# FATTY ACID SYNTHESIS IN THE CELL SAP AND MITOCHONDRIA OF RAT BROWN ADIPOSE TISSUE

Z.DRAHOTA*, P.CORATELLI and C.LANDRISCINA
*Department of Biochemistry, University of Bari, 70126 Bari, Italy*

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## 1. Introduction

Brown adipose tissue is found in hybernating, newborn, and cold-adapted animals. The main physiological function of this tissue is that of thermogenesis, fatty acids being considered as the main source of energy for heat production [1].

Studies on lipogenesis have shown that brown adipose tissue, like white adipose tissue, is an active site for the biosynthesis of fatty acids from glucose [2, 3]. Benjamin et al. [4] and Steiner and Cahill [5] have reported that $^{14}$C-acetate is actively incorporated into fatty acids by rat brown adipose tissue homogenate. With increasing age of the rats, the rate of acetate incorporation remains unchanged in brown adipose tissue, while it is strongly reduced in white adipose tissue [4].

To our knowledge no data are available on fatty acid synthesis in subcellular fractions of brown adipose tissue. In this paper results of fatty acid synthesis in the cell sap and in mitochondria of rat brown adipose tissue are presented. It is shown that in the cell sap *de novo* fatty acid synthesis occurs via malonyl-CoA. On the other hand, in mitochondrial chain elongation of fatty acids takes place and acetyl-CoA acts as the donor of $C_2$ unit for this synthetic activity. Both these activities of the rat brown adipose tissue are higher than those of the liver.

* On leave of absence from the Institute of Physiology, Academy of Sciences, Prague, Czechoslovakia.

## 2. Methods

Male Wistar rats about 30 days old were used. Mitochondria from liver and brown adipose tissue (interscapular region) were prepared essentially according to the procedure of Hogeboom et al. [6]. The cell sap was prepared by centrifuging the post-mitochondrial supernatant at 20,000 *g* for 20 min. The resulting supernatant was centrifuged at 105,000 *g* for 45 min. Mitochondria were suspended in 0.25 M sucrose and disrupted by freeze-thawing. Experimental conditions for studying fatty acid synthesis have been reported previously [7]. Oxygen consumption was measured by a Pt-vibrating electrode at room temperature.

## 3. Results

Table 1 shows the incorporation of 1,3-$^{14}$C-malonyl-CoA into fatty acids in the cell sap of rat brown adipose tissue and liver. The synthetic activity of brown adipose tissue cell sap is about 70% higher than that of liver cell sap. The ratio of total radioactivity to radioactivity in carboxyl carbon of the fatty acids synthesized is about 7.5:1 in both brown adipose tissue and liver cell sap. These data show that, as in liver and white adipose tissue [8, 9], in brown fat supernatant malonyl-CoA is also incorporated into fatty acids by a *de novo* mechanism.

Table 2 presents results of experiments on fatty acid synthesis from 1-$^{14}$C-acetyl-CoA in mitochondria from rat brown adipose tissue and liver. The synthetic activity of brown adipose tissue mitochondria is about

Table 1
Fatty acid synthesis in the cell sap of rat brown adipose tissue and liver.

| Tissue | nmoles 1,3-$^{14}$C-malonyl-CoA incorporated/min/mg protein | TR/CR ratio |
|---|---|---|
| Brown adipose tissue | 3.6 | 7.3:1 |
| Liver | 2.2 | 7.5:1 |

The concentrations of the substrates and cofactors in the incubation mixture were: 1,3-$^{14}$C-malonyl-CoA, 44 μM (0.2 μCi), acetyl-CoA 30 μM, phosphate buffer 60 mM pH 6.5, NADPH 1 mM, $MnCl_2$ 1 mM. Final volume, 1 ml. Incubation time, 10 min in aerobiosis at 37.5°C. Soluble protein, 0.4 mg (liver) and 0.35 mg (brown adipose tissue). TR/CR ratio = ratio of total radioactivity to radioactivity in carboxyl carbon of the synthesized fatty acids.

50% higher than that of liver mitochondria. The ratio of total radioactivity to radioactivity in carboxyl carbon of the synthesized fatty acids about 2.0:1 in both cases. This indicates that, similarly to mitochondria from other tissues [7, 10, 11], mitochondria from brown adipose tissue synthesize fatty acids by chain elongation and utilize acetyl-CoA.

When freshly isolated and disrupted mitochondria are compared (table 2), no considerable difference is observed in the synthetic activity of brown adipose tissue mitochondria, in contrast with liver mitochondria. In fact, by disrupting brown fat mitochondria, an increase of only 1.5-fold in synthetic activity is observed, while in disrupted rat liver mitochondria, the rate of 1-$^{14}$C-acetyl-CoA incorporation into fatty acids is enhanced 4.0-fold with respect to intact mitochondria. As the enzymes responsible of fatty acid synthesis are localized in the inner mitochondrial membrane [7], these observations indicate that brown adipose tissue mitochondria, unlike liver mitochondria, are not entirely impermeable to reduced pyridine nucleotides or acetyl-CoA.

Table 3
NADH oxidation by freshly isolated and disrupted mitochondria of rat brown adipose tissue and liver.

| Tissue | Mitochondria | Oxygen consumption −NADH (A) | +NADH (B) | (B−A) |
|---|---|---|---|---|
| Brown adipose tissue | Freshly isolated | 11 | 34 | 23 |
| | Disrupted | 10 | 190 | 180 |
| Liver | Freshly isolated | 5 | 7 | 2 |
| | Disrupted | 5 | 46 | 41 |

The concentrations of substrates and cofactors in the incubation mixtures were: tris-HCl buffer 15 mM pH 7.4, KCl 80 mM, $MgCl_2$ 6 mM, ATP 1 mM, sucrose 25 mM and NADH 0.5 mM. Mitochondrial protein, 0.75 mg (liver) and 0.6 mg (brown adipose tissue). Final volume, 1 ml. The results are expressed as natoms O consumed/min/mg protein.

In order to obtain insight on the permeability properties of freshly isolated rat brown adipose tissue mitochondria, the oxidation of NADH by these mitochondria, as well as by rat liver mitochondria for comparison, was studied (table 3). Freshly isolated brown adipose tissue mitochondria oxidized NADH at a rate

Table 2
Fatty acid synthesis in freshly isolated and disrupted mitochondria of rat brown adipose tissue and liver.

| Tissue | Mitochondria | nmoles 1-$^{14}$C-acetyl-CoA incorporated/min/mg protein | TR/CR ratio |
|---|---|---|---|
| Brown adipose tissue | Freshly isolated | 0.19 | 1.8:1 |
| | Disrupted | 0.29 | 1.9:1 |
| Liver | Freshly isolated | 0.04 | 1.6:1 |
| | Disrupted | 0.16 | 2.0:1 |

The concentrations of substrates and cofactors in the incubation mixture were: 1-$^{14}$C-acetyl-CoA 28 μM (0.2 μCi), tris buffer 10 mM pH 7.0, KCl 80 mM, ATP 4 mM, NADH 0.5 mM, NADPH 0.5 mM and $MnCl_2$ 1 mM. Final volume, 1 ml. Incubation time, 10 min under $N_2$ at 37°C. Mitochondrial protein, 1.0 mg (liver) and 1.2 mg (brown adipose tissue).

Table 4
Effect of defatted bovine serum albumin on fatty acid synthesis and NADH oxidation in rat brown adipose tissue mitochondria.

| Mitochondria | 1-$^{14}$C-acetyl-CoA incorpo-* tion into fatty acids | TR/CR ratio | Oxygen consumption** −NADH (A) | +NADH (B) | (B−A) |
|---|---|---|---|---|---|
| Freshly isolated | 0.20 | 2.0:1 | 9.2 | 37.4 | 28.2 |
| Disrupted | 0.31 | 1.8:1 | 12.5 | 233 | 221 |
| Freshly isolated + albumin | 0.09 | 1.7:1 | 8.8 | 14.8 | 6.0 |
| Disrupted + albumin | 0.33 | 2.2:1 | 11.7 | 213 | 201 |

The incubation mixture for assaying fatty acid synthesis as indicated in table 2, that for NADH oxidation as in table 3. Where indicated, 6.5 mg of defatted bovine serum albumin [14] were added to the incubation mixture.
* Expressed as nmoles 1-$^{14}$C-acetyl-CoA incorporated/min/mg protein.
** Expressed as natoms O consumed/min/mg protein

of 23 natoms O consumed/min/mg protein; in contrast, freshly isolated liver mitochondria did not oxidize exogenous NADH. After disruption by freeze-thawing, NADH oxidation by liver mitochondria was 41 natoms O consumed/min/mg protein, while in brown adipose tissue mitochondria and 8-fold increase in NADH oxidation was observed.

It has been reported that mitochondria from brown adipose tissue of cold-acclimated rats [12] and from newborn rabbits [13] are not capable of electron-transport-linked phosphorylation. In contrast, Guillory and Racker [14] have demonstrated that, in the presence of defatted bovine serum albumin, brown fat mitochondria from the same animals exhibit a high phosphorylating capacity with P/O ratios comparable to those obtained with mitochondria from other tissues. It therefore seemed useful, in the present investigation, to test the effect of albumin on fatty acid synthesis and NADH oxidation in freshly isolated rat brown adipose tissue mitochondria. Table 4 shows that in the presence of albumin, the amount of 1-$^{14}$C-acetyl-CoA incorporation into fatty acids by freshly isolated rat brown adipose tissue mitochondria is much lower than that observed in the absence of albumin. Similarly no appreciable NADH oxidation is obtained in freshly isolated mitochondria incubated with defatted bovine serum albumin. In addition, in control experiments on oxidative phosphorylation with rat brown adipose tissue mitochondria, in the presence of defatted bovine serum albumin and with succinate or α-oxoglutarate as substrate, P/O ratios similar to those reported by Guillory and Racker [14] are obtained.

## iscussion

The data presented in this paper indicate that cell sap and mitochondria from rat brown adipose tissue synthesize fatty acids by the same mechanism as corresponding subcellular fractions of rat liver. However mitochondria from brown fat exhibit a behaviour different in respect to liver mitochondria in that they appear permeable to NADH, NADPH and acetyl-CoA. This finding supports the previous suggestions of Aldridge and Street that isolated brown fat mitochondria are damaged [15]. As indicated by the effect of albumin, it is possible that this damage is an artifact of isolation due to the high content of free fatty acids [16], which are known to be strong swelling agents [17]. Presumably *in vivo*, in brown fat, the liberation of fatty acids is under hormonal control, e.g. norephinephrine, and does not affect the metabolic behaviour of mitochondria.

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