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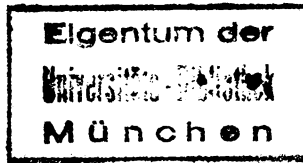

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Enzyme Response to Thyrotoxicosis and Hypothyroidism in Human Liver and Muscle: Comparative Aspects*

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Abstract. 1. Activities of phosphoglucomutase, hexokinase, glucose-6-phosphate dehydrogenase, triosephosphate dehydrogenase, mitochondrial glycerolphosphate dehydrogenase, hexosediphosphatase and phosphoenolpyruvate-carboxykinase, of enzymes involved in the citric acid cycle and connected pathways, of hydroxyacyl-CoA dehydrogenase and carnitine acetyltransferase were determined in biopsy specimens of liver and of tibialis anterior muscle from thyrotoxic and hypothyroid patients and from controls. The results are compared with data obtained from liver and red and white muscle of thyrotoxic rats and guinea pigs. 2. Concomitant with diminished glucose tolerance, the glucokinase activity is decreased in thyrotoxic human liver. The decrease of rat liver glucokinase activity as a response to administered thyroid hormones is found to be dose-dependent. A relationship between the diminished glucose tolerance in thyrotoxicosis and the decrease of glucokinase activity is discussed. The increase of hexokinase activity (isozyme II) is the most interesting finding in thyrotoxic human muscle because of its possible significance with respect to the elevated metabolic rate. The activity of triosephosphate dehydrogenase, (NADP) malate dehydrogenase, phosphoenolpyruvate carboxykinase and carnitine acetyltransferase is markedly enhanced in human thyrotoxic liver, whereas that of phosphoglucomutase is diminished. 3. Mitochondrial glycerolphosphate dehydrogenase, which is known to be markedly increased in the liver and red muscle of thyrotoxic rats, is not increased in the liver and tibialis anterior muscle of thyrotoxic patients, nor in the liver and white muscle of thyrotoxic guinea pigs. The enzyme responses to thyrotoxicosis in human liver and muscle are more similar to those of the guinea pig than of the rat.

Key words: Thyrotoxicosis, hypothyroidism, enzyme activity pattern, comparative study, liver, muscle, human, hexokinase isozymes, glucose tolerance, m-glycerolphosphate dehydrogenase, gluconeogenesis.

Comparative physiological studies on the metabolic effects and the mode of action of thyroid hormones have attracted renewed attention, especially because of recent findings that in mammals there are species specific differences [49, 13, 25, 28]. Lee and Lardy [27] have shown that mitochondrial glycerolphosphate dehydrogenase activity as the rate-limiting step of the glycerolphosphate cycle [10] increases markedly under the influence of thyroid hormones in all organs or tissues of the rat, which also exhibit elevated oxygen consumption. However, the findings of several groups [49, 13, 25, 28] suggest that this correlation between enhanced basal metabolic rate and increased glycerolphosphate dehydrogenase activity does not exist in the guinea pig and rabbit.

As could be shown by Kubista *et al.* [26] on the rat, during thyroid hormone treatment glycerolphosphate dehydrogenase increases more in red than in white muscle, whereas hexokinase is increased to a greater extent in white muscle. For this reason, in this study the metabolic type of muscles investigated is considered. Further differences with an obviously species-specific response of guinea pig, rat and—as will be demonstrated—man to thyroid hormones, exist for (NADP) malate dehydrogenase, glucose-6-phosphate dehydrogenase and (NADP) isocitrate dehydrogenase [49, 13, 28].

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Compared to the numerous studies on enzyme induction by thyroid hormones in experimental animals, little is known about corresponding observations in humans [33]. The present investigation was undertaken in order to study thyroid hormone-induced changes in the enzyme activity patterns of human liver and muscle. Preliminary reports of this study have been presented [34–36, 45].

Materials

Biochemicals were purchased from C. F. Boehringer u. Soehne GmbH., Germany, inosine diphosphate from Calbiochem, $NaH^{14}CO_3$ from NEN-Chemicals. All solutions were prepared in quartz-distilled water.

Methods

Patients

For this study groups of patients with thyrotoxicosis ($N = 31$) and with hypothyroidism ($N = 15$) and euthyroid controls ($N = 27$) were selected during the last $2^1/_2$ years from patients in hospital. These patients were all on regular diet and were receiving no medication for their thyroid disease. Other drugs known to affect metabolism were also excluded if possible [40, 42]. Only unequivocal cases of thyrotoxicosis and hypothyroidism were selected for this study. However, patients with most severe thyrotoxicosis, e.g. threatening thyroid storm, were excluded because of their need for immediate treatment. The

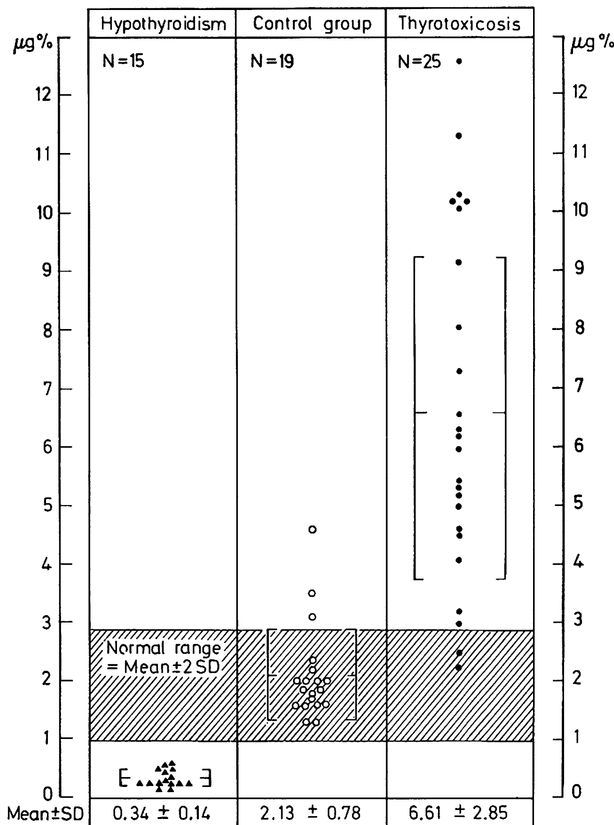

Fig. 1. Free thyroid hormone index (PB $^{127}I \times T_3$-^{125}J) of patients studied. Individual values of the product of PB ^{127}I and "free" T_3-^{125}J from the T_3-test-in vitro are given. The results of the patients: hypothyroidism (▲), control group (○) and thyrotoxicosis (●) are given as mean $\pm$ SD and compared with the normal range of healthy controls [21]: earlier studied (means $\pm$ 2 SD)

group of euthyroid controls was selected from our hospital patients on the basis of the following criteria: absence of overt thyroid abnormality, of overt diabetes mellitus and of obvious liver disease [40].

The hypothyroid patients were found to have: idiopathic myxoedema ($N = 5$), postoperative hypothyroidism ($N = 4$), congenital hypothyroidism ($N = 3$), atrophic autoimmun-thyroiditis ($N = 2$) and secondary hypothyroidism ($N = 1$).

Clinical Analysis

Thyrotoxic patients and euthyroid controls were first examined by means of the clinical diagnostic index for thyrotoxicosis of Crooks *et al.* [14] with minor modifications [44]. In our hands the clinical diagnostic index for hypothyroidism [5] was less useful. The weight of patients is given as a percentage of average weight [19]. The values of PB ^{127}I (autoanalyser) [21], of T_3-tests-*in vitro* (dextran-gel-filtration, automated technique) [21], of free thyroid hormone index (PB $^{127}I \times T_3$-^{125}J) [44, 21], of serum cortisol (fluorimetric method) [43], of serum protein binding of ^3H-cortisol [20], of I.V. glucose tolerance tests and of k_G [15, 16, 42], of insulin efficiency coefficients [40, 32] and of clinical chemical data [29] were determined as described and are summarized in Table 2. Statistical analysis was performed by conventional methods [19, 50].

Biopsies

Liver biopsy was performed according to the method of Menghini [31], using a 1.6 mm needle. The material (wet weight 30–50 mg) was obtained in saline and processed after removal of adherent blood and saline within 10 sec. as follows: a) material for histological examination was fixed in 5% formaline and subjected to routine staining procedures. Histological examination was performed by statistical evaluation of single criteria [40]; b) material for assay of enzyme activities was frozen within 10 sec. in liquid nitrogen and stored in air-tight containers at $-43°$ to -80 °C for less than 2 months. The effect of freezing and storage on the activities of the enzymes studied was investigated (Table 1). Muscle biopsy

Table 1. Effects of freezing and storage on activities of rat liver enzymes. Livers of 2 rats were pooled and analysed either immediately or after 10 weeks storage at -80 °C

	Freshly prepared (μM/h × g w.wt.)	Storage for 10 weeks at -80 °C (μM/h × g w.wt.)
Phosphoglucomutase	4638	4158
Hexokinase	152	90
Glucokinase	163	80
Glucose-6-phosphate dehydrogenase	272	139
Triosephosphate dehydrogenase	4625	4730
Mitochondrial glycerol-phosphate dehydrogenase	60	58
Extramitochondrial glycerol-3-phosphate dehydrogenase	4103	3800
Hexosediphosphatase	405	308
Phosphoenolpyruvate carboxykinase	50	50
Citrate synthase	159	130
(NADP) isocitrate dehydrogenase	1056	1036
Succinate dehydrogenase	800	780
Glutamate dehydrogenase	10289	13741
(NAD) malate dehydrogenase	32246	28479
Aspartate aminotransferase	4332	3040
Hydroxyacyl-CoA dehydrogenase	3200	2900

(m. tibialis ant.) was performed in local anaesthesia (2% novocaine). The material (wet weight 80–250 mg) was processed as described above.

Preparations of Extracts and Homogenates

For tissue extraction, liver biopsy samples weighing less than 30 mg were pooled. Extraction was carried out at +4 °C.

Extraction of Muscle. Homogenization and extraction was performed according to Bass *et al.* [4] in a 19-fold volume (w/v) of 0.1 M phosphate buffer containing 5 mM mercaptoethanol, pH 7.2, with a Polytron PT 10 disintegrator (Fa. Kinematic, Luzern, Switzerland) in four 30 sec. periods with 30 sec. intervals. During disintegration the sample was cooled by an ice-salt mixture. The homogenate was centrifuged for 20 min. at 144.000 × g. The sediment was resuspended 1:15 in 0.05 M phosphate buffer (pH 7.2) by 4 sec. sonification (Sonifier, Branson instruments). For hexokinase extraction, an aliquot of the homogenate was diluted 1:1 with 2% Triton X−100 in 0.1 M phosphate buffer. The mixture was shaken for 30 min. and then centrifuged at 144.000 × g.

Extraction of Liver. Homogenization was performed in eight 10 sec. sonification periods. In some experiments, homogenization was performed with the Polytron PT 10 disintegrator which was specially designed in our workshop for disintegration of small samples (diameter of the head 8 mm). The homogenate was then centrifuged for 20 min. as above.

Enzymes Assays

Phosphoenolpyruvate carboxykinase was measured at 37 °C, all other enzymes at 25 °C. Auxiliary enzymes were dialysed against quartz-distilled water.

Glucokinase [EC 2.7.1.2]. 50 μmoles Triethanolamine-HCl, 5 μmoles EDTA, 8 μmoles $MgSO_4$, 1 μmole dithioerythritol, 0.3 μmoles NADP, 2 μmoles ATP, 100 μmoles D(+)glucose, 10 μg glucose-6-phosphate dehydrogenase in 1.0 ml Final pH 7.6.

Phosphoenolpyruvate Carboxykinase [E.C.4.1.1.32]. A modification of the test (carboxylating reaction) according to Chang and Lane [12] was used. 100 μmoles Imidazole-Cl, 3 μmoles phosphoenolpyruvate, 2 μmoles IDP, 2 μmoles $MgCl_2$, 2.5 μmoles NADH, 1 μmole dithioerythritol, 50 μmoles $NaH^{14}CO_3$ (approximately 50000 cpm/μmole), 50 μg malate dehydrogenase in 1.0 ml. The final pH is 6.8. To overcome phosphoenolpyruvate and IDP consumption by the pyruvate kinase reaction, potassium-free reagents were used. For the same reason, malate dehydrogenase was freed from ammonium sulphate by extensive dialysis against quartz-distilled water. After incubation for 5, 10, 15 and 20 min. at 37 °C the reaction was terminated by addition of 0.5 ml 16% trichloracetic acid. After centrifugation, an aliquot of 0.5 ml was freed from $^{14}CO_2$ activity not incorporated, by drying overnight in a desiccator over $CaCl_2$. The residue was dissolved in 0.5 ml distilled water and counted for acid-stable activity (^{14}C-malate) in a liquid scintillation spectrometer (Packard). Units of enzyme activity are defined as micromoles of bicarbonate fixed per min. Proportionality is given up to 0.003–0.004 units [12].

The activity determinations of the other enzymes measured have been described earlier: Aspartate aminotransferase [E.C. 2.6.1.1], glucose-6-phosphate dehydrogenase [E.C. 1.1.1.49], glycerol-3-phosphate dehydrogenase [E.C. 1.1.1.8], hexokinase [E.C. 2.7.1.1], NADP malate dehydrogenase (decarboxylating) [E.C. 1.1.1.40] and phosphoglucomutase [E.C. 2.7.5.1] [11], citrate synthase [E.C. 4.1.3.7], glutamate dehydrogenase [E.C. 1.4.1.2], 3-hydroxyacyl-CoA dehydrogenase [E.C. 1.1.1.35], NADP isocitrate dehydrogenase [E.C. 1.1.1.42], malate dehydrogenase [E.C. 1.1.1.37] and succinate dehydrogenase [E.C. 1.3.99.1] [8], phosphorylase (a+b) [E.C. 2.4.1.1], triosephosphate dehydrogenase [E.C. 1.2.1.12], lactate dehydrogenase [E.C. 1.1.1.27] and hexosediphosphatase [E.C. 3.1.3.11] [4], mitochondrial glycerolphosphate dehydrogenase [E.C. 1.1.99.5] [26] and carnitine acetyltransferase [E.C. 2.3.1.7] [9].

Protein Determination

Protein was determined with the Biuret method [6].

Electrophoresis

The electrophoresis of hexokinase isoenzymes was performed according to Kubista *et al.* and Katzen and Schimke [26, 24], in 1% agarose in 20 mM sodium barbital buffer and 5 mM mercaptoethanol, pH 8.4 (6 V per cm) at 3 °C. For specific staining the reaction medium of Katzen and Schimke [24] was used.

Results

Clinical Aspects

The group of hypothyroid patients (Table 2) has characteristically low values of the PB ^{127}I, of "free" T_3-^{125}J, of free thyroid hormone index and exhibits elevated serum cholesterol. There is also a significant reduction of I.V. glucose tolerance (k_G) and insulin sensitivity. The thyrotoxic group is characterized by elevated values of PB ^{127}I, of "free" T_3-^{125}J and of free thyroid hormone index (Table 2). The product of fluorimetrically determined serum cortisol and "free" ^{3}H-cortisol is significantly elevated versus control ($p < 0.0025$). Glucose tolerance and insulin sensivity are reduced in thyrotoxic patients. Bromsulphalein retention is significantly increased and histologically, the sinusoids of the entire hepatic lobuli are dilated in the thyrotoxic group [38]. The patients of the control group were selected for biopsy in order to disprove the clinical suspicion of minor hepatic disturbance [40]. For this reason some of their clinical data are obviously not quite normal compared with the normal ranges of healthy persons examined in our laboratory (Table 2).

Table 2. Clinical data of patients studied. The methods employed are described in the text. The values are given as mean ± SD. The normal ranges were calculated from data of normal healthy controls. The significance of the difference of the mean values of the hypothyroid, and thyrotoxic group respectively versus the mean values of the control group is indicated: [a] $=p<0.0005$, [b] $=p<0.0025$, [c] $=p<0.05$

	N	Hypothyroidism	N	Control group	N	Thyrotoxicosis	Normal range of healthy persons
% of average weight	15	110.8 ± 16.2[a]	27	95.9 ± 9.3	31	88.8 ± 11.8[c]	—
Age, years	15	49 ± 16[c]	27	39 ± 13	31	49 ± 13[b]	—
Clinical diagnostic index	—	—	27	1 ± 10	31	32 ± 14[a]	—
PB^{127}I µg/100 ml	15	1.2 ± 0.5[a]	19	5.6 ± 1.7	25	11.4 ± 3.7[a]	3.1–6.8
"Free" T_3–^{125}J %	15	28.6 ± 2.7[a]	26	38.0 ± 4.6	27	56.7 ± 10.5[a]	32–42
Free thyroid hormone index µg/100 ml	15	0.34 ± 0.14[a]	19	2.13 ± 0.78	25	6.61 ± 2.85[a]	0.99–2.85
Cortisol µg/100 ml	9	17.4 ± 2.7[a]	19	11.0 ± 3.8	18	15.0 ± 6.4[c]	9.7–32.0
"Free" ^{3}H-cortisol %	9	13.1 ± 3.5[c]	19	9.8 ± 4.0	18	16.9 ± 8.9[b]	5.0–15.0
Glucose assimilation coeff. k_6	15	1.14 ± 0.32[a]	27	1.66 ± 0.44	31	1.24 ± 0.34[a]	1.1–2.8
Insulin efficiency coeff. mg/mU	14	17.7 ± 18.0	24	22.2 ± 13.6	26	17.2 ± 15.7	~20–70
Cholesterol mg/100 ml	15	324.6 ± 73.1[a]	27	248.8 ± 57.3	31	182.7 ± 38.1[a]	150–240
Aspartate aminotransferase mU/ml	15	11.6 ± 6.9	27	8.8 ± 3.8	27	11.3 ± 4.9[b]	< 12
Alanine aminotransferase mU/ml	15	8.3 ± 6.7	27	8.1 ± 5.5	27	10.9 ± 5.2[c]	< 12
Alk. phosphatase mU/ml	15	23.5 ± 17.5	27	20.0 ± 10.2	31	27.6 ± 13.0[c]	< 30
K^+ *mval/l*	15	4.2 ± 0.3	27	4.2 ± 0.3	27	4.1 ± 0.3	3.6–5.2
Na^+ *mval/l*	15	138.2 ± 2.9	27	140.8 ± 3.5	27	140.7 ± 3.3	135–148
Ca^{++} *mval/l*	15	4.5 ± 0.2	8	4.7 ± 0.2	10	4.9 ± 0.2	4.5–5.3
Bromsulphalein retention (45 min) %	15	7.5 ± 3.8[c]	22	4.9 ± 3.6	21	15.5 ± 10.9[a]	< 5
Widened sinusoids of hepatic lobuli	15	0%	25	22%	30	97%	—

Biochemical Data

Liver

Glycogen Metabolism and Glycolysis. Related to the depletion of liver glycogen [48], in human thyrotoxicosis there was a decrease in the activity of phosphoglucomutase as compared with the control group and hypothyroidism (Table 3).

Hexokinase activity was decreased in human hypothyroidism whereas glucokinase was found to be diminished in thyrotoxicosis. In rat liver a dose-dependent decrease of glucokinase activity was also observed under experimental thyrotoxicosis, as is evident from the data of Table 4. It may be noted, however, that the total activity of the other hexokinase isozymes remains unaffected. The electrophoretic analysis of hexokinase isozymes in thyrotoxic rat liver extracts (Fig. 2a), reveals no detectable activity of isozyme IV (glucokinase).

No unequivocal changes are observed in glucose-6-phosphate dehydrogenase activity. In contrast to the experimental thyrotoxicosis of the rat, there is no increase of glucose-6-phosphate dehydrogenase in thyrotoxic human liver. The data in Table 3 indicate rather a decrease of this enzyme activity.

On the other hand, human thyrotoxicosis causes a significant increase of triosephosphate dehydrogenase activity, whereas in hypothyroidism the activity is decreased.

Glycerin-1P-Cycle. Only slight changes were observed with regard to the key enzyme of the glycerolphosphate cycle [10], i.e. mitochondrial glycerolphosphate dehydrogenase (Table 3 and 7). In thyrotoxicosis as compared with the control group only a slight, statistically insignificant increase is observed. Nevertheless, differences between hypothyroidism and the thyrotoxic group are significant ($p< 0.05$), as are those between the control group and hypothyroidism.

Gluconeogenesis. Of the 2 enzymes representing the pathway of gluconeogenesis (hexosediphosphatase and phosphoenolpyruvate carboxykinase) only phosphoenolpyruvate carboxykinase shows an increase of activity in thyrotoxicosis; it is decreased in hypothyroidism. It should be mentioned, however, that the data given in Table 3 refer to only 2 cases of hypothyroidism and 5 cases of controls.

Citric Acid Cycle and Connected Pathways. (NAD) malate dehydrogenase showed only minor changes in thyrotoxic patients and in hypothyroidism there was only a very slight decrease of this enzyme activity (Table 3). Similar changes were also observed with succinate dehydrogenase. Other enzymes involved in the operation of the citric acid cycle and connected pathways (citrate synthase, (NADP) isocitrate dehydrogenase, glutamate dehydrogenase, asparate aminotransferase) in thyrotoxicosis were similarly characterized by slight decreases in activity.

Fatty Acid Metabolism. Hydroxyacyl-CoA dehydrogenase (fatty acid oxidation) in human liver undergoes no changes in activity at different thyroid hormone levels. On the other hand, carnitine acetyltransferase is markedly increased in thyrotoxicosis and is found at a low activity level in the hypothyroid state (Table 3).

Table 3. Enzyme activities (mean ± SD) in liver biopsy specimens of man. The significance of the differences of the means of enzyme activities is shown by p-values, the asterisk in the line indicating the compared group. Only significant differences are indicated. If SD-values are not given, the biopsy samples have been pooled. (N in parentheses)

	μmoles × h^{-1} × g w. wt.$^{-1}$		
	Hypothyroidsim	Control group	Thyrotoxicosis
Glycogen metabolism			
Phosphoglucomutase	6380 ± 2207 (7)	7373 ± 2406 (18)	4460 ± 2082 (11)
	*		$p < 0.2$
		*	$p < 0.1$
Glycolysis, pentosephosphate shunt			
Hexokinase	59 ± 12 (7)	122 ± 67 (18)	98 ± 45 (14)
	$p < 0.1$		*
	$p < 0.05$	*	
Glucokinase	71 ± 38 (3)	81 ± 57 (9)	45 ± 23 (10)
		*	$p < 0.2$
Glucose-6-phosphate dehydrogenase	33 ± 24 (6)	30 ± 17 (14)	24 ± 13 (10)
Triosephosphate dehydrogenase	4497 ± 982 (7)	4946 ± 853 (20)	6089 ± 482 (15)
	$p < 0.005$		*
	$p < 0.2$	*	
		$p < 0.005$	*
Glycerin-1 P cycle			
Mitochondrial glycerolphosphate dehydrogenase	13 ± 6 (7)	22 ± 11 (17)	25 ± 12 (14)
	$p < 0.05$		*
		$p < 0.2$	*
	$p < 0.05$	*	
Cytosolic glycerol-3-phosphate dehydrogenase	994 ± 308 (7)	1849 ± 575 (16)	1399 ± 801 (10)
Gluconeoegenesis			
Hexosediphosphatase	—	253 ± 19 (5)	279 ± 16 (5)
Phosphoenolpyruvate carboxykinase	594	955 ± 55 (5)	1200 ± 412 (10)
	*		$p < 0.1$
Citric acid cycle and connected pathways			
(NADP) malate dehydrogenase (decarboxylating)	5 (2)	13 ± 3 (5)	19 ± 4 (6)
Citrate synthase	59 ± 6 (7)	93 ± 64 (18)	76 ± 32 (8)
(NADP) isocitrate dehydrogenase	864 ± 239 (7)	1149 ± 651 (18)	682 ± 322 (11)
Succinate dehydrogenase	158 ± 49 (7)	309 ± 216 (17)	303 ± 123 (14)
	$p < 0.02$		*
Glutamate dehydrogenase	6058 ± 2525 (7)	8190 ± 2878 (18)	6333 ± 2749 (11)
Aspartate aminotransferase	2988 ± 1098 (7)	4592 ± 1837 (17)	3512 ± 1508 (11)
(NAD) malate dehydrogenase	9543 ± 2301 (7)	12066 ± 4058 (18)	11620 ± 2800 (11)
	$p < 0.2$		*
Fatty acid metabolism			
3-hydroxyacyl-CoA dehydrogenase	1153 ± 545 (7)	1224 ± 634 (18)	959 ± 736 (11)
Carnitine acetyltransferase	23 (2)	91 (3)	162 ± 31 (3)

The protein values are: hypothyroidism ($N = 7$) 117 ± 41, control group ($N = 20$) 101 ± 41, thyrotoxicosis ($N = 14$) 108 ± 31 mg/g w. wt.

NADP-Dependent Dehydrogenase. Table 5 provides further information on the three investigated NADP-dependent dehydrogenases. The activity levels of glucose-6-phosphate dehydrogenase (NADP) malate dehydrogenase and (NADP) isocitrate dehydrogenase are found at highly different absolute values and various ratios in the livers of rat, man and guinea pig. In addition, the response of these enzymes to thyroid hormones varies greatly in the three species. Obviously, changes observed in rat liver cannot be compared with those in human liver, which resemble rather more closely those in guinea pig liver.

Muscle

In the cases studied so far, mitochondrial glycerol-phosphate dehydrogenase of human thyrotoxic muscle

Table 4. Dose-response of thyroid hormone induced changes in rat liver hexokinase and glucokinase activities. Experiments were performed with four groups of rats each containing 4 animals. A 3.3/1 mixture of L-thyroxine/3,3',5-triiodo-L-thyronine was administered to standard died and the animals were fed for 4 weeks. The control group was on a standard diet. Group A received 3.3 µg hromone mixture daily, group B 16 µg hormone mixture daily, group C 85 µg hormone mixture daily. The daily amount of hormone mixture was completely consumed overnight. During the day a standard diet was administered. At the beginning of the experiment the mean value of the body weight of each group was 155 g. For determination of enzyme activities the livers of each group were pooled. The values for oxygen consumption represent the means of 8 determinations per group (2 determinations per animal).

	Hexokinase (µM/h × w.wt.)	Glucokinase (µM/h × g w.wt.)	Oxygen consumption (ml O_2/h × g body weight) (25 °C)	% increase of body weight/ 4 weeks
Control	152	164	2 ± 0.3	+ 109
Group A	136	116	2.2 ± 0.4	+ 98
Group B	139	76	2.5 ± 1.3	+ 94
Group C	122	32	4.3 ± 1.1	+ 81

Table 5. Influence of thyroid hormones on the activities of NADP-dependent enzymes in the livers of different species. Rats and guinea pigs were given I.P. injections of 25 µg 3,3',5-triiodo-L-thyronine/100 g body weight daily for 6 days. Controls were given I.P. injections of 0.1 ml of 0.9% NaCl-solution daily for 6 days. The livers of 2 rats and 2 guinea pigs were pooled. Human liver values are taken from Table 3

	Glucose-6-phosphate dehydrogenase (µM/h × g w.wt.)		(NADP) malate dehydrogenase (µM/h × g w.wt.)		(NADP) isocitrate dehydrogenase (µM/h g w.wt.)	
	Control	Thyrotoxic	Control	Thyrotoxic	Control	Thyrotoxic
Rat	250	660	60	250	900	1300
Man	32	24	13	19	1100	685
Guinea pig	22	26	4	4	3100	2290

Table 6. Influence of thyrotoxicosis on enzyme activities in human tibialis anterior muscle. The biopsy specimens were stored at −80 °C for several days before extraction. The significance of the difference of the means of hexokinase activities is shown by p-value

	Control group (µM/h × g w.wt.)	N	Thyrotoxicosis (µM/h × g w.wt.)	N
Phosphorylase (a + b)	914 ± 450	3	700 ± 310	6
Hexokinase	62 ± 16 $p < 0.05$	4	103 ± 28 *	7
Triosephosphate dehydrogenase	14162 ± 4065	3	12581 ± 4511	6
Lactate dehydrogenase	10041 ± 4909	3	8362 ± 1732	6
M-glycerolphosphate dehydrogenase	23 ± 0.6	3	24 ± 6	6
3-hydroxyacyl-CoA dehydrogenase	262 ± 133	3	247 ± 151	6
Citrate synthase	132 ± 52	3	152 ± 42	6

failed to increase (Tables 6 and 7). This finding refers to biopsies from m. tibialis anterior.

Results of a comparative study are given in Table 7. These data show the different behaviour of liver and muscle enzyme in rat, guinea pig and man. There is a greater similarity between the changes in the liver of man and those of the guinea pig than between man and the rat. However, this pattern does not hold for the glycerolphosphate dehydrogenase changes observed in skeletal muscle. Only the red muscles of rat and guinea pig show increased glycerolphosphate dehydrogenase activities in thyrotoxicosis.

As shown in Table 6, hexokinase activity of human skeletal muscle is increased in thyrotoxicosis. As is evident from Fig. 2b, this increase is due to isozyme II, whereas isozyme I remains unaffected.

Discussion

Experimental shifts in thyroid hormones cannot necessarily be correlated with clinically occuring variations in the hormones. In spite of these limitations it is obvious that some of the enzyme responses revealed to be main effects in animal experiments are not valid for man. This holds especially for experimentally-induced increases in mitochondrial glycerolphosphate dehydrogenase. In contrast to the marked changes in rat liver and red muscle of rat and guinea pig, there is no increase in mitochondrial glycerolphosphate dehydrogenase in thyrotoxic human liver and muscle (Tables 3, 6 and 7). As a matter of fact, there is also no (or only very little) change of this enzyme activity in guinea pig liver. It appears thus that the thyroid hormone-induced changes of the rat liver enzyme represent a special response which cannot contribute to an understanding of the action of thyroid hormones in the human.

Table 7. Effects of thyrotoxicosis on mitochondrial glycerolphosphate dehydrogenase in rat, guinea pig and human liver, in white (m. rectus femoris) and red (m. soleus) muscles of rat and guinea pig, and in mixed human tibialis anterior muscle[a]

Rats and guinea pigs were given I.P.-injections of 25 μg 3,3′-5-triiodo-L-thyronine/100 g body weight daily for 6 days. Controls were given I.P.-injections of 0.1 ml of 0.9% NaCl-solution daily for 6 days. In experimental thyrotoxicosis livers and the respective muscles of 2 animals were pooled for enzyme activity measurement. Values for human tissues were taken from Tables 3 and 6.

	Rat (μM/h × g w.wt.)			Guinea pig (μM/h × g w.wt.)			Man (μM/h × g w.wt.)	
	m. soleus (red)	m. rectus (white)	liver	m. soleus (red)	m. rectus (white)	liver	m. tib. ant. (mixed)	liver
Control	27	42	49	8	49	33	23	22
Thyrotoxic	41	49	624	15	40	33	24	25

[a] Human tibialis ant. muscle contains 20% white and 80% intermediate or red fibres [39].

Human tibialis anterior muscle is a mixed muscle and contains 20% white muscle fibres, the rest are red or intermediate fibres [39]. This composition is interesting since Kubista *et al.* [26] have found that experimental thyrotoxicosis in rat muscle causes an increase of mitochondrial glycerolphosphate dehydrogenase activity only in red and heart muscle but not in white muscle. With regard to the present results (Tables 6 and 7) it thus appears that human red or intermediate fibres react differently from those of rat and guinea pig, since no increase in mitochondrial glycerolphosphate dehydrogenase was observed in thyrotoxicosis.

Different conclusions must be drawn with regard to the observed fluctuations of liver glucokinase and muscle hexokinase isozyme. II (Fig. 2, Tables 4 and 6). The respective changes of these enzyme activities in the human correlates well with those found in animal experiments. Studying a large group of patients, Dieterle *et al.* [15] have shown that 53% of the cases with thyrotoxicosis showed decreased I.V. glucose tolerance. Hornichter and Brown [22] have demonstrated in the rat a relation between hepatic glucokinase levels and glucose tolerance. It may be suggested, therefore, that a similar relation exists also between the findings of Dieterle *et al.* [15] confirmed in this study (Table 2) and the observed decrease of human liver glucokinase activity in thyrotoxicosis.

The increase of hexokinase in rat skeletal muscle by experimental thyrotoxicosis was demonstrated by several groups [47, 2, 26]. The latter authors have shown that this change does not occur in heart muscle but is restricted in white and red skeletal muscle to an increase of hexokinase isozyme II. The increase of hexokinase activity appears to be of special interest with regard to an increased aerobic oxidative carbohydrate breakdown. Because of the high total weight of muscle, an increased carbohydrate catabolism in muscle could contribute considerably to the metabolism of the whole organism and might cause a significant increase in the basal metabolic rate.

The decrease of glucokinase in the thyrotoxic livers of man and the rat is in good agreement with

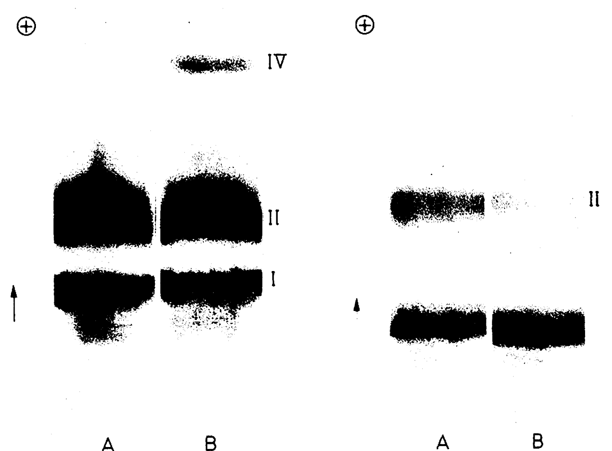

Fig. 2a and b. Specific staining for hexokinase activity in electropherograms of extracts from thyrotoxic and normal human tibialis anterior muscle and from thyrotoxic and normal rat liver. Electrophoresis was performed in 1% agarose gel at pH 8.4, 6 V/cm. a Rat liver. *A* thyrotoxicosis (15 μg 3,3′,5-triiodo-L-thyronine I.P./100 g body weight, daily, for 3 days). B: control (0.1 ml 0.9% NaCl-solution I.P., daily, for 3 days). Tissue extraction (1:3 w/v) was performed in the presence of 10 mM D-glucose. Specific staining was performed at a concentration of 100 mM D-glucose. Running time: 90 min. Staining was interrupted after 60 min. Note absence of isozyme IV in the thyrotoxic liver (isozyme III is not demonstrable at the high glucose concentration applied). b Human tibialis anterior muscle. *A* thyrotoxic. *B* control. The biopsy specimens were stored at −80 °C for several days before extraction. The crude extracts (1:20 w/v) were concentrated 5-fold by suction dialysis in the presence of 2 mM dithioerythritol. Running time: 45 min. Specific staining was performed in the presence of 2 mM D-glucose and was interrupted after 45 min. Note increase of isozyme II in thyrotoxic muscle

the decrease of insulin sensitivity in thyrotoxicosis [15] since the level of active glucokinase is known to be insulin-dependent [41]. The increase of hexokinase isozyme II activity in thyrotoxic skeletal muscle of man and the rat, however, is not consistent with the decrease of insulin sensitivity. Hexokinase isozyme II is known to be stimulated by insulin [e.g. 24].

The rise of hexokinase isozyme II by thyroid hormones may be related to the observations of

Norman and Hiestana [37], who found that D-xylose penetration in skeletal muscle is enhanced under the influence of thyroid hormones. This would indicate an increased influx of glucose into the muscle cell during thyroid hormone treatment. Beyond that, it has been shown that hexokinase isozyme II increases in cultures of mammalian cells when high glucose concentrations are present [23].

The increase of triosephosphate dehydrogenase and phosphoenolpyruvate-carboxykinase occurring in the liver of man under the influence of thyrotoxicosis corresponds to findings in rat liver [1, 3, 7, 30]. In rat liver phosphoenolpyruvate-carboxykinase activity parallels the gluconeogenetic rate [7, 30]. Since triosephosphate dehydrogenase is involved in gluconeogenesis and glycolysis, because of the diminished glucokinase activity and glycogen metabolism it is not anreasonable to assume that the rise of this enzyme observed here is an expression of increased gluconeogenesis rather than increased glycolysis. The question arises, of course, whether the increase in phosphoenolpyruvate-carboxykinase activity is due to elevated cortisol levels. This enzyme is known to be induced by glucocorticoids [46] and elevated free cortisol levels occur in human thyrotoxicosis [20]. Similar findings were obtained in the present study (Table 2).

With respect to the fatty acid metabolism the increased activity of carnitine acetyltransferase in thyrotoxicosis is interesting because of the role of this enzyme in intra-extramitochondrial acetyltransfer (e.g. [9, 18]) and the well-established increase in fatty acid turnover in human thyrotoxicosis [17]. Marked species differences are seen in the pattern of the NADP-dependent dehydrogenase (Table 5). Both with regard to the absolute activities as well as in the differing responses to thyroid hormones, human liver is more similar to the guinea pig than to the rat liver. It must be pointed out, however, that only total activities were measured. Since (NADP) malate dehydrogenase and (NADP) isocitrate dehydrogenase occur both in intra- and extramitochondrial space, it could be that a rise in one compartment might balance a decrease in the other. Hence no net change would be apparent by the assay method employed.

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