relative to DNA content between brown and white cells, there were also comparable differences in the activities of the two 'pathway enzymes'. At 10 weeks of age, differences in both lipogenic rates and activities of the 'pathway-enzyme' activities between the two cell types were less apparent. Table 5 shows that, when compared relative to 'pathway-enzyme' activities, lipogenic rates in brown and white adipocytes at either age were comparable. Thus, relative to their intrinsic lipogenic capability, the brown adipocytes performed as well as the white cells in fatty acid synthesis *de novo*. Table 4 shows that rates of lipogenesis *in vivo* were also similar for the two tissue types when expressed relative to the activities of the 'pathway enzymes'. However, the rates *in vivo* were also comparable between the two tissues on a wet-weight and a DNA basis, and it is therefore concluded that, although metabolically competent (also see Fig. 1), the isolated brown adipocytes are probably not the cells that are most active in lipogenesis in the whole tissue. This would seem to be particularly true for the younger rats. After collagenase disaggregation of brown adipose tissue, the digest is filtered through nylon mesh and then centrifuged to yield a creamy-brown adipocyte top layer, an infranatant and a reddish-brown pellet. Appreciable amounts of undisaggregated tissue are not usually retained on the nylon filter, and after centrifugation floating fat is not usually apparent above the adipocyte layer, suggesting that extensive breakage of cells does not occur. It therefore seems most likely that lipogenically active cells would be found in the infranatant or in the pellet, i.e. they are too dense to float in the aqueous isolation medium. Material from the pellet will incorporate [^{14}C]glucose into fatty acids (E. D. Saggerson, unpublished work), but it is difficult to obtain a maximum estimate of this flux, since this fraction is likely to contain nerve endings releasing noradrenaline. It is presumed that a larger proportion of the available lipogenic cells is recovered in the adipocyte layer with tissue from 10-week-old rats. It is likely that on average these will contain more lipid and be less dense, in view of the decrease in the tissue DNA content per g wet wt. with age (Table 4). It is also possible that the cells would have had a higher lipid content, thereby lessening the disparity between synthesis rates *in vivo* and *in vitro* if the animals had been housed at a higher temperature (e.g. 27–28 °C) rather than the customary 21 °C. Tables 4 and 5 indicate that rates of lipogenesis under what should be reasonably optimal conditions *in vitro* are approx. 50% of those *in vivo* when expressed relative to the activities of the 'pathway enzymes'. It is uncertain to what extent this disparity may reflect the state of viability of the cells or may reflect the absence *in vitro* of all the appropriate substrates and stimulatory factors. It is noteworthy that this disparity is similar in both cell types.

Conclusions

We have shown that a brown-adipocyte fraction from rats maintained at normal temperature will perform sustained synthesis of fatty acids and glycerolipids *in vitro*. With regard to fatty acid synthesis, metabolic flux rates and enzyme activities may not be completely representative of all the lipid-synthesizing cells in the tissue. Lactate appears to be at least as important as glucose as a lipogenic substrate. The cells respond to insulin and noradrenaline and release relatively large amounts of lactate + pyruvate in a manner similar to the tissue *in vivo*. From comparisons of ^{14}C/^{3}H incorporation into fatty acids, no evidence could be found for extensive recycling of acetyl-CoA units between β-oxidation and

fatty acid synthesis. Finally, there is no evidence that glucose (or lactate) may serve as a major fuel for thermogenesis.

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