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Research Article

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In the thyrotoxic group, the decline in FFA concentration which followed glucose administration was accompanied by slight, but statistically significant, decreases in the PBI and both the per cent and absolute concentration of free T_4. Such changes might have been indicative of an increased intensity of T_4 binding secondary to the decrease in FFA. The serum PBI was decreased, however, a change contrary to that which would be expected to follow an increase in the intensity of T_4 binding. Furthermore, comparable changes in free T_4 and PBI did not accompany the decrease in FFA induced by the administration of insulin. Neither manipulation significantly affected the protein binding of endogenous […]

Effect of Physiological Variations in Free Fatty Acid Concentration on the Binding of Thyroxine in the Serum of Euthyroid and Thyrotoxic Subjects

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ABSTRACT The effect of variations in the concentration of free fatty acids (FFA) on the binding of thyroid hormones in serum has been studied in 20 euthyroid subjects and 19 thyrotoxic patients. In the euthyroid group, neither the pronounced decreases in FFA induced by the oral administration of glucose or the intravenous administration of nicotinic acid, nor the marked increases in FFA which followed the administration of nicotinic acid or 2-deoxyglucose were accompanied by significant changes in the per cent of free thyroxine (T_4), the protein-bound iodine (PBI), the per cent of endogenous T_4 bound by the T_4-binding globulin (TBG) or T_4-binding prealbumin (TBPA), or the resin sponge uptake of triiodothyronine (T_3).

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It is concluded that within a wide physiological range of concentration, FFA do not significantly influence the transport of T_4 in the serum of euthyroid subjects. In the serum of patients with thyrotoxicosis, FFA may have a slight effect on the binding of T_4, but the nature of any such effect is obscure, since parallel, rather than contrary changes in PBI and the proportion of free T_4 followed alterations in FFA concentration.

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INTRODUCTION

Hollander and coworkers have presented data which suggest that unesterified or free fatty acids (FFA) in plasma may compete with thyroxine (T_4)-binding sites on serum proteins and may, in this way, affect the intensity of T_4 binding (1). These workers found that when normal subjects were given a fat meal followed by heparin, the expected increase in the concentration of FFA, often to values exceeding 2000 μEq/liter, was accompanied by 2- to 8-fold increases in the proportion of free T_4. Furthermore, generally smaller increases in the per cent of free T_4 could be produced by in vitro enrichment of serum with FFA, when values in excess of about 2000 μEq/liter were achieved. These findings seem consistent with the fact that FFA have been shown to inhibit the binding of T_4 by human serum albumin (2). Conceivably, they could also affect the binding of T_4 by other proteins. As Hollander and coworkers suggested, this effect on binding might provide a mechanism for rapidly changing the quantity of T_4 available to tissues.

Values of the serum FFA in the very high range of concentrations achieved in the studies of Hollander and coworkers are rarely found in the absence of unphysiological experimental manipulation. Hence, we undertook a study of the effect on T_4 binding in serum of changes in FFA within the range of concentrations more likely

to be encountered in normal man and in patients with naturally occurring disease. The results obtained indicate that, within this narrower but more physiologically realistic range of concentrations, FFA have little or no effect on the protein binding or concentration of T_4.

METHODS

Studies were conducted in 12 male and 8 female euthyroid volunteers who ranged in age between 25 and 55 yr. Of these, 16 were nonhospitalized and four were convalescent in-hospital patients. All were free of known disease, save for one subject who was receiving diphenylhydantoin for a convulsive disorder. Similar studies were also carried out in nine male and 10 female patients with thyrotoxicosis, in whom the diagnosis was established on the basis of conventional clinical and laboratory criteria. Most patients were hospitalized for study in either the Harvard Clinical Research Center, Boston City Hospital, or at the St. Elizabeth's Hospital. These patients were kept recumbent after awakening until studies had been completed. A few studies were conducted in out-patients who were rested for at least ½ hr before studies were initiated. All studies were conducted in the morning with the patients in a fasting state.

The following methods were employed to alter the concentration of FFA. In 12 normal and 15 thyrotoxic patients, 75 g of glucose (Glucola, Ames Co., Inc., Elkart, Ind.) was administered by mouth, blood being drawn before glucose feeding and at intervals of ½ hr for 1–4 hr thereafter. Four thyrotoxic patients were given regular insulin (0.1 U/kg) i.v., and blood was drawn before and at 30, 60, and 120 min after insulin administration. Four normal volunteers were given 200 mg of nicotinic acid i.v., blood being obtained before and both 60 and 180 min after the infusion. Finally, in four other normal volunteers, 2-deoxyglucose (60 mg/kg) was administered by continuous infusion over a 30 min period. Blood was drawn immediately before the infusion, 30 min later (immediately after its completion), as well as 120 and 150 min after the infusion was started. Sera were quickly separated and, with rare exception, were analyzed for free T_4 and FFA on the same day as the test. In the few instances in which this was not true, sera were quickly frozen for subsequent analysis. Serum FFA concentration was determined by the modified method of Dole and Meinertz (3, 4). The per cent of free T_4 in serum was measured in undiluted serum by the resin-dialysis method described in detail elsewhere (5). Serum PBI was measured by a modification of the method of Zak (6). Resin sponge uptake of ^{131}I-labeled triiodothyronine was performed at room temperature (Abbott Trisorb, Abbott Laboratories, North Chicago, Ill). The distribution of small, tracer concentrations of T_4 among the serum proteins ("endogenous" distribution) was assessed by reverse-flow electrophoresis in glycine-acetate buffer, pH 8.6. The specimens employed were aliquots of the same sera which had been enriched with ^{131}I-labeled hormone for the measurement of free T_4. Results obtained were analyzed by Students t test and the paired t test, according to methods described by Snedecor (7).

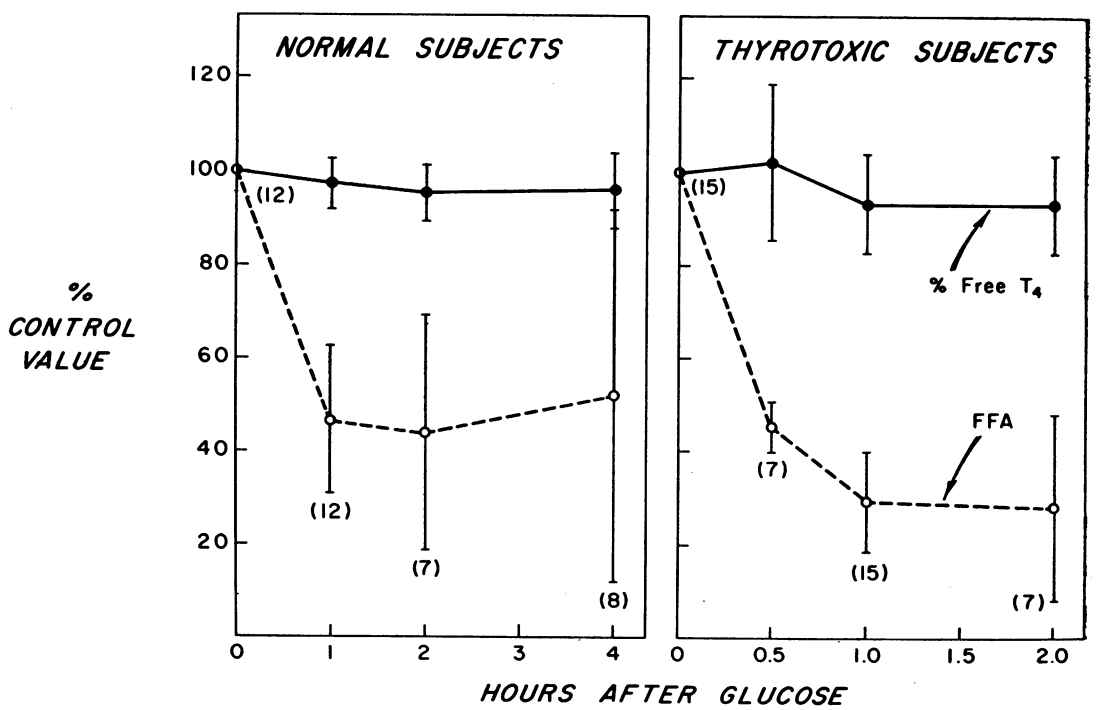

FIGURE 1 The effect of glucose ingestion on the concentration of free fatty acids (FFA) and the per cent of free thyroxine (T_4) in the serum of normal and of thyrotoxic patients. Numbers in parenthesis indicate the number of patients studied at each time period. In control specimens from normal subjects, FFA concentrations averaged 521 ±215 μEq/liter (mean ±SD) and free T_4 0.023 ±0.003% of the total. In control specimens from thyrotoxic patients, FFA concentrations averaged 960 ±331 μEq/liter and free T_4, 0.053 ±0.022% of the total.

RESULTS

Effect of glucose in euthyroid subjects. Glucose (75 g) was administered orally to 12 euthyroid patients who had been fasted overnight. Serum FFA concentrations before glucose administration averaged 521 ±215 μEq/liter (mean ±SD). After administration of glucose, mean FFA decreased to 49% of the control value at 1 hr (12 patients), 44% at 2 hr (seven patients), and 52% at 4 hr (nine patients). Despite these pronounced decreases in serum FFA concentration, the proportion of free T_4 was unchanged, averaging 97, 95, and 97% of control values at 1, 2, and 4 hr, respectively (Fig. 1). Table I presents the results obtained with respect to FFA, protein-bound iodine (PBI), per cent of free T_4, and concentration of free T_4 in control specimens and in those sera which demonstrated the greatest decrease in FFA concentration after the administration of glucose. When analyzed in this manner, the data revealed a mean maximum decrease in FFA of approximately 62%, despite which the PBI and both the per cent and concentration of free T_4 were unchanged (paired *t* test). In the same specimens of serum, the proportion of endogenous T_4 associated with T_4-binding globulin (TBG) averaged 58.8 ±7.8% in control samples and 59.1 ±7.9% in specimens containing the lowest concentration of FFA. The per cent of endogenous T_4 associated with T_4-binding prealbumin (TBPA) in these specimens averaged 31.9 ±5.9 and 31.3 ±6.3, respectively. Values for the resin sponge uptake of T_3 averaged 30.3% in both groups of samples.

Effect of nicotinic acid in euthyroid subjects (Table II). In four euthyroid subjects who had been fasted overnight, serum FFA averaged 569 ±84 μEq/liter. At 60 min after 200 mg of nicotinic acid i.v., mean FFA concentration had decreased to 284 ±70 μEq/liter. In samples obtained 3 hr after nicotinic acid administration, a rebound in FFA to a mean concentration of 1346 ±325 μEq/liter was seen. Values of FFA at all time points were significantly different from one another (paired *t* test). In the same specimens, values for the per cent of free T_4 averaged 0.027 ±0.004, 0.026 ±0.002, and 0.026 ±0.003, respectively. At 60 min after nicotinic acid, the serum PBI had decreased from control values in all patients, the mean decreasing from 5.1 ±1.0 to 4.4 ±0.7 μg/100 ml. By 180 min after nicotinic acid administration, mean PBI had increased to 4.9 ±0.7 μg/100 ml. None of the changes in PBI were significant when judged by the paired *t* test. In the three groups of specimens, small differences in mean values for the concentration of free T_4, of unlikely physiological significance, were seen. The proportion of endogenous T_4 associated with TBG during filter paper electrophoresis averaged 56.5 ±4.8, 55.3 ±5.0, and 55.1 ±6.0% at 0, 60, and 180 min, respectively. In the same specimens, the

TABLE I

Effect of Glucose Administration on Various Indices of Thyroid Hormone Binding in Euthyroid Subjects

Patient	Free fatty acids		PBI		Free T_4		Conc. free T_4	
	Control	After glucose	Control	After glucose	Control	After glucose	Control	After glucose
	μEq/liter		*μg/100 ml*		%		*mμg/100 ml*	
1	445	140	5.2	4.4	0.023	0.021	1.82	1.43
2	491	140	5.0	5.0	0.020	0.018	1.56	1.39
3	257	187	6.0	6.0	0.021	0.022	1.93	2.02
4	673	121	5.2	4.8	0.020	0.021	1.60	1.52
5	234	140	4.8	5.2	0.023	0.022	1.70	1.78
6	410	184	4.8	4.6	0.026	0.023	1.96	1.62
7	398	140	4.8	4.8	0.022	0.022	1.64	1.65
8	384	121	4.4	4.0	0.029	0.027	1.98	1.66
9	633	273	3.8	4.0	0.025	0.022	1.44	1.37
10	702	420	5.6	5.6	0.020	0.021	1.76	1.81
11	995	312	5.0	4.8	0.024	0.023	1.82	1.72
12	634	225	4.0	4.6	0.026	0.026	1.58	1.84
Mean	521	200	4.9	4.8	0.023	0.022	1.73	1.65
SD	215	93	0.6	0.6	0.003	0.002	0.17	0.20
SEMD*	50		0.1		0.0004		0.06	
p value, paired *t*	<0.001		NS		NS		NS	

* Standard error of mean difference.

per cent of endogenous T_4 associated with TBPA averaged 34.9 ±4.9, 35.9 ±5.1, and 36.9 ±5.4, respectively. Values for the resin sponge uptake of T_3 in the three sequential specimens averaged 32.7 ±4.0, 32.1 ±2.9, 31.9 ±4.4%.

Effect of 2-deoxyglucose in euthyroid subjects (Table II). Studies were conducted in four euthyroid subjects who, after an overnight fast, were given 60 mg/kg of 2-deoxyglucose i.v. over a 30 min period. Values for serum FFA averaged 445 ±135 μEq/liter in control specimens, 520 ±270 in specimens obtained at the end of the infusion, 1265 ±200 at 120 min, and 925 ±230 at 150 min after the start of the infusion. Despite these wide differences in FFA, significant differences in PBI and both the per cent and absolute concentration of free T_4 were not seen.[1]

In the four sequential specimens, the per cent of endogenous T_4 associated with TBG averaged 66.2 ±4.5, 64.3 ±2.7, 65.4 ±4.7, and 66.2 ±3.8. In the same specimens, the per cent of endogenous T_4 associated with TBPA averaged 23.4 ±4.7, 25.6 ±1.8, 24.4 ±4.4, and 23.5 ±4.3.[2]

Effect of glucose in thyrotoxic subjects. Studies were conducted in 15 thyrotoxic subjects. After an overnight fast, serum FFA concentrations averaged 960 ±331 μEq/liter, a value significantly greater than that found in the normal subjects studied ($P < 0.001$). After ingestion of 75 g of glucose, mean FFA decreased to 44.5% of the mean control value at 30 min (seven patients), 28.9% at 60 min (15 patients), and 27.5% at 120 min (seven patients) (Fig. 1). Control values for the per cent of free T_4 averaged 0.053 ±0.022, a value significantly higher than that present in the serum of euthyroid subjects ($P < 0.001$). In specimens obtained at 30, 60, and 120 min after the ingestion of glucose, values for the per cent of free T_4 averaged 103, 94, and 96% of control values, respectively. Table III presents the results obtained with respect to FFA, PBI, per cent of free T_4,

[1] One patient in this group displayed a serum PBI of 2.4 μg/100 ml, despite clinical euthyroidism. This can probably be ascribed to the diphenylhydantoin which the patient was taking for a convulsive disorder (8). However, the net effect of this agent on T_4 binding was negligible, since the proportion of free T_4 was not increased, a finding consistent with that reported by Chin and Schussler (9). For this reason, the results obtained in this patient are included in the over-all means, despite the low PBI.

[2] In this group of patients, the proportion of endogenous T_4 bound by TBG was somewhat higher, and that bound by TBPA somewhat lower than was the case in the other euthyroid groups. This probably was a reflection of the fact that studies with 2-deoxyglucose were carried out in convalescent in-hospital patients in whom T_4 binding by TBPA might have been slightly decreased. The remaining euthyroid subjects were nonhospitalized controls.

TABLE II

Effect of Various Agents on Free Fatty Acid Concentrations and Various Indices of Thyroid Hormone Binding in the Serum of Euthyroid or Thyrotoxic Patients

	Free fatty acids			PBI			Free T_4			Conc. free T_4		
Time	Mean	SD	SEMD*	Mean	SD	SEMD	Mean	SD	SEMD	Mean	SD	SEMD
min	*μEq/liter*			*μg/100 ml*				%		*mμg/100 ml*		
Nicotinic acid, 200 mg mg i.v. at 0 time; four euthyroid subjects												
0	569‡	84	32	5.1	1.1	0.4	0.027	0.004	0.003	2.08	0.34	0.24
60	284	70		4.4	0.7		0.026	0.002		1.73	0.41	
180	1346‡	325	170	4.9	0.7	0.2	0.026	0.003	0.001	1.96§	0.23	0.05
2-Deoxyglucose, 60 mg/kg i.v. during 30 min starting at 0 time; four euthyroid subjects												
0	445	135		4.2	1.3		0.024	0.002		1.70	0.55	
30	520	270	69	5.0	0.9	0.3	0.025	0.002	0.0005	1.93	0.14	0.06
120	1265‖	200	42	4.6	1.9	0.4	0.025	0.004	0.001	1.80	0.81	0.19
150	925§	230	115	4.5	1.6	0.3	0.026	0.003	0.0007	1.82	0.64	0.11
Regular insulin, 0.1 U/kg i.v. at 0 time; four thyrotoxic patients												
0	710¶	107	67	12.4	3.9	0.07	0.043	0.011	0.001	8.22	2.82	0.28
30	357	159	242	13.3	4.3		0.042	0.010		8.48	4.09	
120	1015	526		12.6	4.2	0.24	0.046	0.010	0.002	9.03	3.36	0.70

* Standard error of mean difference.
‡ Significantly different from mean in group for which no SEMD shown; paired t test; $P < 0.01$.
§ Significantly different from mean in group for which no SEMD shown; paired t test; $P < 0.02$.
‖ Significantly different from mean in group for which no SEMD shown; paired t test; $P < 0.001$.
¶ Significantly different from mean in group for which no SEMD shown; paired t test; $P < 0.05$.

TABLE III

Effect of Glucose Administration on Various Indices of Thyroid Hormone Binding in Thyrotoxic Subjects

Patient	Free fatty acids		PBI		Free T_4		Conc. free T_4	
	Control	After glucose	Control	After glucose	Control	After glucose	Control	After glucose
	μEq/liter		*μg/100 ml*		%		*mμg/100 ml*	
1	763	243	14.2	13.8	0.052	0.048	11.39	10.18
2	1529	164	19.4	17.6	0.079	0.069	23.62	18.43
3	770	179	9.0	8.6	0.032	0.034	4.50	4.48
4	514	235	8.4	8.6	0.037	0.037	4.72	4.88
5	1151	155	11.6	9.0	0.054	0.053	9.65	7.35
6	970	169	12.4	11.2	0.041	0.040	7.89	6.85
7	858	386	14.8	14.8	0.043	0.040	9.80	9.21
8	986	205	14.0	14.0	0.053	0.041	11.49	8.92
9	865	218	13.0	14.3	0.108	0.096	21.60	21.16
10	1730	470	10.8	9.8	0.064	0.040	10.67	5.99
11	870	212	13.2	13.2	0.042	0.043	8.59	8.71
12	705	332	11.0	11.2	0.031	0.030	5.22	5.25
13	598	190	7.0	6.8	0.036	0.038	3.86	4.03
14	860	320	11.2	9.8	0.039	0.039	6.65	5.82
15	1227	282	15.9	14.8	0.079	0.070	19.42	15.89
Mean	960	251	12.4	11.8	0.053	0.048	10.60	9.14
SD	331	91	3.1	3.1	0.022	0.017	6.24	5.27
SEMD*	82		0.25		0.002		0.46	
P value, paired *t*	<0.001		<0.05		<0.02		<0.01	

* Standard error of mean difference.

and absolute concentration of free T_4 in control specimens and in those sera which displayed the greatest decrease in FFA after glucose administration. When analyzed in this way, the data revealed a mean maximum decrease in FFA of approximately 74%. This major change in FFA was associated with small but significant decreases in serum PBI and in both the per cent and absolute concentration of free T_4. Changes in these functions were of the order of magnitude of 10%, on the average. In the same specimens for which values are shown in Table III, the per cent of endogenous T_4 bound by TBG averaged 47.6 ±7.2 and 48.0 ±6.3, and that bound by TBPA 36.7 ±6.3 and 36.6 ±5.7. Resin sponge uptakes of triiodothyronine (T_3) averaged 49.1 ±7.2 and 49.1 ±7.1%, respectively.

Effect of insulin in thyrotoxic patients (*Table II*). In the four thyrotoxic patients who were given insulin after an overnight fast, values for serum FFA averaged 710 ±107 μEq/liter in control specimens, 357 ±159 in those obtained at 30 min, and 1015 ±526 in those obtained at 120 min. The per cent of free T_4 in control specimens averaged 0.043 ±0.011, a value significantly higher than normal, and was not significantly different 30 and 120 min after insulin administration. Similarly, neither the PBI nor absolute concentration of free T_4 was significantly affected by insulin administration. In control specimens and those obtained at 30 and 120 min after insulin administration, the per cent of endogenous T_4 bound by TBG averaged 47.8 ±9.9, 48.1 ±9.9, and 47.4 ±9.6, respectively, while the per cent bound by TBPA averaged 37.7 ±10.5, 39.9 ±9.9, and 38.2 ±9.4. Resin sponge uptakes of T_3 averaged 49.8 ±8.4, 49.8 ±7.8, and 49.6 ±8.6%.

DISCUSSION

On the basis of studies in five male patients, Hollander and coworkers postulated that plasma FFA, by competing with T_4 for binding sites on serum proteins, may serve to influence the proportion and concentration of free T_4. This conclusion was based on the finding that an increase in the per cent of free T_4 in plasma was associated with the rise in plasma FFA produced by a fat meal followed by heparin (1). The changes in the per cent of free T_4 observed by Hollander and coworkers were so striking (2- to 8-fold) as to leave no doubt as to their validity under the conditions of the experiment. However, two factors led us to wonder whether these findings were truly applicable to the physiological regulation of T_4 binding. First, values of the plasma FFA induced by a fat meal and heparin (greater than 2000 μEq/liter) are markedly higher than those usually en-

countered in clinical practice. Second, it seemed possible that heparin itself might be at least partly responsible for the changes in free T_4 seen, since the red blood cell uptake of T_3 is said to be increased in patients receiving heparin (10). We therefore undertook the present studies in which a variety of other means were used to produce changes in FFA concentrations which, although pronounced, were within the range usually encountered during normal physiological responses or during most disease states. These studies indicate that, within wide limits, pronounced changes in FFA in the serum of normal subjects are not associated with evidence of altered protein binding of T_4. In euthyroid subjects, glucose was employed to decrease serum FFA in 12 instances (11) and nicotinic acid in four (12). Pronounced increases in serum FFA concentration were induced in eight patients, four during the rebound following nicotinic acid administration (12) and four by the administration of 2-deoxyglucose (13). None of these manipulations significantly altered the per cent or absolute concentration of free T_4 or the serum PBI. Furthermore, these wide variations in serum FFA were unaccompanied by significant changes in the in vitro resin sponge uptake of ^{131}I-labeled T_3, or the distribution of endogenous T_4 among the serum proteins, as assessed by filter paper electrophoresis.

In view of these negative findings, similar studies were conducted in patients with active thyrotoxicosis, the rationale being that in this disorder the decreased overall intensity of T_4 binding, reflected in abnormally high values for the proportion of free T_4, might facilitate the demonstration of any changes in free T_4 associated with changes in the concentration of FFA. Furthermore, the increase in fasting serum FFA concentration which occurs during the thyrotoxic state might itself contribute to the increased proportion of free T_4. If this were true, an induced decrease in FFA should be associated with a clear decrease in the per cent of free T_4. In 15 patients with thyrotoxicosis given glucose, the pronounced decrease in FFA induced by glucose (average maximum decrease 74%) was associated with an average decrease of approximately 10% in the proportion of free T_4. Although a decrease in per cent of free T_4 was by no means a constant accompaniment of the decrease in FFA concentration attendant upon glucose administration, the most marked decreases in the per cent of free T_4 were seen in the sera of the three patients in whom the initial FFA concentrations were the highest. Superficially, it might appear that these findings are in support of the hypothesis that FFA inhibit the binding of T_4 in serum and thereby increase the proportion of unbound hormone. However, the decrease in per cent of free T_4 which accompanied the decrease in FFA was also associated with a decrease in PBI. An increase in PBI would have been expected had the decrease in FFA produced an increased intensity of T_4 binding. Hence, it is possible that the decrease in per cent of free T_4 was, in fact, secondary to the decrease in PBI, the explanation for the latter remaining unknown. Moreover, the foregoing changes in free T_4 induced by glucose in thyrotoxic patients may have been fortuitous, since the wide variations in FFA which followed insulin administration were not accompanied by significant changes in the per cent of free T_4, PBI, or absolute concentration of T_4.

Since Hollander and coworkers were able to increase the per cent of free T_4 in serum by in vitro enrichment with FFA, it seems clear that FFA alone (in concentrations in excess of 2000 μEq/liter) are capable of producing this effect. With regard to the increased per cent of free T_4 induced by heparin in vivo, a preliminary communication suggests that heparin itself may alter T_4 binding; when protamine is given before heparin, the increase in FFA is prevented, but the increase in the proportion of free T_4 is not (14). In view of these considerations and of our own findings, we would conclude that within the general range of approximately 200–1500 μEq/liter, FFA have little or no effect on the binding of T_4 in serum. As this is the range of concentrations over which FFA vary in most clinical conditions, it is unlikely that FFA contribute significantly to the regulation of serum free T_4 concentration in most physiological or pathological states.

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