Changes in activities of some enzymes of glycerolipid synthesis in brown adipose tissue of cold-acclimated rats

Andrew C. DARNLEY, Carol A. CARPENTER and E. David SAGGERSON

Department of Biochemistry, University College London, Gower Street, London WC1E 6BT, U.K.

1. Measurements were made of the activities of the following enzymes of glycerolipid synthesis in homogenates of interscapular brown adipose tissue obtained from rats subjected to a 4 °C environment for time periods of 6 h up to 12 days: fatty acyl-CoA synthetase (FAS), mitochondrial and microsomal forms of glycerolphosphate acyltransferase (GPAT), monoacylglycerolphosphate acyltransferase (MGPAT) and Mg^{2+} -dependent phosphatidate phosphohydrolase (PPH). 2. Relative to tissue DNA content, the activities of mitochondrial GPAT, MGPAT and Mg^{2+} -dependent PPH were significantly increased after 1 day of exposure to cold, and continued to increase thereafter. By contrast, FAS and microsomal GPAT activities were unchanged relative to tissue DNA. 3. The time profile of the increase in MGPAT activity correlated well with a concomitant increase in the microsomal marker NADP⁺-cytochrome *c* reductase. Changes in mitochondrial GPAT and in Mg^{2+} -dependent PPH activities were larger in amplitude than that of MGPAT. 4. It is proposed that these selective changes in enzyme activity may be associated with the onset of brown-adipose-tissue hyperplasia or possibly with an increase in triacylglycerol synthesis during cold-acclimation.

INTRODUCTION

The supply of fatty acids to brown adipose tissue is increased during exposure to cold, and uncoupled respiration of these fatty acids is thought to contribute in a major way to thermogenesis by the tissue. Besides using lipolysis as a source, it is now clear that brown adipocytes in cold-acclimated animals obtain fatty acids from two other sources, i.e. from enhanced synthesis *de novo* (Trayhurn, 1979, 1981; Agius & Williamson, 1980, 1981; Buckley & Rath, 1987) and through enhanced activity of lipoprotein lipase (Radomski & Orme, 1971; Matsushita *et al.*, 1980; Carneheim *et al.*, 1984). It was therefore decided to determine whether the expansion of these latter two processes in cold-acclimation was associated with any enhancement of the pathway of glycerolipid synthesis, or whether enhanced fatty acid flux is wholly directed towards oxidation. After less than 24 h of exposure to cold, the triacylglycerol content of brown adipose tissue reaches a minimum (Cameron & Smith, 1964); thereafter some restoration of triacylglycerol occurs, suggesting that the capacity of the glycerolipid synthesis pathway might be expanded. Accordingly we have made measurements of some enzymes of this pathway in brown adipose tissue obtained from rats exposed to cold for up to 12 days. Some dramatic increases in enzyme activities were observed, but, surprisingly, these changes were selective to certain enzymes.

MATERIALS AND METHODS

Chemicals

These were obtained as described by Baht & Saggerson (1988).

Animals

Male Sprague-Dawley rats were bred in the animal colony at University College London. Animals were at all times maintained on a 13 h-light/11 h-dark cycle, with light from 06:00 to 19:00 h, and with Rat and Mouse No. 3 Breeding Diet (Special Diet Services, Witham, Essex, U.K.) supplied *ad libitum*. The diet contained (w/w) 21 % digestible protein, 4 % digestible crude oil and 39 % starches and sugars. The rats were selected for experimentation at 160–190 g body wt., and either were then kept at approx. 21 °C or were transferred to a 4 °C room for various periods of time. Both sets of animals were kept on the same light/dark cycle.

Experimental methods

Rats were killed by cervical dislocation between 11:00 and 12:00 h. Individual interscapular brown adipose tissues were removed, chilled in ice-cold medium consisting of 0.25 M-sucrose/10 mM-Tris/HCl buffer (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol, weighed, and then homogenized in 7–10 ml of the same medium with an Ultra-Turrax tissue disintegrator. The homogenates were left in ice for at least 5 min before the underlying aqueous homogenate was aspirated away from floating fat. Portions of these homogenates were then kept frozen at –70 °C until used for all subsequent analyses.

FAS, Mg^{2+} -dependent PPH and MGPAT were assayed as described by Baht & Saggerson (1988). GPAT activity in homogenates was resolved into mitochondrial and microsomal activities by assaying with 40 μ M-palmitoyl-CoA + *N*-ethylmaleimide or with 120 μ M-oleoyl-CoA respectively (Baht & Saggerson, 1988). Citrate synthase (EC 4.1.3.7) and NADP⁺-cytochrome *c* reductase (EC 1.6.2.4) were assayed spectrophotometrically at 25 °C by

Abbreviations used: FAS, ATP-dependent long-chain fatty acyl-CoA synthetase (EC 6.2.1.3); GPAT, glycerolphosphate acyltransferase (EC 2.3.1.15); MGPAT, monoacylglycerolphosphate acyltransferase (EC 2.3.1.51); PPH, phosphatidate phosphohydrolase (EC 3.1.3.4).

the methods of Shepherd & Garland (1969) and Phillips & Langdon (1962) respectively. Protein was measured by the method of Lowry *et al.* (1951), with fatty-acid-poor bovine albumin as a standard. DNA was measured in defatted homogenates by the method of Switzer & Summer (1971).

RESULTS AND DISCUSSION

Table 1 shows changes in body weights and in tissue parameters when rats were exposed to a 4 °C environment or were maintained at 21 °C. As found by Carneheim *et al.* (1984), there was no decrease in the tissue wet weight on exposure to cold. The total tissue protein content was significantly increased by 69 % within 6 h of exposure to cold. In the cold the tissue protein content then continued to increase after the first day, reaching more than 3 times the initial value after 12 days. After 1 day in the cold the total tissue DNA content began to increase, with significant changes of 1.8- and 2.6-fold being seen after 7 and 12 days respectively, suggesting that cell proliferation was occurring. It should, however, be noted that these measurements do not discriminate between changes in

the DNA content owing to increases in the numbers of adipocytes rather than alterations in other cell types. Animals maintained at 21 °C for 15 days showed a small (46 %) increase in tissue protein content, with no significant change in tissue wet weight or DNA content. Unlike Carneheim *et al.* (1984), we observed a significant initial decrease in body weight on exposure of the rats to the cold. After 3 days normal mean growth rate was resumed (5.8 and 5.6 g/day at 4 °C and 21 °C respectively).

Table 2 shows activity changes for the respective mitochondrial and microsomal marker enzymes citrate synthase and NADP⁺-cytochrome *c* reductase. Both activities were significantly increased per total tissue during cold exposure. Relative to tissue DNA content, however, citrate synthase activity did not change appreciably, whereas, on this basis, NADP⁺-cytochrome *c* reductase activity was increased by 2.6-fold after 7 days at 4 °C. This is noteworthy, and may indicate a general increase in the endoplasmic reticulum of the tissue on cold-acclimation.

Tables 3 and 4 show activity changes for enzymes of glycerolipid synthesis during cold-acclimation. Although

Table 1. Details of animals and interscapular brown adipose tissues

The values are means $\pm$ S.E.M. for *n* individual animals. Significant changes relative to the zero-time value are indicated by: **P* < 0.05, ^b*P* < 0.02, ^c*P* < 0.01, ^d*P* < 0.001.

Time (days)	Temperature (°C)	Change in body wt. (g) compared with zero time	Brown-adipose-tissue wet wt. (g)	Protein (mg per total tissue)	DNA (μg per total tissue)	<i>n</i>
0	21	—	0.51 $\pm$ 0.07	18.3 $\pm$ 3.0	282 $\pm$ 52	6
0.25	4	-11 $\pm$ 2 ^c	0.53 $\pm$ 0.05	30.9 $\pm$ 3.0 ^b	280 $\pm$ 46	4
1	4	-14 $\pm$ 2 ^c	0.62 $\pm$ 0.05	31.4 $\pm$ 1.7 ^c	382 $\pm$ 60	4
3	4	-16 $\pm$ 4 ^a	0.71 $\pm$ 0.04 ^a	36.2 $\pm$ 1.9 ^d	450 $\pm$ 52	4
7	4	+7 $\pm$ 7	0.55 $\pm$ 0.04	50.6 $\pm$ 3.9 ^d	510 $\pm$ 28 ^c	4
12	4	+36 $\pm$ 2 ^d	0.59 $\pm$ 0.05	57.3 $\pm$ 2.4 ^d	734 $\pm$ 54 ^d	4
1	21	+2 $\pm$ 2	—	—	—	6
3	21	+24 $\pm$ 2	—	—	—	6
7	21	+47 $\pm$ 4	—	—	—	6
12	21	+60 $\pm$ 7	—	—	—	6
15	21	+89 $\pm$ 12	0.37 $\pm$ 0.03	26.9 $\pm$ 2.0 ^a	293 $\pm$ 44	6

Table 2. Effects of cold-acclimation on activities of marker enzymes

Activities are expressed as nmol/min and are means $\pm$ S.E.M. for *n* animals. Significant changes relative to the zero-time value are indicated as in Table 1.

Time (days)	Temperature (°C)	Citrate synthase activity			NADP ⁺ -cytochrome <i>c</i> reductase activity			<i>n</i>
		per total tissue	per mg of protein	per 100 μg of DNA	per total tissue	per mg of protein	per 100 μg of DNA	
0	21	33643 $\pm$ 5487	1851 $\pm$ 114	12489 $\pm$ 1290	145 $\pm$ 24	8.24 $\pm$ 0.85	54.4 $\pm$ 6.1	6
0.25	4	41544 $\pm$ 7062	1334 $\pm$ 196	14868 $\pm$ 1403	170 $\pm$ 18	5.52 $\pm$ 0.36 ^b	63.3 $\pm$ 5.2	4
1	4	44265 $\pm$ 5638	1404 $\pm$ 135 ^a	11787 $\pm$ 765	271 $\pm$ 31 ^b	8.60 $\pm$ 0.77	72.7 $\pm$ 6.3	4
3	4	48824 $\pm$ 4619	1340 $\pm$ 69 ^b	10202 $\pm$ 941	521 $\pm$ 72 ^c	14.34 $\pm$ 1.64 ^b	115.7 $\pm$ 8.4 ^d	4
7	4	60784 $\pm$ 7537 ^b	1227 $\pm$ 62 ^c	12238 $\pm$ 1138	724 $\pm$ 63 ^d	14.50 $\pm$ 1.39 ^c	142.8 $\pm$ 12.3 ^d	4
12	4	76287 $\pm$ 2962 ^d	1333 $\pm$ 9 ^c	10497 $\pm$ 468	768 $\pm$ 41 ^d	13.44 $\pm$ 0.69 ^c	105.4 $\pm$ 4.6 ^d	4
15	21	25981 $\pm$ 3674	949 $\pm$ 93 ^d	8450 $\pm$ 969 ^a	173 $\pm$ 19	6.31 $\pm$ 0.32	57.5 $\pm$ 5.0	6

Table 3. Effects of cold-acclimation on FAS and GPAT activities

Activities are expressed as nmol/min and are means $\pm$ S.E.M. for *n* animals. Significant changes relative to the zero-time value are indicated as in Table 1.

Time (days)	Temperature (°C)	FAS activity			Mitochondrial GPAT activity			Microsomal GPAT activity			<i>n</i>
		per total tissue	per mg of protein	per 100 μ g of DNA	per total tissue	per mg of protein	per 100 μ g of DNA	per total tissue	per mg of protein	per 100 μ g of DNA	
0	21	2148 $\pm$ 346	120 $\pm$ 12	808 $\pm$ 106	7.4 $\pm$ 1.4	0.40 $\pm$ 0.03	2.6 $\pm$ 0.3	18.4 $\pm$ 3.9	0.97 $\pm$ 0.10	6.6 $\pm$ 1.0	6
0.25	4	2427 $\pm$ 262	79 $\pm$ 7 ^b	930 $\pm$ 145	7.6 $\pm$ 1.2	0.25 $\pm$ 0.04 ^b	2.8 $\pm$ 0.3	18.8 $\pm$ 2.6	0.63 $\pm$ 0.10 ^a	7.1 $\pm$ 1.0	4
1	4	2635 $\pm$ 170	85 $\pm$ 9 ^a	722 $\pm$ 77	17.6 $\pm$ 2.8 ^c	0.56 $\pm$ 0.07	4.6 $\pm$ 0.4 ^c	23.8 $\pm$ 2.0	0.76 $\pm$ 0.06	6.4 $\pm$ 0.5	4
1	4	2913 $\pm$ 275	82 $\pm$ 10 ^a	677 $\pm$ 110	47.1 $\pm$ 3.8 ^d	1.30 $\pm$ 0.06 ^d	10.6 $\pm$ 0.9 ^d	31.3 $\pm$ 5.7	0.85 $\pm$ 0.12	6.8 $\pm$ 0.8	4
7	4	3619 $\pm$ 410 ^a	71 $\pm$ 6 ^c	714 $\pm$ 90	63.4 $\pm$ 5.1 ^d	1.25 $\pm$ 0.03 ^d	12.5 $\pm$ 0.8 ^d	25.1 $\pm$ 3.7	0.49 $\pm$ 0.04 ^c	4.9 $\pm$ 0.6	4
12	4	4062 $\pm$ 388 ^c	71 $\pm$ 6 ^c	563 $\pm$ 66	68.5 $\pm$ 6.1 ^d	1.19 $\pm$ 0.06 ^d	9.4 $\pm$ 0.5 ^d	39.0 $\pm$ 7.7 ^a	0.69 $\pm$ 0.14	5.5 $\pm$ 1.3	4
15	21	2215 $\pm$ 236	82 $\pm$ 4 ^b	741 $\pm$ 64	6.6 $\pm$ 1.0	0.25 $\pm$ 0.03 ^c	2.3 $\pm$ 0.4	30.6 $\pm$ 2.3 ^a	1.14 $\pm$ 0.06	10.5 $\pm$ 1.1 ^a	6

Table 4. Effects of cold-acclimation on MGPAT and Mg²⁺-dependent PPH activities

Activities are expressed as nmol/min and are means $\pm$ S.E.M. for *n* animals. Significant changes relative to the zero-time value are indicated as in Table 1.

Time (days)	Temperature (°C)	MGPAT activity			Mg ²⁺ -dependent PPH			<i>n</i>
		per total tissue	per mg of protein	per 100 μ g of DNA	per total tissue	per mg of protein	per 100 μ g of DNA	
0	21	871 $\pm$ 149	47.9 $\pm$ 4.6	317 $\pm$ 27	257 $\pm$ 54	13.8 $\pm$ 1.8	93 $\pm$ 14	6
0.25	4	1030 $\pm$ 125	33.3 $\pm$ 2.8 ^a	381 $\pm$ 36	371 $\pm$ 25	12.3 $\pm$ 1.1	142 $\pm$ 19	4
1	4	1579 $\pm$ 121 ^c	50.4 $\pm$ 3.4	427 $\pm$ 31 ^a	765 $\pm$ 109 ^c	24.6 $\pm$ 3.5 ^a	203 $\pm$ 18 ^c	4
3	4	3765 $\pm$ 254 ^d	104.1 $\pm$ 5.2 ^d	851 $\pm$ 52 ^d	1943 $\pm$ 112 ^d	53.7 $\pm$ 1.0 ^d	440 $\pm$ 28 ^d	4
7	4	3530 $\pm$ 323 ^d	69.7 $\pm$ 2.7 ^c	692 $\pm$ 51 ^d	1577 $\pm$ 232 ^d	30.8 $\pm$ 3.3	310 $\pm$ 44 ^c	4
12	4	4853 $\pm$ 403 ^d	84.6 $\pm$ 5.2 ^d	663 $\pm$ 32 ^d	1944 $\pm$ 144 ^d	33.8 $\pm$ 1.2 ^d	266 $\pm$ 15 ^d	4
15	21	913 $\pm$ 146	33.0 $\pm$ 3.6 ^a	289 $\pm$ 21	256 $\pm$ 35	9.3 $\pm$ 0.8 ^a	85 $\pm$ 9	6

Fig. 1. Percentage changes in enzyme activities during cold-acclimation

The values are derived from activities expressed relative to tissue DNA (see Tables 2–4): ▲, mitochondrial GPAT; ○, citrate synthase; □, FAS; ■, Mg²⁺-dependent PPH; ▽, MGPAT; ●, NADP⁺-cytochrome *c* reductase; △, microsomal GPAT.

the activity of the microsomal form of GPAT per total tissue was significantly increased after 12 days in the cold, this change was not appreciably different from the age-dependent increase in this activity observed at 21 °C over 15 days (Table 3; also see Baht & Saggerson, 1988). All other enzyme activities showed significant increases per total tissue on cold-acclimation. For mitochondrial GPAT, MGPAT and Mg²⁺-dependent PPH, these increases were large and rapid and were also observed relative to tissue DNA content even after only 1 day in the cold. On this basis (relative to DNA) the increases in PPH and MGPAT activities reached a peak after approx. 3 days in the cold, whereas the maximum increase in mitochondrial GPAT was reached after approx. 7 days. By contrast, relative to tissue DNA content, FAS activity was unchanged throughout the 12-day period of cold-acclimation.

Fig. 1 shows normalized time profiles for the effect of cold-acclimation on enzyme activities expressed relative to tissue DNA content. This restates the conclusion that, relative to cell number, FAS, microsomal GPAT and citrate synthase activities were unchanged over the period of cold-acclimation. Fig. 1 also shows that the percentage change in MGPAT (the highest enzyme activity in the tissue solely concerned with triacylglycerol assembly; see Baht & Saggerson, 1988) is quite well correlated with the percentage change in the activity of the microsomal marker NADP-cytochrome *c* reductase. Linear-regression analysis of the individual MGPAT and reductase activities measured in this study indicated a correlation coefficient (*r*) of 0.88 (*n* = 32, *P* < 0.001). The implication is that increased MGPAT activity might closely follow some proliferation of the endoplasmic reticulum during cold-acclimation. Fig. 1 also demonstrates that cold-acclimation caused larger percentage increases in mitochondrial GPAT and Mg²⁺-dependent PPH activities than those shown for MGPAT. Nevertheless, there was an excellent linear correlation between the profiles of percentage change for MGPAT and PPH (the two enzymes in the triacylglycerol-synthesis pathway that synthesize and dispose of phosphatidate respectively) with *r* = 0.98 (*n* = 6, *P* < 0.001) and gradient = 2.0. In addition, a plot of the individual activities of these two enzymes expressed relative to

tissue DNA also showed a good linear correlation (*r* = 0.94, *n* = 32, *P* < 0.001).

In conclusion, cold-acclimation over a period of 12 days caused increases in activities of enzymes in the glycerolipid-synthesis pathway. However, the changes were selective and, relative to cell DNA, were only seen for the mitochondrial form of GPAT, for MGPAT, which is essentially microsomal (Baht & Saggerson, 1988), and for Mg²⁺-dependent PPH, which is both microsomal and cytosolic in brown adipocytes (Baht & Saggerson, 1988). It is not known at present whether PPH in brown adipose tissue is 'ambiquitous', i.e. whether or not it shows the phenomenon of translocation between soluble and membrane-bound pools, as is found for the enzyme in liver (Cascales *et al.*, 1984; Pittner *et al.*, 1985; Hopewell *et al.*, 1985) and white adipose tissue (Moller *et al.*, 1981; Moller & Hough, 1982; Taylor & Saggerson, 1986). By contrast, the activities of microsomal GPAT and FAS, which is mainly mitochondrial in brown adipocytes (Baht & Saggerson, 1988), appear to be constitutive. The physiological significance of these selective changes is unclear at present. It is not known whether these relate primarily to brown-adipocyte proliferation or whether they may also be concerned with enhanced glycerolipid synthesis during cold-acclimation. It is noteworthy that the rapid increases in mitochondrial GPAT, MGPAT and Mg²⁺-dependent PPH complement the rapid increase in lipoprotein lipase activity, which reaches a plateau after 1–2 days of exposure to cold (Carneheim *et al.*, 1984). It is also noteworthy that hypothyroidism also causes a substantial increase in NADP⁺-cytochrome *c* reductase and in mitochondrial GPAT in brown adipocytes (Baht & Saggerson, 1988). Further studies should be directed towards an understanding of the hormonal stimuli responsible for the changes seen here, and to establish whether the observed changes reflect increases in amount of enzyme protein or modification of existing enzyme activity.

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