Regulation of fatty acid synthesis and malonyl-CoA content in mouse brown adipose tissue in response to cold-exposure, starvation or re-feeding

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1. The effect of nutritional status on fatty acid synthesis in brown adipose tissue was compared with the effect of cold-exposure. Fatty acid synthesis was measured *in vivo* by $^3\text{H}_2\text{O}$ incorporation into tissue lipids. The activities of acetyl-CoA carboxylase and fatty acid synthetase and the tissue concentrations of malonyl-CoA and citrate were assayed. 2. In brown adipose tissue of control mice, the tissue content of malonyl-CoA was 13 nmol/g wet wt., higher than values reported in other tissues. From the total tissue water content, the minimum possible concentration was estimated to be 30 μM . 3. There were parallel changes in fatty acid synthesis, malonyl-CoA content and acetyl-CoA carboxylase activity in response to starvation and re-feeding. 4. There was no correlation between measured rates of fatty acid synthesis and malonyl-CoA content and acetyl-CoA carboxylase activity in acute cold-exposure. The results suggest there is simultaneous fatty acid synthesis and oxidation in brown adipose tissue of cold-exposed mice. This is probably effected not by decreases in the malonyl-CoA content, but by increases in the concentration of free long-chain fatty acyl-CoA or enhanced peroxisomal oxidation, allowing shorter-chain fatty acids to enter the mitochondria independent of carnitine acyltransferase (overt form) activity.

INTRODUCTION

In the liver, fatty acid synthesis and oxidation (hence ketogenesis) are controlled in a reciprocal manner by the inhibition of the overt form of carnitine acyltransferase (CAT 1) by malonyl-CoA (McGarry *et al.*, 1978; McGarry & Foster, 1979, 1980). The hepatic concentration of malonyl-CoA parallels the rate of fatty acid synthesis in the short term in the rat (Guynn *et al.*, 1972; McGarry *et al.*, 1978), which exhibits rapid rates of fatty acid synthesis, and in the sheep (Brindle *et al.*, 1985), which has a low capacity for fatty acid synthesis.

The sensitivity of CAT 1 to inhibition by malonyl-CoA has also been demonstrated in mitochondria isolated from other tissues. These include the kidneys, oxidative tissues, such as heart and skeletal muscle, and lipogenic tissues, such as white adipocytes and lactating mammary gland (Saggerson & Carpenter, 1981; McGarry *et al.*, 1983). On the basis of these results, it has been suggested that the reciprocal relationship between fatty acid synthesis and oxidation also exists in extra-hepatic tissues. However, measurement of malonyl-CoA content of these tissues has been limited to heart and skeletal muscle and not related directly to rates of fatty acid synthesis (McGarry *et al.*, 1983).

In homeotherms, thermogenesis in brown adipose tissue is associated with high rates of fatty acid oxidation (Nicholls & Locke, 1983). In animals maintained on a low-fat diet, it is also associated with high rates of fatty acid synthesis, in both short-term (Rath *et al.*, 1979) and long-term (Trayhurn, 1979) cold-exposure. CAT 1 in mitochondria from brown adipose tissue is extremely sensitive to inhibition by malonyl-CoA *in vitro* (Sagger-

son & Carpenter, 1982), suggesting that a similar mechanism controls fatty acid metabolism in this tissue.

To explain the apparent paradox of simultaneous fatty acid synthesis and oxidation, we have measured the concentration of malonyl-CoA in brown adipose tissue of cold-exposed mice. This has been compared with changes in response to starvation and re-feeding. The concentration of malonyl-CoA in brown adipose tissue was compared with rates of fatty acid synthesis measured *in vivo*. We have also assayed acetyl-CoA carboxylase and fatty acid synthetase to compare the mechanisms regulating fatty acid synthesis and contents of malonyl-CoA in brown adipose tissue during cold-exposure and altered nutritional states. A preliminary account of some of the results presented in this paper has been presented elsewhere (Buckley & Rath, 1985).

MATERIALS AND METHODS

Animals

Female mice, aged 6–8 weeks, were obtained from a random-bred closed colony maintained at Queen Mary College, into which the *ob* gene had been incorporated. The colony was derived from mice obtained from Imperial College and the Dunn Nutrition Research Centre, Cambridge. All mice were homozygous lean, and maintained on laboratory chow [FFG(M)] obtained from Dixon & Sons, Ware, Herts, U.K. The animal house temperature was maintained at $22 \pm 3^\circ\text{C}$ under a 14 h-light/10 h-dark cycle, the light cycle running from 08:00 to 22:00 h. Cold-exposed mice were maintained in environmental cabinets at $4 \pm 2^\circ\text{C}$ under the same

Abbreviation used: CAT 1, overt form of carnitine acyltransferase.

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light/dark cycle, until immediately before being killed. All mice were separated into individual cages 48 h before the experimental time point. Since the rate of fatty acid synthesis varies diurnally (Hems *et al.*, 1975), experiments were always performed between 09:30 and 10:45 h. Plasma glucose concentration was measured in blood taken by cardiac puncture from mice under diethyl ether anaesthesia, to monitor the nutritional status of the animals. Male BALB/c mice used for the preparation of liver fatty acid synthetase were given by the Animal Unit from the London Hospital Medical School.

Materials

All radioactive materials, $^3\text{H}_2\text{O}$, $\text{NaH}^{14}\text{CO}_3$ and $[1\text{-}^{14}\text{C}]\text{acetyl-CoA}$ were obtained from Amersham International, Amersham, Bucks., U.K. Low-background scintillation vials and reagents for scintillation counting were from the BDH Scintran range. Acetyl-CoA, ATP, NADPH, NADH and glutathione were supplied by

buffer (880 μl) was added to tubes containing 20 μl of either water or malonyl-CoA (0.2 nmol). Tissue extract or standard malonyl-CoA solution (both 100 μl) was added. The assay buffer contained (final concns.) potassium phosphate buffer (200 mM), pH 7.0, dithiothreitol (2.5 mM), EDTA (2 mM), NADPH (0.2 mM) and albumin (1 mg/ml). Each tube also contained 2 nmol of $[1\text{-}^{14}\text{C}]\text{acetyl-CoA}$ (sp. radioactivity > 50 mCi/nmol). The reaction was initiated by the addition of approx. 25 munits of purified fatty acid synthetase and terminated after 45 min by the addition of 25 μl of HClO_4 (70%, w/v). Palmitate was extracted in light petroleum (b.p. 40–60 $^\circ\text{C}$), washed with water, transferred to scintillation vials and dried under N_2 . ^3H content of samples was counted three times in a Packard Tri-Carb liquid-scintillation counter to a minimum of 10000 counts (error 1.25%).

To calculate the malonyl-CoA content of tissues, the degree of dilution of labelled acetyl-CoA was calculated from the quotient:

$$\frac{\text{d.p.m. in standard 0.2 nmol of malonyl-CoA (from standard curve)}}{[\text{d.p.m. in (tissue sample + 0.2 nmol of malonyl-CoA)} - \text{d.p.m. in tissue sample}]}$$

BCL, Lewes, Sussex, U.K. Other enzymes and chemicals were obtained from Sigma, Poole, Dorset, U.K.

Measurement of fatty acid synthesis

The rate of fatty acid synthesis *in vivo* was measured by the incorporation of $^3\text{H}_2\text{O}$ into saponifiable fatty acids as described by Rath *et al.* (1979). Mice were injected intraperitoneally with $^3\text{H}_2\text{O}$ (2–5 mCi in 0.1 ml) 30 min before the experimental time point and maintained at the appropriate environmental temperature for 1 h, before the extraction of tissues.

Assay of tissue intermediates

Mice were lightly anaesthetized in diethyl ether/air and the skin was parted without disturbing the blood supply to the interscapular brown adipose tissue. This was rapidly removed and frozen in liquid N_2 (within 6 s). Weighed tissues were homogenized in 7% (w/v) HClO_4 (100 $\mu\text{g}/500 \mu\text{l}$ for malonyl-CoA, whole tissue in 1 ml for citrate), centrifuged at 10000 g for 1 min, and the infranatant was adjusted to pH 6.0–6.5 with KOH (3.5 M) and K_2HPO_4 (1 M). Total tissue citrate concentration was measured by the method of Moellering & Gruber (1966). The tissue content of malonyl-CoA was determined by a modification of the method of McGarry *et al.* (1978), based on the incorporation of $[^{14}\text{C}]\text{acetyl-CoA}$ into palmitate, catalysed by fatty acid synthetase.

The method of McGarry *et al.* (1978) overcame the problem of dilution of the $[^{14}\text{C}]\text{acetyl-CoA}$ by varying quantities of endogenous tissue acetyl-CoA or other acyl-CoA species that may act as primers for condensation with malonyl-CoA in the fatty acid synthetase reaction, by assaying each sample in the presence and absence of 0.2 nmol of malonyl-CoA. To overcome the problem found by McGarry *et al.* (1978), where the effective specific radioactivity of $[^{14}\text{C}]\text{acetyl-CoA}$ was higher in the blanks without tissues than in samples (which contained endogenous acetyl-CoA or primers), a series of standard malonyl-CoA solutions were prepared, containing 0–0.25 nmol in 100 μl , which were run with each experiment.

Assays were performed at 37 $^\circ\text{C}$, in duplicate. Assay

The sample d.p.m. was multiplied by this dilution factor to give the corrected d.p.m. for malonyl-CoA in sampled tissue extracts. The concentration of malonyl-CoA in tissue extracts was determined by reference back to the standard curve of d.p.m. versus malonyl-CoA concentration.

Fatty acid synthetase required for this assay was purified from livers taken from starved/re-fed male mice by the method of Nepokroeff *et al.* (1975). The specific activity of the purified enzyme was 230 munits/mg of protein for batch 1 and 495 munits/mg of protein for batch 2.

Enzyme assays

Crude enzyme extracts were obtained as described by Halestrap & Denton (1973). After removal and freezing of tissue in liquid N_2 , about 115 mg of tissue/ml was homogenized in potassium phosphate buffer (50 mM), pH 7.3, containing EDTA (2 mM) and glutathione (4 mM). For the assay of fatty acid synthetase, albumin was added to the homogenates to give a final concentration of 10 mg/ml. Homogenates were spun for 1 min at 10000 g, the infranatant was removed by syringe, and assays commenced within 5 min.

Acetyl-CoA carboxylase ('initial' activity) was assayed by the incorporation of $[^{14}\text{C}]\text{bicarbonate}$ into malonyl-CoA by a modification of the method of McCormack & Denton (1977). The concentration of acetyl-CoA was varied between 0.01 and 0.1 mM to determine the K_m , which was found to be 0.11 mM for acetyl-CoA. The activity of acetyl-CoA carboxylase was routinely assayed in the presence of 0.5 mM-acetyl-CoA and 2 mM-citrate. One unit of acetyl-CoA carboxylase activity is defined as the amount that will catalyse the conversion of 1 μmol of substrate/min at 37 $^\circ\text{C}$.

Fatty acid synthetase was assayed by the method of Zammit & Corstorphine (1982). The concentration of malonyl-CoA was varied between 2.7 and 250 μM to determine the K_m . This was found to be 18 μM , which is similar to the values of 10 μM for rat liver (Nepokroeff *et al.*, 1975) and 13 μM for mammary-gland enzyme (Smith & Abraham, 1975). One unit of fatty acid synthetase activity is defined as the amount catalysing

Fig. 1. Standard curve of incorporation of [^{14}C]acetyl-CoA into long-chain fatty acid with malonyl-CoA concentration

A standard curve for a representative batch of [^{14}C]acetyl-CoA used for malonyl-CoA determinations is shown. Three experiments were performed per batch (●, first; ■, second; ▲, third): 1.0 nmol of [^{14}C]acetyl-CoA was added to each tube. By knowing the specific radioactivity of [^{14}C]acetyl-CoA in the batch, the theoretical standard curve for radioactivity (d.p.m.) in palmitic acid versus malonyl-CoA was calculated (broken line).

the malonyl-CoA-dependent oxidation of $1\ \mu\text{mol}$ of NADPH/min at 37°C .

Expression of results

Results are given as means $\pm$ S.E.M. for the numbers of observations given in parentheses. Statistical significance of differences was assessed by Student's unpaired t test.

RESULTS AND DISCUSSION

Malonyl-CoA content of brown adipose tissue

Incorporation of ^{14}C from [^{14}C]acetyl-CoA into palmitate was linear with increasing malonyl-CoA concentration (Fig. 1). In no standard curve did the line go through the origin, but in all experiments in the absence of added malonyl-CoA the ^{14}C incorporation into palmitate was similar. One possible explanation is that the purified mouse liver fatty acid synthetase contained endogenous bound malonyl-CoA (of the order of $2\ \text{mol/mol}$, assuming the enzyme preparation to be about 60% pure). The gradient obtained from each experimental curve was 61–76% less than for the theoretical standard curve calculated from the specific radioactivity of the particular batch of [^{14}C]acetyl-CoA and the stoichiometry of the fatty acid synthetase reaction, suggesting the efficiency of extraction of palmitic acid in the light petroleum was of the same order. This should not affect the calculated values of tissue malonyl-CoA, which were related to the experimental standard curve.

The concentration of malonyl-CoA in brown adipose tissue from fed mice was $13\ \text{nmol/g}$ wet wt. (Table 1), higher than the values reported for other tissues (McGarry *et al.*, 1983). For comparison, the content of malonyl-CoA in liver of control fed mice was also

measured. The value of $4\ \text{nmol/g}$ of tissue (mean of two observations) was similar to those obtained for rat liver (McGarry *et al.*, 1978; Zammit, 1981). From the total water content of brown adipose tissue it is possible to estimate the minimum possible concentration of malonyl-CoA as $30\ \mu\text{M}$. It is likely that this underestimates the cytoplasmic concentration of malonyl-CoA by at least 50%. The proportion of intracellular water in brown adipose tissue is higher than that in white adipose tissue (Czech *et al.*, 1974), which is 17% (Saggerson & Greenbaum, 1970). It is noteworthy that these high concentrations of malonyl-CoA in brown adipose tissue were measured when rates of fatty acid synthesis in the tissue are at their lowest in the 24 h cycle (Hems *et al.*, 1975).

Regulation of fatty acid synthesis in brown adipose tissue

Nutritional status. Fatty acid synthesis in brown adipose tissue was extremely sensitive to changes in nutritional status (Table 1), decreasing to negligible rates on starvation and increasing dramatically (150-fold) after only 2 h re-feeding, in agreement with results of other workers (McCormack & Denton, 1977; Agius & Williamson, 1980; Sugden *et al.*, 1981). The concentration of malonyl-CoA changed rapidly in response to starvation and re-feeding (Table 1), as in other tissues (McGarry *et al.*, 1983). Within 2 h of re-feeding, the concentration of malonyl-CoA had doubled from the starved value to be slightly higher than in fed controls.

A linear relationship was observed ($r = 0.945$) between the rate of fatty acid synthesis and the concentration of malonyl-CoA in brown adipose tissue obtained in different nutritional states, in agreement with results obtained in the rat liver (McGarry & Foster, 1979; Guynn *et al.*, 1972) and sheep liver (Brindle *et al.*, 1985). Contrary to the results obtained for hepatocytes (Brindle *et al.*, 1985), significant amounts of malonyl-CoA were measured with (relatively) low rates of fatty acid synthesis.

Thus the regulation of fatty acid synthesis in brown adipose tissue in response to short-term starvation and re-feeding appears to be similar to the regulation in the liver (Guynn *et al.*, 1972, 1973; Zammit, 1981), white adipose tissue (Stansbie *et al.*, 1976) and mammary gland (Munday & Williamson 1982). It is probable that the rapid changes in acetyl-CoA carboxylase activity (Table 1) result in changes in the concentration of malonyl-CoA and regulate the rate of fatty acid synthesis. These changes were probably effected by changes in plasma insulin concentration [in agreement with McCormack & Denton (1977), Agius & Williamson (1981) and Sugden *et al.* (1981)] stimulated by rapid changes in plasma glucose concentration (Table 1).

Cold-exposure. In contrast, changes in fatty acid synthesis in response to acute cold-exposure did not parallel changes in acetyl-CoA carboxylase activity (Table 2), and no correlation was found between the malonyl-CoA content of the tissues and the observed rate of fatty acid synthesis in cold-exposed mice ($r = 0.0214$).

The rapid decrease in synthesis after acute (2 h) cold-exposure is similar to those reported in rats in response to noradrenaline (Agius & Williamson, 1980; Gibbins *et al.*, 1985). However, in contrast with the findings of Gibbins *et al.* (1985), there was no

Table 1. Effect of nutritional status on brown-adipose-tissue composition, malonyl-CoA and citrate contents, activities of acetyl-CoA carboxylase and fatty acid synthetase, rates of fatty acid synthesis *in vivo* and plasma glucose concentration

Tissue water content of interscapular brown adipose tissue was determined by the weight difference before and after freeze-drying the tissue to constant weight. Statistical significance: * $P < 0.05$, ** $P < 0.001$, compared with controls; † $P < 0.05$ compared with starved mice.

	Nutritional status		
	Fed	Starved 24 h	Starved 24 h/re-fed 2 h
Food intake (g/day)	4.4 ± 0.7 (8)	0	1.8 ± 0.3 (3)†
Plasma glucose concn. (mM)	10.8 ± 0.5 (14)	5.0 ± 1.3 (5)**	10.9 ± 0.8 (5)†
Brown adipose tissue			
Tissue weight (mg)	109 ± 2 (98)	56 ± 2.0 (17)**	69 ± 4 (17)**†
Tissue water (mg/g)	490 ± 2 (7)	342 ± 16 (4)**	440 ± 47 (4)
Citrate (nmol/g)	254 ± 16 (14)	503 ± 33 (4)**	375 ± 55 (4)
Malonyl-CoA (nmol/g)	13 ± 2.3 (22)	8.0 ± 1.0 (4)	18 ± 3.0 (5)†
Acetyl-CoA carboxylase initial activity (munits/g)	410 ± 30 (18)	300 ± 50 (6)	720 ± 140 (5)*†
Fatty acid synthetase (munits/g)	2100 ± 200 (18)	—	—
Fatty acid synthesis (μmol of C ₂ units/h per g)	87 ± 16 (24)	2.3 ± 0.5 (3)**	294 ± 116 (3)*†

† Food intake measured during the 2 h re-feeding period after 24 h starvation.

Table 2. Effect of cold-exposure on brown-adipose-tissue composition, contents of malonyl-CoA and citrate, activities of acetyl-CoA carboxylase and fatty acid synthetase and rates of fatty acid synthesis *in vivo*, food intake and plasma glucose concentration

Statistical significance: * $P < 0.05$, ** $P < 0.001$ compared with controls.

	Period of exposure to 4 °C				
	0	2 h	24 h	48 h	2 weeks
Food intake (g/day)	4.4 ± 0.7 (8)	—	8.0 ± 0.5 (4)**	9.5 ± 0.6 (4)**	8.1 ± 0.4 (10)**
Plasma glucose concn. (mM)	10.8 ± 0.5 (14)	11.2 ± 0.4 (4)	10.3 ± 0.7 (5)	8.1 ± 0.7 (6)*	10.3 ± 1.9 (3)
Brown adipose tissue					
Tissue weight (mg)	109 ± 2 (98)	117 ± 5 (17)	97 ± 3 (35)**	113 ± 3 (36)	154 ± 6 (20)**
Tissue water (mg/g)	490 ± 20 (7)	430 ± 30 (4)	390 ± 20 (4)**	420 ± 3 (3)*	410 ± 20 (5)
Citrate (nmol/g)	254 ± 16 (14)	350 ± 44 (14)	323 ± 40 (8)	339 ± 40 (5)	—
Malonyl-CoA (nmol/g)	13 ± 2.3 (22)	13 ± 2.4 (6)	12 ± 2.0 (4)	6 ± 1.0 (6)*	18 ± 3.5 (3)
Acetyl-CoA carboxylase initial activity (munits/g)	410 ± 30 (18)	575 ± 110 (6)	390 ± 60 (7)	620 ± 70 (4)*	610 ± 50 (6)**
Fatty acid synthetase (munits/g)	2100 ± 200 (18)	—	1700 ± 100 (8)	3000 ± 300 (8)*	2900 ± 600 (8)
Fatty acid synthesis (μmol of C ₂ units/h per g)	87 ± 16 (24)	29 ± 4 (4)**	229 ± 39 (5)**	117 ± 37 (10)*	199 ± 38 (5)**

concomitant fall in the activity of acetyl-CoA carboxylase (Table 2). Noradrenergic stimulation of brown adipose tissue may inhibit acetyl-CoA carboxylase *in vivo* by increasing the cytoplasmic concentration of fatty acyl-CoA as it does in white adipose tissue (Denton & Halperin, 1968), despite the increase in concentration of the activator citrate (Table 2) (Halestrap & Denton, 1974; Carlson & Kim, 1974). However, this would result in a decrease in the concentration of malonyl-CoA in parallel with fatty acid synthesis, since there is unlikely to be a decrease in the activity of fatty acid synthetase within 2 h, whether by changes in enzyme protein (Craig *et al.*, 1972) or by removal of the 4'-phosphopantetheine moiety (Yu & Burton, 1974; Das, 1980).

The malonyl-CoA content of brown adipose tissue was unchanged during the first 24 h of cold-exposure,

although the rate of synthesis apparently decreased after 2 h and increased after 24 h compared with controls. This suggests that synthesis was constant and that the incorporation of ³H from ³H₂O into tissue fatty acids is not a valid measure of fatty acid synthesis under these conditions. However, it is not possible to account for the 7–8-fold variation in labelling of fatty acids during acute cold-exposure simply by variation in the source of hydrogen atoms entering fatty acid during its synthesis. ³H incorporated on to the even-numbered (α) carbons of fatty acid are derived from water and could not be altered. ³H incorporation on to the odd-numbered (β) carbons could be affected by alterations in the contribution of NADPH derived from the malate cycle (which would be labelled) rather than unlabelled from the pentose phosphate pathway (Jungas, 1968), but this

could only account for a variation of approx. 15%, not for the large variations in ^3H incorporation into fatty acid observed.

It is also unlikely that the 7–8-fold variation in the observed rates of synthesis during acute cold-exposure were caused by the uptake of labelled fatty acid from the circulation. Hepatic synthesis increased from 27 ± 3 to 60 ± 5 μmol of C_2 units/h per g (means $\pm$ S.E.M. for nine and three observations respectively) after 24 h cold-exposure (see also Rath *et al.*, 1979) and in cold-acclimated mice (Trayhurn, 1981), when synthesis in brown adipose tissue apparently increased. However, experiments using inhibitors of lipoprotein lipase suggest that hepatic synthesis is unlikely to contribute significantly to the increase in ^3H -labelled fatty acid in brown adipose tissue of cold-exposed mice (Trayhurn, 1979).

The results support the hypothesis that fatty acid synthesis and fatty acid oxidation occur simultaneously in brown adipose tissue, so forming a heat-producing substrate cycle as proposed by Masoro (1963). Newly synthesized fatty acids may be preferentially oxidized, and ^3H incorporation may underestimate synthesis. The relative rates of synthesis and oxidation may depend not only on ambient temperature but also on the housing of animals. It has been reported that the thermogenic activity in brown adipose tissue depends on the housing of animals (Jennings *et al.*, 1986), as does fatty acid synthesis. Mice maintained at 22°C in groups have significantly lower rates of fatty acid synthesis ($P < 0.005$) (27.1 ± 4 μmol of C_2 units/h per g, for six observations) than do mice housed singly (Table 2). In contrast, rates of synthesis after acute (2 h) cold-exposure were similar whether the mice were housed in groups (22.4 ± 6 μmol of C_2 units/h per g, four observations) or singly (Table 2). Thus the response to acute cold-exposure may depend on the maintenance of control animals and hence their relative rates of synthesis and oxidation. This may partially explain the report of an increase in synthesis after 1 h cold-exposure of mice housed in groups (Rath *et al.*, 1979), in contrast with the decrease after 2 h in mice housed singly (Table 2).

After long-term cold-exposure, when fatty acid oxidation is enhanced (Nicholls & Locke, 1983), ^3H incorporation into fatty acids is also increased (to rates approaching those measured in starved/re-fed mice). The increase in fatty acid synthesis may be explained by the increase in activities of acetyl-CoA carboxylase and fatty acid synthetase. McCormack (1982) speculated that insulin may effect this increase in fatty acid synthesis in brown adipose tissue. Although plasma insulin is not increased in cold-adapted mice (Trayhurn, 1981), nor is plasma glucose, despite increased food intake (Table 2; Trayhurn, 1981), there is evidence that cold-acclimation results in increased sensitivity to insulin in brown adipose tissue (Smith *et al.*, 1986).

The results suggest that simultaneous fatty acid synthesis and oxidation does occur in brown adipose tissue of cold-exposed mice, although CAT 1 remains extremely sensitive to inhibition by malonyl-CoA. In cold-exposed mice, the minimum concentrations of malonyl-CoA in brown adipose tissue (between 14 and $44 \mu\text{M}$) were above the concentration of $10 \mu\text{M}$ reported to cause maximum inhibition of CAT 1 from brown adipose tissue *in vitro* in the presence of $40 \mu\text{M}$ -palmitoyl-CoA (Saggerson & Carpenter, 1982). The inhibition of CAT 1 by malonyl-CoA *in vivo* is dependent on the

concentration of free long-chain fatty acyl-CoA present (McGarry *et al.*, 1978; Cook *et al.*, 1978). It is possible that on cold-exposure increased CAT 1 activity and fatty acid oxidation in brown adipose tissue may be effected, not by decreases in the concentration of malonyl-CoA but by increases in the concentration of long-chain fatty acyl-CoA.

An alternative mechanism is that the requirement for CAT 1 is effectively by-passed in brown adipose tissue on cold-exposure. Long-chain fatty acids may undergo partial oxidation in peroxisomes (Nedergaard *et al.*, 1980). The shorter-chain fatty acids so formed could enter the mitochondria independently of CAT 1 activity (McGarry & Foster, 1980). Furthermore, a product of peroxisomal oxidation, acetyl-CoA, could become a substrate for fatty acid synthesis, supplementing that produced by pyruvate dehydrogenase in the mitochondria.

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