A specific L-tri-iodothyronine-binding protein in the cytosol fraction of human breast adipose tissue

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1. Binding of L-tri-[¹²⁵I]iodothyronine to the cytosol fraction of normal human female breast adipose tissue was investigated by the charcoal adsorption method. Equilibrium of binding was reached after 120 s at 25°C. 2. The L-tri-[¹²⁵I]iodothyronine-binding component is a protein; this was confirmed by experiments in which binding was totally lost after heating the cytosol fraction for 10 min at 100°C and in which binding was diminished after treatment with proteolytic enzymes and with thiol-group-blocking reagents. The binding protein was stable at –38°C for several months. 3. It displayed saturability, high affinity (apparent K_d 3.28 nM) and a single class of binding sites. 4. High specificity for L-tri-iodothyronine and L-3,5-di-iodo-3'-isopropylthyronine was observed, whereas other iodothyronines were less effective in displacing L-tri-[¹²⁵I]-iodothyronine from its binding site. 5. The binding of the hormone by the cytosol fraction did not show a pH optimum. 6. When cytosol fractions of adipose tissue from different females were subjected to radioimmunoassay for the determination of thyroxine-binding globulin a value of $0.304 \pm 0.11 \mu\text{g}/\text{mg}$ of cytosol protein (mean $\pm$ S.D., $n = 4$) was obtained; the mean concentration in plasma was $0.309 \pm 0.07 \mu\text{g}/\text{mg}$ of plasma protein (mean $\pm$ S.D., $n = 3$). 7. The K_a value of $6.3 \times 10^8 \text{ M}^{-1}$ of L-tri-[¹²⁵I]iodothyronine for binding to plasma, the similar thermal-inactivation profiles of binding and the reactivity to thiol-group-blocking reagents were some properties common between the binding components from the cytosol fraction and plasma. 8. These results suggest that the cytosol fraction of human female breast adipose tissue contains thyroxine-binding globulin; the protein that binds L-tri-[¹²⁵I]iodothyronine with high affinity and specificity appears to be similar to thyroxine-binding globulin.

The reactivity of the adipose tissue to lipolytic hormones varies among species, and under normal conditions noradrenaline liberated by the sympathetic nervous system is the major lipolytic stimulus (Myers, 1977). Adrenergic receptors on the plasma membranes and the membrane-bound adenylate cyclase are involved in the action of these hormones. The sensitivity of the tissue to lipolytic agents is increased by thyroid hormones (Ingbar & Woeber, 1974; Loeb, 1978); e.g. lipolysis of triacylglycerols is increased in states of thyroid hormone excess (Bray & Goodman, 1965). The increase in the number of β -adrenergic receptors appears to be certain, but whether the activity of the

adenylate cyclase is also affected is still an unsettled issue (Bernal & Degroot, 1980).

In contrast with the above-mentioned effects of thyroid hormones, very little is known about their interaction with intracellular components of adipose tissue, and the effects that follow this interaction. Therefore we decided to study the effects of thyroid hormones on human adipose tissue; in the present paper the characteristics of binding of L-tri-[¹²⁵I]-iodothyronine to the cytosol fraction from normal human breast adipose tissue are reported.

Experimental

Chemicals

L-Tri-[¹²⁵I]iodothyronine (sp. radioactivity 18–30 MBq/nmol) was obtained from New England

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Nuclear Chemicals (Dreieich, Germany). Deoxy-ribonuclease (EC 3.1.22.1), ribonuclease (EC 3.1.27.5) and α -chymotrypsin (EC 3.4.21.1) were from Boehringer (Mannheim, Germany); Pronase P (EC 3.4.21.14), Pronase AS (proteinase V; EC 3.4.21.14) and Nagarse (proteinase VII; EC 3.4.21.14) were from Sigma Chemicals (München, Germany). Thyroid hormones and their analogues were gifts from Dr. E. Scheiffele and Dr. H. Rokos; monospecific anti-(thyroxine-binding globulin) serum from rabbits were given by Dr. M. P. Deparade (Henning-Berlin, Berlin, Germany). L-3,5-Di-iodo-3'-isopropylthyronine was a gift from (the late) Dr. E. C. Jorgensen (San Francisco, CA, U.S.A.).

Preparation of the cytosol fraction

Adipose tissue of the breast was obtained from normal female subjects in the age range 20–43 years; the women sought counsel for mammoplasty. Their endocrine status was normal and their case history did not indicate use of medication. Immediately after removal of the adipose tissue it was transported to the laboratory on crushed ice; the tissue was macroscopically normal. All further procedures were performed at 0–2°C. The tissue was washed several times with Tris buffer I, pH 7.0 (10 mM-Tris/HCl/1.5 mM-EDTA/1 mM-dithioerythritol/0.25 M-sucrose/10 mM-NaOH) to free it of blood and blood clots. Pieces of weight 20–30 g were cut out, wrapped in Parafilm, dropped into liquid N₂ and then stored at –38°C. To prepare the cytosol fraction, the tissue was cut into small pieces and washed again thoroughly several times with Tris buffer I to remove traces of blood. A 50% (w/v) homogenate of the tissue in Tris buffer I was prepared with an Ultra-Turrax homogenizer (Janke und Kunkel), set at maximal speed for 10 s, with intervals of 10 s of cooling; the procedure was repeated three times. The homogenate was centrifuged at 25000 g for 30 min at 2–4°C. The lipid layer in the tubes was pierced with tough plastic tubing fixed to a 20 ml syringe, and the buffer phase was aspirated and transferred to fresh tubes, which were then centrifuged at 150000 g for 30 min in a Beckman L2-65B ultracentrifuge. The supernatant, i.e. the cytosol fraction, contained 2.3–2.6 mg of protein/ml; 1.0 ml portions were frozen in liquid N₂ and kept at –38°C until further use. The cytosol fraction was also prepared with Tris buffer I containing 0.6 M-sucrose to test for leakage of nuclear material. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as standard. Before being used for binding studies the cytosol fraction was treated for 1 h with a suspension of Norit A charcoal (400 mg/10 ml) in Tris buffer I, to remove endogenous L-tri-iodothyronine. The cytosol fraction/charcoal suspension ratio was 1:1 (v/v). The mixture was centrifuged at

10000 g for 10 min and the supernatant was used for binding studies.

Determination of binding of L-tri-[¹²⁵I]iodothyronine

Tubes for incubation were prepared with the appropriate concentration of L-tri-[¹²⁵I]iodothyronine and the additives in a final volume of 200 μ l of Tris buffer II, pH 7.0 (10 mM-Tris/HCl/1.5 mM-EDTA/1 mM-dithioerythritol/1 mM-NaOH). All components were equilibrated for 10 min at 25°C before the incubation; the reaction was started by adding 50 μ l of the cytosol fraction; the final concentration of protein was approx. 580 μ g/ml. Unless otherwise mentioned, the incubation lasted 120 s. The bound hormone was separated from the free hormone by transferring 200 μ l of the incubation mixture to a tube containing 500 μ l of Norit A charcoal suspension in Tris buffer II (200 mg of Norit A charcoal/10 ml) prechilled in an ice/water bath. After 10 s the tubes were centrifuged at 5000 g for 120 s in a Minifuge (Heraeus Christ). Portions (400 μ l) of the supernatant were immediately taken out and transferred to plastic tubes for measuring protein-bound L-tri-[¹²⁵I]iodothyronine in an LKB γ -radiation counter. The counting efficiency was 81%. The absolute decay rate of L-tri-[¹²⁵I]iodothyronine was calculated by the method of Horrocks (1975).

Various concentrations of unlabelled L-tri-iodothyronine or its analogues were prepared by dissolving the appropriate amounts in 10 μ l of 0.1 M-NaOH and diluting them to 10 ml with 0.9% NaCl solution to give a 0.1 mM solution of the respective hormones. The desired concentrations for incubation were obtained by an additional dilution with Tris buffer II; the pH of the final incubation mixture was 7.0. Unless otherwise mentioned, L-tri-iodothyronine or one of its analogues was incubated with 0.18 nM-L-tri-[¹²⁵I]iodothyronine (117000–168000 c.p.m.) for binding or competition studies. For the experiment on the dependence of binding on the concentration of the hormone, increasing concentrations of unlabelled L-tri-iodothyronine prepared by serial dilution were added to tubes containing L-tri-[¹²⁵I]iodothyronine. The concentration of the hormone ranged from 0.18 to 48.9 nM. Bound L-tri-[¹²⁵I]iodothyronine was calculated from the specific radioactivity of the hormone. The affinity of the analogues (K_A) for the L-tri-iodothyronine-binding site in the cytosol fraction is the reciprocal of the concentration of the analogue required for 50% decrease in the binding of L-tri-[¹²⁵I]iodothyronine. The relative affinity of the analogues with respect to the affinity of L-tri-iodothyronine (K_{T3}) is expressed as $100 \times K_A/K_{T3}$.

Thyroid hormones are readily adsorbed on glass- and plastic-ware used for experiments; in order to

assess the adsorption, L-tri-[125 I]iodothyronine in the absence and in the presence of unlabelled L-tri-iodothyronine or the analogues was incubated with the cytosol fraction for the respective time periods; samples along with their pipette tips were taken for measurement of radioactivity. Throughout the concentration range the proportion adsorbed at each concentration was about 17% and was found to be independent of the concentration of protein used. The concentrations of the hormone used for binding to the cytosol fraction were corrected for this adsorption. Simultaneous incubations in the absence of cytosol were performed, since these values (blanks, 0.5–1.0%) were linearly dependent on the amounts of radioactivity used.

Measurement of the concentration of L-tri-iodothyronine in the cytosol fraction

The cytosol fraction was freeze-dried at -30°C ; the residue was extracted three times with 1.0 ml of 90% (v/v) ethanol. The extracts were combined and evaporated under N_2 . The residue was taken up in 4% (w/v) bovine serum albumin; 0.1 ml of this solution was used for the determination of L-tri-iodothyronine with the radioimmunoassay kit [Total T₃, poly(ethylene glycol) method from Serono, Freiburg, Germany]. The sensitivity of the method was about 0.06 ng/ml. From separate samples of the cytosol fraction the recovery of L-tri-iodothyronine, from the use of tracer amounts of L-tri-[125 I]iodothyronine, was determined to be about 50%.

Treatment of the cytosol fraction with hydrolytic enzymes and thiol-group-blocking reagents

The charcoal-treated cytosol fraction (100 μl) was exposed to the enzymes (each 2.5 mg/ml) and to the thiol-group-blocking reagent solutions (10 mM) in Tris buffer II (100 μl). Tris buffer II processed in the same way served as blank; the charcoal-treated cytosol fraction (100 μl) kept with 100 μl of Tris buffer II served as control. After 30 min of incubation at 25°C with the reagents, 50 μl of L-tri-[125 I]iodothyronine (0.18 nM) was added and binding was determined as described above. Binding of L-tri-[125 I]iodothyronine to the enzymes in the absence of the cytosol fraction served as an additional control.

Effect of pH on binding

Tris buffer II, containing 0.2 M-Tris/HCl, in the pH range 5.0–9.0 was used to study the binding of L-tri-[125 I]iodothyronine (0.18 nM) to the cytosol fraction. Specific binding was obtained by subtracting non-specific binding from total binding; the former was determined in parallel incubations with

mixtures that contained a 1000-fold excess of unlabelled L-tri-iodothyronine.

Determination of thyroxine-binding globulin in plasma and in the cytosol fraction

Plasma was obtained from subjects who underwent plastic surgery of the breast. The concentration of thyroxine-binding globulin was determined by radioimmunoassay with commercially available test kits (RIA Henning TBG-RIA double-antibody-technique, Henning-Berlin, Berlin, Germany, and the Corning TBG- 125 I-radioimmunoassay test system, Immo-phase IMA, Abtl. Diagnostika, Giessen, Germany). Since the protein content of the cytosol fraction of adipose tissue was about 2.4 mg/ml, it was necessary to concentrate it in order to attain a concentration comparable with that of plasma. A 25–30-fold concentration was obtained by using a Minicon B 15 concentrator (Amicon, Witten, Germany). Microimmunoelectrophoresis of the cytosol fraction and plasma was performed on agar gel in 0.499 M-Tris (Trizma base; Sigma)/74 mM-boric acid/16 mM-EDTA buffer, pH 8.9, with a monospecific anti-(human thyroxine-binding globulin) serum.

Thermal denaturation of binding components of the cytosol fraction and plasma

Portions (0.5 ml) of the cytosol fraction and plasma were kept for 10 min at 0, 25, 37, 42, 50, 60, 65, 70 or 100°C . The samples were then equilibrated for 10 min at 25°C before binding studies were commenced.

All experiments were repeated two to four times; incubations were in quadruplicate and the coefficient of variation ranged between 2 and 8%.

Results

Among the several different methods described in the literature (Samuels *et al.*, 1974; Defer *et al.*, 1975; Visser *et al.*, 1975; Korček & Tabachnik, 1976; Snyder *et al.*, 1976; Surks & Oppenheimer, 1976; Jaffe & Gold, 1977; Murthy *et al.*, 1978) to measure thyroid hormone binding to cellular components, the charcoal adsorption method used in the present study proved to be the most suitable for investigation of the binding of L-tri-[125 I]iodothyronine to the cytosol fraction of human breast adipose tissue at 25°C .

Time- and temperature-dependence of binding

The time course of binding of L-tri-[125 I]iodothyronine was examined at 0, 25 and 37°C (Fig. 1). At 0 and 25°C binding increased linearly with time up to 25 s; at 25°C equilibrium was reached after

Fig. 1. Time course of binding of L-tri-[125 I]iodothyronine to the cytosol fraction from human breast adipose tissue

The concentration of L-tri-[125 I]iodothyronine was 0.06 nM; the protein concentration in this and all other experiments was approx. 580 μ g/ml. The experiments were performed at 0°C (○), 25°C (●) and 37°C (▲). For full experimental details see the text. All values in this and the other Figures are the means for quadruplicate incubations; vertical bars represent $\pm$ S.D.

120 s. At these temperatures equilibrium was observed up to 3 h (points not shown in Fig. 1). At 37°C maximal binding was observed at 15 s, which was, however, less than the maximal binding at 0 and 25°C at equilibrium. Therefore binding experiments were performed at 25°C and for a period of 120 s. No metabolism of the radioactive hormone was detected during this time period. Binding was linear up to 900 μ g of cytosol protein/ml. When the incubation mixture was treated with charcoal at 0°C to remove free L-tri-[125 I]iodothyronine, it was found that L-tri-[125 I]iodothyronine dissociated from the binding component(s); dissociation of radioactivity as a function of time of exposure of the hormone-cytosol fraction complex to charcoal indicated the presence of only one component from which L-tri-[125 I]iodothyronine dissociated. The amount of bound hormone that dissociated from the hormone-cytosol fraction complex after the addition of charcoal including the centrifugation step was 8.8%; the half-time of dissociation was calculated to be 72 s.

Stability of the binding component

Freezing in liquid N_2 and subsequent storage for 3 months at -38°C did not lead to a decrease in binding or to a change in binding characteristics. When the cytosol fraction was kept at 0°C in the absence of L-tri-iodothyronine for 24 h, binding

Fig. 2. Binding of L-tri-[125 I]iodothyronine to the charcoal-treated cytosol fraction from human breast adipose tissue as a function of hormone concentration

Incubation tubes contained a constant amount of L-tri-[125 I]iodothyronine (concentration 0.18 nM), which corresponded to 168 000 c.p.m., plus different concentrations of unlabelled L-tri-iodothyronine ranging from 0.77 to 48.9 nM prepared by serial dilution. For full experimental details see the text. Corrections for adsorption and dissociation are given in the text. The inset shows the Scatchard plot of the data.

decreased by 28%; at 25°C binding did not decrease up to 30 min. Heating the cytosol fraction in the absence of L-tri-iodothyronine for 10 min at different temperatures led initially to a slow decrease in binding at temperatures up to 50°C, but the rate of decrease accelerated as the temperature was further increased. At 60°C binding was diminished by 50% and was completely lost at 100°C.

Saturability and binding affinity

The binding of L-tri-[125 I]iodothyronine to the cytosol fraction as a function of hormone concentration was saturable (Fig. 2); the Scatchard (1949) plot (Fig. 2, inset) shows the presence of a single class of binding sites in the concentration range 0.18–48.9 nM; the apparent equilibrium dissociation constant, K_d , was 3.28 nM and the binding capacity was 0.98 nM (1.7 pmol/mg of cytosol protein). The binding of L-tri-[125 I]iodothyronine was reversible in that a 1000-fold excess of unlabelled L-tri-iodothyronine displaced 92–95% of L-tri-[125 I]iodothyronine from its binding site.

Effect of thyroid hormone analogues on the binding of L-tri-[125I]iodothyronine

Various analogues of L-tri-iodothyronine were tested for their ability to compete with L-tri-[125 I]-

Table 1. *Relative affinities of iodothyronines for the L-tri-[¹²⁵I]iodothyronine-binding protein from the cytosol fraction of human breast adipose tissue*

The concentration of L-tri-[¹²⁵I]iodothyronine was 0.18 nM. Charcoal-treated cytosol fractions equivalent to about 580 µg of protein/ml were incubated at 25°C for 120 s with L-tri-[¹²⁵I]iodothyronine in the absence and in the presence of the analogues. Separation of the free from the bound hormone and calculation of the relative affinities of the iodothyronines are described in the Experimental section.

Compound	Relative affinity (%)
L-Tri-iodothyronine	100.0
D-3,5,3'-Tri-iodothyronine	17.1
Reverse-tri-iodothyronine	42.8
L-3,5-Di-iodo-3'-isopropylthyronine	90.1
L-Thyroxine	28.4
D-Thyroxine	20.3
L-3,3'-Di-iodothyronine	12.6
L-3,5-Di-iodothyronine	0.9
L-3'-Monoiodothyronine	0.1
3,5,3'-Tri-iodothyroacetic acid	0.8
3,5,3',5'-Tetra-iodothyroacetic acid	1.9

iodothyronine for the binding protein (Table 1). The results on the relative binding affinities demonstrated that unlabelled L-tri-iodothyronine and L-3,5-di-iodo-3'-isopropylthyronine were most effective in displacing L-tri-[¹²⁵I]iodothyronine from its binding site, followed by reverse-tri-iodothyronine, L-thyroxine and D-thyroxine. D-3,5,3'-Tri-iodothyronine and L-3,3'-di-iodothyronine competed with L-tri-[¹²⁵I]iodothyronine almost to the same extent. 3,5,3',5'-Tetra-iodothyroacetic acid, L-3,5-di-iodothyronine, 3,5,3'-tri-iodothyroacetic acid and L-3'-monoiodothyronine were the least effective competitors.

Nature of the binding components

To obtain some insight into the nature of the L-tri-[¹²⁵I]iodothyronine-binding component, the cytosol fraction was treated for 30 min at 25°C with different proteolytic enzymes; Pronase AS, α-chymotrypsin, Pronase P and Nagarse decreased the binding by 80–90%. Deoxyribonuclease increased the binding, whereas ribonuclease decreased it by 57%. The binding of L-tri-[¹²⁵I]iodothyronine was also found to be dependent on the integrity of thiol groups. Among the different thiol-group-blocking reagents used, *p*-chloromercuribenzoate, *N*-ethylmaleimide, 1-fluoro-2,4-dinitrobenzene and sodium ethylmercurithiosalicylate, the last-mentioned inhibited the binding of L-tri-[¹²⁵I]iodothyronine by more than 90%, and was the most effective reagent tested.

Fig. 3. *Effect of pH on the binding of L-tri-[¹²⁵I]iodothyronine to the charcoal-treated cytosol fraction from human breast adipose tissue*

The buffer was Tris buffer II, containing 0.2 M-Tris/HCl; 0.18 nM-L-tri-[¹²⁵I]iodothyronine was incubated in the absence and in the presence of a 1000-fold excess of unlabelled L-tri-iodothyronine. For full experimental details see the text. The curve represents specific binding, which is total binding minus non-specific binding.

Effect of pH on binding

The specific binding (total binding minus non-specific binding) of L-tri-[¹²⁵I]iodothyronine to the cytosol fraction of human breast adipose tissue is noteworthy because it did not have an optimum pH value; it increased 5-fold from pH 7.0 to pH 9.1 (Fig. 3). Binding in the presence of a 1000-fold excess of unlabelled L-tri-iodothyronine remained at a constant low value over the entire pH range.

Examination of the cytosol fraction for thyroxine-binding globulin

Several experiments were performed to find out whether the L-tri-[¹²⁵I]iodothyronine binding component in the cytosol fraction could be thyroxine-binding globulin. Four different cytosol fractions were tested for thyroxine-binding globulin by radioimmunoassay; the concentrations were 0.276, 0.348, 0.429 and 0.166 µg/mg of cytosol protein. The concentrations in plasma from three different subjects, determined simultaneously, were 0.319, 0.344 and 0.286 µg/mg of plasma protein (since the volumes of the cytosol fraction and plasma could not be matched, the results are expressed in terms of mg of protein). With use of the same experimental conditions as those for the cytosol fraction, binding of L-tri-[¹²⁵I]iodothyronine to plasma was

measured. (The non-specific binding determined in the presence of a 1000-fold excess of unlabelled L-tri-iodothyronine was about 7% of the total binding.) The apparent K_a for the binding of L-tri-[125 I]iodothyronine to plasma proteins was found to be $6.3 \times 10^8 \text{ M}^{-1}$. The profiles of binding obtained after subjecting the cytosol fraction and plasma to increasing temperatures in the absence of the hormone were almost identical. The binding of L-tri-[125 I]iodothyronine to plasma treated with Nagarse was found to be $6.3 \pm 0.7\%$ when compared with untreated plasma ($100.0 \pm 8.0\%$); deoxyribonuclease increased binding to $424 \pm 36\%$, whereas ribonuclease decreased binding to $58.8 \pm 6.8\%$.

Discussion

Studies on the binding of hormones to cellular components are usually conducted with tissues from immature animals or after ablation of the hormone-producing glands of mature animals. In the present study, the characteristics of binding of L-tri-[125 I]iodothyronine by the cytosol fraction of breast adipose tissue obtained from healthy female subjects with normal thyroid status were investigated. The concentration of L-tri-iodothyronine in the cytosol fraction determined by radioimmunoassay was found to be $13.2 \pm 2.5 \text{ pg/mg}$ of protein (mean $\pm$ S.D., $n = 3$). To avoid interference of binding of L-tri-[125 I]iodothyronine by the endogenous hormone, it was necessary to remove it; this was achieved by treating the cytosol fraction with charcoal. All experiments were performed with this fraction.

Binding was sensitive to temperature; the progress curve of the binding reaction suggests greater stability at 25°C than at 37°C . The binding component appeared to be stable at low temperatures for a considerable period of time, but lost its binding capability rapidly at elevated temperatures (i.e. above 50°C). The total loss of binding activity at 100°C strongly suggests that the binding component is a protein.

The binding protein possessed high affinity, limited capacity and a single binding site for L-tri-[125 I]iodothyronine. Among the iodothyronines tested for their ability to displace L-tri-[125 I]iodothyronine from its binding site, L-3,5-di-iodo-3'-isopropylthyronine was the most active analogue, with a relative affinity of 90%. Reverse-tri-iodothyronine exhibited considerable displacing capability, with a relative affinity of 42.8%. L-Thyroxine, D-thyroxine, D-tri-iodothyronine and L-3,3'-di-iodothyronine, in that decreasing order, were also competent in displacing L-tri-[125 I]iodothyronine. 3,5,3',5'-Tetra-iodothyroacetic acid, L-3,5-di-iodothyronine, 3,5,3'-tri-iodothyroacetic acid and L-3'-monoiodothyronine were very weak competitors of L-tri-[125 I]iodothyronine. These results do not per-

mit us to arrive at a definitive correlation between the relative binding affinities of iodothyronines to cytosol proteins of adipose tissue of the human and the generally accepted thyromimetic activity as has been proposed from studies with the rat (Jorgensen, 1978). The L-tri-[125 I]iodothyronine-binding protein in the cytosol fraction was sensitive to the action of proteolytic enzymes, in that binding decreased. Deoxyribonuclease increased binding, whereas ribonuclease treatment led to a decrease in L-tri-[125 I]iodothyronine binding. In this connection it is not illogical to presume that L-tri-[125 I]iodothyronine could also be bound by ribonucleoprotein particles originating from the nucleus as a result of homogenization of the tissue. However, cytosol fractions prepared by using mild homogenization procedures, such as disruption in the Potter-Elvehjem homogenizer and increasing the molarity of sucrose in the buffer to 0.6 M in order to avoid leakage of nuclear binding material, did not further decrease binding. The activity of the RNA polymerase, a marker enzyme for the nuclei, was not detected. The increase in binding after deoxyribonuclease treatment of the cytosol fraction and plasma is not explainable at the present time; the decrease in binding after ribonuclease treatment could be due to contamination by proteolytic enzymes. The important result, however, is that the binding of L-tri-[125 I]iodothyronine by the cytosol fraction is not due to nuclear binding material. These results taken together suggest that L-tri-[125 I]iodothyronine binds to a protein in the cytosol fraction of human breast adipose tissue. Integrity of thiol group(s) of the binding protein is an essential factor.

The binding protein behaves quite unusually with respect to changes in pH, in that binding increases when the pH is raised from 7.0 to 9.1. The pH-activity profile might originate from alterations of charges of the binding protein, from ionization of the hydroxy group of L-tri-iodothyronine or as a result of both effects. The pK value for the 4'-hydroxy group of L-tri-iodothyronine is 8.45 (Gemmill, 1955); it seems therefore that the ionized phenolic hydroxy group is important for association of the hormone with the binding protein. This finding is in contrast with the binding of L-tri-iodothyronine to nuclear receptors of rat lung and kidney (Latham *et al.*, 1976; Morishige & Guernsey, 1978), where a sharp decrease in binding was noted around the pK of the hormone, which was attributed to the necessity of intact 4'-hydroxy group.

In these types of studies it is important to exclude binding of the ligand to plasma proteins that may have contaminated the tissue and that may contain components that also bind the hormone with high affinity. For example, L-tri-iodothyronine in plasma is distributed between thyroxine-binding globulin and albumin (DeGroot & Stanbury, 1975). The

affinity of L-tri-iodothyronine for thyroxine-binding globulin and albumin is one-tenth of that of L-thyroxine (K_a values of L-thyroxine, for thyroxine-binding globulin, 2×10^{10} – $4 \times 10^{10} \text{ M}^{-1}$ and 6×10^5 – $16 \times 10^5 \text{ M}^{-1}$ for albumin; Nicoloff, 1978). Purified thyroxine-binding globulin possesses two binding sites for L-tri-iodothyronine at the three different temperatures studied (5, 25 and 37°C ; Korček & Tabachnik, 1976). At 25°C the two K_a values were $6.5(\pm 2.8) \times 10^8 \text{ M}^{-1}$ and $0.43(\pm 0.62) \times 10^8 \text{ M}^{-1}$. L-Tri-iodothyronine and L-thyroxine had the same pH optima of binding, between pH 6.8 and 7.7 (Korček & Tabachnik, 1976). Furthermore, the same authors could show that L-tri-iodothyronine is bound one-twentieth as tightly as L-thyroxine to thyroxine-binding globulin at pH 7.4 at 37°C .

On taking these data into consideration, some of the properties of the cytosol L-tri- $[^{125}\text{I}]$ iodothyronine-binding protein appear to be similar to those of purified thyroxine-binding globulin, such as the K_a value ($3.05 \times 10^8 \text{ M}^{-1}$ at 25°C), an identical pattern of inactivation caused by increased temperatures and the effect of thiol-group-blocking agents; a pH optimum was not present. Microimmunoelectrophoresis of the cytosol fraction gave a single discrete band of immunoprecipitation when a monospecific antiserum to human thyroxine-binding globulin from the rabbit was used. Normal age-matched plasma yielded exactly the same pattern of immunoprecipitation. This result is indicative of the presence of thyroxine-binding globulin in the cytosol fraction. The concentration, as determined by radioimmunoassay, was in the same range as that of thyroxine-binding globulin in plasma, expressed per mg of protein. Contamination of the cytosol fraction by plasma proteins can be excluded, since the tissue was thoroughly washed to free it of blood. The inference that may be drawn from the present study is that a protein in the cytosol fraction of human female breast adipose tissue binds L-tri- $[^{125}\text{I}]$ iodothyronine; this protein may be identical with thyroxine-binding globulin or is a thyroxine-binding-globulin-like molecule. In spite of the similarity in properties between the cytosol L-tri- $[^{125}\text{I}]$ iodothyronine-binding protein and thyroxine-binding globulin, caution should be exercised in concluding that the cytosol protein is in fact thyroxine-binding globulin until unequivocal evidence is obtained to support its identity. Since the liver is the only organ known to produce thyroxine-binding globulin, it will be important to characterize the binding protein in the cytosol fraction of breast adipose tissue in order to understand its nature and physiological role.

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