FATTY ACID BIOSYNTHESIS IN ADIPOSE TISSUE AND LUNG SUBCELLULAR FRACTIONS OF THYROTOXIC RATS

G. V. GNONI, C. LANDRISCINA and E. QUAGLIARIELLO

Centro di Studio sui Mitocondri e Metabolismo Energetico presso l'Istituto di Chimica Biologica dell'Università di Bari, Via Amendola 165/A, 70126 Bari, Italy

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1. Introduction

Both liver and adipose tissue are well-known as active sites of fatty acid synthesis. The regulation of acetyl-CoA carboxylase (EC 6.4.1.2) and fatty acid synthetase by nutritional factors in these two tissues has been found to be basically the same [1]. However, unlike liver, few data are available concerning the regulation of the above enzymatic activities by hormonal factors in adipose tissue. Several reports indicate that abnormal thyroid hormone level alter various enzymatic activities in both liver and other tissues [2–4]. As far as denovo fatty acid biosynthesis is concerned, there is a general agreement on the stimulating effect of hyperthyroidism on the hepatic level. However, data published on the regulation of this synthetic activity in adipose tissue of hyperthyroid animals are few and conflicting [5–7].

For a better understanding of this aspect, de novo fatty acid synthesis by adipose tissue has been studied not only in hyperthyroid rats but in hypothyroid animals as well. Moreover, since mammalian lung actively synthesizes fatty acids [8–10], which represent an important source of pulmonary surfactant phospholipids [11], this investigation was also devoted to establishing the effect of thyrotoxicosis on this activity in rat lung. In addition, insofar as the influence of thyroid hormones on microsomal fatty acid chain elongation synthesis has to date been studied only in liver [12,13], it seemed worthwhile to verify whether thyrotoxicosis has some effect on this synthetic activity in rat adipose tissue and lung as well.

The data obtained indicate that acetyl-CoA carboxylase, fatty acid synthetase and microsomal fatty acid chain elongation synthesis in rat adipose tissue are significantly influenced by the thyrotoxic state, although less so than in liver. In contrast, in rat lung these activities are only slightly affected by thyroid hormones.

2. Materials and methods

Male Wistar rats (200–250 g) fed ad libitum with a standard diet, were used throughout these studies and divided into 3 groups of 4 animals each.

Group 1: Control rats to which only solvent was injected intraperitoneally;

Group 2: Rats made hyperthyroid by 3,3′,5-triiodo-L-thyronine administration at 30 μg . 100 g body wt^{-1} . day^{-1};

Group 3: Rats in which hypothyroidism was induced by administration of 6-*n*-propyl-2-thiouracil (10 mg . 100 g body wt^{-1} . day^{-1}).

The drugs were dissolved in propyleneglycol:NaCl 0.9% (60:40, v/v) and administered with a single daily intraperitoneal injection for 5 consecutive days. The animals were killed by decapitation 24 h after the last administration. Liver, epididymal adipose tissue and lungs were quickly removed, weighed and homogenized in 4 vol. 0.01 M Tris–HCl buffer, containing 0.25 M sucrose and 0.001 M EDTA (TSE). Prior to homogenization, lungs were washed several times in TSE buffer and cut into small pieces, with the homogenate filtered through 2 layers of gauze. Preparation of the liver and lung subcellular fractions was carried out as in [14], and that of epididymal adipose tissue as in [15], with protein determined by the biuret method [16]. $(NH_4)_2SO_4$-precipitated cell sap (40% saturation) was used for acetyl-CoA carboxylase determination, and acetyl-CoA-dependent CO_2 fixa-

Table 1
Effect of the thyrotoxic state on acetyl-CoA carboxylase activity in rat liver, adipose tissue and lung

Animals	Specific activity (nmol [1-^{14}C]malonyl-CoA formed . min^{-1} . mg protein^{-1})		
	Liver	Adipose tissue	Lung
Control	2.24 ± 0.27 (100)	1.75 ± 0.14 (100)	0.46 ± 0.06 (100)
Triiodothyronine treated	8.28 ± 0.71 (370)	2.84 ± 0.23 (162)	0.61 ± 0.05 (133)
Propylthiouracil treated	1.10 ± 0.09 (50)	1.13 ± 0.08 (64)	0.30 ± 0.04 (65)

Data mean of 5 expt ± SD; 4 rats used in each expt. Drugs injected i.p. once daily for 5 consecutive days at the doses indicated in the text. $(NH_4)_2SO_4$ precipitated cell sap protein (40% saturation) used as enzyme source. Values in brackets express the relative specific activity (%); protein (0.25 mg)

tion was assayed as in [17]. Other experimental conditions were as in [13].

3. Results and discussion

The dose of drugs and duration of treatment were chosen to obtain a variation of haematic triiodothyronine level without significantly changing body weight of the treated rats [18], so that the hormonal influence on fatty acid synthesis could not be ascribed to alterations resulting from changes in body weight.

Table 1 shows the effect of the thyrotoxic state on the activity of rat adipose tissue and lung acetyl-CoA carboxylase. It is evident that the specific activity of acetyl-CoA carboxylase in hyperthyroid rats is less stimulated in adipose tissue than in liver (62% *vs* 270%), while in propylthiouracil-treated animals an almost similar inhibition is observed in both tissues. In contrast, rat lung acetyl-CoA carboxylase seems slightly affected by the thyroid hormone level.

Essentially the same behaviour of acetyl-CoA carboxylase is exhibited by fatty acid synthetase in rat adipose tissue and lung following triiodothyronine or propylthiouracil administration (cf. table 2). In fact, this synthetic activity in hyperthyroid rat adipose tissue and lung is stimulated by 68% and 36%, respectively, as compared to the 211% increase which occurs in liver. Likewise, hypothyroidism seems to have only a slight effect on this activity in lung, while in liver and adipose tissue it is reduced by 56% and 40%, respectively.

These results agree with those in [5,6] where both acetyl-CoA carboxylase and fatty acid synthetase and

Table 2
Effect of triiodothyronine or propylthiouracil administration on fatty acid synthetase in rat liver, adipose tissue and lung

Animals	Specific activity [1,3-^{14}C]malonyl-CoA incorporated . min^{-1} . mg protein^{-1})		
	Liver	Adipose tissue	Lung
Control	0.75 ± 0.14 (100)	0.65 ± 0.04 (100)	0.25 ± 0.02 (100)
Triiodothyronine treated	2.33 ± 0.25 (311)	1.09 ± 0.10 (168)	0.34 ± 0.04 (136)
Propylthiouracil treated	0.33 ± 0.05 (44)	0.39 ± 0.02 (60)	0.18 ± 0.02 (72)

Data mean of 5 expt ± SD; 4 animals used in each expt. Triiodothyronine and propylthiouracil administered as indicated in legend to table 1. Values in brackets express the relative specific activity (%); crude cell sap protein (0.40 mg)

Table 3
Effect of triiodothyronine or propylthiouracil administration on rat liver, adipose tissue and lung microsomal fatty acid chain elongation synthesis

Animals	Specific activity (nmol [1,3-^{14}C]malonyl-CoA incorporated . min^{-1} . mg protein^{-1})		
	Liver	Adipose tissue	Lung
Control	0.37 ± 0.04 (100)	0.28 ± 0.04 (100)	0.19 ± 0.02 (100)
Triiodothyronine treated	1.06 ± 0.09 (286)	0.42 ± 0.06 (151)	0.21 ± 0.01 (113)
Propylthiouracil treated	0.24 ± 0.02 (65)	0.19 ± 0.03 (68)	0.14 ± 0.02 (74)

Data mean of 6 expt ± SD; 4 rats used in each expt. Assay done at 37°C for 10 min under N_2 with constant shaking. Incubation vials flushed with N_2 for 10 min prior to assay to achieve anaerobic condition. Animal treatment as indicated in legend to table 1. Values in brackets express the relative specific activity (%); microsomal protein (0.45 mg)

other key lipogenic enzymes [5] were increased in adipose tissue of hyperthyroid animals: findings at odds with [7], according to which de novo fatty acid synthesis in adipose tissue was significantly reduced in hyperthyroid animals. In addition, since the data of tables 1 and 2 indicate that, as in liver, both these activities are also inhibited in adipose tissue of hypothyroid rats, it appears that during thyrotoxicosis the latter tissue exhibits a behaviour similar to that found in the former. In [19] a similar behaviour for fatty acid synthetase in liver and adipose tissue was determined as far as both nutritional factors and the diabetic state and insulin administration were concerned. In [20], using thyroidectomized rats, certain glucose and lipid metabolism enzymes in adipose tissue were also shown to behave similarly to those in liver.

Table 3 reports the effect of the thyrotoxic state on microsomal fatty acid chain elongation synthesis in the 2 tissues under investigation. To date, no data on this synthetic activity in adipose tissue have been reported in either the hyper or hypothyroid state. The interest in investigating this mechanism under these conditions arises from the fact that the microsomal chain elongation synthesis plays an important role in the supplying of fatty acids to adipocyte membrane phospholipids [21]. From the results in table 3 it can be seen that microsomal system in adipose tissue seems to parallel that in liver: in hyperthyroid rats a 51% stimulation of [^{14}C]malonyl-CoA 1,3-incorporation into fatty acids occurs (186% in liver), while a 32% reduction in activity is found in the hypothyroid state (35% in liver).

These results further support the idea that enzymes involved in fatty acid synthesis are subject to similar regulation in liver and adipose tissue. The stimulation observed in hyperthyroid rat adipose tissue could be ascribed to the more rapid mobilization of the newly-formed fatty acids in this tissue. Indeed, lipolysis takes place at a greatly elevated rate in adipose tissue of hyperthyroid animals [22], seeming to indicate a short life of the newly formed lipids as a result of their rapid release in circulation for use in other tissues. A similar correlation has been suggested for the increased activities of glycogenesis and glycogenolysis in hyperthyroid rat liver [23].

Table 3 indicates that, in lung, unlike liver and adipose tissue, microsomal fatty acid chain elongation synthesis is almost unaffected in triiodothyronine-treated rats, perhaps due to the fact that lecithin pulmonary surfactant is essentially made up of dipalmitoyl phosphatidylcholine, with few of the molecular species containing fatty acids longer than palmitic [24]. Thus, it is apparent that the activities of fatty acid synthesizing enzymes are not under quantitatively identical thyroid hormone control in liver and lung, with those of the latter organ varying to only a slight degree when compared to the very large variation evident in those of the former. A similar situation has been found in lung of starved–refed and diabetic animals [10], leading to the conclusion that fatty acid synthesizing enzymes appear to be under much more stringent control in lung than in liver.

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