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Important sites of lipogenesis in the mouse other than liver and white adipose tissue

Marjorie A. HOLLANDS* and M. A. CAWTHORNE*
Beecham Pharmaceuticals, Walton Oaks, Tadworth, Surrey, U.K.

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The musculature of the shoulders and back has been identified as a major site of fatty acid synthesis in mice.

When fatty acid synthesis has been measured *in vivo* in rats and mice, it has been shown that the liver and adipose tissue, which are generally regarded as the two principal lipogenic tissues, contribute only between 10 and 40% of the overall synthetic rate, depending on the experimental conditions and methods used (Baker & Huebotter, 1973; Baker *et al.*, 1978; Bates *et al.*, 1955; Cawthorne & Cornish, 1979; Cornish & Cawthorne, 1978; Jansen *et al.*, 1967; Kannan *et al.*, 1976; Kato, 1969; Shigeta & Shreeve, 1964). Few authors have suggested explanations for this large carcass contribution to the overall lipogenic rate. Kannan *et al.* (1976) measured the rate of fatty acid synthesis *in vivo* from [U-^{14}C]glucose in the popliteal fat-pad and found a much higher rate of synthesis than that of the epididymal fat-pad or the muscle of the hind leg. They proposed that intermuscular fat-pads may be responsible for the high rates of lipogenesis seen in the 'rest of carcass'. In order to investigate their proposals, we have divided the 'rest of carcass' into many constituent parts in order to assess their individual contribution to the total rate of fatty acid synthesis. Furthermore, in order to measure the total fatty acid synthesis irrespective of the carbon source, we have used the ^3H_2O method of assay (Windmueller & Spaeth, 1967; Jungas, 1968).

Experimental

Female CFLP mice were obtained from Anglia Laboratories Ltd., Alconbury, Huntingdon, Cambs., U.K. The mice were kept in a thermostatically controlled room (25.5°C) on a 12h dark/12h light cycle, with the dark phase from 18:00 to 06:00h. The mice were given water and Oxoid pelleted breeders diet (Oxoid Ltd., London SE1, U.K.) *ad libitum*.

* Present address: Beecham Pharmaceuticals, Biosciences Research Centre, Great Burgh, Yew Tree Bottom Road, Epsom, Surrey, U.K.

Measurements of the rates of lipogenesis *in vivo* were made by determining the incorporation into fatty acids of ^3H from ^3H_2O. ^3H_2O (200μCi/mouse) was administered intraperitoneally in 0.1ml of saline (0.9% NaCl) at 10:00h. After 1h, the mice were killed by cervical dislocation and bled from the neck. The carcasses were dissected into 14 components as follows. Mice were skinned and the subcutaneous fat removed. The peritoneal cavity was then opened and all the required tissues and organs removed. The hindlimbs were dissected adjacent to the gluteus maximus muscle. The forelimbs were removed at the shoulder joint. The head was removed, leaving the neck muscles attached to the back. The back region therefore consisted of the spine and the musculature of the back and shoulders. The tissues and organs were finely minced, then saponified in a mixture of aq. 60% (w/v) KOH (1vol.) and ethanol (2vol.). The non-saponifiable lipid was removed by extraction with light petroleum (b.p. 40–60°C). The fatty acids were obtained from the aqueous extract as described by Cawthorne & Cornish (1979) and the radiochemical content was determined in an Intertechnique SL 4000 spectrometer. The rate of fatty acid synthesis in tissues was calculated from the quotient (^3H in fatty acids in d.p.m.)/(specific radioactivity of plasma water expressed as d.p.m. per μg atom of ^3H in total water).

To separate triacylglycerols from phospholipids, the total lipids were extracted from the back muscle (Folch *et al.*, 1957). The lipid fractions were separated by silicic acid chromatography (Van Handel & Zilversmit, 1957). The fractions were then saponified and the fatty acids obtained as described above.

Acetyl-CoA carboxylase activity was measured by the method of Gove & Hems (1978), except that 15mM-$MgCl_2$/22.5mM-potassium citrate rather than 15mM-$MgCl_2$/10mM-potassium citrate was found to be required to achieve complete activation of the enzyme.

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Results and discussion

The major reported sites of fatty acid synthesis in the body are liver and adipose tissue. In the present study, in mice fed *ad libitum*, these two sites only accounted for 22 and 7% respectively of the total synthetic rate (Table 1). This result is in accord with that obtained by most other workers. There were four main sites of high biosynthetic activity in the 'rest of carcass'. The skin and head contributed 13 and 15% respectively and the gut, which was not stripped of adipose tissue, contributed 14%. However, the main contributor was the back region, which was quantitatively almost as active as the liver (Table 1). In contrast, the percentage contribution of the fore- and hind-legs together was only 2% (Table 1). Kannan *et al.* (1976), who used [U-^{14}C]glucose, found that newly synthesized fatty acid in the hind-leg was isolated mainly in the popliteal fat-pad. In view of this observation, we carefully dissected out the muscles of the back region, but no inter-muscular fat-pads were visible. The relatively small contribution of the legs, which contained the popliteal fat-pads, to the overall synthetic rate, coupled with the failure to find any other inter-muscular fat-pads militates against the overall significance of these fat-pads to the total body rate of fatty acid synthesis.

The total lipid ^{3}H-labelled fatty acid content of the back-muscle region could represent the rate of synthesis *de novo* of fatty acids in muscle cells in the back region or it could arise from transfer of fatty acids from other sites of synthesis. Haft (1973) showed that there is relatively little transport of newly synthesized triacylglycerols from liver to peripheral tissues during a 1 h period. In the current studies it was considered that if there were significant transfer of fatty acids from a site of synthesis to the back-muscle region, the time course of incorporation of ^{3}H into fatty acids in the back-muscle region would show a lag phase. Table 2 shows that there is no evidence of a lag phase in the time course; rather, there is a decrease in the rate of incorporation at the later time periods, suggesting that there may be rapid utilization or export of newly synthesized fatty acids. In contrast, fatty acid synthesis in liver showed a linear time course over the 60 min period. These results indicate that the rate of fatty acid synthesis in the back region given in Table 1 is almost certainly an underestimate. Table 2 also shows that 74% of the ^{3}H-labelled fatty acid in the back-muscle region is located in the triacylglycerol fraction.

The postural muscles of the back region are predominantly red, whereas those of the hindlimbs are predominantly white. In additional studies we have found that the rate of incorporation (per g of

Table 1. *Rate of fatty acid synthesis in various tissues and organs in mice*

CFLP female mice (28–32 g) were given 200 μCi of ^3H_2O in 0.1 ml of saline (0.9% NaCl) intraperitoneally at 10:00 h and were killed 1 h later. Blood was obtained from the neck for the determination of the specific radioactivity of plasma water. Results are means ± S.E.M. of five values.

Site	Wet weight (g)	Fatty acid synthesis (μg-atom of ^{3}H incorporated/h per site)	Contribution to total animal rate of fatty acid synthesis(%)
Liver	2.00	13.5 ± 1.7	22
Back*	4.40	12.9 ± 1.7	21
Head	2.89	9.3 ± 2.5	15
Gut	6.07	8.9 ± 0.9	14
Skin	3.62	8.1 ± 0.6	13
White adipose tissue	1.50	4.6 ± 0.6	7
Peritoneum	0.96	2.4 ± 0.2	4
Limbs	4.65	1.4 ± 0.1	2
Brown adipose tissue	0.19	0.6 ± 0.2	1
Kidney	0.45	0.3 ± 0.0	
Brain	0.40	0.1 ± 0.0	
Lungs	0.46	0.2 ± 0.0	1
Diaphragm	0.08	0.2 ± 0.0	
Heart	0.19	0.1 ± 0.0	

* See the text for a description of the composition of this site.

Table 2. *Time course of fatty acid synthesis in muscles of the back region*

CFLP female mice (28–32 g) were given 200 μCi of ^3H_2O in 0.1 ml of saline (0.9% NaCl) intraperitoneally at 10:00 h and were killed after the time periods shown below. Blood was obtained from the neck for the determination of the specific radioactivity of plasma water. The mice were rapidly dissected as described in the text and the back-muscle region stored at −20°C before analysis. Results are means ± S.E.M. of five values.

Time (min)	Fatty acid synthesis (μg-atoms of ^{3}H incorporated per time period)		
	Total	Triacylglycerol fatty acid	Phospholipid fatty acid
15	5.8 ± 0.6	—	—
30	6.0 ± 1.1	—	—
45	7.0 ± 2.0	—	—
60	9.4 ± 2.0	6.4 ± 2.5	2.0 ± 0.5

tissue) of ^3H_2O into fatty acids in psoas muscle, a predominantly red muscle, is from three to ten times greater, depending on the nutritional state of the animals, than in the predominantly white quadriceps muscle, the activity of acetyl-CoA carboxylase in psoas muscle (initial activity 0.103, total activity 0.176nmol of malonyl-CoA formed/min per mg of protein) from mice fed *ad libitum* was four times that found in the quadriceps muscle (initial activity 0.032, total activity 0.042nmol of malonyl-CoA formed/min per mg of protein).

Histochemical (George & Naik, 1958) and biochemical studies (Froberg, 1967) have shown that red muscle contains more triacylglycerol than white muscle. Red muscle, which is rich in narrow muscle fibres with a high content of mitochondria, is well equipped for oxidative metabolism and seems to oxidize more intramuscular triacylglycerol as compared with white muscle (Carlson *et al.*, 1966; Froberg, 1971; Reitman *et al.*, 1973). The finding of high rates of lipogenesis coincident with the known high enzymic capacity of red muscle for fatty acid oxidation raises the possibility of the existence of an acetyl-CoA–fatty acid–triacylglycerol cycle (Newsholme & Crabtree, 1976), which could have implications in metabolic-regulation and heat-production mechanisms.

The cellular origin of the lipogenic enzymes is unknown. Favarger & Gerlach (1973) proposed that pre-adipocytes might be responsible for much of the fatty acid synthesis in the rest of carcass in mice, and we consider it possible that adipocytes, either mature or immature, and either brown or white, may be present within or associated with red muscles.

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