FATTY ACID SYNTHESIS IN VIVO IN BROWN ADIPOSE TISSUE, LIVER AND WHITE ADIPOSE TISSUE OF THE COLD-ACCLIMATED RAT

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1. Introduction

When homeotherms are exposed to a cold environment, heat is produced to maintain the body temperature by shivering and non-shivering mechanisms [1]. In new-born mammals and hibernators it is well-established that brown adipose tissue is the primary site of non-shivering thermogenesis [1–3]. Recent work has indicated [4,5] that contrary to earlier conclusions [1], brown adipose tissue (BAT) is also the main site of non-shivering thermogenesis in adult cold-acclimated non-hibernators. Heat is produced in BAT through a proton short-circuit across the inner mitochondrial membrane, which is sensitive to purine nucleotides, and which allows respiration and heat production to proceed without the generation of ATP [6,7].

The primary substrate for heat production in BAT is fatty acid [2,3,7]. Early studies, both in vivo and in vitro, have suggested that even during cold exposure little fatty acid synthesis occurs in BAT itself, the fatty acids for the tissue being derived from other sources [8–10]. In contrast, a recent study employing tritiated water as a tracer, which unlike other tracers for investigating fatty acid synthesis gives values which are independent of the carbon source, has indicated a high rate of synthesis in vivo in BAT from cold-acclimated rats [11]. It is likely, however, that this study may have substantially underestimated the true capacity for fatty acid synthesis in BAT, since the animals were removed from the cold before the experiment, and acute changes in environmental temperature elicit a rapid response in the metabolic activity of BAT.

In the present experiments fatty acid synthesis has been investigated in vivo in BAT, liver and white adipose tissue of cold-acclimated rats studied both in the cold and following acute exposure to the warm. A comparison has also been made with warm-acclimated animals. The results show that the rate of fatty acid synthesis in BAT is 13.7-times higher in cold-acclimated rats maintained in the cold than in warm-acclimated animals kept in the warm. Fatty acid synthesis is also augmented, but much less so, in the liver and white adipose tissue of cold-acclimated rats in the cold. The transfer of cold-acclimated animals to the warm results in an immediate fall in fatty acid synthesis in BAT, as well as in liver and in white adipose tissue.

2. Materials and methods

2.1. *Animals*

Female hooded rats, aged 2.5–3 months, were caged singly in temperature-controlled cabinets at either 28–29°C (thermoneutrality) or 4°C. The rats were acclimated for 3–4 weeks, and were subject to a 12 h light–12 h dark cycle, the light period running from 08.00–20.00 h. Both water and a standard high carbohydrate/low fat diet (Spillers-Spratts Rodent Breeding Diet 1) were available ad libitum. At the end of the acclimation period the cold-exposed animals were consuming 2.2-times more food than those at thermoneutrality, but there was no difference in the mean body weight of the two groups.

2.2. *Lipogenesis*

Lipogenesis was determined by measuring the

incorporation of tritium from ^3H_2O into tissue fatty acids [12–16]. Tritiated water was obtained from the Radiochemical Centre (Amersham) at spec. act. 5 Ci/ml; immediately before use it was diluted with sterile saline (0.9%, w/v, NaCl).

The rats were taken fully-fed between 09.00 and 09.30 h, injected intra-peritoneally with 2.5 mCi ^3H_2O in 100 μl, then immediately returned to the temperature-controlled cabinets. In the study on the acute effect of temperature change the rats were transferred to the new temperature 1 h before the experiment. One hour after administering ^3H_2O all animals were lightly anaesthetised with diethyl ether, and blood was collected by severing the jugular vein. Interscapular BAT (trimmed free of white adipose tissue), parametrial white adipose tissue (WAT) and the liver were each quickly removed and frozen in liquid nitrogen.

While blood was centrifuged for the collection of plasma, which was then de-proteinised with trichloroacetic acid and recentrifuged. The supernatant was collected in order to determine the specific activity of plasma water. Over 99% of the total radioactivity in the supernatant was in the form of tritiated water.

2.3. *Extraction of fatty acids*

Tissue samples were saponified with ethanolic KOH at 70°C for 2 h, and the fatty acids extracted with light petroleum (b.p. 40–60°C) and washed with water [17]. The light petroleum extracts were evaporated to dryness at room temperature and the fatty acids dissolved in a toluene-based scintillation solution. Incorporation of radioactivity into cholesterol has been discounted since it amounts to < 5% of the incorporation into fatty acids [13,16,17].

Radioactivity was measured in a Packard Tri-Carb 2425 liquid scintillation counter.

2.4. *Expression of results*

Fatty acid synthesis was expressed as μg atoms 'H' incorporated/h by dividing the radioactivity in extracted fatty acids by the specific activity of plasma water; no correction was made for isotope effects. The statistical significance of differences between groups was assessed using Student's unpaired *t*-test.

3. Results

A preliminary study showed that following the injection of ^3H_2O the specific activity of plasma water reached a plateau within 15 min, after which time it remained approximately constant for ⩾ 1 h. In the tissues examined the incorporation of radioactivity into fatty acids was linear in cold-acclimated rats for up to 75 min. In all subsequent experiments the rats were killed 60 min after administering the ^3H_2O.

Table 1 shows the rates of fatty acid synthesis in BAT, WAT and liver of animals studied under various environmental conditions. In warm-acclimated rats maintained in the warm, fatty acid synthesis was

Table 1
Fatty acid synthesis in tissues from warm- and cold-acclimated rats

Group	Temperature		Brown adipose tissue	Liver	White adipose tissue
	Acclim.	Expt.	(μg atoms 'H' incorporated .h^{-1} .g wet wt^{-1})		
A	28	28	16.2 ± 1.7 (14)	15.7 ± 1.5 (14)	4.8 ± 0.3 (8)
B	28	4	15.6 ± 3.5 (8)	17.0 ± 1.9 (8)	5.7 ± 0.3 (8)
C	4	4	222.0 ± 23.6[c] (14)	34.1 ± 4.5[b] (14)	8.3 ± 1.4[a] (8)
D	4	28	43.1 ± 3.9[c,e] (8)	12.1 ± 0.8[d] (8)	6.1 ± 0.7 (8)

[a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$; compared to group A
[d] $p < 0.01$, [e] $p < 0.001$; compared to group C

The results are expressed as mean values ± SE with no. animals in parentheses

similar in BAT and liver, but the rate in these tissues was 3–4-times higher than in WAT. A very high synthesis rate was observed in BAT from cold-acclimated rats studied in the cold. Under these conditions the rate was 13.7-times greater than in warm-acclimated animals in the warm. Hepatic fatty acid synthesis and the synthesis in WAT were also elevated in cold-acclimated rats maintained in the cold, but the increase over the warm-acclimated animals was only 2.2- and 1.7-times, respectively, for the 2 tissues.

When warm-acclimated rats were exposed acutely to the cold, there was no significant increase in fatty acid synthesis in any of the tissues. In contrast, there were substantial reductions in the rates of synthesis in cold-acclimated rats following acute exposure to the warm. Hepatic synthesis and the synthesis in WAT declined to values which were similar to those found in warm-acclimated animals. In BAT, fatty acid synthesis in cold-acclimated rats was reduced on exposure to the warm to only 20% of the value of animals maintained in the cold. This rate was, however, still 2.7-times higher than the rate found in the warm-acclimated group in the warm.

Cold acclimation resulted in hypertrophy of both the BAT and the liver, but a reduction in the amount of parametrial WAT was observed. The quantity of BAT in the cold-acclimated animals was 54% greater than in the warm-acclimated group, while the liver weight was increased by 22%. Table 2 shows the total fatty acid synthesis in each tissue from each group of rats. The results in this table, which take the effect of environmental temperature on tissue weight into account, enable the contribution of each tissue to total fatty acid synthesis to be assessed. The total synthesis in BAT was 20.1-times greater in cold-acclimated rats in the cold than in warm-acclimated animals in the warm, and the total hepatic synthesis was 2.5-fold greater. The total synthesis in parametrial WAT was, however, lower by 50% in the cold-acclimated group compared to the warm.

4. Discussion

The present results demonstrate that in cold-acclimated rats the capacity for fatty acid synthesis in BAT is extremely high, particularly when tissue hypertrophy is taken into account. The rates obtained are > 3-times greater than those reported in cold-acclimated rats studied using ^3H_2O as a tracer [11]. This difference is clearly due to the acute effect of temperature change, since in [11] the rats were removed from the cold prior to the experiment and this results in an immediate fall in fatty acid synthesis in BAT, as well as in other tissues (table 1). Overall, the rates of synthesis in BAT obtained with ^3H_2O are much greater than the apparent rates obtained using [^{14}C]glucose as precursor [8–10]. Since ^3H_2O

Table 2
Total fatty acid synthesis in tissues from warm- and cold-acclimated rats

Group	Temperature		Interscapular brown adipose tissue	Liver	Parametrial white adipose tissue
	Acclim.	Expt.	(μg atoms 'H' incorporated .h^{-1} .tissue^{-1})		
A	28	28	9.5 ± 1.3 (14)	114.5 ± 11.2 (14)	11.5 ± 1.5 (8)
B	28	4	9.8 ± 2.7 (8)	131.8 ± 15.4 (8)	10.8 ± 1.2 (8)
C	4	4	191.0 ± 19.9[b] (14)	286.9 ± 36.7[a] (14)	5.7 ± 0.9[a] (8)
D	4	28	39.9 ± 4.5[b,d] (8)	105.3 ± 6.9[c] (8)	4.4 ± 0.7[b] (8)

[a] $p < 0.01$, [b] $p < 0.001$; compared to group A
[c] $p < 0.01$, [d] $p < 0.001$; compared to group C

The results are expressed as mean values ± SE with the no. animals in parentheses (data obtained by multiplying synthesis/g by the total tissue weight)

measures fatty acid synthesis independently of the carbon source, this indicates that glucose is not a significant substrate for the production of fatty acids in BAT of the cold-acclimated rat. Cold acclimation does, however, greatly stimulate the incorporation of glucose into the glycerol moiety of triglycerides in the tissue [9].

The present experiments substantiate the suggestion that in cold-acclimated rats BAT is a major site for the conversion of carbohydrate to fat [11]. In cold-acclimated rats studied in the cold the rate of fatty acid synthesis in BAT was 6.5-times greater than in the liver and 26.6-times greater than in WAT, but because of the size of the liver the total body synthesis in this organ was greater than the total in interscapular BAT. The interscapular site represents, however, $< 25\%$ of the total BAT in rats [6]. If it is assumed that the fatty acid synthesis rate in the rest of BAT is similar to that of the interscapular site, then the total body synthesis in BAT in cold-acclimated rats is ~3-times the total hepatic synthesis. In contrast, in warm-acclimated rats BAT is a much less important site of fatty acid synthesis, since the total body BAT synthesis would amount to a little $> 33\%$ of the total in the liver.

In conclusion, it should be noted that the activities of pyruvate dehydrogenase and acetyl-CoA carboxylase, key enzymes in fatty acid synthesis, are high in BAT of cold-acclimated rats [11]. The very high fatty acid synthesis rates reported here for the tissue are therefore unlikely to be a reflection of other than true de novo synthesis.

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