EFFECT OF ACUTE CHANGE IN AMBIENT TEMPERATURE ON FATTY ACID SYNTHESIS IN THE MOUSE

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1. Introduction

During cold exposure, homeotherms increase heat production to maintain their body temperature. It is well known that turnover of plasma free fatty acids increases [1] as fatty acids are rapidly mobilized from white adipose tissue and oxidised by several tissues, notably brown adipose tissue and muscle [2,3]. In rats, short-term cold exposure is associated with decreased body fat [4], although body weight and body fat is restored on chronic exposure [5], suggesting there is a lag between increased fatty acid oxidation and any compensatory increase in fatty acid synthesis.

Early experiments in rats which used ^{14}C-labelled acetate or glucose as precursors to measure fatty acid synthesis, indicated that on cold exposure, fatty acid synthesis was increased in brown adipose tissue but not white adipose tissue after 9 days [6]. No increases were reported in fatty acid synthesis in white adipose tissue [7] or the liver [8] after 24 h exposure. These experiments suffer from problems of different isotope dilutions which would result from changes in substrate turnover during cold exposure. Recent work which overcame these problems by monitoring fatty acid synthesis by the incorporation of ^{3}H from $^{3}H_2O$, suggested that after only 1 h cold exposure, synthesis was increased in brown adipose tissue but not in white adipose tissue or liver [9]. The lack of increase in hepatic synthesis in the rat is in contrast to results obtained in the mouse, using the same method, where a rapid increase in fatty acid synthesis was reported after 1 h and 24 h cold exposure [10].

Here we have measured the effect of acute changes in ambient temperature on fatty acid synthesis in vivo in liver, white adipose tissue and brown adipose tissue of conscious mice. The results show that there is a rapid increase in synthesis within 1 h of cold exposure in liver and adipose tissue. A further increase in synthesis is found after 24 h exposure, and this increase is independent of any increase in food intake. Similar increases in fatty acid synthesis occur when mice maintained at thermal neutrality are exposed to an ambient temperature of 22°C, suggesting that the increase in fatty acid synthesis is an acute response to a relative fall in ambient temperature.

2. Materials and methods

2.1. *Animals*

Female mice (10–12 weeks) were obtained from a mixed strain of a random-bred, closed colony maintained at Imperial College, London SW 7. Mice were maintained at 22°C, housed under a 12 h light–12 h dark cycle, the light period running from 07.00–19.00 h. Mice were fed on a standard breeding diet (Oxoid Ltd., London SE1).

When mice were exposed to a cold environment, they were placed in cages in a large light-proof box in a refrigerated room at 4°C. When mice were exposed to 30°C they were placed in cages inside a thermostatically controlled cabinet (designed for liver perfusions). In both cases, animals were subjected to the

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same 12 h light–12 h dark cycle, and allowed food and water ad libitum unless otherwise stated.

2.2. *Fatty acid synthesis*

Experiments were started between 10.00 h and 12.00 h. Before the experimental time point (30 min) mice were injected intraperitoneally with ^3H_2O (2–5 mCi in 0.1 ml). The mice were maintained without water for a further 1 h. They were then bled by cardiac puncture under light diethyl ether anaesthesia, and tissues removed and frozen on a glass plate precooled with solid CO_2. Plasma was prepared to determine the specific radioactivity of water; plasma water was taken as 53 M.

2.3. *Analytical methods*

Frozen tissues were weighed and saponified at 80°C for 3 h, in 5 ml ethanolic KOH. After saponification, 5 ml water was added and non-saponifiable lipids (cholesterol) were extracted with light petroleum ether (b.p. 40–60°C). After acidification, fatty acids were then extracted in 2 × 10 ml washes with petroleum ether. The extracts of fatty acids were evaporated to dryness under nitrogen. Fatty acids were dissolved directly in 10 ml toluene-based scintillant.

2.4. *Calculation of results*

The total rate of fatty acid synthesis (i.e., the rate of conversion of acetyl CoA to fatty acids, independent of precursor source) was calculated from the ^3H content of the fatty acid and the specific radioactivity of plasma ^3H_2O as in [11]. The absolute rates of fatty acid synthesis were calculated using a factor of 2.94 to correct for the discrimination against ^3H compared to ^1H in mouse tissues [12], rather than the value of 2.38 used for the rat [13].

Data from control and experimental animals were compared using Student's *t*-test.

3. Results

When mice maintained at 22°C were exposed to an ambient temperature of 4°C, fatty acid synthesis increased significantly in brown and white adipose tissue and the liver within 1 h of cold exposure, and increased further after 24 h in both types of adipose tissue (table 1).

The increased fatty acid synthesis was associated with a rapid increase in food intake, from 6.1 g/day at 22°C to 10 g/day at 4°C. However, the increase in fatty acid synthesis was independent of the hyperphagia (table 2). Mice exposed to 4°C for 24 h, but fed only 5 g/day showed the same increases in fatty acid synthesis as cold-exposed mice which were fed ad libitum and ate twice as much (table 1).

The increase in fatty acid synthesis was not a response to the absolute temperature to which the animal was exposed, but to the relative temperature change. Mice maintained at 22°C were exposed to an ambient temperature of 30°C for 4 days, then returned to 22°C, and rates of fatty acid synthesis measured at intervals (fig.1.). When the ambient temperature was increased towards thermal neutrality, food intake declined and hepatic fatty acid synthesis tended to decline. When mice were returned to an ambient tem-

Table 1
Fatty acid synthesis in vivo on acute exposure of mice to low ambient temperature

Exposure to 4°C (h)	Fatty acid synthesis (μmol C_2 units . g^{-1} . h^{-1})			No. obs.
	Liver	White parametrial adipose tissue	Brown scapular adipose tissue	
0	14.2 ± 1	5.8 ± 1	36.3 ± 6	11
1	44.6 ± 7[b]	53.4 ± 14[a]	78.1 ± 15[a]	8
24	52.8 ± 5[b]	201 ± 49[b]	219 ± 17[b]	8

[a] $P < 0.05$; [b] $P < 0.001$

Results are expressed as mean ± SEM. Synthesis in cold-exposed mice was significantly different from control animals maintained at 22°C

Table 2
Effect of acute exposure to low ambient temperature on fatty acid synsthesis in vivo in mice pair-fed with mice maintained at 22°C

Exposure to 4°C (h)	Food intake (g/day)	Fatty acid synthesis (μmol C_2 units . g^{-1} . h^{-1})			No. obs.
		Liver	White parametrial adipose tissue	Brown scapular adipose tissue	
0	6.1	17.6 ± 5	9.0 ± 2	47.0 ± 5	3
24	5.0	42.4 ± 6[a]	158 ± 20[b]	161 ± 28[a]	4

[a] $P < 0.05$; [b] $P < 0.001$

Control mice were fed ad libitum. Cold-exposed mice were fed 5 g/24 h. Food (1 g) was given 3 times between 11.00 h and 22.00 h and 2 g were given overnight. Results are expressed as mean ± SEM. Synthesis in pair-fed cold-exposed mice was significantly greater than in mice maintained at 22°C

perature of 22°C, synthesis increased dramatically within 24 h to values ~ 4-times greater than those in mice maintained at 22°C.

4. Discussion

The present results demonstrate that in the mouse a rapid increase in fatty acid synthesis occurs in brown and white adipose tissue and the major lipogenic organ, the liver, in response to acute changes in ambient temperature. These findings differ from results obtained in the rat where no increase in fatty acid synthesis in the liver or white adipose tissue have been reported, although this has not been systematically studied in these tissue [6–8]. The results obtained in brown adipose tissue of the mouse agree with those in the rat [9], where similar increases were reported after acute (1 h) cold exposure.

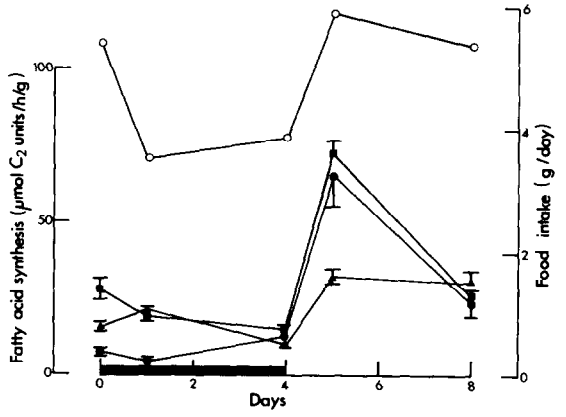

Fig. 1. Effect of acute exposure to high ambient temperature on fatty acid synthesis in vivo. Female mice (10–12 weeks) maintained at 22°C were exposed to an ambient temperature of 30°C and then returned to an ambient temperature of 22°C. Food intake was recorded (○). Animals were housed under the same light–dark cycle and allowed free access to food and water. The shaded area or the abscissa indicates the period of exposure to 30°C. The experimental procedure was the same as in table 1. Fatty acid synthesis was measured in liver (■), white parametrial adipose tissue (●) and brown scapular adipose tissue (▲). Results are the means of 4 obs; bars indicate the SEM.

Hems [10] suggested that the increase in hepatic fatty acid synthesis may be of significance in the acute thermoregulatory response. The apparently different characteristics of the response of fatty acid synthesis in mice compared to rats may reflect a difference in thermoregulatory requirement. The smaller body size and consequent greater capacity for heat loss may necessitate this greater response to cold exposure in mice. This is supported by the fact that the increase in oxygen consumption is greater in mice than in rats on cold exposure [14].

Since the liver is the major site of lipogenesis in this strain of mouse [11,12], the data suggest that after 24 h cold-exposure, fatty acid synthesis in the body is increased ⩾ 4-fold. The question arises of the fate of this newly-synthesised fatty acid. Assuming no changes in body composition takes place, this implies that the net oxidation of fatty acid increased by a similar amount. This is of the same order of increase which has been observed in oxygen consumption on acute cold exposure [14].

Food-restriction did not abolish the increased synthesis in response to low ambient temperature indicating a rapid increase in the proportion of the diet which is channelled into fatty acid synthesis. We suggest that an increase in fatty acid synthesis may be an integral part of the rapid biochemical response to a decrease in ambient temperature, possibly providing a preferred substrate for oxidation. The nervous or hormonal control of these rapid events is not known.

It is noteworthy that the amount of fatty acid synthesis that occurs is not necessarily constant at a certain temperature, but is a function of the temperatures to which the animal has been pre-exposed.

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