

# Fatty Acid-Induced Increase in Serum Dialyzable Free Thyroxine after Physical Exercise: Implication for Nonthyroidal Illness

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**ABSTRACT.** In 14 healthy males, prolonged running exercise resulted in a mean 25% increase in serum free  $T_4$  ( $FT_4$ ) concentration ( $P < 0.001$ ) which was significantly correlated ( $P < 0.01-0.02$ ) with an over 5-fold increase in the concentration of serum FFA and the FFA/albumin molar ratio. Hemoconcentration, as reflected in a mean 19% increase in serum albumin, caused an increase in serum  $T_4$ -binding globulin and therefore also in serum total  $T_4$ . As there was no change in the serum  $T_4/T_4$ -binding globulin molar ratio, the rise in serum  $FT_4$  was probably not caused, or only partly caused, by an exercise-induced shift of  $T_4$  from the extravascular to the intravascular compartment. Neither is it likely that the mean 41% increase in serum TSH observed after exercise, partly owing to hemoconcentration, was the reason for the increase in serum  $FT_4$  and  $T_4$  as there was no

correlation between the increases in the TSH and thyroid hormone levels. Further support for the assumption that the elevation in serum  $FT_4$  after exercise was FFA-induced was provided by the observation that addition of 2.5 mmol/L oleic acid to normal serum *in vitro* resulted in a 33% increase in serum  $FT_4$  ( $P < 0.001$ ). There is an association between increased concentrations of serum  $FT_4$  and unsaturated FFA in patients with various nonthyroidal illnesses according to earlier observations, but it is unlikely, in the light of the present data from healthy subjects, that FFA are directly involved in raising the serum  $FT_4$  concentration in nonthyroidal illnesses patients unless the serum FFA concentration exceeds 2 mmol/L or the FFA/albumin molar ratio rises above 2.5. (*J Clin Endocrinol Metab* 74: 1361-1365, 1992)

**R**AISED free  $T_4$  ( $FT_4$ ) concentrations in serum, as measured by equilibrium dialysis and ultrafiltration methods, in patients with various moderate and severe nonthyroidal illnesses (NTI) have been repeatedly documented for a long time (*e.g.* 1-6), but the mechanism of this phenomenon is still not resolved. There is evidence for increased concentrations of nonesterified FFA being the mediators of the rise in serum  $FT_4$  by displacement of hormone from serum  $T_4$ -binding proteins (7-9). It is known from *in vitro* experiments that unsaturated FFA can displace  $T_4$  from its main transport protein  $T_4$ -binding globulin (TBG) (10-13). Significant positive correlations between the concentrations of  $FT_4$  and oleic (18:1) acid (14-16), and linoleic (18:2) and linolenic (18:3) acids (16) have been observed in NTI patients. A stronger correlation was found between serum  $FT_4$  and the FFA/albumin molar ratio than the FFA concentration demonstrating that the typically low serum albumin levels in NTI facilitate the expression of an FFA-induced increase in  $FT_4$  because albumin is the most important carrier of

FFA (9, 14, 16). Based on data from *in vitro* experiments Mendel *et al.* (17) concluded that the FFA/albumin ratio must reach 5, which is very unusual in NTI (9, 18), before a significant increase in  $FT_4$  can be expected. Our *in vivo* data indicate that an effect on  $FT_4$  may already be discernable when this ratio exceeds 2 (6, 9, 16).

The FFA-induced elevation in serum  $FT_4$  was first observed after administration of heparin (19). Because quite high serum  $FT_4$  levels have been observed in sera from intensive care patients (7, 14, 15), who frequently receive heparin, heparin as a cause of or contributor to the rise in serum  $FT_4$  cannot be excluded (20). Salicylate, furosemide, fenclofenac, and some other drugs can also increase serum  $FT_4$  by displacing  $T_4$  from its protein binding (21, 22). It is therefore important to exclude drug-interference in human studies designed to clarify the degree of elevation of the FFA level and FFA/albumin ratio required for serum  $FT_4$  to increase significantly. It has been reported that serum  $FT_4$  assessed by indirect methods ( $FT_4$  indices and analog-type assays) is increased after prolonged physical exercise (23-25). The purpose of the present study was therefore to use long-term aerobic exercise, which is known to raise serum FFA, as a model for determining whether an increase in

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serum FFA of a magnitude similar to that observed in some NTI patients could cause an elevation of the FT<sub>4</sub> concentration measured by a well validated equilibrium dialysis method.

### Materials and Methods

Informed consent was obtained from 14 healthy males (mean age 24.2 yr, range 19–38) who volunteered for this study, which was approved by the local ethical committee. No subject had a history of thyroid or liver disease. Venous blood samples were collected in the morning after a 30-min rest just before and immediately after the exercise. In subjects numbered 1–7 the exercise was marching with full combat gear (weight 18 kg) for 3 h and in subjects 8–14 the exercise was running on a treadmill for 2 h 15 min. The mean heart rate during exercise in both groups was 75% of maximum. All subjects were used to physical exercise; 1–7 were young men doing their military service and 8–14 were athletes with experience of endurance sports. The serum collected was immediately separated by centrifugation and stored at –20 C.

Serum FT<sub>4</sub> was measured with an equilibrium dialysis method based on direct RIA of the dialyzable free hormone fraction (26). In this method 100 µl serum sample are prediluted with 400 µl 10 mmol/L HEPES (Sigma Chemicals Co., St. Louis, MO) buffer (pH 7.4 at 37 C) containing 106 mmol/L NaCl. Dialysis was performed in specially designed plastic cells at 37 C for 18 h against 5.0 mL HEPES buffer (final sample dilution was 1:55). Serum T<sub>4</sub> was determined by an in-house RIA, serum TSH by an enzyme immunoassay (ES 600, Boehringer Mannheim GmbH, Germany) and serum TBG by RIA (Clinical Assays Inc., Cambridge, MA). Serum FFA were measured fluorometrically with an enzymatic assay (27) and serum albumin with the bromocresol purple method using a Hitachi 747 analyzer (Tokyo, Japan).

Oleic acid, dissolved in ethanol, was added in incremental concentrations (0–5 mmol/L) to a pooled normal serum whereafter determinations of the FT<sub>4</sub> concentration were performed (n = 4). The endogenous FFA concentration in this serum pool was 0.30 mmol/L, and the total T<sub>4</sub> and TBG concentrations were 97 nmol/L and 25.2 mg/L, respectively. The possibility of analytical interference by FFA in the T<sub>4</sub> and TBG assays was checked by adding increasing concentrations of oleic acid to the pooled normal serum; no effect was observed on either assay.

The serum samples were analyzed with the various methods in duplicate and in one batch in order to avoid interassay variation. Intraassay imprecision, calculated as coefficient of variation (%) from multiple determinations (n = 20) of single analyte concentrations similar to those of the samples studied, were as follows: FT<sub>4</sub> 6.9, T<sub>4</sub> 1.9, TSH 4.3, TBG 7.1, FFA 1.3, and albumin 0.6.

Statistical analysis of data was performed with the nonparametric Wilcoxon's test for pair differences and Spearman's coefficient of correlation.

### Results

Data on serum FT<sub>4</sub>, FFA, and albumin levels and FFA/albumin molar ratios before and after physical exercise

are presented in Table 1, and corresponding data on serum T<sub>4</sub>, TBG, and TSH levels and T<sub>4</sub>/TBG molar ratios are shown in Table 2. There was a mean 24.8% increase ( $P < 0.001$ ) in serum FT<sub>4</sub> and a 6.4-fold elevation in serum FFA after exercise. The albumin level rose by a mean of 19.1% and the FFA/albumin ratio was increased 5.3-fold after exercise. The postexercise mean serum TSH level was elevated by 41%. The pre- and postexercise FT<sub>4</sub>/T<sub>4</sub> mean molar ratios ( $\times 10^{-4}$ ) were 1.23 and 1.26, respectively.

A significant correlation was observed for  $\Delta$  FT<sub>4</sub> vs.  $\Delta$  FFA ( $r = 0.66$ ,  $P < 0.02$ ) and for  $\Delta$  FT<sub>4</sub> vs.  $\Delta$  FFA/albumin ratio ( $r = 0.75$ ,  $P < 0.01$ ) (Fig. 1). There was no significant correlation for  $\Delta$  FT<sub>4</sub> vs.  $\Delta$  TSH ( $r = -0.04$ , NS) or for  $\Delta$  T<sub>4</sub> vs.  $\Delta$  FFA ( $r = -0.25$ ; NS).

The following mean serum FT<sub>4</sub> concentrations (+/-SD) were measured after addition of oleic acid (mmol/L in brackets) *in vitro* (n = 4): 11.8 +/- 1.0 pmol/L (0), 12.3 +/- 1.7 pmol/L (0 + 1% ethanol), 10.5 +/- 0.6 pmol/L (0.3), 11.5 +/- 0.6 pmol/L (0.625), 12.0 +/- 0.0 pmol/L (1.25), 16.3 +/- 1.0 pmol/L (2.5) and 20.3 +/- 2.1 pmol/L (5.0). The 32.5% increase in serum FT<sub>4</sub> after the addition of 2.5 mmol/L oleic acid was significant ( $P < 0.001$ ).

### Discussion

During ultrafiltration of serum the concentration of T<sub>4</sub>-binding proteins in the retentate will not change the ambient FT<sub>4</sub> concentration (31) and centrifugal ultrafiltration has therefore been used by us (6) and others (28–30) for the determination of serum FT<sub>4</sub>. Hemoconcentration resembles ultrafiltration although the vascular bed is not entirely protein-impermeable as there is also passage of large molecules from the intravascular to the extravascular compartment in sections of the microvasculature. It is clear, however, that the free hormone concentration in serum is not influenced by the exercise-induced hemoconcentration *per se*.

If the exercise-induced increase in serum FT<sub>4</sub> were due to increased secretion of T<sub>4</sub> from the thyroid because of the small rise in serum TSH, of which approximately half can be explained by hemoconcentration, an increase would be expected in serum total T<sub>4</sub>. The relative increases in serum total T<sub>4</sub> and TBG were similar and therefore there was no change in the mean T<sub>4</sub>/TBG molar ratio indicating that the rise in serum T<sub>4</sub> was a consequence of hemoconcentration and not of an influx of hormone into the blood. Hemoconcentration will actually cause a proportionally increased net binding affinity and consequently a decrease in the free/bound hormone ratio. The unchanged ratio between free and bound hormone after exercise was therefore an unexpected finding indicating inhibition of T<sub>4</sub> to protein binding, pre-

TABLE 1. Serum FT<sub>4</sub>, FFA, and albumin concentrations and FFA/albumin molar ratios before (B) and after (A) a prolonged physical exercise in 14 males

Subject no.	FT <sub>4</sub> B	(pmol/L) A	FFA B	(mmol/L) A	Albumin B	(g/L) A	FFA/Alb B	(mol/mol) A
1	13	13	0.43	1.55	46	53	0.62	1.96
2	15	21	0.33	2.32	49	55	0.45	2.85
3	16	18	0.48	1.80	50	52	0.64	2.31
4	15	17	0.35	1.86	47	56	0.50	2.21
5	11	14	0.58	2.30	48	53	0.81	2.91
6	17	19	0.14	1.49	42	49	0.22	2.04
7	13	17	0.36	2.62	47	55	0.51	3.20
8	10	12	0.04	0.73	44	48	0.06	1.01
9	13	18	0.36	2.44	45	54	0.54	3.01
10	11	14	0.24	2.50	44	54	0.36	3.09
11	10	13	0.34	2.59	44	56	0.52	3.08
12	10	12	0.25	2.24	42	52	0.40	2.87
13	11	17	0.52	2.48	38	50	0.91	3.31
14	10	14	0.23	2.57	38	57	0.40	3.02
Mean	12.5	15.6	0.33	2.11	44.6	53.1	0.50	2.63
SD	2.4	2.8	0.15	0.55	3.7	2.7	0.22	0.64

TABLE 2. Serum T<sub>4</sub>, TBG, and TSH concentrations and T<sub>4</sub>/TBG molar ratios before (B) and after (A) a prolonged physical exercise in 14 males

Subject no.	T <sub>4</sub> B	(nmol/L) A	TBG B	(mg/L) A	T <sub>4</sub> /TBG B	(mol/mol) A	TSH B	(mU/L) A
1	99	121	25	28	0.24	0.26	1.3	3.4
2	87	104	17	18	0.31	0.35	1.6	2.0
3	99	104	26	26	0.23	0.24	2.0	1.8
4	104	123	27	29	0.24	0.26	6.7	8.4
5	106	136	30	34	0.22	0.24	1.0	1.4
6	125	144	32	36	0.24	0.24	1.1	1.3
7	97	107	25	28	0.24	0.23	1.9	3.3
8	120	129	29	33	0.25	0.24	1.2	1.9
9	93	125	27	38	0.21	0.20	1.2	2.1
10	104	125	28	33	0.23	0.23	0.8	1.1
11	85	108	19	24	0.27	0.27	1.3	1.7
12	90	124	31	44	0.18	0.17	1.0	1.9
13	115	155	29	38	0.24	0.25	1.4	1.7
14	105	137	24	30	0.26	0.28	1.3	1.4
Mean	102	124	26.4	31.4	0.240	0.247	1.7	2.4
SD	12	15	4.3	6.6	0.030	0.041	1.5	1.9

sumably by FFA. The absence of an association between raised serum TSH and FT<sub>4</sub> levels is further evidence against a TSH-induced accelerated thyroïdal secretion as the reason for the rise in serum FT<sub>4</sub>. Neither did Krotkiewski *et al.* (32) observe an increase in serum T<sub>4</sub> corrected for hemoconcentration in subjects studied after a 60-min bicycle ergometer exercise, although they also recorded a small significant increase in the mean serum TSH level.

The significant correlation between the rise in serum FT<sub>4</sub> and FFA indicates that displacement of T<sub>4</sub> from TBG by FFA, a phenomenon well known from earlier *in vitro* experiments (10–13) and patients given heparin, also an *in vitro* effect (20), could be the mechanism of the increase in serum FT<sub>4</sub> in the subjects studied after prolonged heavy exercise. If the increase in serum FT<sub>4</sub>

was caused by a shift of hormone from the intracellular to the extracellular compartment because of displacement of T<sub>4</sub> from cellular stores by FFA, or some other mechanism, there should be an increase in the T<sub>4</sub>/TBG ratio. However, the possibility of release of T<sub>4</sub> from intracellular stores cannot be rejected on the basis of the unchanged T<sub>4</sub>/TBG molar ratio observed, as competition between FFA and T<sub>4</sub> for binding sites on serum TBG will reduce the T<sub>4</sub>/TBG ratio, the net effect possibly resulting in an unaltered ratio. Therefore the possibility of displacement of T<sub>4</sub> from both intra- and extravascular binding sites must be recognized. This could also explain why an expected negative correlation between serum T<sub>4</sub> and FFA could not be detected, although there was a trend. The conclusion that displacement of T<sub>4</sub> from proteins by FFA is a likely mechanism for the 25% rise

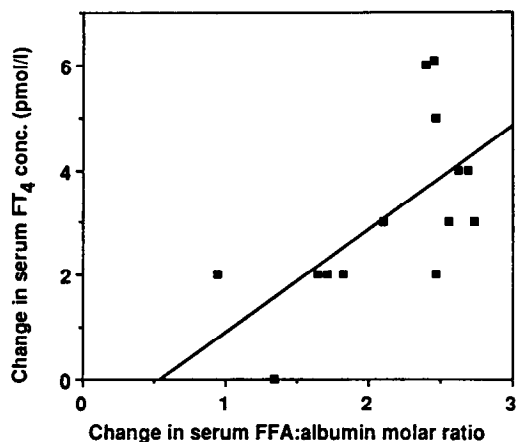


FIG. 1. Relationship between changes in the  $FT_4$  concentration, as determined by direct equilibrium dialysis, and changes in the FFA/albumin molar ratio in sera of males during a prolonged heavy running exercise. Linear regression analysis:  $Y = -1.02 + 1.95X$  ( $r = 0.75$ ,  $P < 0.01$ ,  $n = 14$ ).

in serum  $FT_4$  during exercise is supported by the observation that addition of 2.5 mmol/L oleic acid to serum *in vitro* resulted in an elevation in the  $FT_4$  level of a similar magnitude.

In a study of NTI patients we observed interrelated elevations in serum  $FT_4$  and FFA (and FFA/albumin ratio) (9) of a degree similar to those after physical exercise indicating that the increase in serum  $FT_4$  might have been caused by the rise in serum FFA. It should also be pointed out that displacement of  $T_4$  from protein binding sites increases  $T_4$  clearance, which has the effect of normalizing the  $FT_4$  level at a lower total  $T_4$  concentration. Even if displacement of FFA from intracellular stores may prevent the return of the  $FT_4$  level to normal this should be a temporary effect. Therefore the present observation of a FFA-induced increase in the serum  $FT_4$  level during exercise apparently has implication only for acute NTI. It is our experience that elevated dialyzable  $FT_4$  concentrations are found more often in acute than chronically sick euthyroid patients (9, 33).

Because evidence for the presence of inhibitors of  $T_4$ -protein binding could not be found by Mendel *et al.* (34) in their recent *in vitro* experiments on NTI sera, a cause-and-effect relationship between the alterations in  $FT_4$  and FFA levels does not necessarily exist. Drug effects are probably also of importance for the increase in serum  $FT_4$  reported in NTI. Particularly the possibility of an interaction between FFA and drugs must be accounted for as it has been reported that unsaturated FFA in concentrations that have no direct effect on  $T_4$ -binding can augment inhibition of  $T_4$ -binding by drugs such as furosemide and fenclofenac (35).

It is concluded that the observed increase in serum  $FT_4$  during physical exercise is possibly an effect of the elevation of serum FFA to about 2 mmol/L. Therefore

only in those NTI patients with an equal or higher increase in FFA (or a FFA/albumin molar ratio exceeding ~2.5) could unsaturated fatty acids be directly involved in raising serum  $FT_4$ .

## References

1. Oppenheimer JH, Squef R, Surks MI, Hauer H. Binding of thyroxine by serum proteins evaluated by equilibrium dialysis and electrophoretic techniques. Alterations in nonthyroidal illness. *J Clin Invest.* 1963;42:1769-82.
2. Ingbar SH, Braverman LE, Dawber NA, Lee GY. A new method for measuring the free thyroid hormone in human serum and an analysis of the factors that influence its concentration. *J Clin Invest.* 1965;44:1679-89.
3. Lutz J, Gregerman RI, Spaulding SW, Hornick RB, Dawkins Jr AT. Thyroxine binding proteins, free thyroxine and thyroxine turnover interrelationships during acute infectious illness in man. *J Clin Endocrinol Metab.* 1972;35:230-49.
4. Liewendahl K, Tikanoja S, Helenius T, Välimäki M. Discrepancies between serum free triiodothyronine and free thyroxine as measured by equilibrium dialysis and analog radioimmunoassay in nonthyroidal illnesses. *Clin Chem.* 1984;30:760-2.
5. Nelson JC, Tomei RT. The effect of serum dilution on free thyroxine ( $T_4$ ) concentration in the low  $T_4$  syndrome of nonthyroidal illness. *J Clin Endocrinol Metab.* 1985;61:239-46.
6. Tikanoja SH, Liewendahl BK. New ultrafiltration method for free thyroxine compared with equilibrium dialysis in patients with thyroidal dysfunction and nonthyroidal illness. *Clin Chem.* 1990;36:800-4.
7. Chopra IJ, Huang T-S, Beredo A, Solomon DH, Chua Teco GN, Mead JF. Evidence for an inhibitor of extrathyroidal conversion of thyroxine to 3,5,3'-triiodothyronine in sera of patients with nonthyroidal illnesses. *J Clin Endocrinol Metab.* 1985;60:666-72.
8. Vermaak WJH, Kalk WJ, Kuyl JM, Smit AM. Fatty acid induced changes in circulating total and free thyroid hormones: *in vivo* effects and methodological artefacts. *J Endocrinol Invest.* 1986;9:121-6.
9. Liewendahl K, Tikanoja S, Mähönen H, Helenius T, Välimäki M, Tallgren LG. Concentrations of iodothyronines in serum of patients with chronic renal failure and other nonthyroidal illnesses: role of free fatty acids. *Clin Chem.* 1987;33:1382-86.
10. Liewendahl K, Helenius T. Effect of fatty acids on thyroid function tests *in vitro* and *in vivo*. *Clin Chim Acta.* 1976;72:301-13.
11. Shaw W, Powell J, Hubert IL, Spierto FW. A comparison of the interference of fatty acids in the competitive binding radioassay and radioimmunoassay for serum  $T_4$ . *Clin Chim Acta.* 1976;73:25-9.
12. Tabachnick M, Korcek L. Effect of long-chain fatty acids on the binding of thyroxine and triiodothyronine to human thyroxine-binding globulin. *Biochem Biophys Acta.* 1986;881:292-6.
13. Bregengård C, Kirkegaard C, Faber J, Poulsen S, Siersbaek-Nielsen K, Friis T. The influence of free fatty acids on the free fraction of thyroid hormones in serum estimated by ultrafiltration. *Acta Endocrinol (Copenh).* 1987;116:102-7.
14. Chopra IJ, Chua Teco GN, Mead JF, Huang T-S, Beredo A, Solomon DH. Relationship between serum free fatty acids and thyroid hormone binding inhibitor in nonthyroidal illnesses. *J Clin Endocrinol Metab.* 1985;60:980-4.
15. Herrmann J, Allasso G, Beyer M, Heinen E, Römisch J, Weyer P. Thyroid hormone binding inhibitor (THBI) mainly associated with serum oleic acid concentration. *Horm Metab Res.* 1985;17:426-7.
16. Tikanoja SH, Joutti A, Liewendahl BK. Association between increased concentrations of free thyroxine and unsaturated free fatty acids in non-thyroidal illnesses: role of albumin. *Clin Chim Acta.* 1989;179:33-44.
17. Mendel CM, Frost PH, Cavalieri RR. Effect of free fatty acids on the concentration of free thyroxine in human serum: the role of albumin. *J Clin Endocrinol Metab.* 1986;63:1394-99.
18. Nicolson RE, Reilly CP, Pannall PR, Esposito L, Wellby ML. Do

- nonesterified fatty acids displace thyroxine from its plasma binding sites in severe nonthyroidal illnesses? *Clin Chem*. 1989;35:931-4.
19. Hollander CS, Scott RL, Burgess JA, Rabinowitz D, Merimee TJ, Oppenheimer JH. Free fatty acids: a possible regulator of free thyroid hormone levels in man. *J Clin Endocrinol Metab*. 1967;27:1219-23.
  20. Mendel CM, Frost PH, Kunitake ST, Cavalieri RR. Mechanism of the heparin-induced increase in the concentration of free thyroxine in plasma. *J Clin Endocrinol Metab*. 1987;65:1259-64.
  21. Larsen PR. Salicylate-induced increases in free triiodothyronine in human serum. *J Clin Invest*. 1972;51:1125-34.
  22. Lim C-F, Bai Y, Topliss DJ, Barlow JW, Stockigt JR. Drug and fatty acid effects on serum thyroid hormone binding. *J Clin Endocrinol Metab*. 1988;67:628-8.
  23. Refsum HE, Strømme SB. Serum thyroxine, triiodothyronine and thyroid stimulating hormone after prolonged heavy exercise. *Scand J Clin Lab Invest*. 1979;39:455-9.
  24. Sander M, Röcker L. Influence of marathon running on thyroid hormones. *Int J Sports Med*. 1988;9:123-6.
  25. Pakarinen A, Alén M, Häkkinen K, Komi P. Serum thyroid hormones, thyrotropin and thyroxine binding globulin during prolonged strength training. *Eur J Appl Physiol*. 1988;57:394-8.
  26. Helenius T, Liewendahl K. Improved dialysis method for free thyroxine in serum compared with five commercial radioimmunoassays in nonthyroidal illnesses and subjects with abnormal concentrations of thyroxine-binding globulin. *Clin Chem*. 1983;29:816-22.
  27. Shimizu S, Inoue K, Tani Y, Yamada H. Enzymatic microdetermination of serum free fatty acids. *Anal Biochem*. 1979;98:341-5.
  28. Faber J, Rogowski P, Kirkegaard C, Siersbaek-Nielsen K, Friis T. Serum free T<sub>4</sub>, T<sub>3</sub>, rT<sub>3</sub>, 3,3'-diiodothyronine and 3',5'-diiodothyronine measured by ultrafiltration. *Acta Endocrinol (Copenh)*. 1984;107:357-65.
  29. Lee LA, Mooney RA, Woolf PD. Clinical utility of measuring free thyroxine and free triiodothyronine in serum of critically ill patients by ultrafiltration. *Clin Chem*. 1986;32:797-800.
  30. Weeke J, Boye N, Ørskov H. Ultrafiltration method for direct radioimmunoassay measurement of free thyroxine and free triiodothyronine in serum. *Scand J Clin Lab Invest*. 1986;46:381-9.
  31. Ekins R. Measurement of free hormones in blood. *Endocr Rev*. 1990;11:5-46.
  32. Krotkiewski M, Sjöström L, Sullivan P-A et al. The effect of acute and chronic exercise on thyroid hormones in obesity. *Acta Med Scand*. 1984;216:269-75.
  33. Välimäki M, Liewendahl K, Nikkanen P, Pelkonen R. Hormonal changes in severely uncontrolled type 1 (insulin-dependent) diabetes mellitus. *Scand J Clin Lab Invest*. 1991;51:385-93.
  34. Mendel CM, Laughton CW, McMahon FA, Cavalieri RR. Inability to detect an inhibitor of thyroxine-serum protein binding in sera from patients with nonthyroid illness. *Metabolism*. 1991;40:491-502.
  35. Lim C-F, Curtis AJ, Barlow JW, Topliss DJ, Stockigt JR. Interactions between oleic acid and drug competitors influence specific binding of thyroxine in serum. *J Clin Endocrinol Metab*. 1991;73:1106-10.